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Air Quality Criteria for Ozone and Related Photochemical Oxidants

Volume I of III

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Preface

In 1971, the U.S. Environmental Protection Agency (EPA) promulgated National Ambient Air Quality Standards (NAAQS) to protect the public health and welfare from adverse effects of photochemical oxidants. In 1979, the chemical designation of the standards was changed from photochemical oxidants to ozone (O_3). This document focuses primarily on the scientific air quality criteria for O_3 and, to a lesser extent, on those for other photochemical oxidants such as hydrogen peroxide and the peroxyacyl nitrates.

The EPA promulgates the NAAQS on the basis of scientific information contained in air quality criteria issued under Section 108 of the Clean Air Act. The previous O_3 criteria document, *Air Quality Criteria for Ozone and Other Photochemical Oxidants*, was released in August 1986 and a supplement, *Summary of Selected New Information on Effects of Ozone on Health and Vegetation*, was released in January 1992. These documents were the basis for a March 1993 decision by EPA that revision of the existing 1-h NAAQS for O_3 was not appropriate at that time. That decision, however, did not take into account some of the newer scientific data that became available after completion of the 1986 criteria document. The purpose of this revised air quality criteria document for O_3 and related photochemical oxidants is to critically evaluate and assess the latest scientific data associated with exposure to the concentrations of these pollutants found in ambient air. Emphasis is placed on the presentation of health and environmental effects data; however, other scientific data are presented and evaluated in order to provide a better understanding of the nature, sources, distribution, measurement, and concentrations of O_3 and related photochemical oxidants and their precursors in the environment. Although the document is not intended to be an exhaustive literature review, it is intended to cover all pertinent literature available through 1995.

This document was prepared and peer reviewed by experts from various state and Federal governmental offices, academia, and private industry and reviewed in several public meetings by the Clean Air Scientific Advisory Committee. The National Center for Environmental Assessment (formerly the Environmental Criteria and Assessment Office) of EPA's Office of Research and Development acknowledges with appreciation the contributions provided by these authors and reviewers as well as the diligence of its staff and contractors in the preparation of this document at the request of the Office of Air Quality Planning and Standards.

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1

Executive Summary

1.1 Introduction

Air Quality Criteria for Ozone and Related Photochemical Oxidants evaluates the latest scientific information useful in deriving criteria that form the scientific basis for U.S. Environmental Protection Agency (EPA) decisions regarding the National Ambient Air Quality Standards (NAAQS) for ozone (O_3). This Executive Summary concisely summarizes key conclusions from the document, which comprises nine chapters. Following this Executive Summary is a brief Introduction (Chapter 2) containing information on the legislative and regulatory background for review of the O_3 NAAQS, as well as a brief discussion of the issues presented and the format for their discussion in the document. Chapter 3 provides information on the chemistry, sources, emissions, measurement, and transport of O_3 and related photochemical oxidants and their precursors, whereas Chapter 4 covers environmental concentrations, patterns, and exposure estimates of O_3 and oxidants. Chapter 5 deals with environmental effects, and Chapters 6, 7, and 8 discuss animal toxicological studies, human health effects, and extrapolation of animal toxicological data to humans, respectively. The last chapter, Chapter 9, provides an integrative, interpretative characterization of health effects associated with exposure to O_3. The following sections conform to the chapter organization of the criteria document.

1.2 Legislative and Regulatory Background

The photochemical oxidants found in ambient air in the highest concentrations are O_3 and nitrogen dioxide (NO_2). Other oxidants, such as hydrogen peroxide (H_2O_2) and peroxyacyl nitrates, also have been observed, but in lower and less certain concentrations. In 1971, EPA promulgated NAAQS to protect the public health and welfare from adverse effects of photochemical oxidants, at that time, defined on the basis of commercially available measurement methodology. After 1971, however, O_3-specific commercial analytical methods became available, as did information on the concentrations and effects of the related non-O_3 photochemical oxidants. As a result, the chemical designation of the standards was changed in 1979 from photochemical oxidants to O_3.

The EPA is required under Sections 108 and 109 of the Clean Air Act to evaluate periodically the air quality criteria that reflect the latest scientific information relevant to review of the O_3 NAAQS. These air quality criteria are useful for indicating the kind and extent of all identifiable effects on public health or welfare that may be expected from the presence of O_3 and related photochemical oxidants in ambient air. The last O_3 criteria

document was released in 1986, and a supplement was released in 1992. These documents were the basis for a March 1993 decision by EPA that revision of the existing 1-h NAAQS for O_3 was not appropriate at that time. That decision, however, did not take into consideration more recent scientific information that has been published since the last literature review in early 1989. The purpose of this revised criteria document, therefore, is to summarize the pertinent information contained in the previous O_3 criteria document and to critically evaluate and assess the more recent scientific data associated with exposure to O_3 and, to a lesser extent, to H_2O_2 and the peroxyacyl nitrates, particularly peroxyacetyl nitrate (PAN). This document will be used by EPA's Office of Air Quality Planning and Standards to provide a staff paper assessing the most significant scientific information and presenting staff recommendations on whether revisions to the O_3 NAAQS are appropriate.

1.3 Tropospheric Ozone and Its Precursors

Introduction

Ozone is found in the stratosphere, the "free" troposphere, and the planetary boundary layer (PBL) of the earth's atmosphere. In the PBL, background O_3 occurs as the result of (1) the intrusions of stratospheric O_3 into the "free" troposphere and downward transport into the PBL, and (2) photochemical reactions of methane (CH_4), carbon monoxide (CO), and nitrogen oxides (NO_x). These processes contribute to the background O_3 near the surface. The major source of O_3 in the PBL is the photochemical process involving anthropogenic and biogenic emissions of NO_x with the many classes of volatile organic compounds (VOCs).

The topics considered in this section of the document include: tropospheric O_3 chemistry; meteorological influences on O_3 formation and transport; precursor VOC and NO_x emissions, ambient concentrations of VOCs and NO_x, and source apportionment and reconciliation of measured VOC ambient concentrations with emission inventories; O_3 air quality models; and analytical methods for oxidants and precursors.

Tropospheric Ozone Chemistry

Ozone occurs in the stratosphere as the result of chemical reactions initiated by short-wavelength radiation from the sun. In the "free" troposphere, O_3 occurs as the result of incursions from the stratosphere; upward venting from the PBL (the layer next to the surface of the earth) through certain cloud processes; and photochemical formation from precursors, notably CH_4, CO, and NO_x.

The photochemical production of O_3 and other oxidants found at the surface of the earth (in the PBL, troposphere, or ambient air [used interchangeably in this summary]) is the result of atmospheric physical processes and complex, nonlinear chemical processes involving two classes of precursor pollutants: (1) reactive anthropogenic and biogenic VOCs and (2) NO_x. The only significant initiator of the photochemical production of O_3 in the polluted troposphere is the photolysis of NO_2, yielding nitric oxide (NO) and a ground-state oxygen atom that reacts with molecular oxygen to form O_3. The O_3 thus formed reacts with NO, yielding oxygen and NO_2. These cyclic reactions attain equilibrium in the absence of VOCs. However, in the presence of VOCs, which are abundant in polluted ambient air, the equilibrium is upset, resulting in a net increase in O_3. Methane is the chief VOC found in the free troposphere and in most "clean" areas of the PBL. The VOCs found in polluted

ambient air are much more complex and more reactive than CH_4, but, as with CH_4, their atmospheric oxidative degradation is initiated through attack on the VOCs by hydroxyl (OH) radicals. As in the CH_4 oxidation cycle, the conversion of NO to NO_2 during the oxidation of VOCs is accompanied by the production of O_3 and the efficient regeneration of the OH radical. The O_3, PAN, and higher homologues formed in polluted atmospheres increase with the NO_2/NO concentration ratio.

At night, in the absence of photolysis of reactants, the simultaneous presence of O_3 and NO_2 results in the formation of the nitrate (NO_3) radical. Reactions with NO_3 radicals appear to constitute major sinks for alkenes, cresols, and several other compounds, although the chemistry is not well characterized.

Most inorganic gas-phase processes (i.e., the nitrogen cycle and its interrelationships with O_3 production) are well understood. The chemistry of the VOCs in ambient air is not as well understood. It is well known, however, that the chemical loss processes of gas-phase VOCs include reaction with OH and NO_3 radicals and O_3, and photolysis. Reaction with the OH radical is the only important atmospheric reaction (loss process) for alkanes, aromatic hydrocarbons, and the higher aldehydes and ketones that lack $>C=C<$ bonds; and the only atmospheric reaction of alcohols and ethers. Photolysis is the major loss process for formaldehyde and acetone. Reactions with OH and NO_3 radicals and with O_3 are all important loss processes for alkenes and for carbonyls containing $>C=C<$ bonds.

Uncertainties in the atmospheric chemistry of the VOCs can affect quantification of the NO-to-NO_2 conversion and of O_3 yields, and can present difficulties in representation of chemical mechanisms, products, and product yields in O_3 air quality models. Major uncertainties in understanding the atmospheric chemistry of the VOCs with NO_x in both urban and rural atmospheres include chemistry of alkyl nitrate formation, mechanisms and products of $>$C4 *n*-alkanes and branched alkanes, mechanisms and products of alkene-O_3 reactions, and mechanisms and products of aromatic hydrocarbons.

It should be noted that the atmospheric chemical processes involved in the photooxidation of certain higher molecular weight VOCs and in the formation of O_3 also can lead to the formation of particulate-phase organic compounds. The OH radicals produced not only can oxidize VOCs to particulate-phase organic compounds but also can react with NO_2 and sulfur dioxide (SO_2) to form nitric acid (HNO_3) and sulfuric acid (H_2SO_4), respectively, portions of which become incorporated into aerosols as particulate nitrate and sulfate.

Meteorological Influences on Ozone Formation and Transport

The surface energy (radiation) budget of the earth strongly influences the dynamics of the PBL. The redistribution of energy through the PBL creates thermodynamic conditions that influence vertical mixing. Growing evidence indicates that the strict use of mixing heights in modeling is an oversimplification of the complex processes by which pollutants are redistributed within urban areas, and that it is necessary to treat the turbulent structure of the atmosphere directly and acknowledge the vertical variations in mixing. Energy balances therefore require study so that more realistic simulations can be made of the structure of the PBL.

Day-to-day variability in O_3 concentrations depends heavily on day-to-day variations in meteorological conditions, including temperature, solar radiation, and the degree of mixing that occurs between release of a pollutant or its precursors and their arrival at a

receptor; the occurrence of inversion layers (layers in which temperature increases with height above ground level); and the transport of O_3 left overnight in layers aloft and subsequent downward mixing of that O_3 to the surface.

The transport of O_3 and its precursors beyond the urban scale (≤ 50 km) to neighboring rural and urban areas has been well documented. Episodes of high O_3 concentrations in urban areas are often associated with high concentrations of O_3 in the surroundings. Areas of O_3 accumulation usually are characterized by synoptic-scale subsidence of air in the free troposphere, resulting in development of an elevated inversion layer; relatively low wind speeds associated with the weak horizontal pressure gradient around a surface high pressure system; a lack of cloudiness; and high temperatures.

Ultraviolet (UV) radiation from the sun plays a key role in initiating the photochemical processes leading to O_3 formation and affects individual photolytic reaction steps. Still, there is little empirical evidence in the literature linking day-to-day variations in observed UV radiation levels to variations in O_3 levels. An association, however, between tropospheric O_3 concentrations and temperature has been demonstrated. Empirical data from four urban areas, for example, show an apparent upper bound on O_3 concentrations that increases with temperature. A similar qualitative relationship exists at a number of rural locations.

The relationship between wind speed and O_3 buildup varies from one part of the country to another.

Statistical techniques (e.g., regression techniques) can be used to help identify real trends in O_3 concentrations, both intra- and interannual, by normalizing meteorological variability.

Precursors

Volatile Organic Compound Emissions

Hundreds of VOCs, usually containing from 2 to 12 carbon atoms, are emitted by evaporative and combustion processes from a large number of source types. Total U.S. anthropogenic VOC emissions in 1991 were estimated at 21.0 Tg; the two largest source categories were (1) industrial processes (10.0 Tg) and (2) transportation (7.9 Tg). Emissions of VOCs from highway vehicles accounted for almost 75% of the transportation-related emissions; studies have shown that the majority of these VOC emissions come from about 20% of the automobiles in service, many, perhaps most, of which are older cars that are poorly maintained. The accuracy of VOC emission estimates is difficult to determine for both stationary and mobile sources.

Vegetation emits significant quantities of VOCs into the atmosphere, chiefly monoterpenes and isoprene, but also oxygenated VOCs, according to recent studies. The most recent biogenic VOC emissions estimate for the United States showed annual emissions of 29.1 Tg/year.

Although the biogenic VOC emission estimates exceed the anthropogenic estimates, the biogenic emissions are more diffusely distributed than the anthropogenic emissions, which tend to be concentrated in population centers. However, the large uncertainties in both biogenic and anthropogenic VOC emission inventories prevent establishing the relative contributions of these two categories.

Nitrogen Oxides Emissions

Anthropogenic NO_x is associated with combustion processes. The primary pollutant emitted is NO, formed at high combustion temperatures from nitrogen and oxygen in the air and from nitrogen in the combustion fuel. Emissions of NO_x in 1991 in the United States totaled 21.39 Tg. The two largest single NO_x emission sources are electric power generating plants and highway vehicles. Because a large proportion of anthropogenic NO_x emissions come from distinct point sources, published annual estimates are thought to be much more reliable than VOC estimates.

Natural NO_x sources include stratospheric intrusion, oceans, lightning, soil, and wildfires. Lightning and soil emissions are the only two significant natural sources of NO_x in the United States. It is estimated that combined natural sources contribute about 2.2 Tg of NO_x to the troposphere over the continental United States; however, uncertainties in natural NO_x emission inventories are much greater than those for anthropogenic NO_x emissions.

Concentrations of Volatile Organic Compounds in Ambient Air

The VOCs most frequently analyzed in ambient air are the nonmethane hydrocarbons (NMHCs). Morning (6:00 to 9:00 a.m.) concentrations most often have been measured because of the use of morning data in the Empirical Kinetic Modeling Approach (EKMA) and in air quality simulation models.

Concurrent measurements of anthropogenic and biogenic NMHCs have shown that biogenic NMHCs usually constituted much less than 10% of the total NMHCs. For example, average isoprene concentrations ranged from 0.001 to 0.020 ppm carbon (C) and terpenes from 0.001 to 0.030 ppm C.

Concentrations of Nitrogen Oxides in Ambient Air

Measurements of NO_x made in 22 and 19 U.S. cities in 1984 and 1985, respectively, showed median 6:00-to-9:00 a.m. NO_x concentrations ranging from 0.02 to 0.08 ppm in most of these cities. Nonurban NO_x concentrations, reported as average seasonal or annual NO_x, range from <0.005 to 0.015 ppm.

Ratios of Concentrations of Nonmethane Organic Compounds to Nitrogen Oxides

Ratios of 6:00-to-9:00 a.m. nonmethane organic compounds (NMOC) to NO_x are higher in southeastern and southwestern U.S. cities than in northeastern and midwestern U.S. cities, according to data from EPA's multicity studies conducted in 1984 and 1985. Rural NMOC/NO_x ratios tend to be higher than urban ratios. The NMOC/NO_x ratios trended downward to well below 10 in the South Coast Air Basin and in cities in the eastern United States during the 1980s. Based on these low ratios, hydrocarbon control should be more effective than NO_x control within a number of cities. Morning (6:00-to-9:00 a.m.) NMOC/NO_x ratios are used in the EKMA type of trajectory model. The correlation of NMOC/NO_x ratios with maximum 1-h O_3 concentrations, however, was weak in a recent analysis.

Source Apportionment and Reconciliation

Source apportionment (regarded as synonymous with receptor modeling) refers to determining the quantitative contributions of various sources of VOCs to ambient air pollutant concentrations. Source reconciliation refers to the comparison of measured ambient

VOC concentrations with emissions inventory estimates of VOC source emission rates for the purpose of validating the inventories.

Recent findings have shown that vehicle exhaust was the dominant contributor to ambient VOCs in seven of eight U.S. cities studied. Whole gasoline contributions were estimated to be equal to vehicle exhaust in one study and to 20% of vehicle exhaust in a second study.

Estimates of biogenic VOCs at a downtown site in Atlanta, GA, in 1990 indicated a lower limit of 2% (24-h average) for the biogenic percentage of total ambient VOCs at that location (isoprene was used as the biogenic indicator species). The percentage varies during the 24-h period because of the diurnal (e.g., temperature, light intensity) dependence of isoprene concentrations.

Source reconciliation data have shown disparities between emission inventory estimates and receptor-estimated contributions. For biogenics, emission estimates are greater than receptor-estimated contributions. The reverse has been true for natural gas contributions estimated for Los Angeles, CA; Columbus, OH; and Atlanta; and for refinery emissions in Chicago, IL.

Ozone Air Quality Models

Models and Their Components

Photochemical air quality models are used to predict how O_3 concentrations change in response to prescribed changes in source emissions of NO_x and VOCs. These models operate on sets of input data that characterize the emissions, topography, and meteorology of a region and produce outputs that describe air quality in that region.

Two kinds of photochemical models are recommended in guidelines issued by EPA: (1) the use of EKMA is accepted under certain circumstances, and (2) the grid-based Urban Airshed Model (UAM) is recommended for modeling O_3 over urban areas. The 1990 Clean Air Act Amendments mandate the use of three-dimensional (grid-based) air quality models such as UAM in developing state implementation plans for areas designated as "extreme", "severe", "serious", or "multistate moderate". General descriptions of EKMA and grid-based models were given in the 1986 EPA criteria document for O_3.

The EKMA-based method for determining O_3 control strategies has limitations, the most serious of which is that predicted emissions reductions are critically dependent on the initial NMHC/NO_x ratio used in the calculations. This ratio cannot be determined with any certainty and is expected to be quite variable in time and space in an urban area.

Spatial and temporal characteristics of VOC and NO_x emissions are major inputs to a grid-based photochemical air quality model. Greater accuracy in emissions inventories is needed for biogenics and for both mobile and stationary source components. Grid-based air quality models also require as input the three-dimensional wind field for the photochemical episode being simulated.

A chemical kinetic mechanism, representing the important chemical reactions that occur in the atmosphere, is used in an air quality model to estimate the net rate of formation of each pollutant simulated as a function of time.

Dry deposition is an important removal process for O_3 on both urban and regional scales and is included in all urban- and regional-scale models. Wet deposition is generally not included in urban-scale photochemical models, because O_3 episodes do not occur during periods of significant clouds or rain.

Concentration fields of all species computed by the model must be specified at the beginning of the simulation ("initial conditions"). These initial conditions are determined mainly with ambient measurements, either from routinely collected data or from special studies; but interpolation can be used to distribute the surface ambient measurements.

Use of Ozone Air Quality Models

Photochemical air quality models are used for control strategy evaluation by first demonstrating that a past episode or episodes can be simulated adequately. The hydrocarbon or NO_x emissions or both are reduced in the model inputs, and the effects of these reductions on O_3 in the region are assessed. The adequacy of control strategies based on grid-based models depends, in part, on the nature of input data for simulations and model validation, on input emissions inventory data, and on the mismatch between the spacial output of the model and the current form of the NAAQS for O_3. Uncertainties in models obviously can affect their outputs. Uncertainties exist in all components of grid-based O_3 air quality models: emissions, meteorological modules, chemical mechanisms, deposition rates, and determination of initial conditions.

Grid-based models that have been widely used to evaluate control strategies for O_3 or acid deposition, or both, are the UAM, the California Institute of Technology/Carnegie Institute of Technology model, the Regional Oxidant Model, the Acid Deposition and Oxidant Model, and the Regional Acid Deposition Model. The UAM (Version IV) is the grid model approved nationwide for control strategy development at this time.

Despite the many uncertainties in photochemical air quality modeling, including emission inventories, these models are essential for regulatory analysis and solving the O_3 problem. Grid-based O_3 air quality modeling is superior to the available alternatives for O_3 control planning, but the chances of its incorrect use must be minimized.

Analytical Methods for Oxidants and Their Precursors

Oxidants

Current methods used to measure O_3 are chemiluminescence (CL); UV absorption spectrometry; and newly developed spectroscopic and chemical approaches, including chemical approaches applied to passive sampling devices (PSDs) for O_3.

The CL method has been designated as the reference method by EPA. Detection limits of 0.005 ppm and a response time of <30 s are typical of currently available commercial instruments. A positive interference from atmospheric water vapor was reported in the 1970s and recently has been confirmed. Proper calibration can minimize this source of error.

Commercial UV photometers for measuring O_3 have detection limits of about 0.005 ppm and a response time of <1 min. Because the measurement is absolute, UV photometry is also used to calibrate O_3 methods. A potential disadvantage of UV photometry is that atmospheric constituents that absorb 254-nm radiation, the wavelength at which O_3 is measured, will cause a positive interference in O_3 measurements. Interferences have been reported in two recent studies, but assessment of the potential importance of such interferences (e.g., toluene, styrene, cresols, nitrocresols) is hindered by lack of absorption spectra data in the 250-nm range and by lack of aerometric data for the potentially interfering species. There also can be some interference from water, possibly from the condensation of moisture in sampling lines.

Calibration of O_3 measurement methods (other than PSDs) is done by UV spectrometry or by gas-phase titration (GPT) of O_3 with NO. Ultraviolet photometry is the reference calibration method approved by EPA. Ozone is unstable and must be generated in situ at time of use to produce calibration mixtures.

Peroxyacetyl nitrate and the higher peroxyacyl nitrates normally are measured by gas chromatography (GC) using an electron capture detector. Detection limits have been extended to 1 to 5 ppt. The preparation of reliable calibration standards is difficult because PAN is unstable, but several methods are available.

Volatile Organic Compounds

The method recommended by EPA for total NMOC measurement involves the cryogenic preconcentration of NMOCs and the measurement of the revolatilized NMOCs using flame ionization detection (FID). The primary technique for speciated NMOC/NMHC measurements is cryogenic preconcentration followed by GC-FID. Systems for sampling and analysis of VOCs have been developed that require no liquid cryogen for operation.

Stainless steel canisters have become the containers of choice for collection of whole-air samples for NMHC/NMOC data. Calibration procedures for NMOC instrumentation require the generation, by static or dynamic systems, of dilute mixtures at concentrations expected to occur in ambient air.

Preferred methods for measuring carbonyl species (aldehydes and ketones) in ambient air are spectroscopic methods; on-line colorimetric methods; and, the most common method currently in use for measuring gas-phase carbonyl compounds in ambient air, the high-performance liquid chromatography method, which employs 2,4-dinitrophenylhydrazine derivatization in a silica gel cartridge. Use of an O_3 scrubber has been recommended to prevent interference by O_3 in this method in ambient air.

Oxides of Nitrogen

Nitric oxide and NO_2 comprise the NO_x compounds involved as precursors to O_3 and other photochemical oxidants.

The most common method of NO measurement is the gas-phase CL reaction with O_3, which is essentially specific for NO. Commercial NO monitors have detection limits of a few parts per billion by volume (ppbv) in ambient air but may not have sensitivity sufficient for surface measurements in rural or remote areas or for airborne measurements. Direct spectroscopic methods for NO exist that have very high sensitivity and selectivity for NO, but their complexity, size, and cost restrict these methods to research applications. No PSDs exist for measurement of NO.

Chemiluminescence analyzers are the tools of choice for NO_2 measurement, even though they do not measure NO_2 directly. Minimum detection levels for NO_2 have been reported to be 5 to 13 ppb, but more recent evaluations have indicated detection limits of 0.5 to 1 ppbv. Reduction of NO_2 to NO is required for measurement. These analyzers actually measure NO_y (NO_x + PAN + HNO_3 + other reactive nitrogen species); however, for most urban atmospheres, NO_x is the predominant species measured diurnally.

Several spectroscopic approaches to NO_2 detection have been developed but share the drawbacks of spectroscopic NO methods. Passive samplers for NO_2 exist but are still in the developmental stage for ambient air monitoring.

Calibration of methods for NO measurement is done using standard cylinders of NO in nitrogen. Calibration of methods for NO_2 measurement include use of cylinders of NO_2 in nitrogen or air, use of permeation tubes, and GPT.

1.4 Environmental Concentrations, Patterns, and Exposure Estimates

Ozone is measured at concentrations above the minimum detectable level at all monitoring locations in the world. In this section, hourly average concentration and exposure information is summarized for urban, rural forested, and rural agricultural areas in the United States.

Because O_3 from urban area emissions is transported to rural downwind locations, elevated O_3 concentrations can occur at considerable distances from urban centers. Urban O_3 concentration values are often depressed because of titration by NO. Because of the absence of chemical scavenging, O_3 tends to persist longer in nonurban areas than in urban areas, and nonurban exposures may be higher than those in urban locations.

Trends

Ozone hourly average concentrations have been recorded for many years by the state and local air pollution agencies who report their data to EPA. The 10-year (1983 to 1992) composite average trend for the second highest daily maximum hourly average concentration during the O_3 season shows that the 1992 composite average for the trend sites was 21% lower than the 1983 average. The 1992 value was the lowest composite average of the 10-year period and was significantly less than each of the previous nine years, 1983 to 1991. The relatively high O_3 concentrations in 1983 and 1988 likely were attributable, in part, to hot, dry, stagnant conditions in some areas of the country, which were especially conducive to O_3 formation.

From 1991 to 1992, the composite mean of the second highest daily maximum 1-h O_3 concentrations decreased 7%, and the composite average of the number of estimated exceedances of the O_3 standard decreased by 23%. Nationwide VOC emissions decreased 3% from 1991 to 1992. The composite average of the second daily maximum concentrations decreased in 8 of the 10 EPA regions from 1991 to 1992, and remained unchanged in Region VII. Except for Region VII, the 1992 regional composite means were lower than the corresponding 1990 levels. Although meteorological conditions in the east during 1993 were more conducive to O_3 than those in 1992, the composite mean level for 1993 was the second lowest composite average of the decade, 1984 to 1993.

Surface Concentrations

Published data provide evidence showing the occurrence at some sites of multihour periods within a day of O_3 at levels of potential health effects. Although most of these analyses were made using monitoring data collected from sites in or near nonattainment areas, in one analysis of five sites (two in New York state, two in rural California, and one in rural Oklahoma), none of which was in or near a nonattainment area, O_3 concentrations showed only moderate peaks but showed multihour levels above 0.1 ppm.

A small amount of the O_3 concentration measured at a monitoring site is produced by sources distant to the photochemical reactions occurring on an urban or regional scale. Typical sources include stratospheric intrusions into the troposphere, photochemical production by the $CH_4/CO/NO_x$ cycle in the troposphere, and transport of very distant anthropogenic or biogenic VOCs and NO_x. The specific concentrations of this "background" O_3 vary with averaging times ranging from the daily 1-h maximum to daily, monthly, seasonal, or annual values. The background concentrations also vary with geographical region and with elevation of the monitoring site.

On the basis of O_3 data from isolated monitoring sites, EPA has indicated that a reasonable estimate of O_3 background concentration near sea level in the United States is from 0.020 to 0.035 ppm for an annual average, 0.025 to 0.045 ppm for an 8-h daily summer seasonal average, and from 0.03 to 0.05 ppm for the average summertime 1-h daily maximum. This estimate includes a 0.005 to 0.015 ppm O_3 contribution from stratospheric intrusions into the troposphere.

Diurnal Variations

Diurnal patterns of O_3 may be expected to vary with location, depending on the balance among the many factors affecting O_3 formation, transport, and destruction. Although they vary with locality, diurnal patterns of O_3 typically show a rise in concentration from low levels, or levels near minimum detectable amounts, to an early afternoon peak. The diurnal pattern of concentrations can be ascribed to three simultaneous processes: (1) downward transport of O_3 from layers aloft, (2) destruction of O_3 through contact with surfaces and through reaction with NO at ground level, and (3) in situ photochemical production of O_3.

Seasonal Patterns

Seasonal variations in O_3 concentrations in urban areas usually show the pattern of high O_3 in the late spring or in the summer and low levels in the winter; however, weather conditions in a given year may be more favorable for the formation of O_3 and other oxidants than during the prior or following year.

Average O_3 concentrations tend to be higher in the second versus the third quarter of the year for many isolated rural sites. This observation has been attributed to either stratospheric intrusions or an increasing frequency of slow-moving, high-pressure systems that promote the formation of O_3. However, for several clean rural sites, the highest exposures have occurred in the third quarter rather than in the second. For rural O_3 sites in the southeastern United States, the daily maximum 1-h average concentration was found to peak during the summer months.

Spatial Variations

Concentrations of O_3 vary with altitude and with latitude. There appears to be no consistent conclusion concerning the relationship between O_3 exposure and elevation.

Indoor Ozone

Until the early 1970s, very little was known about the O_3 concentrations experienced inside buildings; to date, the database on this subject is not large, and a wide range of indoor/outdoor O_3 concentration relationships can be found in the literature

(reported indoor/outdoor values for O_3 are highly variable). Indoor/outdoor O_3 concentration ratios generally fall in the range from 0.1 to 0.7 and indoor concentrations of O_3 almost invariably will be less than outdoors.

Estimating Exposure

Both fixed-site monitoring information and human exposure models are used to estimate risks associated with O_3 exposure. Because, for most cases, it is not possible to estimate population exposure solely from fixed-station data, several human exposure models have been developed. These models also contain submodels depicting the sources and concentrations likely to be found in each microenvironment, including indoor, outdoor, and in-transit settings. Two distinct types of O_3 exposure models exist: (1) those that focus narrowly on predicting indoor O_3 levels and (2) those that focus on predicting O_3 exposures on a community-wide basis. These latter models and their distinguishing features are:

1. pNEM/O_3 based on the National Air Quality Standards Exposure Model (NEM) series of models
   - Uses mass-balance approach and seasonal considerations for I/O ratio estimation.
   - Variables affecting indoor exposure obtained by Monte Carlo sampling from empirical distributions of measured data.
2. Systems Applications International (SAI)/NEM
   - More districts and microenvironments and more detailed mass-balance model than pNEM/O_3.
   - Human activity data outdated and inflexible.
3. Regional Human Exposure Model (REHEX)
   - More detailed geographic resolution than NEM.
   - Uses California-specific activity data and emphasizes in-transit and outdoor microenvironments.
4. Event probability exposure model (EPEM)
   - Estimates probability that a randomly selected person will experience a particular exposure regime.
   - Lacks multiday continuity.

Few data are available for individuals using personal exposure monitors. Results from a pilot study demonstrated that fixed-site ambient measurements may not adequately represent individual exposures. Models based on time-weighted indoor and outdoor concentrations explained only 40% of the variability in personal exposures.

Peroxyacyl Nitrates

Peroxyacetyl nitrate and peroxypropionyl nitrate (PPN) are the most abundant of the non-O_3 oxidants in ambient air in the United States, other than the inorganic nitrogenous oxidants such as NO_2, and possibly HNO_3. Most of the available data on concentrations of PAN and PPN in ambient air are from urban areas. The levels to be found in nonurban areas will be highly dependent on the transport of PAN and PPN or their precursors from urban areas, because the concentrations of the NO_x precursors to these compounds are considerably lower in nonurban areas than in urban areas.

Co-occurrence

Studies of the joint occurrence of gaseous NO_2/O_3 and SO_2/O_3 at rural sites have concluded that the periods of co-occurrence represent a small portion of the potential plant-growing period. For human ambient exposure considerations, in most cases, the simultaneous co-occurrence of NO_2/O_3 and SO_2/O_3 was infrequent. Some researchers have reported the joint occurrence of O_3, nitrogen, and sulfur in forested areas, combining cumulative exposures of O_3 with data on dry deposition of sulfur and nitrogen. One study reported that several forest landscapes with the highest dry deposition loadings of sulfur and nitrogen tended to experience the highest average O_3 concentrations and largest cumulative exposure. Although the authors concluded that the joint concentrations of multiple pollutants in forest landscapes were important, nothing was mentioned about the hourly co-occurrences of O_3 and SO_2 or O_3 and NO_2. Acid sulfates, which are usually composed of H_2SO_4, ammonium bisulfate, and ammonium sulfate, have been measured at a number of locations in North America. The potential for O_3 and acidic sulfate aerosols to co-occur at some locations in some form (i.e., simultaneously, sequentially, or complex-sequentially) is real and requires further characterization. For human ambient exposures, the simultaneous co-occurrence of NO_2 and O_3 was infrequent.

In one study, the relationship between O_3 and hydrogen ions in precipitation was explored using data from sites that monitored both O_3 and wet deposition simultaneously and within one minute latitude and longitude of each other. It was reported that individual sites experienced years in which both hydrogen ion deposition and total O_3 exposure were at least moderately high. With data compiled from all sites, it was found that relatively acidic precipitation occurred together with relatively high O_3 levels approximately 20% of the time, and highly acidic precipitation occurred together with a high O_3 level approximately 6% of the time. Sites most subject to relatively high levels of both hydrogen ions and O_3 were located in the eastern part of the United States, often in mountainous areas.

The co-occurrence of O_3 and acidic cloudwater in high-elevation forests has been characterized. The frequent O_3-only and pH-only single-pollutant episodes, as well as the simultaneous and sequential co-occurrences of O_3 and acidic cloudwater, have been reported. Both simultaneous and sequential co-occurrences were observed a few times each month above cloud base.

1.5 Environmental Effects of Ozone and Related Photochemical Oxidants

Ozone is the gaseous pollutant most injurious to agricultural crops, trees, and native vegetation. Exposure of vegetation to O_3 can inhibit photosynthesis, alter carbon (carbohydrate) allocation, and interfere with mycorrhizal formation in tree roots. Disruption of the important physiological processes of photosynthesis and carbon allocation can suppress the growth of crops, trees, shrubs, and herbaceous vegetation by decreasing their capacity to form the carbon (energy) compounds needed for growth and maintenance and their ability to absorb the water and mineral nutrients that they require from the soil. In addition, loss of vigor impairs the ability of trees and crops to reproduce and increases their susceptibility to insects and pathogens. The following section summarizes key environmental effects associated with O_3 exposure.

Effects on Agroecosystems

Methodologies Used in Vegetation Research

Most of the knowledge concerning the effects of O_3 on vegetation comes from the exposure-response studies of important agricultural crop plants and some selected forest and urban tree species, mostly as seedlings. A variety of methodologies have been used, ranging from field exposures without chambers to open-top chambers and to exposures conducted in chambers under highly controlled conditions. In general, the more controlled conditions are most appropriate for investigating specific responses and for providing the scientific basis for interpreting and extrapolating results. The greatest body of knowledge is from OTC studies.

Mode of Action

Leaves are important regulators of plant stress and function. Stress resulting from exposure to O_3 produces a leaf-mediated response. Effects expressed within cells in the leaf (i.e., inhibition of photosynthesis) affect a plant's carbon (energy) budget. Plant processes are impaired only by the O_3 that enters the plant through the stomata (opening in the leaves). An effect will occur only if sufficient O_3 reaches sensitive sites within the leaf cells. The uptake and movement of O_3 to sensitive cellular sites within a leaf are subject to various biochemical and physiological controls. Leaf injury will not be detected if the rate of uptake is small enough for the plant to detoxify or metabolize O_3 and its derivatives, or the plant is able to repair or compensate for the impact at a rate equal to or greater than the rate of uptake. Impairment of leaf cellular processes is the basis for all other plant effects. The diurnal pattern of stomatal opening plays a critical role in O_3 uptake, particularly at the canopy level.

Visible injury is usually the first observable indication of cellular response; injury can occur, however, with no visible effects. Early senescence of leaves or needles is also a result of cellular response. Impairment of cellular processes inhibits the rate of photosynthesis, reduces carbon (sugars, carbohydrate) production, and alters carbon allocation, causing a shift in growth pattern that favors shoots over roots. The reduced allocation of carbon to leaf repair and new leaf formation limits the availability of carbon for reproduction; stem and root growth; and, particularly, the formation of the mycorrhizae on roots necessary for nutrient and water uptake. Reduction of plant vigor by O_3 can result in mortality, particularly when plant susceptibility to insects and pathogens is increased.

Factors That Modify Plant Response

Plant response to O_3 exposure is influenced by a variety of biological, chemical, and physical factors. When determining the impact of O_3 exposure on plants, both the influence of environmental factors on plant response and the effects of O_3 on that response must be considered. Biological factors within plants that affect their response to stresses include, genetic composition, stage of development, and the diurnal pattern of stomatal opening. Genotype significantly influences plant sensitivity to O_3. Individuals, varieties, and cultivars of a species are known to differ greatly in their responses to a given O_3 exposure. Genotype also influences the ability of plants to compete with one another for space, nutrients, light, and water.

The magnitude of response of a particular species, variety, or cultivar depends on a number of environmental factors. The plant's present and past environmental milieu, which includes the temporal exposure pattern and stage of development, dictates the plant response.

The corollary is also true: exposure to O_3 can modify plant response to other environmental variables. Available light, temperature, atmospheric turbulence and moisture, in both the atmosphere and soil; soil nutrition; and exposure to and interaction with other pollutants such as agricultural chemical sprays also influence the magnitude of plant response.

Drought can reduce visible injury and the adverse effects of O_3 on growth and yield of crops. However, in the case of crops, drought, per se, much more adversely affects yield than the effects of O_3. Ozone, on the other hand, tends to reduce the water-use efficiency of well-watered crops. In some plants, O_3 exposure reduces cold/winter hardiness. Although exposure to O_3 tends to reduce attacks by obligate pathogens, susceptibility of plants to facultative pests and pathogens increases.

Effects-Based Air Quality Exposure Indices

Environmental scientists for many years have attempted to characterize and mathematically represent plant exposures to O_3. A variety of averaging times have been used. Although most studies have characterized exposure by using mean concentrations, such as seasonal, monthly, weekly, daily, or peak hourly means, other studies have used cumulative measures (e.g., the number of hours above selected concentrations). None of these statistics completely characterizes the relationships among O_3 concentration, exposure duration, interval between exposures, and plant response.

The use of a mean concentration with long averaging times implies that all concentrations of O_3 are equally effective in causing plant responses and minimizes the contributions of the peak concentrations to the response. Ozone effects are cumulative; therefore, exposure duration should be included in any index if it is to be biologically relevant. Present evidence suggests that cumulative effects of episodic exposures to either peak or mid-range concentrations, or both, can play an important role in producing growth responses. The key to plant response is timing because peak and mid-range concentrations do not occur at the same time. Potentially, the greatest effect of O_3 on plants will occur when stomatal conductance is greatest. When peaks occur at the time of greatest stomatal conductance, the effect of mid-range concentrations will not be observable. Atmospheric conductivity also strongly influences plant response because O_3 must be in contact with the leaf surface if it is to be taken up by a plant. Effects on vegetation appear when the amount of pollutant entering exceeds the ability of the plant to repair or compensate for the impact. Increasing uptake of O_3 will inhibit photosynthesis and result in increased reductions in biomass production.

An index of ambient exposures that relates well to plant response should incorporate, directly or indirectly, environmental influences (e.g., temperature, humidity, soil-moisture status) and exposure dynamics. Peak indices (e.g., second highest daily maximum) imply that a single high-concentration exposure (1- or 8-h concentration) during the course of a 70- to 120-day growing season is related to eventual yield or growth reductions. On the other hand, mean indices (e.g., 7-h seasonal mean) imply that duration of the exposure is not important, and that all concentrations have equal effect on plants. Neither of these indices relates ambient O_3 concentrations to biological effects on plants because these indices do not consider the duration of exposure. An index that cumulates all hourly concentration during the season and gives greater weight to higher concentrations appears to be a more appropriate index for relating ambient exposures to growth or yield effects.

No experimental studies have been designed specifically to evaluate the adequacy of the various peak-weighted indices that have been proposed. In retrospective analyses in which O_3 is the primary source of variation in response, year-to-year variations in plant response are minimized by peak-weighted, cumulative exposure indices. However, a number of different forms of peak-weighted, cumulative indices have been examined for their ability to properly order yield responses from the large number of studies of the National Crop Loss Assessment Network (NCLAN) program. These exposure indices (i.e., SUM00, SUM06, SIGMOID, W126) all performed equally well, and it is not possible to distinguish among them on the basis of statistical fits of the data. The biological basis for these indices has not been determined.

Exposure Response of Plant Species

The emphasis of experimental studies usually has been on the more economically important crop plants and tree species, as seedlings. Crop species usually are monocultures that are fertilized and, in many cases, watered. Therefore, because crop plants are usually grown under optimal conditions, their sensitivity to O_3 exposures can vary from that of native trees, shrubs, and herbaceous vegetation.

The concept of limiting values was used in both the 1978 and 1986 criteria documents to summarize visible foliar injury. Limiting values are defined as concentrations and durations of exposure below which visible injury does not occur. The limit for visible injury indicating reduced plant performance was an O_3 exposure of 0.05 ppm for several hours per day for more than 16 days. When the exposure period was decreased to 10 days, the O_3 concentration required to cause injury was increased to 0.10 ppm. A short, 6-day exposure further increased the concentration to 0.30 ppm. These exposure and concentration periods apply for those crops where appearance or aesthetic value (e.g, spinach, cabbage, lettuce) is considered important. Limiting values for foliar injury to trees and shrubs range from 0.06 to 0.10 ppm for 4 h.

The following assertions can be made based on information from the 1986 criteria document, its 1992 supplement, and literature published since 1986. Ambient O_3 concentrations in several regions of the country are high enough to impair growth and yield of sensitive plant species. This clearly is indicated by comparison of data obtained from crop yield in charcoal-filtered and unfiltered (ambient) exposures. These elevated levels are further supported by data from studies using chemical protectants. These response data make possible the extrapolation to plants not studied experimentally. Both approaches mentioned above indicate that effects occur with only a few exposures above 0.08 ppm. Data from regression studies conducted to develop an exposure-response function for estimating yield loss indicated that at least 50% of the species and cultivars tested could be predicted to exhibit a 10% yield loss at 7-h seasonal mean O_3 concentrations of 0.05 ppm or less.

Effects on Natural Ecosystems

The responses of the San Bernardino mixed forest of Southern California to 50 or more years of chronic ozone exposures based on many studies, present a classic example of ecosystem response to severe stress. Data from an inventory conducted from 1968 through 1972 indicated that for 5 mo of each year, trees were exposed to O_3 concentrations greater than 0.08 ppm for more than 1,300 h. Concentrations rarely decreased below 0.05 ppm at

night near the crest of the mountain slope, approximately 5,500 ft. In addition, during the years 1973 to 1978, average 24-h O_3 concentrations ranged from a background of 0.03 to 0.04 ppm in the eastern part of the San Bernardino Mountains to a maximum of 0.10 to 0.12 ppm in the western part during May through September.

Plants accumulate, store, and use the energy in carbon compounds (sugars) produced during photosynthesis to build their structures and to maintain the physiological processes necessary for life. The patterns of carbon allocation to roots, stems, and leaves directly influence growth. The strategy for carbon allocation changes during the life of a plant, as well as with environmental conditions. Mature trees have a higher ratio of respiration to photosynthetic tissue. Impairment of photosynthesis shifts carbon allocation from growth and maintenance to repair; increased respiration can result in resource imbalances. The significant changes observed in the San Bernardino forest ecosystem were a possible outcome of the combined influences of O_3 on carbon, water, and nutrient allocation.

The biochemical changes within the leaves of ponderosa and Jeffrey pine in the San Bernardino forest, expressed as visible foliar injury, premature needle senescence, reduced photosynthesis, and reduced carbohydrate production and allocation, resulted in reduced tree vigor, growth, and reproduction. Reduced vigor increases susceptibility of trees to insect pests and fungal pathogens. Premature needle senescence alters microorganismal succession on confer needles and changes the detritus-forming process and associated nutrient cycling.

Altered carbon allocation is important in the formation of mycorrhizae (fungus roots), which are an extremely important but unheralded component of all ecosystems; the majority of all plants depend on them because they are integral in the uptake of mineral nutrients and water from the soil. Carbon-containing exudates from the roots are necessary for the formation of mycorrhizae. Reduced carbon allocation to plant roots affects mycorrhizal formation and impacts plant growth. Exposure to ozone, therefore, affects plant growth both above and below ground.

Small changes in photosynthesis or carbon allocation can alter profoundly the structure of a forest. Ecosystem responses to stress begin with the response of the most sensitive individuals of a population. Stresses, whose primary effects occur at the molecular level (within the leaves), must be propagated progressively through more integrated levels of organ physiology (e.g., leaf, branch, root) to whole plant physiology, then to populations within the stand (community), and finally to the landscape level to produce ecosystem effects. Only a small fraction of stresses at the molecular level become disturbances at the tree, stand, or landscape level. The time required for a stress to be propagated from one level to the next (it can take years) determines how soon the effects of the stress can be observed or measured.

The primary effect of O_3 on ponderosa and Jeffrey pine, two of the more susceptible members of the San Bernardino forest community, was that the trees were no longer able to compete effectively for essential nutrients, water, light, and space. Decline in the sensitive trees, a consequence of altered competitive conditions, permitted the enhanced growth of more tolerant species. Removal of the ecosystem dominants at the population level changed its structure and altered the processes of energy flow and nutrient cycling, returning the ecosystem to a less complex stage.

The San Bernardino Mountains continue to experience exposure to O_3; however, there has been a gradual decline in concentrations and length of exposure. Ozone concentrations of 0.06 ppm or higher of varying durations capable of causing injury to trees

in forest ecosystems have been observed during the past 5 years in the Sierra Nevada Mountains and the Appalachian Mountains from Georgia to Maine. Visible injury to forest trees and other vegetation in these areas has been observed.

Injury to sensitive trees from exposure to ozone concentrations 0.06 ppm or greater in the Sierra Nevada Mountains and the Appalachian Mountains has never had the impact on these ecosystems that it did on the San Bernardino forest. Forest stands differ greatly in age, species composition, stability, and capacity to recover from disturbance. In addition, the position in the stand or community of the most sensitive species is extremely important. Ponderosa and Jeffrey pine were the dominant species in the San Bernardino forest. Removal of populations of these trees altered both ecosystem structure and function. Both the Sierra Nevada Mountains and the Appalachian Mountains are biologically more diverse. Removal of sensitive individuals of eastern white pine and black cherry has not visibly altered the forest ecosystems along the Appalachian Mountains, possibly because of the absence of population changes in these species. Decline and dieback of trees on Mt. Mitchell, NC, and Camel's Hump, VT, cannot be related solely to O_3 injury.

Effects on Agriculture, Forestry, and Ecosystems: Economics

A number of economic assessments of the effects of O_3 on agriculture have been performed over the last decade. All use NCLAN response data to predict crop yield changes. Although these studies employ somewhat different economic assessment methodologies, each shows national-level economic losses to major crops in excess of $1 billion (1990 dollars) from exposure to ambient concentrations of O_3. These studies also evaluate the sensitivity of the economic estimates to uncertainties in data, including the NCLAN response data. The economic assessment models used could be adapted to future O_3-crop yield response findings, if available.

The plant science literature shows that O_3 adversely influences physiological performance of both urban and native tree species; the limited economic literature also demonstrates that changes in growth have economic consequences. However, the natural science and economic literature on the topic are not yet mature enough to conclude unambiguously that ambient O_3 is imposing economic costs. The economic effects of O_3 on ecosystems have not yet been addressed in the published literature. There is, however, an emerging interest in applying economic concepts and methods to the management of ecosystems.

Effects on Materials

Over four decades of research show that O_3 damages certain materials such as elastomers, textile fibers, and dyes. The amount of damage to actual in-use materials and the economic consequences of that damage are poorly characterized.

Natural rubber and synthetic polymers of butadiene, isoprene, and styrene, used in products like automobile tires and protective outdoor electrical coverings, account for most of the elastomer production in the United States. The action of O_3 on these compounds is well known, and concentration-response relationships have been established and corroborated by several studies. These relationships, however, must be correlated with adequate exposure information based on product use. For these and other economically important materials, protective measures have been formulated to reduce the rate of oxidative damage. When antioxidants and other protective measures are incorporated in elastomer production, the

O_3-induced damage is reduced considerably, although the extent of reduction differs widely according to the material and the type and amount of protective measures used.

Both the type of dye and the material in which it is incorporated are important factors in the resistance of a fabric to O_3. Some dyed fabrics, such as royal blue and red rayon-acetate and plum cotton are resistant to O_3. On the other hand, anthraquinone dyes on nylon fibers are sensitive to fading by O_3. Field studies and laboratory work show a positive association between O_3 levels and dye fading of nylon materials. At present, the available research is insufficient to quantify the amount of damaged materials attributable to O_3 alone.

The degradation of fibers from exposure to O_3 is poorly characterized. In general, most synthetic fibers, such as modacrylic and polyester, are relatively resistant, whereas cotton, nylon, and acrylic fibers have greater but varying sensitivities to O_3. Ozone reduces the breaking strength of these fibers, and the degree of strength reduction depends on the amount of moisture present. The limited research in this area indicates that O_3 in ambient air may have a minimal effect on textile fibers, but additional research is needed to verify this conclusion.

A number of artists' pigments and dyes are sensitive to O_3 and other oxidants; in particular, many organic pigments are subject to fading or other color changes when exposed to O_3. Although most, but not all, modern fine arts paints are more O_3 resistant, many older works of art are at risk of permanent damage due to O_3-induced fading.

A great deal of work remains to be done to develop quantitative estimates of the economic damage to materials from photochemical oxidants. Most of the available studies are outdated in terms of O_3 concentrations, technologies, and supply-demand relationships. Additionally, little is known about the physical damage functions, so cost estimates have been simplified to the point of not properly recognizing many of the scientific complexities of the impact of O_3.

1.6 Toxicological Effects of Ozone and Related Photochemical Oxidants

Respiratory Tract Effects of Ozone

Biochemical Effects

Knowledge of molecular targets provides a basis for understanding mechanisms of effects and strengthening animal-to-human extrapolations. Ozone reacts with polyunsaturated fatty acids and sulfhydryl, amino, and some electron-rich compounds. These elements are shared across species. Several types of reactions are involved, and free radicals may be created. Based on this knowledge, it has been hypothesized that the O_3 molecule is unlikely to penetrate the liquid linings of the respiratory tract (RT) to reach the tissue, raising the possibility that reaction products exert effects.

In acute and short-term exposure studies, a variety of lung lipid changes occur, including an increase in arachidonic acid, the further metabolism of which produces a variety of biologically active mediators that can affect host defenses, lung function, the immune system, and other functions.

The level of lung antioxidant metabolism increases after O_3 exposure, probably as a result of the increase in the number of Type 2 cells, which are rich in antioxidant enzymes.

Collagen (the structural protein involved in fibrosis) increases in O_3-exposed lungs in a manner that has been correlated to structural changes (e.g., increased thickness of the

tissue between the air and blood after prolonged exposure). Some studies found that the increased collagen persists after exposure ceases.

Generally, O_3 enhances lung xenobiotic metabolism after both short- and long-term exposure, possibly as a result of morphological changes (increased numbers of nonciliated bronchiolar epithelial cells). The impact of this change is dependent on the xenobiotics involved; for example, the metabolism of benzo[*a*]pyrene to active metabolites was enhanced by O_3.

Lung Inflammation and Permeability Changes

Elevated concentrations of O_3 disrupt the barrier function of the lung, resulting in the entry of compounds from the airspaces into the blood and the entry of serum components (e.g., protein) and white blood cells (especially polymorphonuclear leukocytes [PMNs]) into the airspaces and lung tissue. This latter impact reflects the initial stage of inflammation. These cells can release biologically active mediators that are capable of a number of actions, including damage to other cells in the lung. In lung tissue, this inflammation also can increase the thickness of the air-blood barrier.

Increases in permeability and inflammation have been observed at levels as low as 0.1 ppm O_3 (2 h/day, 6 days; rabbits). After acute exposures, the influence of the time of exposure (from two to several hours) increases as the concentration of O_3 increases. Long-term exposure effects are discussed under lung morphology.

The impacts of these changes are not fully understood. At higher O_3 concentrations (e.g., 0.7 ppm, 28 days), the diffusion of oxygen into the blood decreases, possibly because the air-blood barrier is thicker; cellular death may result from the enzymes released by the inflammatory cells; and host defense functions may be altered by mediators.

Effects on Host Defense Mechanisms

Exposure to elevated concentrations of ozone results in alterations of all defense mechanisms of the RT, including mucociliary and alveolobronchiolar clearance, functional and biochemical activity of the alveolar macrophage (AM), and immunologic competence. These effects can cause susceptibility to bacterial respiratory infections.

Mucociliary clearance, which removes particles and cellular debris from the conducting airways, is slowed by acute, but not repeated exposures to O_3. Ciliated epithelial cells that move the mucous blanket are altered or destroyed by acute and chronic exposures. Neonatal sheep exposed to O_3 do not have normal development of the mucociliary system. Such effects could prolong the retention of unwanted substances (e.g., inhaled particles) in the lungs, allowing them to exert their toxicity for a longer period of time.

Alveolar clearance mechanisms, which center on the functioning of AMs, are altered by O_3. Short-term exposure to levels as low as 0.1 ppm O_3 (2 h/day, 1 to 4 days; rabbits) accelerates clearance, but longer exposures do not. Even so, after a 6-week exposure of rats to an urban pattern of O_3, the retention of asbestos fibers in a region protected by alveolar clearance is prolonged.

Alveolar macrophages engulf and kill microbes, as well as clear the deeper regions of the lungs of nonviable particles; AMs also participate in immunological responses, but little is known about the effects of O_3 on this function. Acute exposures of rabbits to levels as low as 0.1 ppm O_3 decrease the ability of AMs to ingest particles. This effect is displayed in decreases in the ability of the lung to kill bacteria after acute exposure of mice to levels as low as 0.4 ppm O_3.

Both the pulmonary and systemic immune system are affected by O_3, but in a poorly understood way. It appears that the part of the immune system dependent on T-cell function is more affected than is the part dependent on B-cell function.

Dysfunction of host defense systems results in enhanced susceptibility to bacterial lung infections. For example, acute exposure to O_3 concentrations as low as 0.08 ppm for 3 h can overcome the ability of mice to resist infection with streptococcal bacteria, resulting in mortality. However, more prolonged exposures (weeks, months) do not cause greater effects on infectivity.

Effects on antiviral defenses are more complex and less well understood. Only high concentrations (1.0 ppm O_3, 3 h/day, 5 days; mice) increase viral-induced mortality. Apparently, O_3 does not impact antiviral clearance mechanisms. Although O_3 does not affect acute lung injury from influenza virus infection, it does enhance later phases of the course of an infection (i.e., postinfluenzal alveolitis).

Morphological Effects

Elevated concentrations of O_3 cause similar types of alterations in lung structure in all laboratory animal species studied, from rats to monkeys. In the lungs, the most affected cells are the ciliated epithelial cells of the airways and Type 1 epithelial cells of the gas-exchange region. In the nasal cavity, ciliated cells are also affected.

The centriacinar region (CAR; the junction of the conducting airways and gas-exchange regions) is the primary target, possibly because this area receives the greatest dose of O_3. The ciliated cells can be killed and replaced by nonciliated cells (i.e., cells not capable of clearance functions that also have increased ability to metabolize some foreign compounds). Mucous-secreting cells are affected, but to a lesser degree. Type 1 cells, across which gas exchange occurs, can be killed; they are replaced by Type 2 cells, which are thicker and produce more lipids. An inflammatory response also occurs in the tissue. The tissue is thickened further in later stages when collagen (a structural protein increased in fibrosis) and other elements accumulate. Although fibrotic changes have been observed in the CAR, they have not been distributed throughout the whole lung.

The distal airway is remodeled; more specifically, bronchiolar epithelium replaces the cells present in alveolar ducts. Concurrent inflammation may play a role. This effect has been observed at 0.25 ppm O_3 (8 h/day, 18 mo) in monkeys; at a higher concentration, this remodeling persists after exposure stops.

The progression of effects during and after a chronic exposure is complex. Over the first few days of exposure, inflammation peaks and then drops considerably, plateauing for the remainder of exposure, after which it largely disappears. Epithelial hyperplasia increases rapidly over the first few days and rises slowly or plateaus thereafter; when exposure ends, it begins to return toward normal. In contrast, fibrotic changes in the tissue between the air and blood increase very slowly over months of exposure, and, after exposure ceases, the changes sometimes persist or increase.

The pattern of exposure can make a major difference in effects. Monkeys exposed to 0.25 ppm O_3 (8 h/day) every other month of an 18-mo period had equivalent changes in lung structure, more fibrotic changes, and more of certain types of pulmonary function changes than did monkeys exposed every day over the 18 mo. From this work and rat studies, it appears that natural seasonal patterns may be of more concern than more continuous exposures. Thus, long-term animal studies with uninterrupted exposures may underestimate some of the effects of O_3.

The morphologic lesions described in early publications on laboratory animals exposed to O_3 do not meet the current criteria for emphysema of the type seen in human lungs.

Effects on Pulmonary Function

Pulmonary function changes in animals resemble those observed in humans after acute exposure.

During acute exposure, the most commonly observed alterations are increased frequency of breathing and decreased tidal volume (i.e., rapid, shallow breathing). This has been reported at exposures as low as 0.2 ppm O_3 for 3 h (rats). Typically, higher concentrations (around 1 ppm) are required to affect breathing mechanics (compliance and resistance). Extended characterizations of pulmonary function show types of changes generally seen in humans. For example, there are decreased lung volumes at levels $\geq$0.5 ppm O_3 (a few hours; rats).

When rats are exposed to O_3 for 2 h/day for 5 days, the pattern of attenuation of pulmonary function responses is similar to that observed in humans. Other biochemical indicators of lung injury did not return to control values by Day 5, and morphological changes increased in severity over the period of exposure. Thus, attenuation did not result in protection against all the effects of O_3.

Long-term exposures have provided mixed results on pulmonary function, including no or minimal effects, restrictive effects, and obstructive effects. When changes occurred and postexposure examinations were performed, pulmonary function recovered.

Genotoxicity and Carcinogenicity of Ozone

The chemical reactivities of O_3 give it the potential to be a genotoxic agent.

In vitro studies are difficult to interpret because the culture systems used allowed the potential formation of artifacts, and high or very high concentrations of O_3 often were used. Generally, in these studies, O_3 causes DNA strand breaks, sometimes is weakly mutagenic, and causes cellular transformation and chromosomal breakage. The latter finding has been investigated in vivo, with mixed results in animals.

The few earlier long-term carcinogenic studies in laboratory animals, with or without coexposure to known carcinogens, are either negative or ambiguous.

The National Toxicology Program (NTP) completed chronic rat and mouse cancer bioassays using commonly accepted experimental approaches and designs. Both male and female rats and mice were studied. Animals were exposed for 2 years (6 h/day, 5 days/week) to 0.12, 0.5, and 1.0 ppm O_3 or for a lifetime to the same levels (except 0.12 ppm). Following their standard procedures for determination of weight-of-evidence for carcinogenicity, the NTP reported "no evidence" in rats, "equivocal evidence" in male mice, and "some evidence" in female mice. The increases in adenomas and carcinomas were observed only in the lungs. There was no concentration response. One of the reasons for the designation of "some evidence" in female mice was that when the 2-year and lifetime exposure studies were combined, there was a statistically significant increase in total tumors at 1.0 ppm. Lung tumors from control and O_3-exposed mice also were examined for the presence of mutated Ha-ras oncogenes. Although the types of mutations found were similar in both groups, a higher incidence of mutations was found in lung tumors from the O_3-exposed mice. At the present time, however, there is inadequate information to provide

mechanistic support for the finding in mice. Thus, the potential for animal carcinogenicity is uncertain.

In a companion NTP study, male rats were treated with a tobacco carcinogen and exposed for 2 years to 0.5 ppm O_3. Ozone did not affect the response and therefore had no tumor promoting activity.

Systemic Effects of Ozone

Ozone causes a variety of effects on tissues and organs distant from the lung. Because O_3 itself is not thought to penetrate the lung, these systemic effects are either secondary to lung alterations or result from reaction products of O_3. Effects have been observed on clinical chemistry, white blood cells, red blood cells, the circulatory system, the liver, endocrine organs, and the central nervous system. Most of these effects cannot be interpreted adequately at this time and have not been investigated in humans, but it is of interest to note that O_3 exposures causing effects on the RT of animals cause a wide array of effects on other organs also.

Several behavior changes occur in response to O_3. For example, 0.12 ppm O_3 (6 h, rats) decreases wheel-running activity, and 0.5 ppm (1 min) causes mice to avoid exposure. These effects are not fully understood, but they may be related to lung irritation or decreased ability to exercise.

Although cardiovascular effects, such as slowed heart rate and decreased blood pressure, occur in O_3-exposed rats, some observed interactions with thermoregulation prevent qualitative extrapolation of these effects to humans at this time.

Developmental toxicity studies in pregnant rats summarized in the 1986 O_3 criteria document showed that levels up to about 2.0 ppm O_3 did not cause birth defects. Rat pups from females exposed to 1.0 ppm O_3 during certain periods of gestation weighed less or had delays in development of behaviors (e.g., righting, eye opening). No "classical" reproductive assays with O_3 were found.

Other studies have indicated that O_3 can affect some endocrine organs (i.e., pituitary-thyroid-adrenal axis, parathyroid gland). It appears that the liver has less ability to detoxify drugs after O_3 exposure, but assays of liver enzymes involved in xenobiotic metabolism are inconsistent.

Interactions of Ozone with Other Co-occurring Pollutants

Animal studies of the effects of O_3 in combination with other air pollutants show that antagonism, additivity, and synergism can result, depending on the animal species, exposure regimen, and health endpoint. Thus, these studies clearly demonstrate the major complexities and potential importance of interactions but do not provide a scientific basis for predicting the results of interactions under untested ambient exposure scenarios.

1.7 Human Health Effects of Ozone and Related Photochemical Oxidants

This section summarizes key effects associated with exposure to O_3, the major component of photochemical oxidant air pollution that is clearly of most concern to the health of the human population. Another, often co-occurring photochemical oxidant component of

"smog" is PAN, but this compound has been demonstrated to be primarily responsible for induction of smog-related eye irritation (stinging of eyes). Limited pulmonary function studies have shown no effects of PAN at concentrations below 0.13 to 0.30 ppm, which are much higher than the generally encountered ambient air levels in most cities.

Controlled Human Studies of Acute Ozone Effects

Effects on Lung Function

Controlled studies in healthy adult subjects have demonstrated O_3-induced decrements in pulmonary function, characterized by alterations in lung volumes and flow and airway resistance and responsiveness. Respiratory symptoms, such as cough and pain on deep inspiration, are associated with these changes in lung function.

Ozone-induced decreases in lung volume, specifically forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1), largely can be attributed to decreases in inspiratory capacity (the ability to take a deep breath), although at higher exposure concentrations, there is clearly an additional component that is not volume dependent. Lung volumes recover to a large extent within 2 to 6 h; normal baseline function typically is reestablished within 24 h, but not fully with more severe exposures.

Ozone causes increased airway resistance and may cause reductions in expiratory flow and the FEV_1/FVC ratio.

Ozone causes an increase in airway responsiveness to nonallergenic stimuli (e.g., histamine, methacholine) in healthy and asthmatic subjects. There is no clear evidence of a relationship between O_3-induced lung volume changes and changes in airway responsiveness.

Inflammation and Host Defense Effects

Controlled studies in healthy adult subjects also indicate that O_3 causes an inflammatory response in the lungs characterized by elevated levels of PMNs, increased epithelial permeability, and elevated levels of biologically active substances (e.g., prostaglandins, proinflammatory mediators, cytokines).

Inflammatory responses to O_3 can be detected within 1 h after a single 1-h exposure with exercise to concentrations ≥ 0.3 ppm; the increased levels of some inflammatory cells and mediators persist for at least 18 h. The temporal response profile is not defined adequately, although it is clear that the time course of response varies for different mediators and cells.

Lung function and respiratory symptom responses to O_3 do not seem to be correlated with airway inflammation.

Ozone also causes inflammatory responses in the nose, marked by increased numbers of PMNs and protein levels suggestive of increased permeability.

Alveolar macrophages removed from the lungs of human subjects after 6.6 h of exposure to 0.08 and 0.10 ppm O_3 have a decreased ability to ingest microorganisms, indicating some impairment of host defense capability.

Ozone Exposure-Response Relationships

Functional, symptomatic, and inflammatory responses to O_3 increase with increasing exposure dose of O_3. The major determinants of the exposure dose are O_3 concentration (C), exposure duration (T), and the amount of ventilation ($\dot{V}_E$).

Exercise increases response to O_3 by increasing $\dot{V}_E$ (greater mass delivered), tidal volume, inspiratory flow (greater percentage delivery), and the intrapulmonary O_3 concentration.

Repeated daily exposures to relatively high levels of O_3 doses ($C \times T \times \dot{V}_E$) causing substantial reductions in FEV_1 ($\geq$20% decrement) typically cause exacerbation of the lung function and respiratory symptom responses on the second exposure day. However, attenuation of these responses occurs with continued exposures for a few days. Most inflammatory responses also attenuate; for example, the PMN influx is absent after five consecutive exposures.

Multihour exposures (e.g., for up to 7 h) to O_3 concentrations as low as 0.08 ppm cause small but statistically significant decrements in lung function, increases in respiratory symptoms, and increases in PMNs and protein levels. Ozone C is a more important factor than exercise $\dot{V}_E$ or T in predicting responses to multihour low-level O_3 exposure. There is clear evidence of a response plateau in terms of lung volume response to prolonged O_3 exposure. This evidence suggests that for a given combination of exercise and O_3 concentration (i.e., dose rate), there is a response plateau; continued exposure (i.e, increased T) at that dose rate will not increase response. Therefore, quantitative extrapolation of responses to longer exposure durations is not valid.

Mechanisms of Acute Pulmonary Responses

The mechanisms leading to the observed pulmonary responses induced by O_3 are beginning to be better understood. The available descriptive data suggest a number of mechanisms leading to the alterations in lung function and respiratory symptoms, including O_3 delivery to the tissue (i.e., the inhaled concentration, breathing pattern, airway geometry; O_3 reactions with the airway lining fluid and epithelial cell membranes; local tissue responses, including injury and inflammation; and stimulation of neural afferents (bronchial C-fibers) and the resulting reflex responses and symptoms. The cyclooxygenase inhibitors block production of prostaglandin E_2 and interleukin-6 as well as reduce lung volume responses; however, these drugs do not reduce inflammation and levels of cell damage markers such as lactate dehydrogenase.

Effects on Exercise Performance

Maximal oxygen uptake, a measure of peak exercise performance capacity, is reduced in healthy young adults if preceded by O_3 exposures sufficient to cause marked changes in lung function (i.e., decreases of at least 20%) and increased subjective symptoms of respiratory discomfort. Limitations in exercise performance may be related to increased symptoms, especially those related to breathing discomfort.

Factors Modifying Responsiveness to Ozone

Many variables have the potential for influencing responsiveness to O_3; however, most are addressed inadequately in the available clinical data to make definitive conclusions.

Active smokers are less responsive to O_3 exposure, which may reverse following smoking cessation, but these results should be interpreted with caution.

The possibility of age-related differences in response to O_3 has been explored, although young adults historically have provided the subject population for controlled human studies. Children and adolescents have lung volume responses to O_3 similar to those of young adults, but lack respiratory symptoms. Pulmonary function responsiveness in adults

appears to decrease with age, whereas symptom rates remain similar to young adults. Group mean lung function responses of adults over 50 years of age are less than those of children, adolescents, and young adults.

The available data have not demonstrated conclusively that men and women respond differently to O_3. Likewise, pulmonary function responses of women have been compared during different phases of the menstrual cycle, but the results are conflicting. If gender differences exist for lung function responsiveness to O_3, they are not based on hormonal changes, differences in lung volume, or the ratio of FVC to $\dot{V}_E$.

There is no compelling evidence, to date, suggesting that any ethnic or racial groups have a different distribution of responsiveness to O_3.

Seasonal and ambient factors may vary responsiveness to O_3, but further research is needed to determine how they affect individual subjects. Individual sensitivity to O_3 may vary throughout the year, related to seasonal variations in ambient O_3 concentrations.

The specific inhalation route appears to be of minor importance in exercising adults. Exposure to O_3 by oral breathing (i.e., mouthpiece) yields results similar to exposure by oronasal breathing (i.e., chamber exposures).

Population Groups at Risk from Ozone Exposure

Population groups that have demonstrated increased responsiveness to ambient concentrations of O_3 consist of exercising healthy and asthmatic individuals, including children, adolescents, and adults.

Available evidence from controlled human studies on subjects with preexisting disease suggests that mild asthmatics have similar lung volume responses, but greater airway resistance responses to O_3 than nonasthmatics; and that moderate asthmatics may have, in addition, greater lung volume responses than nonasthmatics.

Of all the other population groups studied, those with preexisting limitations in pulmonary function and exercise capacity (e.g., chronic obstructive pulmonary disease, chronic bronchitis, ischemic heart disease) would be of primary concern in evaluating the health effects of O_3. Unfortunately, limitations of subject selection, standardized methods of subject characterization, and range of exposure hamper the ability to make definitive conclusions regarding the relative responsiveness of most chronic disease subjects.

Effects of Ozone Mixed with Other Pollutants

No significant enhancement of respiratory effects has been demonstrated consistently for simultaneous exposures of O_3 mixed with SO_2, NO_2, H_2SO_4, HNO_3, particulate aerosols, or combinations of these pollutants. It is fairly well established that simultaneous exposure of healthy adults and asthmatics to mixtures of O_3 and other pollutants for short periods of time (<2 h) induces pulmonary function responses not significantly different from those following O_3 alone when studies are conducted at the same O_3 concentration. Exposure to PAN has been reported to induce greater pulmonary function responses than exposure to O_3 alone, but at PAN concentrations (>0.27 ppm) much higher than ambient levels. Unfortunately, only a limited number of pollutant combinations and exposure protocols have been investigated, and subject groups are small and are representative of only small portions of the general population. Thus, much is unknown about the relationships between O_3 and the complex mix of pollutants found in the ambient air.

Prior exposure to O_3 in asthmatics may cause an increase in response to other pollutant gases, especially SO_2. Likewise, prior exposure to other pollutants can enhance responses to O_3 exposure.

Controlled Human Studies of Ambient Air Exposures

Mobile laboratory studies of lung function and respiratory symptoms in a local subject population exposed to ambient photochemical oxidant pollution provide quantitative information on exposure-response relationships for O_3. A series of these studies from Los Angeles has demonstrated pulmonary function decrements at mean ambient O_3 concentrations of 0.14 ppm in exercising healthy adolescents and increased respiratory symptoms and pulmonary function decrements at 0.15 ppm in heavily exercising athletes and at 0.17 ppm in lightly exercising healthy and asthmatic subjects. Comparison of the observed effects in exercising athletes with controlled chamber studies at comparable O_3 concentrations showed no significant differences in lung function and symptoms, suggesting that coexisting ambient pollutants have a minimal contribution to the measured responses under typical summer ambient conditions in Southern California.

Field and Epidemiology Studies of Ambient Air Exposures

Individual-level field studies and aggregate-level time-series studies have addressed the acute effects of O_3 on lung function decrements and increased morbidity and mortality in human populations exposed to real-world conditions of O_3 exposure.

Camp and exercise studies of lung function provide quantitative information on exposure-response relationships linking lung function declines with O_3 exposure occurring in ambient air. Combined statistical analysis of six recent camp studies in children yields an average relationship between decrements in FEV_1 and previous-hour O_3 concentration of −0.50 mL/ppb. Two key studies of lung function measurements before and after well-defined outdoor exercise events in adults have yielded exposure-response slopes of −0.40 and −1.35 mL/ppb. The magnitude of pulmonary function declines with O_3 exposure is consistent with the results of controlled human studies.

Daily life studies support a consistent relationship between O_3 exposure and acute respiratory morbidity in the population. Respiratory symptoms (or exacerbation of asthma) and decrements in peak expiratory flow rate are associated with increasing ambient O_3, particularly in asthmatic children; however, concurrent temperature, particles, acidity (hydrogen ions), aeroallergens, and asthma severity or medication status also may contribute as independent or modifying factors. Aggregate results show greater responses in asthmatic individuals than in nonasthmatics, indicating that asthmatics constitute a sensitive group in epidemiologic studies of oxidant air pollution.

Summertime daily hospital admissions for respiratory causes in various locations of eastern North America have consistently shown a relationship with ambient levels of O_3, accounting for approximately one to three excess respiratory hospital admissions per hundred parts per billion O_3 per million persons. This association has been shown to remain even after statistically controlling for the possible confounding effects of temperature and copollutants (e.g., hydrogen ions, sulfate, and particles less than 10 μm), as well as when considering only concentrations below 0.12 ppm O_3.

Many of the time-series epidemiology studies looking for associations between O_3 exposure and daily human mortality have been difficult to interpret because of

methodological or statistical weaknesses, including the a failure to account for other pollutant and environmental effects. One of the two most useful new studies on O_3-mortality found a small but statistically significant association in Los Angeles when peak 1-h maximum O_3 concentrations reached concentrations greater than 0.2 ppm during the study period. A second study in regions with lower ($\leq$0.15 ppm) maximum 1-h O_3 concentrations (St. Louis, MO, and Kingston-Harriman, TN) did not detect a significant O_3 association with mortality.

Only suggestive epidemiologic evidence exists for health effects of chronic ambient O_3 exposure in the population. All of the available studies of chronic respiratory system effects in exposed children and adults are limited by a simplistic assignment of exposure or by their inability to isolate potential effects related to O_3 from those of other pollutants, especially particles.

1.8 Extrapolation of Animal Toxicological Data to Humans

There have been significant advances in O_3 dosimetry since 1986 that better enable quantitative extrapolation with marked reductions in uncertainty. Experiments and models describing the uptake efficiency and delivered dose of O_3 in the RT of animals and humans are beginning to present a clearer picture than has existed previously.

The total RT uptake efficiency of rats at rest is approximately 50%. Within the RT of the rat, 50% of the O_3 taken up by the RT is removed in the head, 7% in the larynx/trachea, and 43% in the lungs.

In humans at rest, the total RT uptake efficiency is between 80 and 95%. Total RT uptake efficiency falls as flow increases. As tidal volume increases, uptake efficiency increases and flow dependence lessens. Pulmonary function response data and O_3 uptake efficiency data in humans generally indicate that the mode of breathing (oral versus nasal versus oronasal) has little effect on upper RT or on total RT uptake efficiency, although one study suggests that the nose has a higher uptake efficiency than the mouth.

When all of the animal and human in vivo O_3 uptake efficiency data are compared, there is a good degree of consistency across data sets. This agreement raises the level of confidence with which these data sets can be used to support dosimetric model formulations.

Several mathematical dosimetry models have been developed since 1986. Generally, the models predict that net O_3 dose to lung lining fluid plus tissue gradually decreases distally from the trachea toward the end of the tracheobronchial region and then rapidly decreases in the pulmonary region.

When the dose of O_3 to lung tissue is computed theoretically, it is found to be very low in the trachea; to increase to a maximum in the terminal bronchioles of the first generation pulmonary region; and then to decrease rapidly, moving further into the pulmonary region. The increased tidal volume and flow, associated with exercise in humans, shifts O_3 dose further into the periphery of the lung and causes a disproportionate increase in distal lung dose.

Predictions of delivered dose have been used to investigate both acute and chronic O_3 responses in the context of intra- and interspecies comparisons. In the case of intraspecies comparisons, for example, the distribution of predicted O_3 tissue dose to a

ventilatory unit in a rat as a function of distance from the bronchoalveolar duct junction is very consistent with the distribution of alveolar wall thickening. In the case of interspecies comparisons (using the delivered O_3 dose to the proximal alveolar regions), although the functional responses (e.g., rapid, shallow breathing) differ markedly between rats and humans, there is similarity of acute dose-response patterns in inflammation (influx of cells and protein) among species, with humans and guinea pigs more responsive than rats and rabbits, and similarity of chronic dose-response patterns for increased alveolar interstitial thickness in the CAR of the lung, with monkeys being more responsive than humans and rats less responsive. In other words, the quantitative relationship between animal and human responses is dependent on the animal species and the endpoint.

In summary, there is an emerging consistency among a variety of O_3 dosimetry data sets and between the experimental data and theoretical predictions of O_3 dose. The convergence of experimental data with theoretical predictions lends a degree of confidence to the use of theoretical models to predict total and regional O_3 dose. The use of O_3 dosimetry data and models is beginning to provide a useful extrapolation of effects between animals and humans. The data and models have thus far helped demonstrate that humans may be more responsive to O_3 than rats, but less responsive than monkeys with respect to acute and chronic inflammatory responses. However, the monkey, with its similarity to the human in distal airway structure, provides chronic effects data that may best reflect the degree to which a comparably exposed human would respond. These findings, therefore, suggest that long-term exposure to O_3 could impart a chronic effect in humans.

1.9 Integrative Summary of Ozone Health Effects

This section summarizes the primary conclusions derived from an integration of the known effects of O_3 provided by animal toxicological, human clinical, and epidemiological studies.

1. What are the effects of short-term (< 8-h) exposures to ozone?

Recent epidemiology studies addressing the effects of short-term ambient exposure to O_3 in the population have yielded significant associations with a wide range of health outcomes, including lung function decrements, aggravation of preexisting respiratory disease, increases in daily hospital admissions and emergency department visits for respiratory causes, and increased mortality. Results from lung function epidemiology studies generally are consistent with the experimental studies in laboratory animals and humans.

Short-term O_3 exposure of laboratory animals and humans causes changes in pulmonary function, including tachypnea (rapid, shallow breathing), decreased lung volumes and flows, and increased airway responsiveness to nonspecific stimuli. Increased airway resistance occurs in both humans and laboratory animals, but typically at higher exposure levels than other functional endpoints. In addition, adult human subjects experience O_3-induced symptoms of airway irritation such as cough or pain on deep inspiration. The changes in pulmonary function and respiratory symptoms occur as a function of exposure concentration, duration, and level of exercise. Adult human subjects with mild asthma have responses in lung volume and airway responsiveness to bronchoconstrictor drugs that are qualitatively similar to those of nonasthmatics. Respiratory symptoms are also similar, but wheezing is a prevalent symptom in O_3-exposed asthmatics in addition to the other

demonstrated symptoms of airway irritation. Airway resistance, however, increases relatively more in asthmatics from an already higher baseline. Recovery from the effects of O_3 on pulmonary function and symptoms is usually complete within 24 h of the end of exposure, although other responses may persist somewhat longer.

- An association between daily mortality and O_3 concentration for areas with high O_3 levels (e.g., Los Angeles) has been suggested, although the magnitude of such an effect is unclear.
- Increased O_3 levels are associated with increased hospital admissions and emergency department visits for respiratory causes. Analyses from data in the northeastern United States suggest that O_3 air pollution is associated with a substantial portion (on the order of 10 to 20%) of all summertime respiratory hospital visits and admissions.
- Pulmonary function in children at summer camps in southern Ontario, Canada, in the northeastern United States, and in Southern California is associated with O_3 concentration. Meta-analysis indicates that a 0.5-mL decrease in FEV_1 is associated with a 1-ppb increase in O_3 concentration. For preadolescent children exposed to 120 ppb (0.12 ppm) ambient O_3, this amounts to an average decrement of 2.4 to 3.0% in FEV_1. Similar responses are reported for children and adolescents exposed to O_3 in ambient air or O_3 in purified air for 1 to 2 h while exercising.
- Pulmonary function decrements generally are observed in healthy subjects (8 to 45 years of age) after 1 to 3 h of exposure as a function of the level of exercise performed and the O_3 concentration inhaled during the exposure. Group mean data from numerous controlled human exposure and field studies indicate that, in general, statistically significant pulmonary function decrements beyond the range of normal measurement variability (e.g., 3 to 5% for FEV_1) occur
  (1) at >0.50 ppm O_3 when at rest,
  (2) at >0.37 ppm O_3 with light exercise (slow walking),
  (3) at >0.30 ppm O_3 with moderate exercise (brisk walking),
  (4) at >0.18 ppm O_3 with heavy exercise (easy jogging), and
  (5) at >0.16 ppm O_3 with very heavy exercise (running).
  Smaller group mean changes (e.g., $<5\%$) in FEV_1 have been observed at lower O_3 concentrations than those listed above. For example, FEV_1 decrements have been shown to occur with very heavy exercise in healthy adults at 0.15 to 0.16 ppm O_3, and such effects may occur in healthy young adults at levels as low as 0.12 ppm. Also, pulmonary function decrements have been observed in children and adolescents at concentrations of 0.12 and 0.14 ppm O_3 with heavy exercise. Some individuals within a study may experience FEV_1 decrements in excess of 15% under these exposure conditions, even when the group mean decrement is less than 5%.
- For exposures of healthy subjects performing moderate exercise during longer duration exposures (6 to 8 h), 5% group mean decrements in FEV_1 were observed at
  (1) 0.08 ppm O_3 after 5.6 h,
  (2) 0.10 ppm O_3 after 4.6 h, and
  (3) 0.12 ppm O_3 after 3 h.

For these same subjects, 10% group mean FEV_1 decrements were observed at 0.12 ppm O_3 after 5.6 and 6.6 h. As in the shorter duration studies, some individuals experience changes larger than those represented by the group mean changes.

- An increase in the incidence of cough has been reported at O_3 concentrations as low as 0.12 ppm in healthy adults during 1 to 3 h of exposure with very heavy exercise. Other respiratory symptoms, such as pain on deep inspiration, shortness of breath, and lower respiratory scores (a combination of several symptoms), have been observed at 0.16 to 0.18 ppm O_3 with heavy and very heavy exercise. Respiratory symptoms also have been observed following exposure to 0.08, 0.10, and 0.12 ppm O_3 for 6.6 h with moderate levels of exercise.
- Increases in nonspecific airway responsiveness in healthy adults have been observed after 1 to 3 h of exposure to 0.40 but not 0.20 ppm O_3 at rest and have been observed at concentrations as low as 0.18 but not to 0.12 ppm O_3 during exposure with very heavy exercise. Increases in nonspecific airway responsiveness during 6.6-h exposures with moderate levels of exercise have been observed at 0.08, 0.10, and 0.12 ppm O_3.

Short-term O_3 exposure of laboratory animals and humans disrupts the barrier function of the lung epithelium, permitting materials in the airspaces to enter lung tissue, allowing cells and serum proteins to enter the airspaces (inflammation), and setting off a cascade of responses.

- Increased levels of PMNs and protein in lung lavage fluid have been observed following exposure of healthy adults to 0.20, 0.30, and 0.40 ppm with very heavy exercise and have not been studied at lower concentrations for 1- to 3-h exposures. Increases in lung lavage protein and PMNs also have been observed at 0.08 and 0.10 ppm O_3 during 6.6-h exposures with moderate exercise; lower concentrations have not been tested.

Short-term O_3 exposure of laboratory animals and humans impairs AM clearance of viable and nonviable particles from the lungs and decreases the effectiveness of host defenses against bacterial lung infections in animals and perhaps in humans. The ability of AMs to engulf microorganisms is decreased in humans exposed to 0.08 and 0.10 ppm O_3 for 6.6 h with moderate exercise.

2. What are the effects of repeated, short-term exposures to ozone?

During repeated short-term exposures, some of the O_3-induced responses are partially or completely attenuated. Over a 5-day exposure, pulmonary function changes are typically greatest on the second day, but return to control levels by the fifth day of exposure. Most of the inflammatory markers (e.g., PMN influx) also attenuate by the fifth day of exposure, but markers of cell damage (e.g., lactate dehydrogenase enzyme activity) do not attenuate but continue to increase. Attenuation of lung function decrements is reversed following 7 to 10 days without O_3. Some inflammatory markers also are reversed during this time period, but others still show attenuation even after 20 days without O_3. The mechanisms and impacts involved in attenuation are not known, although animal studies show that the underlying cell damage continues throughout the attenuation process.
In addition, attenuation may alter the normal distribution of O_3 within the lung, allowing

more O_3 to reach sensitive regions, possibly affecting normal lung defenses (e.g., PMN influx in response to inhaled microorganisms).

3. What are the effects of long-term exposures to ozone?

Available data indicate that exposure to O_3 for months and years causes structural changes in several regions of the RT, but effects may be of the greatest importance in the CAR (where the alveoli and conducting airways meet); this region typically is affected in most chronic airway diseases of the human lung. This information on O_3 effects in the distal lung is extrapolated from animal toxicological studies because, to date, comparable data are not available from humans. The apparent lack of reversal of effects during periods of clean air exposure raises concern that seasonal exposures may have a cumulative impact over many years. The role of adaptive processes in this response is unknown but may be critically dependent on the temporal frequency or profile of exposure. Furthermore, the interspecies diversity in apparent sensitivity to the chronic effects of O_3 is notable, with the rat representing the lower limit of response, and the monkey the upper limit. Epidemiological studies attempting to associate chronic health effects in humans with long-term O_3 exposure provide only suggestive evidence that such a linkage exists.

Long-term exposure of one strain of female mice to high O_3 levels (1 ppm) caused a small, but statistically significant increase in lung tumors. There was no concentration-response relationship, and rats were not affected. Genotoxicity data are either negative or weak. Given the nature of the database, the effects in one strain of mice cannot yet be extrapolated qualitatively to humans. Ozone (0.5 ppm) did not show tumor-promoting activity in a chronic rat study.

4. What are the effects of binary pollutant mixtures containing ozone?

Combined data from laboratory animal and controlled human exposure studies of O_3 support the hypothesis that coexposure to pollutants, each at low-effect levels, may result in effects of significance. The data from human studies of O_3 in combination with NO_2, SO_2, H_2SO_4, HNO_3, or CO show no more than an additive response on lung spirometry or respiratory symptoms. The larger number of laboratory animal studies with O_3 in mixture with NO_2 and H_2SO_4 show that effects can be additive, synergistic, or even antagonistic, depending on the exposure regimen and the endpoint studied. This issue of exposure to copollutants remains poorly understood, especially with regard to potential chronic effects.

5. What population groups are at risk as a result of exposure to ozone?

Identification of population groups that may show increased sensitivity to O_3 is based on their biological responses to O_3, preexisting lung disease (e.g., asthma), activity patterns, personal exposure history, and personal factors (e.g., age, nutritional status).

The predominant information on the health effects of O_3 noted above comes from clinical and field studies on healthy, nonsmoking, exercising subjects, 8 to 45 years of age. These studies demonstrate that, among this group, there is a large variation in sensitivity and responsiveness to O_3, with at least a 10-fold difference between the most and least responsive individuals. Individual sensitivity to O_3 also may vary throughout the year, related to seasonal variations in ambient O_3 exposure. The specific factors that contribute to this large intersubject variability, however, remain undefined. Although differences in response may be due to the dosimetry of O_3 in the RT, available data show little difference on O_3 deposition in the lungs for inhalation through the nose or mouth.

Daily life studies reporting an exacerbation of asthma and decrease in peak expiratory flow rates, particularly in asthmatic children, appear to support the controlled studies; however, those studies may be confounded by temperature, particle or aeroallergen exposure, and asthma severity of the subjects or their medication use. In addition, field studies of summertime daily hospital admissions for respiratory causes show a consistent relationship between asthma and ambient levels of O_3 in various locations in the northeastern United States, even after controlling for independent contributing factors. Controlled studies on mild asthmatics suggest that they have similar lung volume responses but greater airway resistance changes to O_3 than nonasthmatics. Furthermore, limited data from studies of moderate asthmatics suggest that this group may have greater lung volume responses than nonasthmatics.

Other population groups with preexisting limitations in pulmonary function and exercise capacity (e.g., chronic obstructive pulmonary disease, chronic bronchitis, ischemic heart disease) would be of primary concern in evaluating the health effects of O_3. Unfortunately, not enough is known about the responses of these individuals to make definitive conclusions regarding their relative responsiveness to O_3. Indeed, functional effects in these individuals with reduced lung function may have greater clinical significance than comparable changes in healthy individuals.

Currently available data follow on personal factors or personal exposure history known or suspected of influencing responses to O_3.

- Human studies have identified a decrease in pulmonary function responsiveness to O_3 with increasing age, although symptom rates remain similar. Toxicological studies are not easily interpreted but suggest that young animals are not more responsive than adults.
- Available toxicological and human data have not demonstrated conclusively that males and females respond differently to O_3. If gender differences exist for lung function responsiveness to O_3, they are not based on differences in baseline pulmonary function.
- Data are not adequate to determine whether any ethnic or racial group has a different distribution of responsiveness to O_3. In particular, the responses of nonwhite asthmatics have not been investigated.
- Information derived from O_3 exposure of smokers is limited. The general trend is that smokers are less responsive than nonsmokers. This reduced responsiveness may wane after smoking cessation.
- Although nutritional status (e.g., vitamin E deficiency) makes laboratory rats more susceptible to O_3-induced effects, it is not clear if vitamin E supplementation has an effect in human populations. Such supplementation has no or minimal effect in animals. The role of such antioxidant vitamins in O_3 responsiveness, especially their deficiency, has not been well studied.

Based on information presented in this document, the population groups that have demonstrated increased responsiveness to ambient concentrations of O_3 consist of exercising, healthy and asthmatic individuals, including children, adolescents, and adults.

2

Introduction

The photochemical oxidants found in ambient air in the highest concentrations are ozone (O_3) and nitrogen dioxide (NO_2). Other oxidants, such as hydrogen peroxide (H_2O_2) and the peroxyacyl nitrates, also have been observed, but in lower and less certain concentrations. In 1971, the U.S. Environmental Protection Agency (EPA) promulgated National Ambient Air Quality Standards (NAAQS) to protect the public health and welfare from adverse effects of photochemical oxidants. The 1971 photochemical oxidant standards were promulgated on the basis of (1) commercially available measurement methodology,[1] (2) uncertainties over the concentrations of O_3 and non-O_3 photochemical oxidants in the atmosphere resulting from the nonspecificity of the measurement methodology, and (3) uncertainties regarding the health and welfare effects of the non-O_3 photochemical oxidants found in ambient air. After 1971, however, O_3-specific commercial analytical methods became available, as did additional information on concentrations and effects of the non-O_3 photochemical oxidants. As a result, the chemical designation of the standards was changed in 1979 from photochemical oxidants to O_3. This document focuses primarily on the scientific air quality criteria for O_3 and, to a lesser extent, on those for H_2O_2 and the peroxyacyl nitrates, particularly peroxyacetyl nitrate. The scientific air quality criteria for NO_2 are discussed in a separate document (U.S. Environmental Protection Agency, 1993).

The previous O_3 air quality criteria document (AQCD), *Air Quality Criteria for Ozone and Other Photochemical Oxidants* (U.S. Environmental Protection Agency, 1986) was released by EPA in August 1986 and a supplement, *Summary of Selected New Information on Effects of Ozone on Health and Vegetation* (U.S. Environmental Protection Agency, 1992), was released in January 1992. These documents were the basis for a March 1993 decision by EPA that revision of the existing 1-h NAAQS for O_3 was not appropriate at that time. That decision did not take into account some of the newer scientific data that became available after completion of the 1986 criteria document. The purpose of this document is to summarize the air quality criteria for O_3 available in the published literature through early 1995. This review was performed in accordance with provisions of the Clean Air Act (CAA) to provide the scientific basis for periodic reevaluation of the O_3 NAAQS.

This chapter provides a general introduction to the legislative and regulatory background for decisions on the O_3 NAAQS, as well as a general summary of the organization, content, and major scientific topics presented in this document.

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[1]The term "photochemical oxidants" historically has been defined as those atmospheric pollutants capable of oxidizing neutral iodide ions (U.S. Environmental Protection Agency, 1978). A number of oxidants other than O_3 are measured, qualitatively if not quantitatively, by potassium iodide methods.

2.1 Legislative Background

Two sections of the CAA govern the establishment,review, and revision of the NAAQS. Section 108 (U.S. Code, 1991) directs the Administrator of EPA to identify certain ubiquitous pollutants that may reasonably be anticipated to endanger public health or welfare and to issue air quality criteria for them. These air quality criteria are to reflect the latest scientific information useful in indicating the kind and extent of all identifiable effects on public health or welfare that may be expected from the presence of the pollutant in ambient air.

Section 109(a) of the CAA (U.S. Code, 1991) directs the Administrator of EPA to propose and promulgate primary and secondary NAAQS for pollutants identified under Section 108. Section 109(b)(1) defines a primary standard as one the attainment and maintenance of which, in the judgment of the Administrator and based on the criteria and allowing for an adequate margin of safety, are requisite to protect the public health. The secondary standard, as defined in Section 109(b)(2), must specify a level of air quality the attainment and maintenance of which, in the judgment of the Administrator and based on the criteria, are requisite to protect the public welfare from any known or anticipated adverse effects associated with the presence of the pollutant in ambient air.

Section 109(d) of the CAA (U.S. Code, 1991) requires periodic review and, if appropriate, revision of existing criteria and standards. Thus, the Administrator may find that EPA's review and revision of criteria make appropriate the proposal of new or revised standards. Alternatively, the Administrator may find that revision of the standards is inappropriate and conclude the review by leaving the existing standards unchanged.

2.2 Regulatory Background[2]

On April 30, 1971, EPA promulgated primary and secondary NAAQS for photochemical oxidants under Section 109 of the CAA (Federal Register, 1971). These standards were set at an hourly average of 0.08 ppm total photochemical oxidants not to be exceeded more than 1 h/year. On April 20, 1977, EPA announced (Federal Register, 1977) the first review and updating of the 1970 *Air Quality Criteria for Photochemical Oxidants* in accordance with Section 109(d) of the CAA. In preparing a revised AQCD, EPA made two external review drafts of the document available for public comment, and these drafts were peer reviewed by the Subcommittee on Scientific Criteria for Photochemical Oxidants of EPA's Science Advisory Board (SAB). A final revised AQCD for O_3 and other photochemical oxidants was published on June 22, 1978.

Based on the 1978 revised AQCD and taking into account the advice and recommendations of the Subcommittee and the comments received from the public, EPA announced (Federal Register, 1979) a final decision to revise the NAAQS for photochemical oxidants on February 8, 1979. The final ruling revised the level of the primary standard from 0.08 to 0.12 ppm, set the secondary standard identical to the primary standard, changed the chemical designation of the standards from photochemical oxidants to O_3, and revised the

[2]This text is excerpted and adapted from the *Proposed Decision on the National Ambient Air Quality Standards for Ozone* (Federal Register, 1992a).

definition of the point at which the standard is attained to "when the expected number of days per calendar year with maximum hourly average concentrations above 0.12 ppm is equal to or less than one" (see Table 2-1).

Table 2-1. National Ambient Air Quality Standards for Ozone[a]

Date of Promulgation	Primary and Secondary NAAQS	Averaging Time
February 8, 1979	0.12 ppm[b] (235 $\mu g/m^3$)	1 h[c]

[a]See Appendix A for abbreviations and acronyms.
[b]1 ppm = 1,962 $\mu g/m^3$, 1 $\mu g/m^3$ = 5.097×10^{-4} ppm at 25 °C, 760 mm Hg.
[c]The standard is attained when the expected number of days per calendar year with a maximum hourly average concentration above 0.12 ppm (235 $\mu g/m^3$) is equal to or less than one.

On March 17, 1982, in response to requirements of Section 109(d) of the CAA, EPA announced (Federal Register, 1982) that it was undertaking plans to revise the existing 1978 AQCD for O_3 and other photochemical oxidants, and, on August 22, 1983, it announced (Federal Register, 1983) that review of the primary and secondary NAAQS for O_3 had been initiated. Public peer-review workshops on draft chapters of a revised AQCD were held December 15 through 17, 1982, and November 16 through 18, 1983. The EPA considered comments made at both workshops in preparing the first external review draft that was made available (Federal Register, 1984) on July 24, 1984, for public review.

On February 13, 1985 (Federal Register, 1985), and on April 2, 1986 (Federal Register, 1986), EPA announced two public meetings of the Clean Air Scientific Advisory Committee (CASAC) of EPA's SAB to be held March 4 through 6, 1985, and April 21 and 22, 1986, respectively. At these meetings, CASAC reviewed external review drafts of the revised AQCD for O_3 and other photochemical oxidants. After completion of this review, CASAC sent the EPA Administrator a closure letter, dated October 22, 1986, indicating that the document "represents a scientifically balanced and defensible summary of the extensive scientific literature." The EPA released the final draft document in August 1986.

The first draft of the Staff Paper "Review of the National Ambient Air Quality Standards for Ozone: Assessment of Scientific and Technical Information" was reviewed by CASAC at a public meeting on April 21 and 22, 1986. At that meeting, CASAC recommended that new information on prolonged exposure effects of O_3 be considered in a second draft of the Staff Paper prior to closure. The CASAC reviewed this second draft and also a presentation of new and emerging information on the health and welfare effects of O_3 at a public review meeting held on December 14 and 15, 1987. The CASAC concluded that sufficient new information existed to recommend incorporation of relevant new data into a supplement to the 1986 AQCD (O_3 supplement) and in a third draft of the Staff Paper.

A draft O_3 supplement, *Summary of Selected New Information on Effects of Ozone on Health and Vegetation: Draft Supplement to Air Quality Criteria for Ozone and Other Photochemical Oxidants*, and the revised Staff Paper were made available to CASAC and to the public for review in November 1988. The O_3 supplement reviewed and evaluated selected literature concerning exposure- and concentration-response relationships observed for

health effects in humans and experimental animals and for vegetation effects. This literature appeared as peer-reviewed journal publications or as proceedings papers from 1986 through late 1988.

On December 14 and 15, 1988, CASAC held a public meeting to review these documents. The CASAC sent the EPA Administrator a closure letter dated May 1, 1989, indicating that the draft O_3 supplement, along with the 1986 AQCD, and the draft Staff Paper "provide an adequate scientific basis for the EPA to retain or revise the primary and secondary standards of ozone." The CASAC concluded that it would be some time before enough new information on the health effects of multihour and chronic exposure to O_3 would be published in scientific journals to receive full peer review and, thus, be suitable for inclusion in a criteria document. The CASAC further concluded that such information could better be considered in the next review of the O_3 NAAQS. A final version of the O_3 supplement has been published (U.S. Environmental Protection Agency, 1992).

On October 22, 1991, the American Lung Association and other plaintiffs filed suit to compel EPA to complete its review of the criteria and standards for O_3. On May 4, 1992, the U.S. District Court for the Eastern District of New York issued an order requiring the Administrator of EPA to sign a proposed decision on whether to revise the standards for O_3 by August 1, 1992, and to sign EPA's final decision by March 1, 1993.

On August 1, 1992, the Administrator signed a proposed decision not to revise the existing NAAQS for O_3 (Federal Register, 1992a), then, on March 1, 1993, signed EPA's final decision, concluding that revision of the NAAQS was inappropriate at that time (Federal Register, 1993a). For reasons indicated in the proposed and final decisions, the March 1993 decision did not take into consideration a number of recent studies on the health and welfare effects of O_3 that had been published since the last literature review in early 1989. The EPA estimated that approximately 3 years would be necessary to (1) incorporate the new studies into a revised criteria document, (2) complete mandated CASAC review, (3) evaluate the significance of the key information for regulatory decision-making purposes, and (4) publish a proposed decision on the O_3 NAAQS in the *Federal Register*.

The EPA intends to complete the current review of the criteria and standards for O_3 as rapidly as possible. Accordingly, the National Center for Environmental Assessment (formerly the Environmental Criteria and Assessment Office [ECAO]) of EPA's Office of Research and Development, located in Research Triangle Park, NC, has given very high priority to review and revision of the air quality criteria for O_3. The ECAO began by announcing the commencement of the review and identification of new information (Federal Register, 1992b). After assessing and evaluating pertinent new studies, ECAO prepared a preliminary draft of a revised AQCD that was reviewed in a series of expert peer reviewed workshops (Federal Register, 1993b,c). Comments received at the workshops were used to revise the preliminary draft for external review (Federal Register, 1994a). Public peer review meetings were held by CASAC to provide advice on the scientific and technical adequacy of the external review draft (Federal Register, 1994b) and the subsequent revised draft (Federal Register, 1995). The final document was prepared on the basis of comments received from the public and CASAC reviews and provides a scientific basis for review of the existing O_3 standards. The EPA's Office of Air Quality Planning and Standards (OAQPS) is completing its preparation of a draft staff paper assessing the most significant information contained in this AQCD and presenting staff recommendations on whether revisions to the NAAQS for O_3 are appropriate. After reviews of the draft staff paper by the

public and CASAC, the Administrator will decide whether to propose revisions to the O_3 NAAQS.

2.3 Summary of Major Scientific Topics Presented

A number of separate topics and issues are addressed in this O_3 criteria document. Some of the key questions addressed are highlighted below by document section.

2.3.1 Air Chemistry

- What concerns still exist regarding precision and accuracy of measurements of O_3 and its precursors?
- What is the order of magnitude of current estimates of natural emissions of O_3 precursors and emissions from anthropogenic sources and their relevance to tropospheric O_3 photochemistry?
- What new scientific information exists on the roles of meteorologic and climatologic factors in O_3 formation and transport?
- Are the reaction pathways of all major precursors to O_3 understood? Have all major reaction products been identified? How are the reactions and products represented in air quality models?
- What is the status of development, application, evaluation, and verification of air quality models?

2.3.2 Air Quality

- What are the trends and geographic differences in O_3 concentrations across the United States?
- What are diurnal and seasonal patterns of 1-h average O_3 concentrations for urban and nonurban sites and for attainment versus nonattainment areas?
- What is known about patterns of co-occurrence of O_3 with other pollutants in the atmosphere?
- What O_3 exposure assessment data are available for agricultural crops and forests?
- To what level and to what extent are humans typically exposed to O_3 in the course of normal, everyday activities?

2.3.3 Environmental Effects

- What are the effects of ambient O_3 concentrations on vegetation (i.e., agricultural and horticultural crops; urban landscape trees, shrubs, and flowers; forest tree species)?
- What characteristics of air quality (e.g., summary statistics) are relevant to these effects on vegetation?
- What are the long-term effects of O_3 exposures on natural ecosystems?
- Is there important new information on the effects of O_3 on nonbiological materials?

2.3.4 Health Effects

- What O_3 concentration and exposure duration relationships exist for effects on lung structure, function, and host defense mechanisms, and what are the important modifiers of these effects?
- What are the mechanisms of O_3-induced lung injury?
- Can dosimetry models predict human population responses to O_3 on the basis of laboratory animal data?
- Does long-term exposure to O_3 lead to the development of chronic lung disease or to an increased frequency or exacerbation of other chronic respiratory outcomes?
- What segments of the population are most susceptible to effects from exposure to O_3?

2.4 Organization and Content of the Document

This document critically evaluates and assesses scientific information on the health and welfare effects associated with exposure to the concentrations of O_3 and related photochemical oxidants present in ambient air. Although the document is not intended to be an exhaustive literature review, it is intended to selectively cover the pertinent literature through 1995. The references cited in the document should be reflective of the state of knowledge on those issues most relevant to review of the NAAQS for O_3, now set at 0.12 ppm for 1 h. Although emphasis is placed on the presentation of health and welfare effects data, other scientific data will be presented and evaluated in order to provide a better understanding of the nature, sources, distribution, measurement, and concentrations of O_3 and related photochemical oxidants in ambient air, as well as the characterization of population exposure to these pollutants.

To aid in the development of this document, summary tables of the relevant published literature have been provided to supplement a selective discussion of the literature. Most of the scientific information selected for review and comment in the text comes from the more recent literature published since completion of the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986). Some of these newer studies were reviewed briefly in the supplement to that document (U.S. Environmental Protection Agency, 1992), but more intense evaluation of these studies has been included. Other studies, however, are included if they contain unique data, such as the documentation of a previously unreported effect or of a mechanism of an effect, or if they were multiple-concentration studies designed to provide exposure-response relationships. Emphasis is placed on studies conducted at or near O_3 concentrations found in ambient air. For animal toxicology studies, typically only those studies conducted at less than 1 ppm O_3 are considered. Studies that are presented in the previous criteria document and whose data were judged to be significant because of their usefulness in deriving the current NAAQS are discussed briefly in the text. Other, older studies also are discussed in the text if they were judged to be (1) open to reinterpretation because of newer data or (2) potentially useful in deriving revised standards for O_3. The reader should, however, consult the more extensive discussion of these "key" studies in the previous document. Generally, only published information that has undergone scientific peer review is included in the criteria document.

Certain issues of direct relevance to standard setting are not explicitly addressed in this document, but instead are analyzed in documentation prepared by OAQPS as part of its regulatory review process. Such issues include (1) determining what constitutes an "adverse effect" and delineation of particular adverse effects that the primary and secondary NAAQS are intended to protect against, (2) exposure assessment, (3) assessment of consequent risks based on health and exposure analyses, and (4) factors to be considered in determining an adequate margin of safety. Key points and conclusions from such analyses are summarized in the Staff Paper prepared by OAQPS and reviewed by CASAC and the public. Although scientific data contribute significantly to decisions regarding the above issues, their resolution cannot be achieved solely on the basis of experimentally acquired information. Final decisions on items 1 and 4 are made by the EPA Administrator, as mandated by the CAA.

A fourth issue directly pertinent to standard setting is identification of populations at risk, which is basically a determination by EPA of the subpopulations to be protected by the promulgation of a given standard. This issue is addressed only partially in the criteria document. For example, information is presented on factors, such as preexisting disease, that biologically may predispose individuals and subpopulations to more severe effects from exposures to O_3. The identification of a population at risk, however, requires information above and beyond data on biological predisposition, such as information on levels of exposure, activity patterns, and personal habits. Such information is included in the Staff Paper developed by OAQPS.

Finally, the O_3 air quality document considers only the scientific and technical issues that are important for standard setting, not those issues relative to implementation of the O_3 NAAQS. For example, certain issues related to the control strategies for attainment of the standard and to possible atmospheric consequences of control strategy design are not discussed in this document. This limitation also includes discussion of impacts consequent to possible changes in the O_3 NAAQS. These issues would be better addressed in regulatory impact analyses or cost-benefit analyses that may be prepared as part of the O_3 NAAQS decision package.

This document is structured as follows: Chapter 1 (executive summary and conclusions) provides a concise presentation of key information and conclusions from all subsequent chapters. This is followed by this brief introduction (Chapter 2) containing information on the legislative and regulatory background for review of the O_3 NAAQS, as well as an overview of the organization of this document. Chapter 3 provides information on the chemistry, sources, emissions, measurement, and transport of O_3 and related photochemical oxidants and their precursors, and Chapter 4 covers environmental concentrations, patterns, and exposure estimates of O_3 and oxidant air quality. This is followed by Chapter 5, which deals with environmental effects of O_3 and related photochemical oxidants. Chapters 6, 7, and 8 discuss animal toxicological studies, human health effects, and extrapolation of animal toxicological data to humans, respectively. Finally, Chapter 9, provides an integrative and interpretive evaluation of health effects associated with exposure to O_3.

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3

Tropospheric Ozone and Its Precursors

3.1 Introduction

Ozone (O_3) and other oxidants found in ambient air, such as peroxyacyl nitrates (PANs) and hydrogen peroxide (H_2O_2), are formed as the result of atmospheric physical and chemical processes involving two classes of precursor pollutants, volatile organic compounds (VOCs) and nitrogen oxides (NO_x). The formation of O_3 and other oxidants from these precursors is a complex, nonlinear function of many factors, including temperature, the intensity and spectral distribution of sunlight, atmospheric mixing and related meteorological conditions, the concentrations of the precursors in ambient air and the ratio between VOC and NO_x, and the reactivity of the organic precursors.

An understanding of the atmospheric chemistry and meteorological parameters and processes responsible for the formation and occurrence of elevated concentrations of O_3 in ambient air is basic to the formulation of strategies and techniques for its abatement. Such an understanding is required for representing those parameters and processes adequately in predictive models used to determine the emission reductions needed for complying with the National Ambient Air Quality Standards (NAAQS) for O_3. In addition, the identification and quantification of O_3 precursors in ambient air are essential, along with emission inventories or emission models, for the development, verification, and refinement of photochemical air quality models and for comparisons of ambient concentrations with emission inventories (source reconciliation), as a check on the accuracy of measurements and of inventories.

Product identification and quantification of yields, in both chambers and ambient air, are helpful in the verification of photochemical air quality models and in testing theoretical chemical mechanisms. Likewise, product identification and quantification are useful in determining the need for research on the potential effects of the simultaneous or sequential co-occurrence with O_3 and related oxidants of multiple air pollutants.

The ability to measure O_3 and its precursors, its reaction products, and the products of the atmospheric reactions of its respective precursors is essential for understanding the atmospheric chemistry of O_3 formation, verifying chemical mechanisms and models, quantifying emission rates, and adequately characterizing exposure-response factors for both biological and nonbiological receptors.

For these reasons, this chapter presents information on a broad range of topics. The chapter describes the chemical processes by which O_3 and other photochemical oxidants are formed in ambient air (Section 3.2). The chapter also characterizes the nature of the precursors in terms of their sources and emissions into the atmosphere and their

concentrations in ambient air (Section 3.4), as well as the methods by which their concentrations in ambient air are measured (Section 3.5).

In addition to information on the chemistry of oxidants and their precursors, this chapter includes a discussion of meteorological processes (Section 3.3) that contribute to the formation of O_3 and other oxidants and govern their transport and dispersion. Finally, an overview is given (Section 3.6) of models of the relationships between precursor emissions and O_3 formation in the atmosphere.

Readers are referred to other sources (e.g., Finlayson-Pitts and Pitts, 1986; Seinfeld, 1986; the U.S. Environmental Protection Agency, 1986a; National Research Council, 1991) for additional information on the chemical and physical aspects of photochemical air pollution.

3.2 Tropospheric Ozone Chemistry

3.2.1 Background Information

Ozone is formed photochemically in the stratosphere and transported downward, resulting in the presence of O_3 in the natural or "clean" troposphere. The presence of O_3 in the clean troposphere, in the absence of perturbations caused by human activities, is highly important because O_3 is a precursor to the hydroxyl (OH) radical, the key intermediate species in the tropospheric degradation of VOCs emitted into the atmosphere. Although O_3 at relatively low concentrations is an integral part of the clean troposphere, its presence at higher concentrations is detrimental.

The chemical processes occurring in the atmosphere that lead to the formation of O_3 and other photochemical air pollutants are complex. Tropospheric O_3 is formed as a result of (1) the emissions of NO_x and VOCs into the atmosphere from anthropogenic and natural sources, (2) the transport of these emissions and their reaction products, and (3) chemical reactions occurring in the atmosphere concurrent with transport and dispersion of the emissions. These processes lead to the formation of O_3 and other photochemical oxidants, such as peroxyacetyl nitrate (PAN), nitric acid (HNO_3), and sulfuric acid (H_2SO_4), and to other compounds, such as particulate matter and formaldehyde (HCHO) and other carbonyl compounds. Additionally, deposition of gases and particles along the trajectory of an air parcel occurs, reducing the concentrations of precursors and products in the atmosphere, but possibly leading to adverse impacts on the earth's environment.

The basic process leading to the photochemical formation of O_3 in the troposphere involves the photolysis of nitrogen dioxide (NO_2) to yield nitric oxide (NO) and a ground-state oxygen atom, $O(^3P)$,

$$NO_2 + h\nu \rightarrow NO + O(^3P), \tag{3-1}$$

which then reacts with molecular oxygen to form O_3:

$$O(^3P) + O_2 + M \rightarrow O_3 + M, \text{ where } M = \text{air}. \tag{3-2}$$

The NO and O_3 react to reform NO_2:

$$NO + O_3 \rightarrow NO_2 + O_2 . \quad (3\text{-}3)$$

The presence of reactive VOCs leads to the conversion of NO to NO_2 without the intermediary of O_3 (Reaction 3-3), and the photolysis of NO_2 then leads to the formation of elevated levels of O_3:

$$NO \xrightarrow{VOC} NO_2 . \quad (3\text{-}4)$$

The photochemical cycles leading to O_3 production are best understood through a knowledge of the chemistry of the atmospheric oxidation of methane (CH_4), which can be viewed as being the chemistry of the clean or unpolluted troposphere (although this is a simplification because vegetation releases large quantities of complex VOCs into the atmosphere). Although the chemistry of the VOCs emitted from anthropogenic and biogenic sources in polluted urban and rural areas is more complex, a knowledge of the CH_4 oxidation reactions aids in understanding the chemical processes occurring in the polluted atmosphere because the underlying chemical principles are the same.

This section first describes the structure of the atmosphere, followed by discussions of the formation of the OH radical and of tropospheric NO_x chemistry. The photochemical formation of tropospheric O_3 from the oxidation of CH_4 then is discussed in some detail because the CH_4 oxidation cycle serves as a model for the photochemical formation of O_3. In Section 3.2.4, the chemistry of the major classes of nonmethane VOCs and the formation of O_3 from these VOCs are discussed. Finally, in Section 3.2.5, a brief account of the photochemical formation of aerosols is given because the same processes that lead to the formation of elevated levels of O_3 result in the formation of both particulate matter (leading to visibility degradation) and atmospheric acidity.

3.2.2 Structure of the Atmosphere

Earth's atmosphere is composed of a number of layers (McIlveen, 1992). For the purposes of this chapter, those of concern are the troposphere and the stratosphere, and the boundary between them, the tropopause.

The troposphere extends from the earth's surface to the tropopause ($\approx$10 to 18 km altitude, depending on latitude and season). The altitude of the tropopause is greatest in the tropics and lowest in the wintertime polar regions, with an average altitude of $\approx$14 km. The temperature in the troposphere decreases with increasing altitude from an average of 290 K at the earth's surface to $\approx$210 to 220 K at the tropopause, and the pressure decreases from $\approx$760 torr at the earth's surface to $\approx$100 torr at the tropopause.

The stratosphere extends from the tropopause to an altitude of $\approx$50 km. In the stratosphere, the temperature increases with increasing altitude from $\approx$210 to 220 K at the tropopause to $\approx$270 K at the top of the stratosphere. The pressure in the stratosphere decreases with increasing altitude from $\approx$100 torr at the tropopause to $\approx$1 torr at the top of the stratosphere.

3.2.2.1 Vertical and Horizontal Mixing in the Atmosphere

In the troposphere, temperature generally decreases with increasing altitude. As will be discussed in Section 3.3, the lowest 1 to 2 km of the troposphere is influenced by the planetary boundary layer (PBL) and, in certain locales, by inversion layers. These boundary and inversion layers inhibit the vertical movement of pollutants into the free troposphere. Above inversion and boundary layers, vertical mixing in the "free" troposphere has a time scale of $\approx$10 to 30 days (Langner et al., 1990; World Meteorological Organization, 1990a).

Because temperature increases with increasing altitude in the stratosphere, vertical mixing in the stratosphere is slow, with a time scale of months to a few years.

Horizontal mixing in the troposphere occurs both within and between the hemispheres. The time scale for mixing between the Northern and Southern Hemispheres is $\approx$1 year (Cicerone, 1989; Singh and Kanakidou, 1993). Transport within a hemisphere is more rapid (Graedel et al., 1986a), and local, regional, and global transport distances of $<$100 km, 100 to 1,000 km, and $>$1,000 km, respectively, are observed. For a wind speed of 15 km h^{-1} ($\approx$4 m s^{-1}), transport times over these local, regional, and global distances are a few hours, a few hours to a few days, and $\geq$10 days, respectively.

3.2.2.2 Formation of Stratospheric Ozone

At altitudes between approximately 20 and 35 km, the stratosphere has a layer of air containing O_3 at mixing ratios up to approximately 10 ppm. The sun emits radiation $\gtrsim$170 nm, and this radiation impacts the upper levels of the atmosphere. The bulk composition of the atmosphere (78.1% nitrogen [N_2], 21.0% molecular oxygen [O_2], 0.9% argon [Ar], 0.03% carbon dioxide [CO_2], with variable trace gas concentrations) is invariant up to at least 50 km (McIlveen, 1992). The shorter wavelength radiation (175 to 240 nm) is absorbed by O_2 in the stratosphere, leading to dissociation into two ground-state oxygen atoms, $O(^3P)$,

$$O_2 + h\nu \rightarrow 2\ O(^3P), \tag{3-5}$$

followed by the reaction of $O(^3P)$ atoms with O_2 in the presence of a third body, M, to form O_3:

$$O(^3P) + O_2 + M \rightarrow O_3 + M, \text{ where } M = \text{air}. \tag{3-2}$$

Ozone also photolyzes, at wavelengths $<$360 nm (DeMore et al., 1992),

$$O_3 + h\nu \rightarrow O_2 + O, \tag{3-6}$$

where the oxygen atom produced can be in the ground state, $O(^3P)$, or electronically excited state, $O(^1D)$. The $O(^1D)$ atoms produced are deactivated to the ground-state $O(^3P)$ atom by N_2, O_2, CO_2, and Ar:

$$O(^1D) + M \rightarrow O(^3P) + M, \text{ where } M = N_2, O_2, Ar, \text{ and } CO_2. \quad (3\text{-}7)$$

The reaction of $O(^3P)$ atoms with O_3 is the termination step of this reaction sequence,

$$O(^3P) + O_3 \rightarrow 2\ O_2. \quad (3\text{-}8)$$

These reactions, called the Chapman reactions (Chapman, 1930), are responsible for the layer of O_3 found in the stratosphere. Because the stratospheric O_3 layer absorbs the sun's radiation below $\approx$290 nm, only radiation of wavelengths $\gtrsim$290 nm can penetrate into the troposphere and impact the earth's surface. Any depletion of the stratospheric O_3 layer allows shorter wavelength ultraviolet radiation (<320 nm) to be transmitted through the stratosphere and into the troposphere.

In addition to the biological effects expected from increased ultraviolet B (UV-B) radiation (290 to 320 nm), increased penetration of UV-B into the troposphere can lead to changes in tropospheric O_3. Model calculations indicate that O_3 in the troposphere could increase with increasing UV-B in urban and rural areas impacted by anthropogenic NO_x emissions (Gery et al., 1988; Liu and Trainer, 1988; Thompson et al., 1989; Thompson, 1992) but could decrease with increasing UV-B in remote tropospheric areas characterized by low NO_x levels (Liu and Trainer, 1988; Thompson et al., 1989). Besides the implications of long-term trends in stratospheric O_3 concentrations leading to corresponding changes in the intensity of UV-B radiation impacting the troposphere, short-term changes (including daily changes) in stratospheric O_3 levels lead to short-term changes in the rates of photolysis of several important species. These include the photolysis of formaldehyde to produce radicals and of O_3 to form the OH radical. These changes in photolysis rates affect the formation rates and ambient concentrations of key radical intermediates, specifically of the OH radical, in the troposphere. Information concerning such short-term changes in stratospheric O_3 concentrations is needed as input to urban and regional airshed computer models of photochemical air pollution formation.

In the clean atmosphere, stratospheric O_3 also is influenced by the emission of nitrous oxide (N_2O) from soils and oceans (World Meteorological Organization, 1992). Because N_2O is chemically inert in the troposphere and does not photolyze (Prinn et al., 1990), it therefore is transported into the stratosphere, where it undergoes photolysis and also reacts with $O(^1D)$ atoms (DeMore et al., 1992; Atkinson et al., 1992a). The reaction of N_2O with the $O(^1D)$ atom is the major source of stratospheric NO, which then participates in a series of reactions known as the NO_x catalytic cycle (Crutzen, 1970; Johnston, 1971).

$$NO + O_3 \rightarrow NO_2 + O_2 \quad (3\text{-}3)$$

$$NO_2 + O(^3P) \rightarrow NO + O_2 \quad (3\text{-}9)$$

Net:

$$O(^3P) + O_3 \rightarrow 2\ O_2$$

The Chapman reactions and the NO_x catalytic cycle reactions control the O_3 concentrations in the lower clean stratosphere.

Additional reaction sequences leading to the removal of stratospheric O_3 arise from the ClO_x and BrO_x catalytic cycles, which result when chlorine (Cl)- and bromine (Br)-containing organic compounds are emitted into the atmosphere. These O_3-depleting compounds include the chlorofluorocarbons (CFCs), hydrochlorofluorocarbons (HCFCs), carbon tetrachloride (CCl_4), methyl chloroform, halons, and methyl bromide (Anderson et al., 1991; Rowland, 1990, 1991; World Meteorological Organization, 1992). Analogous to N_2O, the CFCs, CCl_4, and certain halons (CF_3Br and CF_2ClBr) are inert in the troposphere and are transported into the stratosphere, where they photolyze to generate Cl or Br atoms (World Meteorological Organization, 1992). Methyl bromide and the HCFCs react to a large extent in the troposphere, so that only a fraction of these Cl- and Br-containing species that are emitted into the troposphere are transported into the stratosphere (World Meteorological Organization, 1990b, 1992).

3.2.3 Background Ozone in the Troposphere

As noted in Section 3.2.1, O_3 is present in the troposphere even in the absence of human activities. Although this "natural" O_3 has received widely varying estimates in the literature, there has not been an attempt to standardize the definition because natural background O_3 is a multidimensional and complex concept. Concentrations of background O_3 can vary with temperature; wind speed and direction; vertical motion; geographical location, including latitude and altitude; and season of the year. Because of the decrease of total pressure with increasing altitude, the O_3 concentration in the clean troposphere may be taken to be reasonably independent of altitude at $\approx 7 \times 10^{11}$ molecules cm^{-3}.

For purposes of this document, the primary focus is on the background O_3 concentration near the surface over the United States during the O_3 season. However, the length of the O_3 season varies from state-to-state, depending predominantly on latitude (see Chapter 4). These variations in O_3 season affect the determination of which seasonal averaging period to apply when estimating O_3 concentrations. Based on available assessments of O_3 monitoring measurements, a daytime, 7-h (0900 to 1559 hours), seasonal (April to October) average O_3 concentration of 25 to 45 ppb can be assumed (Altshuller and Lefohn, 1996) as an estimated background concentration (see Chapter 4). Although one component of this background is of natural origin, the other can be attributed to anthropogenic contributions associated with long-range transport of O_3. This assumption is consistent with the relatively long lifetime of O_3 in the troposphere, which can be as long as 30 to 60 days.

The background of O_3 can be attributed to the following sources: downward transport of stratospheric O_3 through the free troposphere to near ground level, in situ O_3 production from methane emitted from swamps and wetlands reacting with natural NO_x emitted from soils and lighting strikes and from the downward transport of NO from the stratosphere into the troposphere, and in situ production of O_3 from the reactions of biogenic VOCs with natural NO_x (National Research Council, 1991). Another source to be considered is the long-range transport of O_3 from distant pollutant sources.

It is important to appreciate that NO_x has a limited lifetime, often estimated to be as short as 6 h in plumes (Altshuller, 1986) and possibly up to 1 to 2 days under less polluted conditions. Because of this relatively short lifetime, the NO_x emitted from cultivated areas in the United States as a result of agricultural practices may not survive long

enough to interact with substantial emissions of biogenic VOCs in distant forested areas. There also is no direct way to distinguish natural NO_x from anthropogenic NO_x in a rural or remote location.

3.2.3.1 Tropospheric Hydroxyl Radicals

It is now recognized that the key reactive species in the troposphere is the OH radical, which is responsible for initiating the degradation reactions of almost all VOCs. In the presence of NO, these OH radical reactions with VOCs lead to the formation of O_3 and, hence, to O_3 concentrations above those encountered in the clean troposphere. The OH radical is produced from the ultraviolet (UV) photolysis of O_3. Ozone photolyzes in the UV radiation at wavelengths <320 nm to generate the electronically excited $O(^1D)$ atom (DeMore et al., 1992; Atkinson et al., 1992a),

$$O_3 + h\nu \rightarrow O_2 + O(^1D). \tag{3-6a}$$

The $O(^1D)$ atoms either are deactivated to the ground state $O(^3P)$ atom by Reaction 3-7 or they react with water vapor to form the OH radical:

$$O(^1D) + H_2O \rightarrow 2\ OH. \tag{3-10}$$

The $O(^3P)$ atoms formed directly in the photolysis of O_3 or formed from deactivation of $O(^1D)$ atoms (Reaction 3-7) reform O_3 through Reaction 3-2. At room temperature and 50% relative humidity, 0.2 OH radicals are formed per $O(^1D)$ atom generated from the photolysis of O_3. Hydroxyl radical production from Reactions 3-6a and 3-10 is balanced by reaction of the OH radical with carbon monoxide (CO) and CH_4. Because the water vapor mixing ratio decreases with increasing altitude in the troposphere (Logan et al., 1981; World Meteorological Organization, 1992) and the O_3 mixing ratio generally increases with increasing altitude, the OH radical concentration is expected to be reasonably independent of altitude (Dentener and Crutzen, 1993).

A knowledge of ambient tropospheric OH radical concentrations is needed for an understanding of tropospheric chemistry and to reliably calcuate the lifetimes of chemical compounds. Because OH and hydroperoxyl (HO_2) radicals are interrelated through a series of reactions (Section 3.2.3.3), concurrent measurements of OH and HO_2 radical concentrations improve the knowledge of tropospheric chemistry. Only in the past few years have measurements been made of lower tropospheric OH radical concentrations (see, for example, Felton et al., 1990; Hofzumahaus et al., 1991; Eisele and Tanner, 1991; Mount and Eisele, 1992; Comes et al., 1992; Hard et al., 1992). The limited data available show that, as expected, the OH radical concentrations exhibit a diurnal profile, with daytime maximum concentrations of several times 10^6 molecules cm^{-3}. A global, annually, seasonally, and diurnally averaged tropospheric OH radical concentration also can be derived from the estimated emissions and measured atmospheric concentrations of methylchloroform (CH_3CCl_3) and the rate constant for the reaction of the OH radical with CH_3CCl_3 (its major tropospheric loss process). Using this method, Prinn et al. (1992) have derived a 24-h average OH radical concentration of 8×10^5 molecules cm^{-3} (equivalent to a 12-h daytime average of 1.6×10^6 molecules cm^{-3} [$\approx$0.1 ppt]). Ambient air measurements of the decay

of nonmethane hydrocarbons in urban plumes (Blake et al., 1993) give OH radical concentrations of similar magnitude to those derived from direct tropospheric measurements and globally averaged estimates.

3.2.3.2 Tropospheric Nitrogen Oxides Chemistry

The presence of NO_x is necessary for the formation of O_3 from the oxidation of CH_4 and other VOCs. Sources of tropospheric NO_x include downward transport from the stratosphere, in situ formation from lightning (National Research Council, 1991; World Meteorological Organization, 1992) (see Section 3.4.1.2), and emission from soils (National Research Council, 1991; World Meteorological Organization, 1992). Recent measurements show that the NO_x concentrations over maritime areas increase slightly with increasing altitude, from $\approx$15 ppt in the marine boundary layer (Carroll et al., 1990) to $\approx$30 to 40 ppt at 3 to 7 km altitude (Ridley et al., 1989; Carroll et al., 1990). Significantly higher NO_x concentrations ($\approx$100 ppt) have been observed in the boundary layer over relatively unpolluted continental areas (Carroll et al., 1990), with the NO_x concentrations decreasing with increasing altitude to $\approx$50 ppt at 3 to 7 km (Ridley et al., 1989; Carroll et al., 1990).

In the troposphere, NO, NO_2, and O_3 are interrelated by the following reactions:

$$NO + O_3 \rightarrow NO_2 + O_2 \tag{3-3}$$

$$NO_2 + h\nu \rightarrow NO + O(^3P) \tag{3-1}$$

$$O(^3P) + O_2 + M \rightarrow O_3 + M. \tag{3-2}$$

Because Reaction 3-2 is fast (the lifetime of an $O(^3P)$ atom at 298 K and 760 torr of air is $\approx 10^{-5}$ s), the O_3 concentration at photoequilibrium is given by

$$[O_3] = J_1[NO_2]/k_3[NO], \tag{3-11}$$

where J_1 and k_3 are the photolysis rate of NO_2 ($\approx$0.5 min^{-1} for an overhead sun) and the rate constant for the reaction of NO with O_3, respectively.

There are other important reactions involving NO_x. The reaction of NO_2 with O_3 leads to the formation of the nitrate (NO_3) radical,

$$NO_2 + O_3 \rightarrow NO_3 + O_2, \tag{3-12}$$

which in the lower troposphere is nearly in equilibrium with dinitrogen pentoxide (N_2O_5):

$$NO_3 + NO_2 \overset{M}{\rightleftharpoons} N_2O_5. \tag{3-13, -3-13}$$

However, because the NO_3 radical photolyzes rapidly (with a lifetime of ≈ 5 s for an overhead sun [Atkinson et al., 1992a]),

$$NO_3 + h\nu \rightarrow NO + O_2 \quad (10\%) \qquad (3\text{-}14a)$$

$$NO_3 + h\nu \rightarrow NO_2 + O(^3P) \quad (90\%), \qquad (3\text{-}14b)$$

its concentration remains low during daylight hours, but can increase after sunset to nighttime concentrations of $<5 \times 10^7$ to 1×10^{10} molecules cm^{-3} (<2 to 430 ppt) over continental areas influenced by anthropogenic emissions of NO_x (Atkinson et al., 1986). Nitrate radical concentrations over marine areas are low because NO_x concentrations are low over lower tropospheric marine areas (Noxon, 1983), and an NO_3 radical mixing ratio of 0.25 ppt has been measured at 3 km altitude in Hawaii (Noxon, 1983). Atkinson (1991) has suggested the use of a 12-h nighttime average NO_3 radical concentration of 5×10^8 molecules cm^{-3} in the lower troposphere over continental areas, with an uncertainty of a factor of ≈ 10.

The tropospheric chemical removal processes for NO_x involve the daytime reaction of NO_2 with the OH radical and the nighttime wet and dry deposition of N_2O_5 to produce HNO_3.

$$OH + NO_2 \xrightarrow{M} HNO_3 \qquad (3\text{-}15)$$

$$N_2O_5 \xrightarrow{H_2O} HNO_3 \qquad (3\text{-}16)$$

The gas-phase reaction of the OH radical with NO_2 is the major and ultimate removal process for NO_x in the troposphere. This reaction removes radicals (OH and NO_2) and competes with the reaction of the OH radical with VOCs. Gaseous HNO_3 formed from Reaction 3-15 undergoes wet and dry deposition, including combination with gaseous ammonia (NH_3) to form particulate phase ammonium nitrate ($N_2H_4O_3$). The tropospheric lifetime of NO_x due to chemical reaction (mainly Reaction 3-15) is ≈ 1 to 2 days. The tropospheric NO_x reactions are shown schematically in Figure 3-1. It should be noted that OH radicals also can react with NO to produce nitrous acid (HNO_2):

$$OH + NO \xrightarrow{M} HNO_2. \qquad (3\text{-}17)$$

In urban areas, HNO_2 also can be formed during nighttime hours (Harris et al., 1982; Pitts et al., 1984a; Rodgers and Davis, 1989), apparently from the heterogeneous hydrolysis of NO_2 or NO_x, or both (Sakamaki et al., 1983; Pitts et al., 1984b; Svensson et al., 1987;

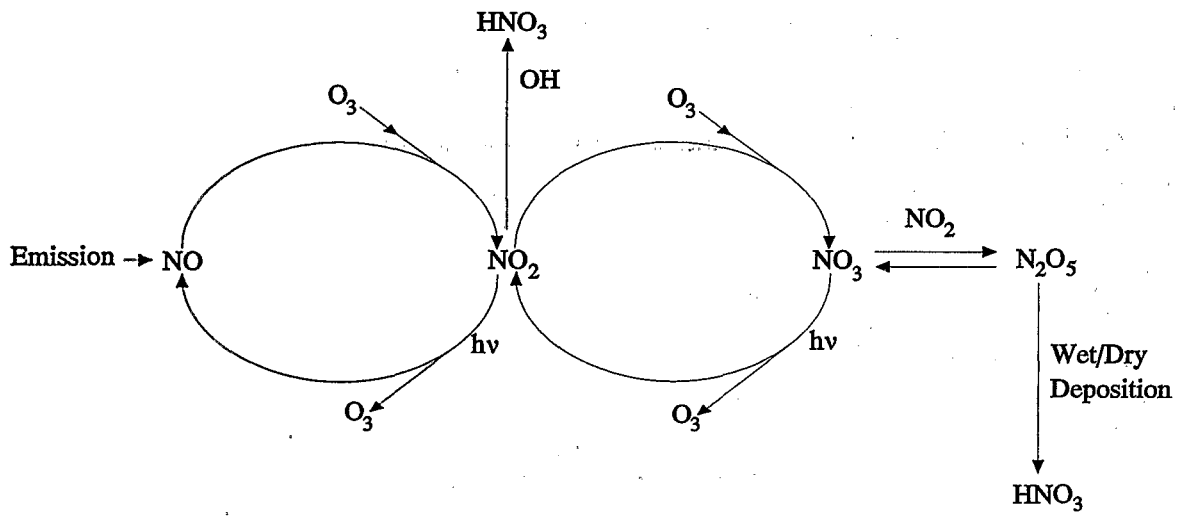


Figure 3-1. The cyclic reactions of tropospheric nitrogen oxides.

Jenkin et al., 1988; Lammel and Perner, 1988; Notholt et al., 1992a,b). The photolysis of HNO_2 during the early morning hours,

$$HNO_2 + h\nu \rightarrow OH + NO, \tag{3-18}$$

thus can become an important source of OH radicals, leading to the rapid initiation of photochemical activity (Harris et al., 1982).

In the troposphere, the initially emitted NO is converted to NO_x ($NO + NO_2$), then to reservoir and termination species (PAN and its homologues, organic nitrates, HNO_3, and particulate nitrate). These reservoir and termination species are referred to as NO_z. The term "NO_y" refers to the total amount of nitrogen, with $NO_y = (NO_x + NO_z)$. Parrish et al. (1993) have investigated the partitioning between the individual nitrogen-containing species at several rural sites in the eastern United States, and Trainer et al. (1993) and Olszyna et al. (1994) have shown that, in rural areas in the eastern United States, there is a good correlation between the O_3 levels and NO_y. Trainer et al. (1993) further showed that O_3 levels correlate even better with NO_z than with NO_y, as may be expected because NO_z quantifies the amount of initially emitted NO that has been processed photochemically, forming O_3 in the process.

3.2.3.3 The Methane Oxidation Cycle

Methane is emitted into the atmosphere from swamps and wetlands, as well as from ruminants (Fung et al., 1991a; World Meteorological Organization, 1992). The major tropospheric removal process for CH_4 is by reaction with the OH radical, with the CH_4 lifetime equal to

$$(k_{21}\,[OH])^{-1}, \tag{3-19}$$

where k_{21} is the rate constant for Reaction 3-21, and [OH] is the (variable) atmospheric OH radical concentration. The calculated lifetime of CH_4 in the troposphere is $\approx$10 to 12 years. As for other saturated organic compounds, the OH radical reaction with CH_4 proceeds by hydrogen (H)-atom abstraction from the carbon (C)-H bonds to form the methyl (CH_3) radical:

$$OH + CH_4 \rightarrow H_2O + \dot{C}H_3. \tag{3-20}$$

In the troposphere, the methyl radical reacts solely with O_2 to yield the methyl peroxy ($CH_3O_2^{\cdot}$) radical (Atkinson et al., 1992a):

$$\dot{C}H_3 + O_2 \overset{M}{\rightarrow} CH_3O_2^{\cdot}. \tag{3-21}$$

In the troposphere, the methyl peroxy radical can react with NO, NO_2, HO_2 radicals, and other organic peroxy ($RO_2^{\cdot}$) radicals, with the reactions with NO and HO_2 radicals being the most important (see, for example, World Meteorological Organization, 1990b). The reaction with NO leads to the formation of the methoxy ($CH_3\dot{O}$) radical,

$$CH_3O_2^{\cdot} + NO \rightarrow CH_3\dot{O} + NO_2. \tag{3-22}$$

The reaction with the HO_2 radical leads to the formation of methyl hydroperoxide (CH_3OOH),

$$CH_3O_2^{\cdot} + HO_2 \rightarrow CH_3OOH + O_2, \tag{3-23}$$

which can photolyze or react with the OH radical (Atkinson et al., 1992a):

$$CH_3OOH + h\nu \rightarrow CH_3\dot{O} + OH, \tag{3-24}$$

$$OH + CH_3OOH \rightarrow H_2O + CH_3O_2^{\cdot} \tag{3-25a}$$

$$OH + CH_3OOH \rightarrow H_2O + \dot{C}H_2OOH \xrightarrow{\text{fast}} HCHO + OH. \tag{3-25b}$$

Methyl hydroperoxide also undergoes wet and dry deposition or incorporation into cloud water. The lifetime of CH_3OOH in the troposphere due to photolysis and reaction with the

OH radical is calculated to be ≈ 2 days. Methyl hydroperoxide is then a temporary sink of radicals, with its wet or dry deposition being a tropospheric loss process for radicals.

The only important reaction for the methoxy radical in the troposphere is with O_2 to form HCHO and the HO_2 radical,

$$CH_3\dot{O} + O_2 \rightarrow HCHO + HO_2. \tag{3-26}$$

Formaldehyde is a "first-generation" product that reacts further, by photolysis:

$$HCHO + h\nu \rightarrow H_2 + CO \quad (55\%) \tag{3-27a}$$

$$HCHO + h\nu \rightarrow H + H\dot{C}O \quad (45\%), \tag{3-27b}$$

where the percentages are for overhead sun conditions (Rogers, 1990) and also by reaction with the OH radical,

$$OH + HCHO \rightarrow H_2O + H\dot{C}O. \tag{3-28}$$

In the troposphere, the H atom and $H\dot{C}O$ (formyl) radical produced in these processes react solely with O_2 to form the HO_2 radical:

$$H + O_2 + M \rightarrow HO_2 + M \tag{3-29}$$

$$H\dot{C}O + O_2 \rightarrow HO_2 + CO. \tag{3-30}$$

The lifetimes of HCHO due to photolysis and OH radical reaction are ≈ 4 h and 1.5 days, respectively, leading to an overall lifetime of ≈ 3 h for overhead sun conditions.

The final step in the oxidation of CH_4 in the earth's atmosphere involves the oxidation of CO by reaction with the OH radical (the only tropospheric reaction of CO) to form CO_2:

$$OH + CO \rightarrow H + CO_2 \tag{3-31}$$

$$H + O_2 + M \rightarrow HO_2 + M. \tag{3-29}$$

The lifetime of CO in the lower troposphere is ≈ 2 mo.

The overall reaction sequence leading to CO_2 formation, through the HCHO and CO intermediate products, is shown in Figure 3-2.

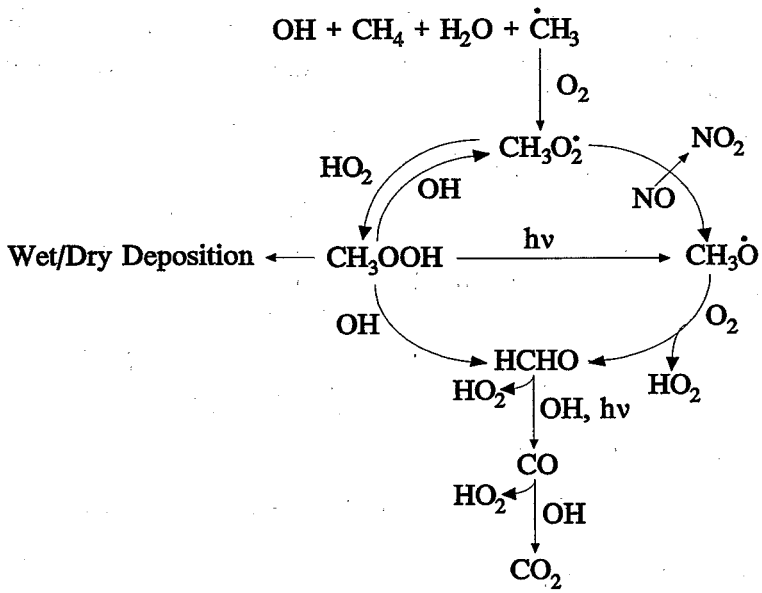


Figure 3-2. Atmospheric reactions in the complete oxidation of methane.

There is competition between NO and the HO_2 radical for reaction with the CH_3O_2 radical, and the reaction route depends on the rate constants for these two reactions and the tropospheric concentrations of HO_2 radicals and NO. The rate constants for the reaction of the CH_3O_2 radicals with NO (Reaction 3-22) and HO_2 radicals (Reaction 3-23) are of comparable magnitude (Atkinson et al., 1992a). Based on the expected HO_2 radical concentration in the troposphere, Logan et al. (1981) calculated that the reaction of the CH_3O_2 radical with NO dominates for NO mixing ratios of >30 ppt (equivalent to an NO concentration of $>7 \times 10^8$ molecules cm^{-3} in the lower troposphere). For NO mixing ratios <30 ppt, the reaction of the CH_3O_2 radical with HO_2 dominates.

Hydroperoxy radicals formed from, for example, Reactions 3-26, 3-29, and 3-30 can react with NO, O_3, or themselves, depending mainly on the concentration of NO. The reaction with NO leads to regeneration of the OH radical,

$$HO_2 + NO \rightarrow OH + NO_2, \tag{3-32}$$

whereas the reactions with O_3 and HO_2 radicals lead to a net destruction of tropospheric O_3:

$$HO_2 + HO_2 \rightarrow H_2O_2 + O_2 \tag{3-33}$$

$$HO_2 + O_3 \rightarrow OH + 2\ O_2. \tag{3-34}$$

This net loss of tropospheric O_3 occurs because the photolytic production of the OH radical from O_3, via the intermediary of the $O(^1D)$ atom, represents a loss process for tropospheric O_3. Hence, the absence of any O_3 formation from the CH_4 oxidation cycle is equivalent to a net O_3 loss. Using the rate constants reported for Reactions 3-32 and 3-34 (Atkinson et al., 1992a) and the tropospheric O_3 mixing ratios given above, it is calculated that the HO_2 radical reaction with NO dominates over reaction with O_3 for NO mixing ratios >10 ppt. The rate constant for Reaction 3-33 is such that an NO mixing ratio of this magnitude also means that the HO_2 radical reaction with NO dominates over the self-reaction of HO_2 radicals.

There are therefore two regimes, depending on the fate of HO_2 and CH_3O_2 radicals: (1) a high-NO regime in which HO_2 and CH_3O_2 radicals react with NO to convert NO to NO_2, regenerate the OH radical, and, through the photolysis of NO_2, produce O_3; and (2) a low-NO regime in which HO_2 and CH_3O_2 radicals combine (Reaction 3-23), and HO_2 radicals undergo self-reaction and react with O_3 (Reactions 3-33 and 3-34), leading to a net destruction of O_3 and inefficient OH radical regeneration (see also Ehhalt et al., 1991; Ayers et al., 1992).

Under high-NO conditions, the oxidation of CH_4 leading to the formation of HCHO can be written as the net reaction,

$$OH + CH_4 + 2\ NO + 2\ O_2 = H_2O + HCHO + 2\ NO_2 + OH, \qquad (3\text{-}35)$$

indicating the conversion of two molecules of NO to NO_2 and regeneration of the OH radical. Because NO_2 photolyzes to form O_3 in the presence of O_2,

$$NO_2 + h\nu \xrightarrow{O_2} NO + O_3\ , \qquad (3\text{-}1,\ 3\text{-}2)$$

the oxidation of CH_4 to HCHO under high-NO conditions can be written as

$$OH + CH_4 + 4\ O_2 = H_2O + HCHO + 2\ O_3 + OH, \qquad (3\text{-}36)$$

showing the formation of O_3 from CH_4 oxidation in the troposphere. The reaction cycles oxidizing CH_4 to HCHO, converting NO to NO_2, and forming O_3 are shown schematically in Figure 3-3.

In a similar manner, under high-NO conditions, the photolysis of HCHO and its reaction with the OH radical is given approximately by

$$0.2\ OH + HCHO + 0.92\ NO \xrightarrow{O_2} CO + 0.44\ H_2 + 0.2\ H_2O + 0.92\ NO_2 + 0.92\ OH. \qquad (3\text{-}37)$$

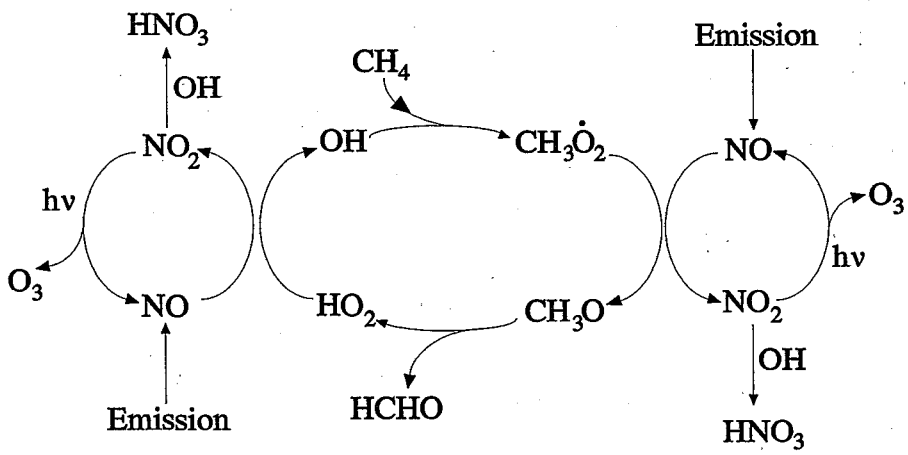


Figure 3-3. Cyclic reactions of methane oxidation to formaldehyde, conversion of nitric oxide to nitrogen dioxide, and concomitant formation of ozone in the atmosphere.

Formaldehyde photooxidation is thus a source of HO_2 radicals (and of OH radicals in high-NO conditions) (Ehhalt et al., 1991), especially in urban areas where the concentration of HCHO is elevated because it is produced during the oxidation of anthropogenic nonmethane VOCs (Finlayson-Pitts and Pitts, 1986).

Nitric oxide mixing ratios are sufficiently low in the lower troposphere over marine areas that oxidation of CH_4 will lead to a net destruction of O_3 (low-NO conditions), as discussed by Carroll et al. (1990) and Ayers et al. (1992). However, in the upper troposphere and over continental areas impacted by NO_x emissions from combustion sources, NO mixing ratios are high enough (high NO-conditions) for CH_4 oxidation to lead to net O_3 formation (Carroll et al., 1990; World Meteorological Organization, 1992).

3.2.3.4 Cloud Processes in the Methane-Dominated Troposphere

In addition to the dry and wet deposition of certain products of the NO_x-CH_4-air photooxidation (e.g., wet and dry deposition of HNO_3 and CH_3OOH [Atkinson, 1988, and references therein; Hellpointner and Gäb, 1989]), cloud processes can have significant effects on the gas-phase chemistry of the clean troposphere (Lelieveld and Crutzen, 1990, 1991; Warneck, 1991, 1992). Lelieveld and Crutzen (1990, 1991) have postulated from modeling studies that the uptake of HCHO, HO_2 radicals, and N_2O_5 into clouds can decrease markedly the production of O_3. The incorporation of HCHO into cloudwater removes HCHO from the gas phase and, hence, reduces the gas-phase formation of HO_2. The uptake of HO_2 radicals into cloudwater has the same effect. Moreover, the aqueous-phase reactions of $CH_2(OH)_2$ (the hydrated form of HCHO) lead to the formation of O_2^-, which reacts with dissolved O_3 to act as a sink for O_3 during cloudy periods. During nighttime, N_2O_5 formed in the gas phase from Reactions 3-3, 3-12, and 3-13 can be readily incorporated into cloudwater with hydrolysis to HNO_3, precluding the reformation of NO_x during the following day from Reactions -3-13 and 3-14. Dentener and Crutzen (1993) have also concluded from a computer modeling study that the heterogeneous reactions of NO_3 radicals and N_2O_5 on

aerosols can have significant effects on global O_3 mixing ratios and on OH concentrations by reducing NO_x levels.

The net effect of these cloud processes is to reduce the gas-phase concentrations of HCHO, NO_x, HO_x, and O_3. Additional, but related, processes can occur in the polluted troposphere (see, for example, Jacob et al., 1989; Dentener and Crutzen, 1993; Section 3.2.5).

3.2.4 Photochemistry of the Polluted Atmosphere

Human activities lead to the emissions of NO_x ($NO + NO_2$) and both CH_4- and nonmethane organic compounds (NMOC) into the atmosphere (Table 3-1). Methane emissions are important on a global scale (World Meteorological Organization, 1992), whereas nonmethane VOC emissions are most important in urban and regional areas. In addition to the emissions of nonmethane VOCs from anthropogenic sources, large quantities of biogenic nonmethane VOCs (mainly of isoprene [2-methyl-1, 3-butadiene] and monoterpenes, [$C_{10}H_{16}$]) are emitted, both in polluted and nonpolluted areas, into the atmosphere from vegetation (see, for example, Isidorov et al., 1985; Lamb et al., 1987; Arey et al., 1991a,b).

Table 3-1. Estimated Emissions of Methane, Nonmethane Organic Compounds, Nitrous Oxide, and Nitrogen Oxides into the Earth's Atmosphere from Biogenic and Anthropogenic Sources[a]

	Emissions (Tg/year[b])	
Chemical	Biogenic Sources	Anthropogenic Sources
CH_4[c]	≈150	≈350
NMOC[d]	≈1,000	≈100
N_2O (as N)[e]	≈7	≈6
NO_x (as N)[f]	≈10	≈40

[a]See Appendix A for abbreviations and acronyms.
[b]Teragram = 10^{12} g, or ≈10^6 metric tons.
[c]Fung et al. (1991a); World Meteorological Organization (1992). Emissions from ruminants, rice paddies, and biomass burning are considered as anthropogenic emissions.
[d]Logan et al. (1981); World Meteorological Organization (1992), with biogenic emissions being assumed to be 50% isoprene and 50% monoterpenes.
[e]Prinn et al. (1990).
[f]National Research Council (1991); World Meteorological Organization (1992); biogenic sources ≈50% from soils and ≈50% from lightning.

Analogous to the photooxidation of CH_4, the interaction of NO_x with nonmethane VOCs from anthropogenic and biogenic sources under the influence of sunlight leads to the formation of photochemical air pollution (National Research Council, 1991). In urban areas, emissions of NO_x and VOCs from human activities (combustion sources, including transportation; industrial sources; solvent usage; landfills; etc.) dominate over biogenic

sources (National Research Council, 1991; Chameides et al., 1992). However, the emissions of VOCs from vegetation have been implicated in the formation of photochemical air pollution in urban (Chameides et al., 1988; 1992) as well as rural areas (Trainer et al., 1987; Roselle et al., 1991; Chameides et al., 1992).

In essence, the chemistry of the polluted urban and regional atmosphere is an extension of that of the clean, CH_4-dominated troposphere, with a number of additional complexities due to the number and types of VOCs emitted from anthropogenic and biogenic sources. At least in certain urban areas, the NMOC content of ambient air is similar to the composition of typical gasolines (Mayrsohn and Crabtree, 1976; Mayrsohn et al., 1977; Harley et al., 1992; see Section 3.4.3). For example, gasolines typically consist of $\approx$55 to 65% alkanes, $\approx$5 to 10% alkenes, and $\approx$25 to 35% aromatic hydrocarbons (Lonneman et al., 1986; Sigsby et al., 1987), whereas in Los Angeles, CA, the ambient urban air composition is $\approx$50 to 55% alkanes, $\approx$5 to 15% alkenes, $\approx$25 to 30% aromatic hydrocarbons, and $\approx$5 to 15% carbonyls (Grosjean and Fung, 1984; California Air Resources Board, 1992). Emissions of NO_x and VOCs are dealt with in detail in Section 3.4.1.

3.2.4.1 Tropospheric Loss Processes of Volatile Organic Compounds

The chemical loss processes of gas-phase VOCs include photolysis and chemical reaction with the OH radical during daylight hours, reaction with the NO_3 radical during nighttime hours, and reaction with O_3, which often is present throughout the 24-h period (Atkinson, 1988).

As discussed earlier, photolysis of chemical compounds in the troposphere is restricted to the wavelength region above $\approx$290 nm. Because of the strength of chemical bonds, the tropospheric wavelength region in which photolysis can occur extends from $\approx$290 to 800 nm, and this wavelength region often is referred to as the "actinic" region. For photolysis to occur, a chemical compound must be able to absorb radiation in the actinic region (and hence have a nonzero absorption cross-section, σ_λ, in this wavelength region). Having absorbed radiation, a chemical compound must then undergo chemical change (i.e., have a nonzero quantum yield, ϕ_λ, for photodissociation or photoisomerization). The quantum yield, ϕ_λ, is defined as (number of molecules of the chemical undergoing change)/(number of photons of light absorbed). The photolysis rate, $k_{photolysis}$, for the process,

$$C + h\nu \rightarrow \text{products} \tag{3-38}$$

is given by

$$k_{photolysis} = \int J_\lambda \, \sigma_\lambda \, \phi_\lambda \, d\lambda, \tag{3-39}$$

where J_λ is the radiation flux at wavelength λ, and σ_λ and ϕ_λ are the absorption cross-section and photolysis quantum yield, respectively, at wavelength λ. Photolysis is therefore a pseudo-first-order process (depending on the radiation flux and spectral distribution) and the lifetime of a chemical with respect to photolysis is given by

$$\tau_{photolysis} = k_{photolysis}^{-1}. \quad (3\text{-}40)$$

For the reaction of a VOC with a reactive species, X (for tropospheric purposes, X = OH, NO_3, and O_3), the lifetime for the reaction process, C + X → products, is given by

$$\tau_X = (k_X[X])^{-1} \quad (3\text{-}41)$$

and depends on the concentration of the reactive species X and the rate constant (k_X) for reaction of the VOC with X. In general, the ambient atmospheric concentrations of OH radicals, NO_3 radicals, and O_3 are variable, depending on time of day, season, latitude, altitude, etc. For the purpose of comparing lifetime calculations for various classes of VOCs, average ambient tropospheric concentrations of these three species often are used. The concentrations used here have been presented in the sections above and are OH radicals, a 12-h average daytime concentration of 1.6×10^6 molecule cm^{-3} (equivalent to a 24-h average concentration of 8×10^5 molecule cm^{-3}) (Prinn et al., 1992); NO_3 radicals, a 12-h nighttime average concentration of 5×10^8 molecule cm^{-3} (Atkinson, 1991); and O_3, a 24-h average of 7×10^{11} molecule cm^{-3} (30 ppb) (Logan, 1985).

The major classes of VOCs are the alkanes, alkenes (including alkenes from biogenic sources), aromatic hydrocarbons, carbonyl compounds, alcohols, and ethers (see California Air Resources Board, 1992). The calculated lifetimes with respect to the individual atmospheric loss processes of compounds representing a range of reactivities in each class are given in Table 3-2. Note that the lifetimes given are dependent on the reaction rate constants and the assumed ambient concentrations of OH radicals, NO_3 radicals, and O_3. Uncertainties in the ambient concentrations of the reactive species translate directly into corresponding uncertainties in the lifetimes.

The following brief discussions of the tropospheric chemistry of the important classes of VOCs are based on the recent review and evaluation article of Atkinson (1994), which should be consulted for further details of the tropospheric reactions of VOCs.

Alkanes

Because gasoline and diesel fuels contain alkanes of carbon number C_4 to $\geq C_{15}$, a large number of alkanes are present in ambient air (see, for example, Grosjean and Fung, 1984; California Air Resources Board, 1992; Section 3.4). Table 3-2 shows that the only important tropospheric loss process for the alkanes is by reaction with the OH radical, with calculated lifetimes of the C_3 to C_{10} alkanes ranging from ≈ 1 to 15 days. As for methane, the OH radical reaction proceeds by H-atom abstraction from the various C-H bonds. The nighttime reactions of the NO_3 radical with alkanes (calculated to be generally of minor importance, but see Penkett et al. [1993]) also proceed by initial H-atom abstraction. For an alkane (RH), the initially formed radical is an alkyl radical (R),

$$OH + RH \rightarrow H_2O + \dot{R}, \quad (3\text{-}42)$$

Table 3-2. Calculated Tropospheric Lifetimes of Selected Volatile Nonmethane Organic Compounds Due to Photolysis and Reaction with Hydroxyl and Nitrate Radicals and with Ozone[a]

	Lifetime Due to Reaction with			
Organic	OH	NO_3	O_3	$h\nu$
n-Butane	5.7 days	2.8 years	>4,500 years	
2-Methylbutane	3.7 days	290 days	>4,500 years	
n-Octane	1.7 days	250 days	>4,500 years	
Ethane	1.7 days	230 days	10 days	
Propene	6.6 h	4.9 days	1.6 days	
Isoprene	1.7 h	0.8 h	1.3 days	
Limonene	1.0 h	3 min	2.0 h	
Benzene	12 days	>4 years	>4.5 years	
Toluene	2.4 days	1.9 years	>4.5 years	
m-Xylene	7.4 h	200 days	>4.5 years	
Formaldehyde	1.5 days	80 days	>4.5 years	4 h
Acetaldehyde	11 h	17 days	>4.5 years	6 days
Acetone	66 days	—	>4.5 years	60 days
2-Butanone	13 days	—	>4.5 years	
Methanol	15 days	>77 days	—	
Ethanol	4.4 days	>50 days	—	
Methyl *t*-butyl ether	4.9 days	—	—	
Ethyl *t*-butyl ether	1.6 days	—	—	
Methylglyoxal	10 h	—	>4.5 years	2 h

[a]See Appendix A for abbreviations and acronyms.

Sources: Lifetimes resulting from reaction with OH, NO_3, and O_3 were calculated using rate constants given in Atkinson and Carter (1984) and Atkinson (1989, 1991, 1994); data for photolysis lifetimes are from Horowitz and Calvert (1982), Meyrahn et al. (1982, 1986), Plum et al. (1983), and Rogers (1990). The OH radical, NO_3 radical, and O_3 concentrations used (molecule cm^{-3} were: OH, 12-h average of 1.6×10^6; NO_3, 12-h average of 5×10^8; O_3, 24-h average of 7×10^{11}.

which rapidly adds O_2 to form an alkyl peroxy radical ($RO_2^{\cdot}$),

$$\dot{R} + O_2 \xrightarrow{M} RO_2^{\cdot}, \tag{3-43}$$

with the simplest of the $RO_2^{\cdot}$ radicals being the methylperoxy radical, described in Section 3.2.3.3 dealing with methane oxidation. Alkyl peroxy radicals can react with NO, NO_2, and HO_2 radicals, and other organic peroxy radicals ($R'O_2^{\cdot}$):

$$RO_2^{\cdot} + NO \rightarrow R\dot{O} + NO_2 \tag{3-44a}$$

$$RO_2^{\cdot} + NO_2 \rightleftarrows ROONO_2 \tag{3-45}$$

$$RO_2^{\cdot} + HO_2 \rightarrow ROOH + O_2 \tag{3-46}$$

$$RO_2^{\cdot} + R'O_2^{\cdot} \rightarrow R\dot{O} + R'\dot{O} + O_2 \tag{3-47a}$$

$$RO_2^{\cdot} + R'O_2^{\cdot} \rightarrow \alpha \text{ carbonyl} + (1 - \alpha)\ ROH + O_2 + \text{products of } R'O_2^{\cdot}\ . \tag{3-47b}$$

The reactions with organic peroxy radicals are expected to be of less importance in the troposphere than the other reactions listed. Because low NO conditions occur even in air masses in urban areas, the HO_2 radical reactions with RO_2 radicals and the subsequent chemistry must be considered. However, because of space constraints and a general lack of knowledge concerning the tropospheric chemistry of RO_2 radicals under low-NO conditions, only the reactions occurring under high-NO conditions are presented and discussed here. For the $\geq C_3$ alkyl peroxy radicals, in addition to the reaction pathway leading to NO-to-NO_2 conversion (Reaction 3-44a), a second reaction pathway leading to formation of an alkyl nitrate becomes important:

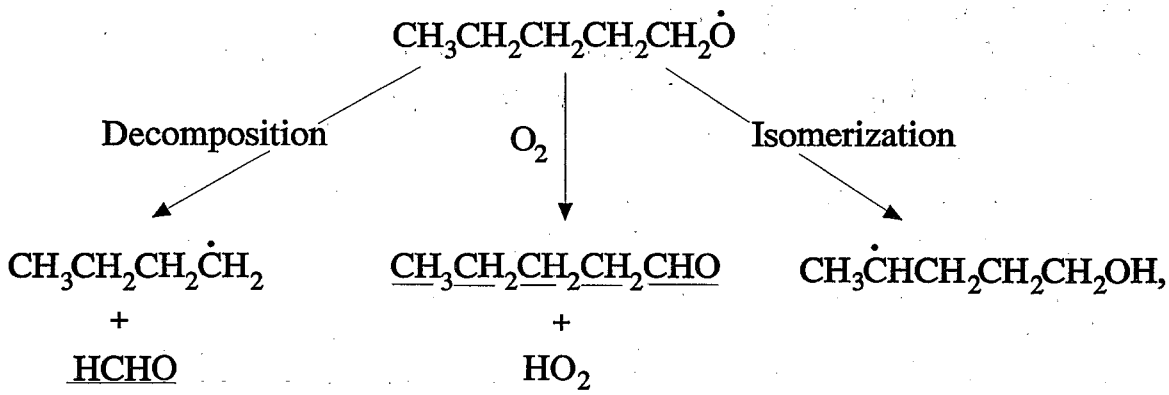
$$RO_2^{\cdot} + NO \xrightarrow{M} RONO_2. \tag{3-44b}$$

For a given alkyl peroxy radical, the alkyl nitrate yield increases with increasing pressure and with decreasing temperature (Carter and Atkinson, 1989a).

Analogous to the case for the methoxy radical, those alkoxy radicals ($RO_2^{\cdot}$) formed from the higher alkanes that have an abstractable H atom can react with O_2 to form the HO_2 radical and a carbonyl; for example,

$$(CH_3)_2CH\dot{O} + O_2 \rightarrow CH_3C(O)CH_3 + HO_2. \tag{3-48}$$

In addition, unimolecular decomposition by C-C bond scission and unimolecular isomerization via a six-member transition state (Atkinson and Carter, 1991; Atkinson, 1994) can be important for the larger alkoxy radicals. For example, the following chemistry can occur for the 1-pentoxy radical:



with the alkyl radicals C_4H_9 and $HOCH_2CH_2CH_2\dot{C}HCH_3$ undergoing further reaction.

The majority of the reaction rate constants and reaction pathways in the alkane degradation schemes are arrived at by analogy from the chemistry of C_1-C_3 alkyl, alkyl peroxy, and alkoxy radicals (Atkinson, 1990, 1994; Carter, 1990; Atkinson et al., 1992a). A number of areas of uncertainty still exist for the tropospheric chemistry of the alkanes (Atkinson, 1993). These include the relative importance of alkoxy radical reaction with O_2, decomposition and isomerization, and the reactions occurring subsequent to the isomerization reaction; the formation of alkyl nitrates from the reactions of the peroxy radicals with NO; and reactions of the alkyl peroxy radicals with HO_2 and other peroxy radicals, reactions that can be important in the nonurban troposphere.

Alkenes (Anthropogenic and Biogenic)

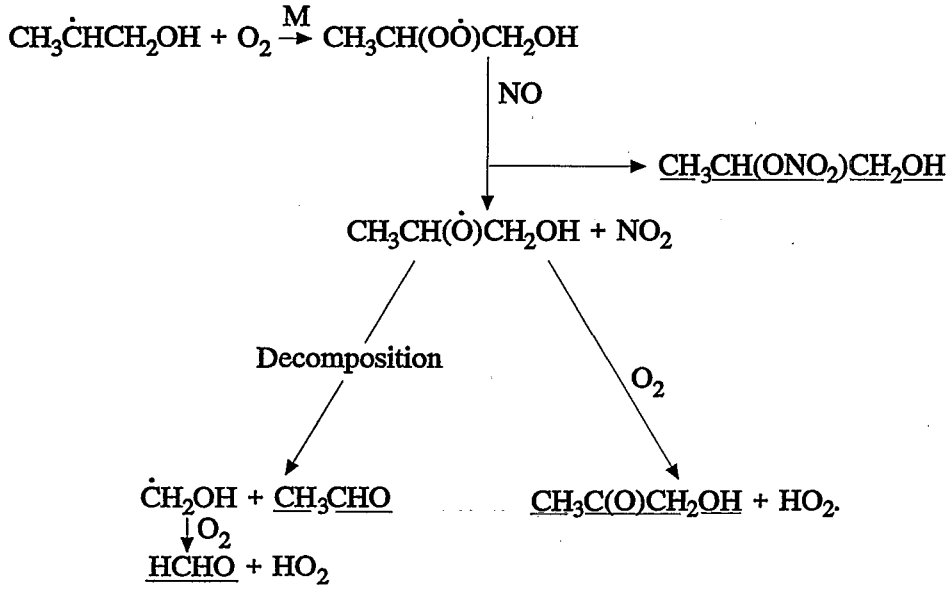
The alkenes emitted from anthropogenic sources are mainly ethene, propene, and the butenes, with lesser amounts of the $\geq C_5$ alkenes. The major biogenic alkenes emitted from vegetation are isoprene (2-methyl-1,3-butadiene) and $C_{10}H_{16}$ monoterpenes (Isidorov et al., 1985; Winer et al., 1992), and their tropospheric chemistry is currently the focus of much attention (see, for example, Hatakeyama et al., 1989, 1991; Arey et al., 1990; Tuazon and Atkinson, 1990a; Pandis et al., 1991; Paulson et al., 1992a,b; Paulson and Seinfeld, 1992a; Zhang et al., 1992; Hakola et al., 1993, 1994).

As evident from Table 3-2, the alkenes react with OH and NO_3 radicals and O_3. All three processes are important atmospheric transformation processes, and all three reactions proceed by initial addition to the $>C=C<$ bonds. These reactions are briefly discussed below.

Hydroxyl Radical Reactions. As noted above, the OH radical reactions with the alkenes proceed mainly by OH radical addition to the $>C=C<$ bonds. For example, the OH radical reaction with propene leads to the formation of the two OH-containing radicals,

$$OH + CH_3CH=CH_2 \xrightarrow{M} CH_3CH(OH)\dot{C}H_2 + CH_3\dot{C}HCH_2OH. \qquad (3\text{-}49)$$

The subsequent reactions of these radicals are similar to those of the alkyl radicals formed by H-atom abstraction from the alkanes. Taking the $CH_3\dot{C}HCH_2OH$ radical as an example, under high-NO conditions, the following chemistry occurs:



The underlined species represent products that, although stable, can undergo further reaction; and, hence, they can lead to "second-generation" products. For the simple $\leq C_4$ alkenes, the intermediate OH-containing radicals appear to undergo mainly decomposition at room temperature and atmospheric pressure of air. Hence, for propene, the "first-generation" products of the OH radical reaction in the presence of NO are HCHO and CH_3CHO, irrespective of which OH-containing radical is formed.

However, this is not the case for the more complex alkenes of biogenic origin. The product studies of Tuazon and Atkinson (1990a) and Paulson et al. (1992a) for the OH radical reaction with isoprene in the presence of NO_x show that the products expected from reaction schemes analogous to that shown above for propene (i.e., HCHO + methyl vinyl ketone [$CH_3C(O)CH{=}CH_2$] and HCHO + methacrolein [$CH_2{=}C(CH_3)CHO$], arising from initial OH radical addition to the $CH_2{=}CH$- and $CH_2{=}C<$ bonds, respectively) do not account for the entire reaction pathways. The product yields obtained from the studies of Tuazon and Atkinson (1990a) and Paulson et al. (1992a) are methyl vinyl ketone, 34%; methacrolein, 24%; 3-methylfuran, 5%; organic nitrates, $\approx$12%; and unidentified carbonyl compounds, $\approx$25% (Tuazon and Atkinson, 1990a; Paulson et al., 1992a; Atkinson, 1994). The HCHO yield was consistent with being a co-product formed with methyl vinyl ketone and methacrolein (Tuazon and Atkinson, 1990a). Aerosol formation for isoprene photooxidation has been shown to be negligible under atmospheric conditions (Pandis et al., 1991; Zhang et al., 1992).

To date, few quantitative product studies have been carried out for the monoterpenes (Arey et al., 1990; Hatakeyama et al., 1991; Hakola et al., 1993, 1994). Arey et al. (1990) and Hakola et al. (1993, 1994) have observed the C_7-C_{10} carbonyl compounds, as had been based on the analogous reaction scheme shown above for propene, but with total carbonyl formation yields of $\leq$50%. These data (Arey et al., 1990; Hakola et al., 1993, 1994) indicate the formation of other products in significant, and often dominant, yields. Hatakeyama et al. (1991) used Fourier transform infrared (FTIR) absorption spectroscopy and reported carbonyl compounds to be formed in high yield from α-pinene and β-pinene, in apparent disagreement with the data of Arey et al. (1990) and

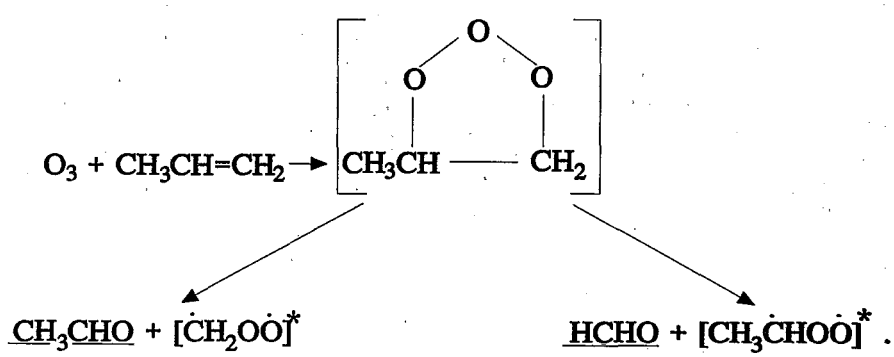
Hakola et al. (1994). Although Hatakeyama et al. (1991) ascribed these carbonyl products to those expected from oxidative cleavage of the $>C=C<$ bonds, it is possible that the yields reported for these carbonyls included contributions by other, as yet unidentified, carbonyl-containing products.

Nitrate Radical Reactions. The NO_3 radical reactions proceed by reaction schemes generally similar to the OH radical reactions, except that, when NO_3 radicals are present, NO concentrations are low (see above) and $RO_2^{\cdot}$ + $RO_2^{\cdot}$ and $RO_2^{\cdot}$ + HO_2 radical reactions are expected to dominate over $RO_2^{\cdot}$ + NO reactions. For propene, the initial reaction is

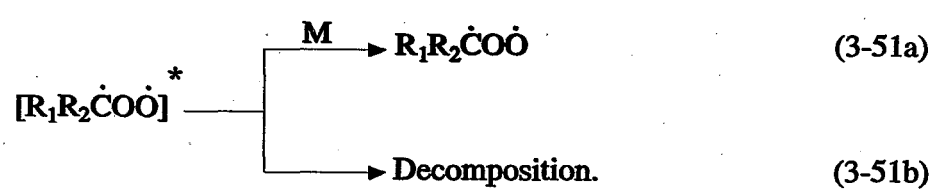
$$NO_3 + CH_3CH=CH_2 \rightarrow CH_3\dot{C}HCH_2ONO_2 \text{ and } CH_3CH(ONO_2)\dot{C}H_2\,, \qquad (3\text{-}50)$$

followed by a series of reactions that are expected (Atkinson, 1991) to lead to the formation of, among others, carbonyls and nitrato-carbonyls (for example, HCHO, CH_3CHO, $CH_3CH(ONO_2)CHO$, and $CH_3C(O)CH_2ONO_2$ from propene). Few data are presently available concerning the products and detailed mechanisms of NO_3-alkene reactions (Atkinson, 1991, 1994, and references therein). In particular, the reaction products and mechanisms for the NO_3 radical reactions with isoprene and the monoterpenes are still not quantitatively understood (Kotzias et al., 1989; Barnes et al., 1990; Hjorth et al., 1990; Skov et al., 1992).

Ozone Reactions. The O_3 reactions also proceed by addition of O_3 to the alkene, to form an energy-rich ozonide that rapidly decomposes to form carbonyls and energy-rich biradicals ([ ]*):



The energy-rich biradicals, $[\dot{C}H_2O\dot{O}]^*$ and $[CH_3\dot{C}H\dot{O}O]^*$, undergo collisional stabilization or decomposition:



There are still significant uncertainties concerning the reactions of the energy-rich biradicals (Horie and Moortgat, 1991; Atkinson, 1990, 1994), with recent studies showing the production of OH radicals in high yields for several alkenes (Niki et al., 1987; Paulson et al., 1992a; Atkinson et al., 1992b; Paulson and Seinfeld, 1992b; Atkinson and Aschmann, 1993).

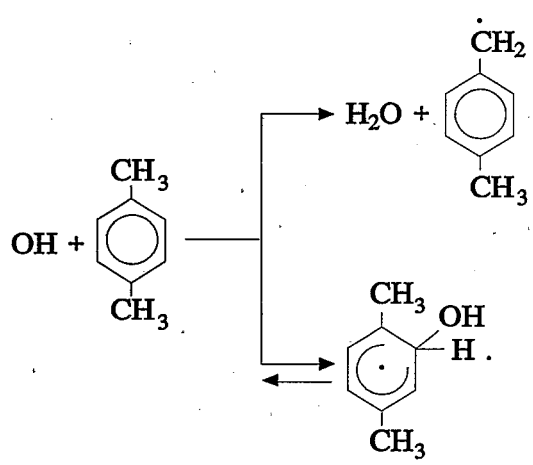
For isoprene, the major products are methacrolein and methyl vinyl ketone (Kamens et al., 1982; Niki et al., 1983; Paulson et al., 1992b). Paulson et al. (1992b) derived an OH radical and an $O(^3P)$ atom formation yield of 0.68 ± 0.15 and 0.45 ± 0.2, respectively, from the O_3 reaction with isoprene, indicating the dominance of secondary reactions. However, Atkinson et al. (1992b) derived a significantly lower OH radical formation yield of 0.27 (uncertain to a factor of ≈ 1.5). Clearly, further studies of this important reaction are needed.

The only quantitative studies of the gas-phase O_3 reactions with the monoterpenes are those of Hatakeyama et al. (1989) for α- and β-pinene and Hakola et al. (1993, 1994) for a series of monoterpenes. Additionally, Atkinson et al. (1992b) derived OH radical formation yields from these reactions under atmospheric conditions.

Several groups (Gäb et al., 1985; Becker et al., 1990, 1993; Simonaitis et al., 1991; Hewitt and Kok, 1991) have reported the formation of H_2O_2 and organic peroxides from O_3 reactions with alkenes. However, there are significant disagreements in the quantitative results reported by Becker et al. (1990, 1993) and Simonaitis et al. (1991).

Aromatic Hydrocarbons

The chemistry of aromatic hydrocarbons is one of the major sources of uncertainty in the atmospheric chemistry of VOCs (National Research Council, 1991; Atkinson, 1994). The most abundant aromatic hydrocarbons in urban atmospheres are benzene, toluene, the xylenes, and the trimethylbenzenes (Grosjean and Fung, 1984; California Air Resources Board, 1992). As shown in Table 3-2, the only tropospherically important loss process for benzene and the alkyl-substituted benzenes is by reaction with the OH radical. For the alkyl-substituted benzenes, the OH radical reactions proceed by two pathways: (1) H-atom abstraction from the C-H bonds of the alkyl substituent groups and (2) OH radical addition to the aromatic ring, as shown below, for *p*-xylene:

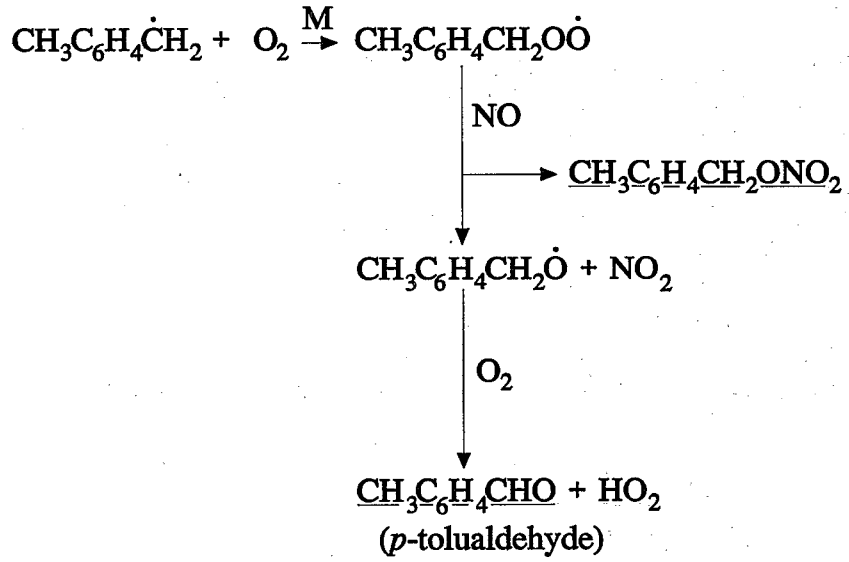


(3-52a)

(3-52b)

with the OH radical addition pathway being reversible above ≈ 325 K (Atkinson, 1989).

The radical formed in Reaction 3-52a reacts analogously to an alkyl radical (Atkinson, 1994), leading, in the presence of NO, to aromatic aldehydes and organic nitrates.



The OH-containing radical formed in Reaction 3-52b can undergo reaction with both NO_2 and O_2. Knispel et al. (1990) reported rate constants for the reactions of NO_2 and O_2 with the OH-containing radicals formed from benzene and toluene. The magnitude of the rate constants they obtained implies that, in the troposphere, the major reactions of these radicals will be with O_2. Laboratory studies of the formation of selected products from the gas-phase reactions of the OH radical with toluene, *o*-xylene, and 1,2,3-trimethylbenzene (Atkinson and Aschmann, 1994), and, in particular, of the formation of the ring-cleavage product 2,3-butanedione from *o*-xylene, are consistent with the kinetic data of Knispel et al. (1990). Thus, at least for the monocyclic aromatic hydrocarbons such as benzene, toluene, and the xylenes, the OH-aromatic adducts formed in Reaction 3-52b react with O_2 under atmospheric conditions. However, care must be taken in using product yields obtained in the laboratory at higher than ambient NO_2 concentrations because data may be influenced by the NO_2 reaction and, hence, may not be applicable to the O_2 reaction with the OH-aromatic adduct. Clearly, the products formed and their yields from the O_2 and NO_2 reactions with the OH-aromatic adducts need to be determined, and the detailed reaction mechanisms elucidated.

Despite these uncertainties, however, products from the OH radical addition pathway have been identified, and their formation yields determined (Atkinson, 1994, and references therein). The major products identified from the OH radical addition pathway are phenolic compounds (e.g., phenol from benzene and *o*-, *m*-, and *p*-cresol from toluene) and α-dicarbonyls (glyoxal, methylglyoxal, and 2,3-butanedione) resulting from the cleavage of the aromatic ring (see, for example, Atkinson, 1990, 1994, and references therein). Significant fractions ($\geq$50% for benzene, toluene, and the xylenes) of the reaction products are, however, still not accounted for.

Carbonyl Compounds

As noted above, the OH radical reactions with the alkanes, alkenes, and aromatic hydrocarbons lead, often in large yield, to the formation of carbonyl compounds. Likewise, carbonyls are formed during the reactions of NO_3 radicals and O_3 with alkenes. As a first approximation, the carbonyl compounds of tropospheric interest are HCHO (see Section 3.2.3.3), acetaldehyde, and the higher aliphatic aldehydes; benzaldehyde; acetone, 2-butanone, and the higher ketones; and simple dicarbonyls such as glyoxal, methylglyoxal, and 2,3-butanedione.

The tropospheric photooxidation of isoprene leads to the formation of methyl vinyl ketone ($CH_3C(O)CH{=}CH_2$) and methacrolein ($CH_3C(CHO){=}CH_2$). The OH radical-initiated reactions of these two carbonyl compounds in the presence of NO_x have been studied by Tuazon and Atkinson (1989, 1990b).

The tropospherically important loss processes of the carbonyls not containing $>C{=}C<$ bonds are photolysis and reaction with the OH radical. As shown in Tables 3-2, photolysis is a major tropospheric loss process for the simplest aldehyde (HCHO) and the simplest ketone ($CH_3C(O)CH_3$), as well as for the dicarbonyls. For the higher aldehydes and ketones, the OH radical reactions are calculated to be the dominant gas-phase loss process (Table 3-2). For CH_3CHO, the reaction proceeds by H-atom abstraction from the -CHO group to form the acetyl ($CH_3\dot{C}O$) radical,

$$OH + CH_3CHO \rightarrow H_2O + CH_3\dot{C}O, \tag{3-53}$$

which rapidly adds O_2 to form the acetyl peroxy radical:

$$CH_3\dot{C}O + O_2 \overset{M}{\rightarrow} CH_3C(O)O\dot{O}. \tag{3-54}$$

This O_2 addition pathway is in contrast to the reaction of O_2 with the formyl ($H\dot{C}O$) radical formed from HCHO, which reacts by an H-atom abstraction pathway (Reaction 3-30). The acetyl peroxy radical reacts with NO and NO_2,

$$\begin{array}{l} CH_3C(O)O\dot{O} + NO \rightarrow CH_3C(O)\dot{O} + NO_2 \\ \qquad\qquad\qquad\qquad\quad \Big\downarrow \text{fast} \\ \qquad\qquad\qquad\qquad\quad \dot{C}H_3 + CO_2 \end{array} \tag{3-55}$$

$$CH_3C(O)O\dot{O} + NO_2 \overset{M}{\rightleftarrows} CH_3C(O)OONO_2, \tag{3-56, -3-56}$$

with the NO_2 reaction forming the thermally unstable PAN. The higher aldehydes also lead to PANs (Roberts, 1990); for example, propionaldehyde reactions lead to the formation of

peroxypropionyl nitrate (PPN). Although the rate constant at atmospheric pressure for the thermal decomposition of PAN (Atkinson et al., 1992a) is such that the lifetime of PAN, with respect to thermal decomposition, is $\approx$30 min at 298 K in the lower troposphere, the thermal lifetime of PAN is calculated to be several hundred years in the upper troposphere. Reaction with OH radicals or photolysis, or both, therefore will dominate as the loss processes of PANs in the upper troposphere (Atkinson et al., 1992a).

The transport of PAN out of urban areas into colder air masses (e.g., to higher altitude) leads to PAN becoming a temporary reservoir of NO_x, allowing the long-range transport of NO_x to less polluted areas. Release of NO_2 in these less polluted areas via Reaction -3-56, with subsequent photolysis of NO_2, then leads to O_3 formation and the pollution of remote areas.

Because the $\dot{C}H_3$ radical formed from the NO reaction with $CH_3C(O)O\dot{O}$ leads to HCHO formation, the OH radical reaction with CH_3CHO subsequently leads to the formation of HCHO. The same process occurs for propionaldehyde, which reacts to form CH_3CHO and then HCHO. Benzaldehyde appears to behave as a phenyl-substituted aldehyde, with respect to its OH radical reaction, and the analog to PAN is then peroxybenzoyl nitrate, PBzN ($C_6H_5C(O)OONO_2$).

The formation of HCHO from CH_3CHO and of CH_3CHO and then HCHO from propionaldehyde are examples of "cascading", in which the photochemical degradation of emitted VOCs leads to the formation of further VOCs, typically containing fewer carbon atoms than the precursor VOC. This process continues until the degradation products are removed by wet and dry deposition or until CO or CO_2 are the degradation products. The reactions of each of these VOCs (i.e., the initially emitted VOC and its first-, second-, and successive-generation products), in the presence of high concentrations of NO, can lead to the formation of O_3.

As discussed in Section 3.2.3.3 for HCHO, the photolysis of carbonyl compounds can lead to the formation of new radicals that result in enhanced photochemical activity. The OH radical reactions of the ketones are generally analogous to the reaction schemes for the alkanes and aldehydes.

Alcohols and Ethers

A number of alcohols and ethers are used in gasolines and in alternative fuels. The alcohols include methanol, ethanol, and *tert*-butyl alcohol, and the ethers include methyl *tert*-butyl ether (MTBE) and ethyl *tert*-butyl ether (ETBE). Table 3-2 shows that in the troposphere these VOCs react only with the OH radical. The relatively long calculated lifetime of methanol in the troposphere (15 days), due to reaction with the OH radical (Table 3-2), suggests that methanol also will be removed from the troposphere by wet and dry deposition and that these physical loss processes may dominate the OH radical reactions proceded by H-atom abstraction from the C-H bonds (and to a minor extent from the O-H bonds in the alcohols), for example, for methanol,

$$OH + CH_3OH \rightarrow H_2O + CH_3O \qquad (15\%) \quad (3\text{-}57a)$$

$$OH + CH_3OH \rightarrow H_2O + CH_2OH. \qquad (85\%) \quad (3\text{-}57b)$$

In the troposphere, both of the CH_3O and CH_2OH radicals react only with O_2 to form HCHO.

$$CH_3O + O_2 \rightarrow HCHO + HO_2 \tag{3-58}$$

$$CH_2OH + O_2 \rightarrow HCHO + HO_2 \tag{3-59}$$

The overall reaction is then

$$OH + CH_3OH + O_2 \rightarrow H_2O + HCHO + HO_2. \tag{3-60}$$

The reaction sequence for ethanol is similar (Atkinson, 1994). Product studies of the OH radical-initiated reactions of MTBE and ETBE in the presence of NO_x have been carried out by Japer et al. (1990), Smith et al. (1991a, 1992), Tuazon et al. (1991), and Wallington and Japar (1991). The major products from MTBE are *tert*-butyl formate, HCHO, and methyl acetate [$CH_3C(O)OCH_3$] and, from ETBE, *tert*-butyl formate, *tert*-butyl acetate, HCHO, CH_3CHO, and ethyl acetate. The available product data and the reaction mechanisms have been reviewed by Atkinson (1994), and that reference should be consulted for further details.

In addition to the use of alcohols and ethers in gasolines and alternative fuels, unsaturated alcohols have been reported as emissions from vegetation (Arey et al., 1991a; Goldan et al., 1993), and kinetic and product studies have begun to be reported for these biogenic VOCs (Grosjean et al., 1993a).

Primary Products and Areas of Uncertainty for the Tropospheric Degradation Reactions of Volatile Organic Compounds

The tropospheric degradation reactions of the alkanes, alkenes (including those of biogenic origin), aromatic hydrocarbons, carbonyls (often formed as products of the degradation reactions of alkanes, alkenes, and aromatic hydrocarbons), and other oxygenates have been briefly discussed above. A more lengthy and detailed discussion of the atmospheric chemistry of alkanes, alkenes, aromatic hydrocarbons, and oxygen- and nitrogen-containing organic compounds emitted into the atmosphere from anthropogenic and biogenic sources and of their atmospheric transformation products is given in the review and evaluation of Atkinson (1994), which also provides an assessment of the uncertainties in the product yields and the reaction rate constants. The first-generation products of the alkanes, alkenes, and aromatic hydrocarbons follow (unfortunately, complete product distributions have not been obtained for most of the VOCs studied).

Alkanes.

- Carbonyl compounds (i.e., aldehydes and ketones) are formed as major products for the smaller ($\leq C_4$) alkanes.
- Alkyl nitrates are formed from the $\geq C_3$ alkanes studied to date. The yields increase with the size of the alkane from $\approx 4\%$ for propane to $\approx 30\%$ for *n*-octane.

- δ-Hydroxycarbonyls are expected to be formed after the alkoxy radical isomerization reaction. To date, no direct evidence for the formation of these compounds exists. For the larger alkanes, the formation yields of these compounds could be high.
- Alkyl hydroperoxides are formed under low-NO conditions.
- Alkyl peroxynitrates ($ROONO_2$) are formed but have short lifetimes (a few seconds at 298 K) due to thermal decomposition.
- Alcohols are formed from the combination reactions of the peroxy radicals under low-NO experimental conditions. These compounds are expected to be formed in low concentrations in the troposphere.

The major uncertainties in the atmospheric chemistry of the alkanes concern the formation of $RONO_2$ from the reactions of the peroxy radicals with NO (Reaction 3-44b) and the reactions of the alkoxy radicals in the troposphere. These uncertainties affect the amount of NO to NO_2 conversion occurring and, hence, the amounts of O_3 that are formed during the NO_x-air photooxidations of the alkanes.

Alkenes.

- Carbonyl compounds (aldehydes and ketones) are formed as major products of the OH radical, NO_3 radical, and O_3 reactions.
- Organic acids are formed from the O_3 reactions, but probably in low yields.
- Hydroxynitrates and nitratocarbonyls are formed from the OH radical reactions and NO_3 radical reactions, respectively. The hydroxynitrates are formed in low yields from the OH radical reactions, whereas the nitratocarbonyls may be major products of the NO_3 radical reactions.
- Hydroxycarbonyls and carbonyl-acids are also expected to be formed, although few, if any, data exist to date.
- Decomposition products are produced from the initially energy-rich biradicals formed in the O_3 reactions; these include CO, CO_2, esters, hydroperoxides, and, in the presence of NO_x, peroxyacyl nitrates ($RC(O)OONO_2$ and PANs).

The major areas of uncertainty concern the products and mechanisms of the O_3 reactions (in particular, the radical yields from these reactions that affect the O_3 formation yields from the NO_x-air photooxidations of the alkenes) and the reaction products and mechanisms of the OH radical reactions with the alkenes containing more than four carbon atoms.

Aromatic Hydrocarbons.

- Phenolic compounds, such as phenol and cresols, have been shown to be major products of the atmospheric reactions of the aromatic hydrocarbons under laboratory conditions.
- Aromatic aldehydes, such as benzaldehyde, are formed in $\leq 10\%$ yield.
- α-Dicarbonyls, such as glyoxal, methylglyoxal, and biacetyl, are formed in fairly high (10 to 40%) yields. These dicarbonyls photolyze rapidly to form radicals and, therefore, are important products with respect to the photochemical activity of the aromatic hydrocarbons.
- Unsaturated carbonyl or hydroxycarbonyl compounds are formed, although there is little direct information concerning the formation of these products.

There is a lack of knowledge of the detailed reaction mechanisms and products for the aromatic hydrocarbons under tropospheric conditions (i.e., for the NO_x concentrations encountered in urban and rural areas). It is possible that the products observed in laboratory

studies and their formation yields are not representative of the situation in the troposphere. This then leads to an inability to formulate detailed reaction mechanisms for the atmospheric degradation reactions of the aromatic hydrocarbons, and the chemical mechanisms used in urban airshed models then must rely heavily on environmental (or "smog") chamber data.

Oxygenated Compounds.

- The products observed from the atmospheric photooxidations of oxygenated organics are carbonyls, organic acids (e.g., RC(O)OH), esters, alcohols, and, in the presence of NO_x, PANs.

The major area of uncertainty concerns the importance of photolysis of carbonyl compounds in the troposphere, and the products formed. In particular, there is a lack of information concerning the absorption cross-sections and photoodissociation quantum yields for most of the aldehydes and ketones other than HCHO, CH_3CHO, and CH_3COCH_3.

3.2.4.2 Chemical Formation of Ozone in Polluted Air

Major Steps in Ozone Formation

As discussed earlier, NO_x and VOCs interact under the influence of sunlight to form O_3 and other photochemical air pollutants. The major steps in this process are the conversion of NO to NO_2 by peroxy radicals, with the photolysis of NO_2 leading to O_3 production. In the absence of a VOC, Reactions 3-1 through 3-3,

$$NO + O_3 \rightarrow NO_2 + O_2 \qquad (3\text{-}3)$$

$$NO_2 + h\nu \xrightarrow{O_2} NO + O_3, \qquad (3\text{-}1,\ 3\text{-}2)$$

do not lead to any net formation of O_3. The reaction of a VOC with the OH radical, or its photolysis, leads to the formation of HO_2 and organic peroxy (RO_2) radicals, which react with NO under high-NO conditions:

$$VOC(+\ OH,\ h\nu) \xrightarrow{O_2} R\dot{O}_2 \qquad (3\text{-}61)$$

$$R\dot{O}_2 + NO \rightarrow R\dot{O} + NO_2 \qquad (3\text{-}44a)$$

$$NO_2 + h\nu \xrightarrow{O_2} NO + O_3 \qquad (3\text{-}1,\ 3\text{-}2)$$

Net:

$$VOC(+\ OH,\ h\nu) \xrightarrow{O_2} R\dot{O}_2 + O_3. \qquad (3\text{-}62)$$

with the alkoxy (RO) radical producing further HO_2 or RO_2 radicals or both, and, hence, resulting in further production of O_3. This process is shown schematically in Figure 3-4.

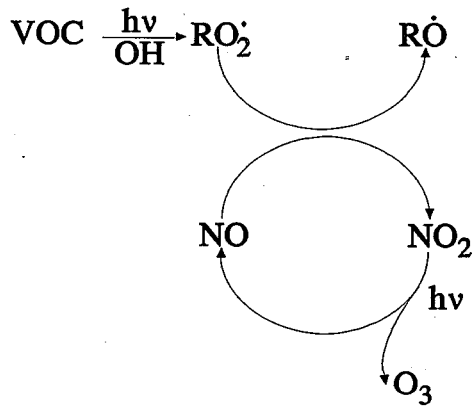


Figure 3-4. Major steps in production of ozone in ambient air (R = H, alkyl or substituted alkyl, or acyl).

The general time-concentration profiles for selected species during irradiation of an NO_x-VOC-air mixture are shown in Figure 3-5 for a constant light intensity and in Figure 3-6 for diurnally varying light intensity. These general features of an NMOC-NO_x-air irradiation are described by the reactions described below.

The conversion of NO to NO_2 occurs through the oxidation reactions:

$$\text{organic}(+\ OH,\ h\nu,\ O_3) \rightarrow R\dot{O}_2 \qquad (3\text{-}63)$$

$$R\dot{O}_2 + NO \rightarrow R\dot{O} + NO_2 \qquad (3\text{-}44a)$$

$$R\dot{O} \xrightarrow{O_2} \text{carbonyl} + HO_2 \qquad (3\text{-}64)$$

$$HO_2 + NO \rightarrow OH + NO_2. \qquad (3\text{-}32)$$

The maximum concentration of NO_2 is less than the initial NO + NO_2 concentration because NO_2 is removed through the reaction

$$OH + NO_2 \xrightarrow{M} HNO_3. \qquad (3\text{-}15)$$

This reaction removes radicals (OH and NO_2) and NO_x from the system. In addition to the removal of NO_x through Reaction 3-15, NO_x also can be removed through the formation of temporary reservoir species such as organic nitrates (Reaction 3-44b) and PAN (Reaction 3-56).

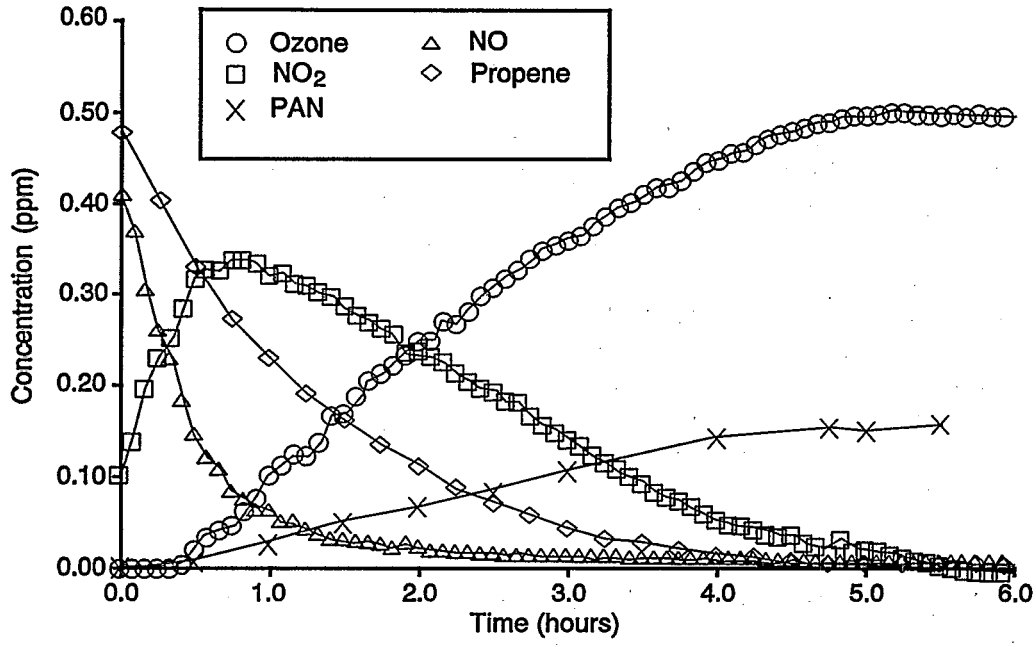


Figure 3-5. Time-concentration profiles for selected species during irradiations of a nitrogen oxide-propene-air mixture in an indoor chamber with constant light intensity.

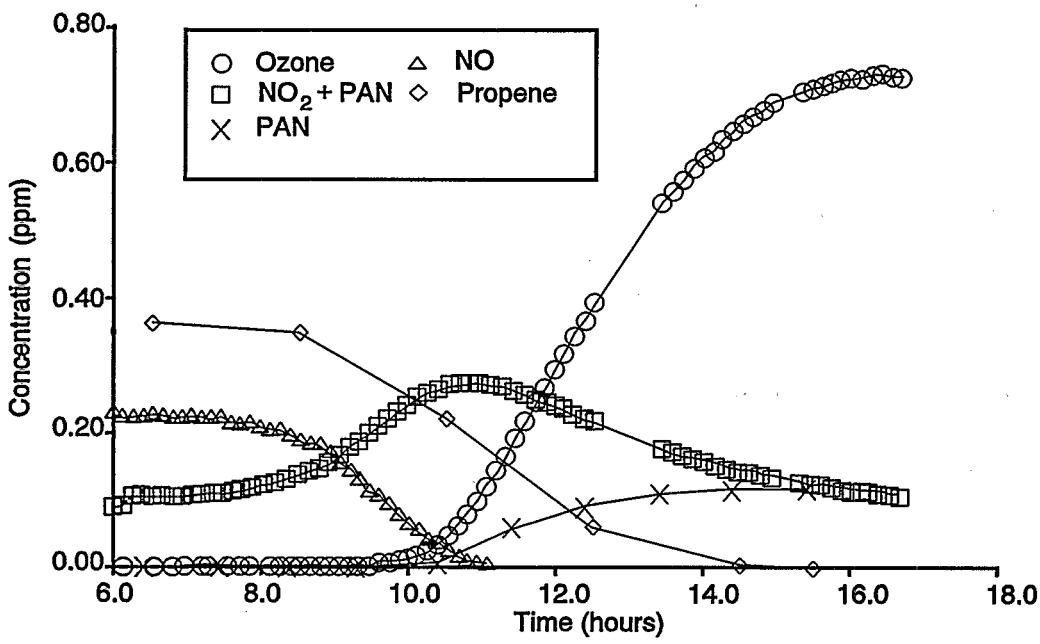


Figure 3-6. Time-concentration profiles for selected species during irradiations of a nitrogen oxide-propene-air mixture in an outdoor chamber with diurnally varying light intensity.

$$RO_2 + NO \rightarrow RONO_2 \quad (3\text{-}44b)$$

$$CH_3C(O)OO + NO_2 \rightarrow CH_3C(O)OONO_2 \quad (3\text{-}56)$$

The O_3 concentration increases with the NO_2/NO concentration ratio, and O_3 formation ceases when NO_2 (and hence NO_x) has been removed by reaction.

Formation of PAN occurs by Reaction 3-56. Because of Reactions 3-55 and 3-56, the PAN concentration also increases with the NO_2/NO concentration ratio, and PAN formation also ceases when NO_x has been depleted.

The removal processes for NO_x are by reaction of NO_2 with the OH radical to form HNO_3 (Reaction 3-16), the formation of organic nitrates from the $RO\dot{O}$ + NO Reaction 3-44b pathway, and the formation of PAN through Reaction 3-56. The initially present NO_x is converted to organic nitrates, HNO_3, and thermally unstable PANs. At ambient temperature, the PANs will gradually thermally decompose to yield NO_2 and the acylperoxy radicals; hence, the ultimate fate of NO_x will be to form HNO_3 and organic nitrates.

Effects of Varying Initial Nitrogen Oxide and Nonmethane Volatile Organic Compound Concentrations

As discussed in Section 3.2.4.2, NO_x and VOC interact in sunlight to form O_3 and other photochemical air pollutants. The formation of O_3 from the NO_x and VOC precursors is nonlinear with respect to the precursor emissions (or ambient concentrations). As discussed in detail in Section 3.6, computer models incorporating emissions, meteorology, and chemistry are necessary for a full understanding of the complexities of the NO_x-VOC-O_3 system. The major reactions in irradiated VOC-NO_x-air mixtures (see Section 3.2.4.2) include

$$OH + VOC \xrightarrow{O_2} RO_2, \quad (3\text{-}61)$$

which is in competition with Reaction 3-15,

$$OH + NO_2 \rightarrow HNO_3, \quad (3\text{-}15)$$

which removes both radicals and NO_x.

$$RO_2 + NO \rightarrow RO + NO_2 \quad (3\text{-}44a)$$

$$NO_2 + h\nu \xrightarrow{O_2} NO + O_3 \quad (3\text{-}1, 3\text{-}2)$$

$$NO + O_3 \rightarrow NO_2 + O_2 \quad (3\text{-}3)$$

Based on these reactions, as the VOC/NO_x ratio decreases, Reaction 3-15 competes more successfully with Reaction 3-61 in removing radicals from the system for a constant source

of OH radicals, and, thus, slows down the formation of O_3 (and vice-versa). The ultimate amount of O_3 formed depends on the NO_2 available for photochemical processing through Reactions 3-44a, 3-1, 3-2, and 3-3. Because of the diurnal light intensity variation and the length of daylight available for reactions and photolysis to occur, O_3 formation from VOC-NO_x-air mixtures, especially as a function of the VOC/NO_x ratio, is complex and depends on the time or distance scale being considered (for example, transport of urban plumes to rural areas) (Wolff, 1993; Finlayson-Pitts and Pitts, 1993). In general, reducing the VOC/NO_x ratio by reducing VOC emissions slows down the formation rate of O_3, leading to lower O_3 levels in urban areas and in areas downwind of urban complexes. Reduction of NO_x emissions leads to a more rapid formation of O_3, although with less O_3 formed, and, hence, inner-urban areas may experience higher O_3 levels with NO_x control than with VOC control, whereas suburban and rural areas may have lower O_3 levels with NO_x control (Trainer et al., 1993; Olszyna et al., 1994).

Effects of Biogenic Nonmethane Volatile Organic Compound Emissions

Biogenic VOC emissions can be important in urban and rural areas (Trainer et al., 1987; Chameides et al., 1988, 1992; Roselle et al., 1991) and can contribute to O_3 formation in much the same way as anthropogenic VOCs. Modeling simulations in which urban biogenic VOC emissions are first included and then excluded from the calculations generally indicate little effect of the biogenic emissions on the predicted O_3 levels. This is not unexpected from the shape of the O_3 isopleths at high VOC/NO_x ratios (Chameides et al., 1988; Section 3.6). However, results of modeling studies in which anthropogenic VOC emissions are removed from the simulations (but anthropogenic NO_x emissions are left unaltered) suggest that anthropogenic NO_x together with biogenic VOCs may form sufficient O_3 to exceed the NAAQS, at least in certain areas (Chameides et al., 1988). Thus, for the anthropogenic and biogenic VOC emissions considered by Chameides et al. (1988) for Atlanta, GA, changes in either the anthropogenic VOC emissions or biogenic VOC emissions have little effect on O_3 levels. Therefore, as discussed for the Atlanta region (Chameides et al., 1988), NO_x control may be more favorable than VOC control in urban areas with substantial biogenic NMOC emissions.

Although it is known that isoprene is reactive with respect to the formation of O_3 (Section 3.2.4.3) and that the monoterpenes react rapidly with OH radicals, NO_3 radicals, and O_3, the O_3-forming potentials of the various monoterpenes emitted into the atmosphere are not known.

3.2.4.3 Hydrocarbon Reactivity with Respect to Ozone Formation

As discussed in Section 3.2.4, VOCs are removed and transformed in the troposphere by photolysis and by chemical reaction with OH radicals, NO_3 radicals, and O_3. In the presence of sunlight, the degradation reactions of the VOCs lead to the conversion of NO to NO_2 and the formation of O_3 and various organic products. However, different VOCs react at differing rates in the troposphere because of their differing tropospheric lifetimes (Table 3-2). The lifetimes of most VOCs with respect to reaction with OH radicals and O_3 are in the range ≈ 1 h to ≈ 10 years. In large part, because of these differing tropospheric lifetimes and rates of reaction, VOCs exhibit a range of reactivities with respect to the formation of O_3 (Altshuller and Bufalini, 1971, and references therein).

A number of "reactivity scales" have been developed over the years (see, for example, Altshuller and Bufalini, 1971, and references therein; Darnall et al., 1976),

including the rate of VOC disappearance in NO_x-VOC-air irradiations, the rate of NO to NO_2 conversion in NO_x-VOC-air irradiations, O_3 formation in NO_x-single VOC-air irradiations, eye irritation, and the rate constant for reaction of the VOC with the OH radical. It appears, however, that a useful definition of "reactivity" is that of "incremental reactivity" (*IR*), defined as the amount of O_3 formed per unit of VOC added or subtracted from the VOC mixture in a given air mass under high-NO conditions (Carter and Atkinson, 1987, 1989b):

$$IR = \Delta[O_3]/\Delta[\mathrm{VOC}], \tag{3-65}$$

at the limit of $\Delta[\mathrm{VOC}] \rightarrow 0$. The concept of incremental reactivity and some further details of this approach are illustrated by the general reaction mechanism for the photooxidation of an alkane, RH:

$$\mathrm{OH} + \mathrm{RH} \rightarrow \mathrm{H_2O} + \dot{\mathrm{R}} \tag{3-42}$$

$$\dot{\mathrm{R}} + \mathrm{O_2} \rightarrow \mathrm{R\dot{O}_2} \tag{3-43}$$

$$\mathrm{R\dot{O}_2} + \mathrm{NO} \rightarrow \mathrm{R\dot{O}} + \mathrm{NO_2} \tag{3-44a}$$

$$\mathrm{R\dot{O}} \rightarrow \text{carbonyl} + \mathrm{HO_2} \tag{3-65}$$

$$\mathrm{HO_2} + \mathrm{NO} \rightarrow \mathrm{OH} + \mathrm{NO_2}. \tag{3-32}$$

The net reaction,

$$\mathrm{OH} + \mathrm{RH} + 2\ \mathrm{NO} \xrightarrow{\mathrm{O_2}} \text{carbonyl} + 2\ \mathrm{NO_2} + \mathrm{OH}, \tag{3-66}$$

can be viewed as involving the two separate reaction sequences:

(1) the formation of organic peroxy ($\mathrm{R\dot{O}_2}$) radicals from the reactions,

$$\begin{array}{ll} & \mathrm{OH} + \mathrm{RH} \rightarrow \mathrm{H_2O} + \dot{\mathrm{R}} \\ & \dot{\mathrm{R}} + \mathrm{O_2} \rightarrow \mathrm{R\dot{O}_2} \\ \hline \text{Net:} & \mathrm{OH} + \mathrm{RH} \rightarrow \mathrm{R\dot{O}_2}, \end{array}$$

and (2) the conversion of NO to NO_2 and the formation of O_3 and other products,

$$\dot{RO}_2 + NO \rightarrow \dot{RO} + NO_2$$

$$\dot{RO} \rightarrow \text{carbonyl} + HO_2$$

$$HO_2 + NO \rightarrow OH + NO_2$$

$$\text{Net:} \quad \dot{RO}_2 + 2\,NO \rightarrow \text{carbonyl} + 2\,NO_2 + OH\,.$$

The photolysis of NO_2 then leads to O_3 formation (Reactions 3-1 and 3-2). The first reaction sequence determines how fast $\dot{RO}_2$ radicals are generated from the VOC, which is called "kinetic reactivity" (Carter and Atkinson, 1989b). For the case given above, where the only reaction of the VOC is with the OH radical, the kinetic reactivity depends solely on the OH radical reaction rate constant. The second reaction sequence, leading to NO to NO_2 conversion, regeneration of OH radicals, and the formation of product species, determines the efficiency of formation of O_3 from the $\dot{RO}_2$ radicals formed from the first reaction sequence and is termed "mechanistic reactivity" (Carter and Atkinson, 1989b). The second reaction sequence can be represented as

$$\dot{RO}_2 + \alpha NO \rightarrow \beta\, NO_2 + \gamma OH + \delta \text{ products.} \tag{3-67}$$

In general, the faster a VOC reacts in the atmosphere, the higher the incremental reactivity. However, the chemistry subsequent to the initial reaction does affect the O_3-forming potential of the VOC. Thus, the existence of NO_x sinks in the reaction mechanism (low values of β or values of α-$\beta > 0$) leads to a decrease in the amount of O_3 formed. Examples of NO_x sinks are the formation of organic nitrates and PANs (which are also sinks for radicals). The generation or loss of radical species can lead to a net formation or net loss of OH radicals ($\gamma > 1$ or $\gamma < 1$, respectively). This, in turn, leads to an enhancement or suppression of radical concentrations in the air parcel and to an enhancement or suppression of the overall reactivity of all VOCs in that air parcel by affecting the rate of formation of $\dot{RO}_2$ radicals.

These effects vary in importance depending on the VOC/NO_x ratio. Nitrogen oxides sinks are most important at high VOC/NO_x ratios (NO_x-limited), affecting the maximum O_3 formed; although the formation or loss of OH radicals is most important at low VOC/NO_x ratios, affecting the initial rate at which O_3 is formed (Carter and Atkinson, 1989b). In addition to depending on the VOC/NO_x ratio (Table 3-3), incremental reactivity depends on the composition of the VOC mixture and on the physical conditions encountered by the air mass (including the dilution rate, light intensity, and spectral distribution (Carter and Atkinson, 1989b; Carter, 1991).

Table 3-3. Calculated Incremental Reactivities of Selected Volatile Organic Compounds as a Function of the Volatile Organic Compound/Nitrogen Oxide Ratio for an Eight-Component Volatile Organic Compound Mixture[a] and Low-Dilution Conditions[b]

	VOC/NO_x Ratio (ppmC/ppm)			
NMOC	4	8	16	40
CO	0.011	0.022	0.016	0.005
Ethane	0.024	0.041	0.018	0.007
n-Butane	0.10	0.16	0.069	0.019
n-Octane	0.068	0.12	0.027	−0.031
Ethene	0.85	0.90	0.33	0.14
Propene	1.28	1.03	0.39	0.14
trans-2-Butene	1.42	0.97	0.31	0.054
Benzene	0.038	0.033	−0.002	−0.002
Toluene	0.26	0.16	−0.036	−0.051
m-Xylene	0.98	0.63	0.091	−0.025
Formaldehyde	2.42	1.20	0.32	0.051
Acetaldehyde	1.34	0.83	0.29	0.098
Methanol	0.12	0.17	0.066	0.029
Ethanol	0.18	0.22	0.065	0.006
Urban Mix	0.41	0.32	0.088	0.011

[a]Eight-component VOC mixture used to simulate NMOC emissions in an urban area.
[b]See Appendix A for abbreviations and acronyms.

Source: Carter and Atkinson (1989b).

The O_3-forming potentials of large numbers of VOCs, including emissions from automobiles using gasoline and various alternative fuels such as methanol and ethanol blends and compressed natural gas, have been investigated by airshed computer models (Chang and Rudy, 1990; Chang et al., 1991a; Derwent and Jenkin, 1991; Andersson-Sköld et al., 1992; McNair et al., 1992, 1994; Carter, 1994). Consistent with the modeling studies of Carter and Atkinson (1987, 1989b), these computer-modeling studies show that VOCs differ significantly in terms of their O_3-forming potential, for single-day as well as multiday conditions (Derwent and Jenkin, 1991; Andersson-Sköld et al., 1992; Carter, 1994; McNair et al., 1994). However, there are some differences between the O_3-forming potentials derived by Derwent and Jenkin (1991) for multiday transport conditions over Europe and by Carter (1994) for 1-day urban airshed "maximum incremental reactivity" conditions, especially for HCHO, an important direct emission and atmospheric transformation product of most VOCs.

Recent modeling studies have been carried out by Carter (1994) and McNair et al. (1994) to determine the O_3-forming potential of alternative fuels. Emissions from vehicles

using 85% methanol and 15% gasoline (M-85) were shown to have ≈40% of the O_3-forming potential of emissions from gasoline-fueled vehicles. Emissions from vehicles using liquefied petroleum gas (LPG) and compressed natural gas (CNG) were shown to have O_3-forming potentials that are ≈50% and ≈18%, respectively, of the O_3-forming potential of emissions from gasoline-fueled vehicles (McNair et al., 1994).

3.2.5 Photochemical Production of Aerosols

The chemical processes involved in the formation of O_3 and other photochemical pollutants from the interaction of NO_x and VOCs lead to the formation of OH radicals and oxidized VOC reaction products that often are of lower volatility than the precursor VOC. The OH radicals that oxidize the VOCs and lead to the generation of RO_2 radicals and conversion of NO to NO_2 (with subsequent photolysis of NO_2 form O_3) also react with NO_2 and sulfur dioxide (SO_2) to form HNO_3 and H_2SO_4, respectively, which can become incorporated into aerosols as particulate nitrate (NO_3^-) and sulfate (SO_4^{2-}). The low-volatility VOC reaction products can condense onto existing particles in the atmosphere to form secondary organic aerosol matter. Hence, O_3 formation, acid formation, and secondary aerosol formation in the atmosphere are so related that controls aimed at reducing O_3 levels can impact (positively or negatively) acid and secondary aerosol formation in the atmosphere.

3.2.5.1 Phase Distributions of Organic Compounds

Chemical compounds are emitted into the atmosphere in both gaseous and particle-associated forms. The emissions from combustion sources (e.g., vehicle exhaust) are initially at elevated temperature, and compounds that may be in the particle phase at ambient atmospheric temperature may be in the gas phase when emitted. In addition, atmospheric reactions of gas-phase chemicals can lead to the formation of products that then condense onto particles, or self-nucleate (Pandis et al., 1991; Wang et al., 1992; Zhang et al., 1992). Measurements of ambient atmospheric gas- and particle-phase concentrations of several classes of organic compounds indicate that the phase distribution depends on the liquid-phase vapor pressure, PL (Bidleman, 1988; Pankow and Bidleman, 1992). The available experimental data and theoretical treatments show that, as a rough approximation, organic compounds with PL > 10^{-6} torr at ambient temperature are mainly in the gas phase (Bidleman, 1988). As expected, the gas-particle phase distribution in the atmosphere depends on the ambient temperature, with the chemical being more particle-associated at lower temperatures. The gas-to-particle adsorption-desorption process can be represented as,

$$A + TSP \rightleftarrows F, \tag{3-68}$$

where A is the gas-phase compound, F is the particle-phase compound, and TSP is the total suspended particulate matter. The relationship among these three species is expressed using a particle-gas partition coefficient, K:

$$K = F/(TSP)A. \tag{3-69}$$

Because K is a constant at a given temperature, if TSP increases (for example, in going from a clean remote atmosphere to an urban area), F/A also must increase and the chemical becomes more particle-associated (Pankow and Bidleman, 1991, 1992).

Gaseous and particulate species in the atmosphere are subject to wet and dry deposition. Dry deposition refers to the uptake of gases and particles at the earth's surface by vegetation, soil, and water, including lakes, rivers, oceans, and snow-covered ground. Wet deposition refers to the removal of gases and particles from the atmosphere through incorporation into rain, fog, and cloud water, followed by precipitation to the earth's surface. These processes are discussed further in Section 3.6.

For gases, dry deposition is important primarily for HNO_3, SO_2, and H_2O_2 as well as for O_3 and PAN, whereas wet deposition is important for water-soluble gases such as HNO_3, H_2O_2, phenols, and, under atmospheric conditions, SO_2. Dry deposition of particles depends on the particle size; those of a mean diameter of ≈ 0.1 to 2.5 μm have lifetimes with respect to dry deposition of ≈ 10 days (Graedel and Weschler, 1981; Atkinson, 1988), sufficient for long-range transport. However, particles are efficiently removed from the atmosphere by wet deposition (Bidleman, 1988).

Particles can form in the atmosphere by condensation or by coagulation, generally occurring by coagulation in urban and rural areas. The photooxidation reactions of VOCs typically lead to the formation of more oxidized and less volatile product species. When the vapor pressures exceed the saturated vapor pressure (i.e., vapor pressure $< 10^{-6}$ Torr), the products will become particle-associated (Pandis et al., 1991, 1992). Accumulation-size particles are in the size range 0.08 to 2.5 μm diameter (Whitby et al., 1972).

In urban areas, the major sources of particulate matter (Larson et al., 1989; Solomon et al., 1989; Wolff et al., 1991; Hildemann et al., 1991a,b; Rogge et al., 1991, 1993; Chow et al., 1993) are

- direct emissions of elemental carbon from, for example, diesel-powered vehicles (Larson et al., 1989);
- direct emissions of primary organic carbon from, for example, meat cooking operations, paved road dust, and wood-burning fireplaces and other combustion sources (Hildemann et al., 1991a,b; Rogge et al., 1991, 1993);
- secondary organic material formed in the atmosphere from the atmospheric photooxidations of gas-phase NMOC (Turpin and Huntzicker, 1991; Pandis et al., 1992);
- the conversion of NO and NO_2 to HNO_3, followed by neutralization by NH_4 or through combination with other cations to form aerosol nitrates:

$$NH_3(gas) + HNO_3(gas) \rightleftarrows NH_4NO_3(aerosol); \qquad (3\text{-}69a)$$

- the conversion of SO_2 (and other sulfur-containing species) to H_2SO_4, which has sufficiently low volatility to move to the aerosol phase; and
- emission into the atmosphere of "fine dust", for example, crustal material.

Because the fine-particle size range is the same magnitude as the wavelength of visible light, particulate matter present in the atmosphere leads to light scattering and absorption, and hence to visibility reduction (Larson et al., 1989; Eldering et al., 1993).

3.2.5.2 Acid Deposition

As noted above, the chemical processes involved in the formation of O_3 and other photochemical pollutants from the interaction of NMOC and NO_x also lead to the formation of acids in the atmosphere. The two major acidic species in ambient air are HNO_3 and H_2SO_4, arising from the atmospheric oxidation of NO_x and SO_2, respectively. Reduced sulfur compounds emitted from biogenic sources and certain anthropogenic sources also may lead to SO_2 or sulfonic acids (Tyndall and Ravishankara, 1991).

The major sulfur-containing compound emitted into the atmosphere from anthropogenic sources is SO_2. In the troposphere, the important loss processes of SO_2 are dry deposition (Atkinson, 1988, and references therein), reactions within cloud water, and gas-phase reaction with the OH radical. The rate constant for the reaction of SO_2 with the OH radical is such that the lifetime of SO_2 with respect to gas-phase reaction with the OH radical is $\approx$15 days. The reaction proceeds by (Stockwell and Calvert, 1983; Atkinson et al., 1992a)

$$OH + SO_2 \xrightarrow{M} HOSO_2 \tag{3-70}$$

$$HOSO_2 + O_2 \rightarrow HO_2 + SO_3 \tag{3-71}$$

$$SO_3 + H_2O \rightarrow H_2SO_4. \tag{3-72}$$

The reaction of SO_3 with water vapor is slow in the gas-phase (Atkinson et al., 1992a) and, hence, this may be a heterogeneous reaction. Because of its low vapor pressure, H_2SO_4 exists in the aerosol or particle phase in the atmosphere.

Dry deposition is an important atmospheric loss process for SO_2, because SO_2 has a fairly long lifetime, due to gas-phase chemical processes, and also has a high deposition velocity. A lifetime, in relation to dry deposition of 2 to 3 days, appears reasonable (Schwartz, 1989).

Sulfur dioxide is not very soluble in pure water (Schwartz, 1989). However, the presence of pollutants such as H_2O_2 or O_3, in the aqueous phase, displaces the equilibrium and allows gas-phase SO_2 to be incorporated into cloud, rain, and fog water, where it is oxidized rapidly (Schwartz, 1989; Pandis and Seinfeld, 1989, and references therein):

$$SO_2(gas) \rightleftarrows SO_2(aqu) \tag{3-73}$$

$$SO_2(aqu) + H_2O \rightleftarrows HSO_3^- + H^+ \rightleftarrows SO_3^{2-} + 2\,H^+ \tag{3-74}$$

$$HSO_3^- + H_2O_2 \rightarrow SO_4^{2-} + H^+ + H_2O \tag{3-75}$$

$$SO_3^{2-} + O_3 \rightarrow SO_4^{2-} + O_2. \tag{3-76}$$

In addition, aqueous sulfur can be oxidized in a process catalyzed by transition metals such as iron(III) (Fe^{3+}) and manganese(II) (Mn^{2+}) (Graedel et al., 1986b; Weschler et al., 1986; Pandis and Seinfeld, 1989).

$$SO_3^{2-} + 1/2\ O_2 \rightarrow SO_4^{2-} \tag{3-77}$$

The oxidation rate of aqueous sulfur by O_3 decreases as the pH decreases (i.e., as the acidity increases) and this oxidation route is therefore self-limiting and generally of minor importance in the atmosphere. The oxidation of SO_2 by H_2O_2 appears to be the dominant aqueous-phase oxidation process of SO_2 (Chandler et al., 1988; Gervat et al., 1988; Schwartz, 1989; Pandis and Seinfeld, 1989; Fung et al., 1991b), although the transition metal-catalyzed oxidation of SO_2 may also be important (Jacob et al., 1989). It should be noted that aqueous-phase H_2O_2 arises, in part, from the absorption of HO_2 radicals and H_2O_2 into the aqueous phase, with HO_2 radicals being converted into H_2O_2 (Zuo and Hoigne, 1993).

The oxidation of SO_2 to sulfate in clouds and fogs is often much faster than the homogeneous gas-phase oxidation of SO_2 initiated by reaction with the OH radical. The gas-phase oxidation rate is $\approx$0.5 to 1% h^{-1}, whereas the aqueous-phase (cloud) oxidation rate may be as high as 10 to 50% h^{-1} (Schwartz, 1989).

The oxidation of NO_x to HNO_3 and nitrates was discussed in Section 3.2.3. During daylight hours, oxidation occurs by the gas-phase reaction of NO_2 with the OH radical:

$$OH + NO_2 \overset{M}{\rightarrow} HNO_3, \tag{3-15}$$

with the lifetime of NO_2 due to Reaction 3-15 calculated to be $\approx$1.4 days. Nitric acid is removed from the troposphere by wet and dry deposition, with wet deposition being efficient. During nighttime hours, NO_2 can be converted into NO_3 radicals and N_2O_5:

$$NO_2 + O_3 \rightarrow NO_3 + O_2 \tag{3-12}$$

$$NO_3 + NO_2 \overset{M}{\rightleftarrows} N_2O_5, \tag{3-13, -3-13}$$

with N_2O_5 undergoing wet or dry deposition, or both. The reader is referred to Schwartz (1989) for further discussion of the conversion of NO_x to NO_3 and HNO_3 and of acid deposition.

3.3 Meteorological Processes Influencing Ozone Formation and Transport

Day-to-day variability in O_3 concentrations is, to a first approximation, the result of day-to-day variations in meteorological conditions. This section presents a succinct overview of those atmospheric processes that affect the concentrations of O_3 and other oxidants in urban and rural areas. Included in this list of processes are the vertical structure and dynamics of the PBL; transport processes, including thermally-driven mesoscale circulations such as lake and sea breeze circulations; complex terrain effects on transport and dispersion; vertical exchange processes; deposition and scavenging; and meteorological controls on biogenic emissions and dry deposition.

3.3.1 Meteorological Processès

3.3.1.1 Surface Energy Budgets

Knowledge of the surface energy budget is fundamental to an understanding of the dynamics of the PBL. The PBL is defined as that layer of the atmosphere in contact with the surface of the earth and directly influenced by the surface characteristics. In combination with synoptic winds, it provides the forces for the vertical fluxes of heat, mass, and momentum. The accounting of energy inputs and outputs provides a valuable check on modeled PBL dynamics.

Figure 3-7 illustrates the surface radiation budget for short-wave (wavelength roughly $<0.4\ \mu m$) and long-wave radiation. The radiation budget for the surface can be described in terms of its components as

$$Q_{sfc} = K\downarrow - K\uparrow + L\downarrow - L\uparrow + Q_H + Q_E . \qquad (3\text{-}78)$$

where $K\downarrow$ is the incoming short-wave radiation, $K\uparrow$ is the outgoing short-wave radiation, $L\downarrow$ is the incoming long-wave radiation from the atmosphere, $L\uparrow$ is the outgoing long-wave radiation, and Q_H and Q_E are the heat flux and latent heat flux to the soil, respectively. On a global annual average, Q_{sfc} is assumed to be near zero (i.e., the planet is not heating or cooling systematically, an assumption clearly being questioned with the growing debate on climatic change). On a day-to-day basis, however, Q_{sfc} will certainly vary from zero and will cause changes in surface temperature. Cloud cover, for example, will reduce the amount of short-wave radiation reaching the surface and will modify all the subsequent components of the radiation budget. Moreover, the redistribution of energy through the PBL creates thermodynamic conditions that influence vertical mixing. The treatment of energy budgets has been attempted on the scale of individual urban areas. These studies are summarized by Oke (1978).

For many of the modeling studies of the photochemical production of O_3, the vertical mixing has been parameterized by a single, well-mixed layer. However, because this is a great simplification of a complex structure, and because the selection of rate and extent of vertical mixing may influence local control options, future modeling and observational studies need to address the energy balances so that more realistic simulations can be made of the structure of the PBL.

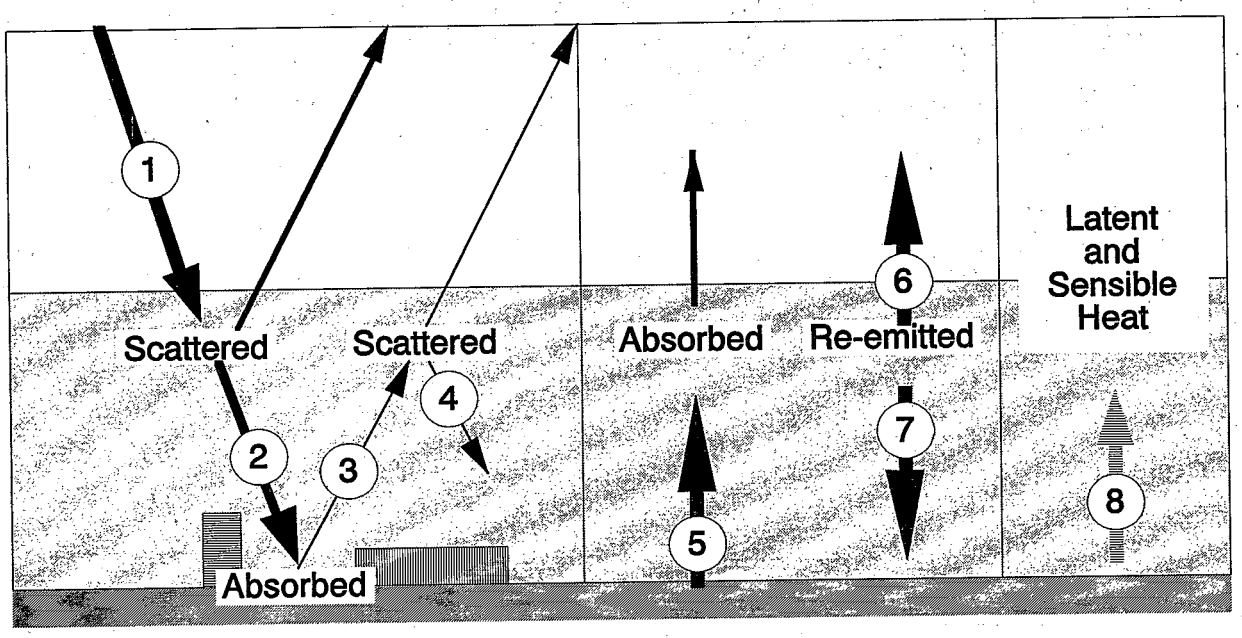


Figure 3-7. ***Surface radiation budget for short- and long-wave radiation. The surface radiation budget is driven by the input of short-wave radiation (1). This direct input is reduced by scatter (2) and absorption passing through the atmosphere. The amount that remains can be absorbed or reflected at the surface. The reflected light (3) also can be scattered back to the surface (4). The short-wave energy absorbed at the surface will ultimately be emitted back to the atmosphere as long-wave radiation (5). The atmosphere absorbs much of this radiation and radiates it back to the surface (7) and out to space (6). This energy cycle is completed as some of the absorbed energy is transmitted to the atmosphere as sensible and latent heat (8).***

3.3.1.2 Planetary Boundary Layer

The concentration of an air pollutant depends significantly on the degree of mixing that occurs between the time a pollutant or its precursors are emitted and the arrival of the pollutant at the receptor. Atmospheric mixing is the result of either mechanical turbulence, often associated with wind shear, or thermal turbulence associated with vertical redistribution of heat energy. The potential for thermal turbulence can be characterized by atmospheric stability. The more stable the air layer, the more work is required to move air vertically.

As air is moved vertically through the atmosphere, as might happen in a convective thermal, its temperature will decrease with height as the result of adiabatic expansion. It is the comparison of how the temperature *should* change with height in the absence of external heating or cooling against the *actual* temperature change with height that is a measure of atmospheric stability. Those layers of the atmosphere where temperature increases with height (inversion layers) are the most stable as air, cooling as it rises, becomes denser than its new warmer environment. In an atmospheric layer with relatively

low turbulence, pollutants do not redistribute vertically as rapidly as they do in an unstable layer. Also, because a stable layer has a relatively low rate of mixing, pollutants in a lower layer will not mix through it to higher altitudes.

The stability of the atmosphere is often measured through computation of potential temperature as

$$\theta = \left[\frac{P}{P_o}\right]^{-R/c_p}, \tag{3-79}$$

where θ is the virtual potential temperature, P is the pressure of the air parcel, P_o is the reference pressure to which the air parcel will be moved (usually 1,000 mb), R is the gas law constant, and c_p is the specific heat of air at constant pressure. The faster θ increases with height, the less the potential for mixing.

A stable layer can also act as a trap for air pollutants lying beneath it. Hence, an elevated inversion is often referred to as a "trapping" inversion. On the other hand, if pollutants are emitted into a stable layer aloft, such as might occur from an elevated stack, the lack of turbulence will keep the effluents from reaching the ground while the inversion persists.

Traditionally, atmospheric mixing has been treated through use of a *mixing height*, which is defined as the base of an elevated inversion layer. In this model, the O_3 precursors are mixed uniformly through the layer below the mixing height. As this layer grows, it both entrains remnant O_3 from previous days and redistributes fresh emissions aloft. The vertical mixing profile through the lower layers of the atmosphere is assumed to follow a typical and predictable cycle on a generally clear day. In such a situation, a nocturnal surface inversion would be expected to form during the night as $L\uparrow$ exceeds $L\downarrow$. This surface layer inversion persists until surface heating becomes significant, probably 2 or 3 h after sunrise. Pollutants initially trapped in the surface inversion may cause relatively high, local concentrations, but these concentrations will decrease rapidly when the surface inversion is broken by surface heating. The boundary formed between the rising, cooling air of the growing mixing layer and that of the existing PBL is often sharp and can be observed as an elevated temperature inversion.

Elevated temperature inversions, when the base is above the ground, are also common occurrences (Hosler, 1961; Holzworth, 1964, 1972). This condition can form simply as the result of rapid vertical mixing from below, but is exacerbated in regions of subsiding air when the sinking air warms to a point at which it is warmer than the rising and cooling underlying air. Because these circumstances are associated with specific synoptic conditions, they are less frequent than the ubiquitous nighttime radiation inversion.
An elevated inversion is, nevertheless, a very significant air pollution feature, because it may persist throughout the day and, thus, restrict vertical mixing.

When compared to a source near the surface and the effects of a radiation (surface) inversion, the pollutant dispersion pattern is quite different for an elevated source plume trapped in a layer near the base of an elevated inversion. This plume will not be in contact with the ground surface in the early morning hours because there is no mixing through the surface radiation inversion. Thus, the elevated plume will not affect surface pollutant concentrations until the mixing processes become strong enough to reach the

altitude of the plume. At that time, the plume may be mixed downward quite rapidly in a process called *fumigation*. During fumigation, surface O_3 concentrations will increase if the morning O_3 concentration is higher aloft than at the ground and if insufficient scavenging by NO occurs at ground-level. In fact, the rapid rise in O_3 concentrations in the morning hours is often the result of vertical (downward) transport from an elevated reservoir of O_3. After this initial increase, surface concentrations can continue to increase as a result of photochemistry or transport of O_3-rich air to the receptor.

When surface heating decreases in the late afternoon and early evening, the surface inversion will form again under most conditions. The fate of the elevated inversion is less clear, however. Although O_3 and its precursors have been mixed vertically, the reduction of turbulence and mixing at the end of the daylight hours leaves O_3 in a remnant layer that is often without a well-defined thermodynamic demarcation. This layer is then transported through the night, often to regions far removed from pollution sources, where its pollutants can influence concentrations at remote locations the next morning, as mixing entrains the elevated remnant layer. This overnight transport can be aided by the development of a nocturnal jet that forms many nights at the top of the surface inversion layer.

Geography can have a significant impact on the dispersion of pollutants (such as along the coast of an ocean or one of the Great Lakes). Near the coast or shore, the temperatures of land and water masses can be different, as can the temperature of the air above these land and water masses. When the water is warmer than the land, there is a tendency toward reduction in the frequency of surface inversion conditions inland over a relatively narrow coastal strip (Hosler, 1961). This in turn tends to increase pollutant dispersion in such areas. The opposite condition also occurs if the water is cooler than the land, as in summer or fall. Cool air near the water surface will tend to increase the stability of the boundary layer in the coastal zone, and thus decrease the mixing processes that act on pollutant emissions. These conditions occur frequently along the New England coast (Hosler, 1961). Similarly, pollutants from the Chicago area have been observed to be influenced by a stable boundary layer over Lake Michigan (Lyons and Olsson, 1972). This has been observed especially in summer and fall when the lake surface is most likely to be cooler than the air that is carried over it from the adjacent land.

Sillman et al. (1993) investigated abnormally high concentrations of O_3 observed in rural locations on the shore of Lake Michigan and on the Atlantic coast in Maine, at a distance of 300 km or more from major anthropogenic sources. A dynamical-photochemical model was developed that represented formation of O_3 in shoreline environments and was used to simulate case studies for Lake Michigan and the northeastern United States. Results suggest that a broad region with elevated O_3, NO_x, and VOC forms as the Chicago plume travels over Lake Michigan, a pattern consistent with observed O_3 at surface monitoring sites. Near-total suppression of dry deposition of O_3 and NO_x over the lake is needed to produce high O_3. Results for the East Coast suggest that the observed peak O_3 can be reproduced only by a model that includes suppressed vertical mixing and deposition over water, 2-day transport of a plume from New York, and superposition of the New York and Boston, MA, plumes. Hence, the thermodynamics associated with the water bodies seem to play a significant role in some regional-scale episodes of high O_3 concentrations.

There is concern that the strict use of mixing height unduly simplifies the complex atmospheric processes that redistribute pollutants within urban areas. There is growing evidence that some O_3 precursors may not be evenly redistributed over some urban areas.

These are cases where the sources are relatively close to the urban area and atmospheric mixing is not strong enough to redistribute the material over a short travel time. In these cases, it is necessary to treat the turbulent structure of the atmosphere directly and acknowledge the vertical variations in mixing. Methods that are being used to investigate these processes include the use of a diffusivity parameter to express the potential for mixing as a function of height. A simple expression of how the mean concentration, $\overline{\chi}$, changes with time, t, in an air parcel, assuming all concentrations are homogeneous in the horizontal, is

$$\frac{d\overline{\chi}}{dt} = -\frac{\partial \overline{w'\chi'}}{\partial z}, \tag{3-80}$$

where $\overline{w'\chi'}$ is the vertical *turbulent eddy flux* of pollutant. The term on the right hand side of the equation changes mean concentration through flux divergence (i.e., turbulence either disperses the pollutant to or from the point being considered). The problem with this representation is that the flux divergence term is virtually impossible to measure directly.

The turbulent eddy flux needed to understand the vertical distribution of O_3 and its precursors often is parameterized in photochemical models, if included at all, through use of *eddy diffusivity*. The eddy diffusivity is set using an analogy to mixing length theory as

$$\overline{w'\chi'} = K_c \frac{\partial \overline{\chi}}{\partial z}, \tag{3-81}$$

which allows estimation of flux divergence from measured or estimated vertical gradients in concentration and estimation of the eddy diffusivity. The selection of diffusivity is often somewhat arbitrary, but can be related to the eddy diffusivity for heat or momentum, depending on circumstances. Large values result in rapid mixing. Thus, the appropriate selection of eddy diffusivity is necessary to simulate whether elevated plumes will enter an urban airshed.

The use of an eddy diffusivity approach to turbulent diffusion assumes local down-gradient diffusion, a situation not always realistic in the atmosphere. It is important to note that eddy diffusivity is not valid under convective conditions because counter-gradient flows occur (Sun, 1986). Eddy diffusivity also does not work in the presence of multiple stable layers. Moreover, the form of the eddy diffusivity used in the existing air quality models is rather arbitrary. More research will be needed to remove this arbitrariness.

Another method used for convective situations is a technique called "large-eddy simulation", employed to recreate the probability of redistribution within the mixing height. This method explicitly simulates the larger eddies occurring under convective situations. These techniques require meteorological information that is not normally available from the National Weather Service but that is now becoming available as part of several O_3 field experiments.

3.3.1.3 Cloud Venting

Vertical redistribution of O_3 out of the PBL is achieved by the venting of pollutants in clouds. Clouds represent the top-most reaches of thermals of air rising through

the PBL and can act as chemical reactors for soluble pollutants, returning the "processed" air to the PBL. They also can result in physical redistribution of O_3 and its precursors from the PBL if convection is sufficiently vigorous (Greenhut, 1986; Dickerson et al., 1987). Clouds also act to influence photolysis rates and chemical transformation rates.

Greenhut (1986) showed that the net O_3 flux in the cloud layer was a linear function of the difference in O_3 concentration between the boundary and cloud layers. Ozone fluxes between clouds were usually smaller than those found within clouds, but the slower rate is at least partially offset by the larger region of cloud-free air relative to cloudy air.

Large clouds, such as cumulonimbus, offer considerably more potential for redistribution of O_3 and its precursors. Additionally, the cumulonimbus clouds also are associated with precipitation, a scavenger of pollutants, and with lightning, a potential source for NO_x. Using CO as a tracer, Dickerson et al. (1987) and Pickering et al. (1990) have illustrated the redistribution potential of cumulonimbus cloud systems. Lyons et al. (1986) provided an illustration of the potential for groups of cumulonimbus clouds to vent the polluted boundary layer.

The role of cloud venting is thought to be largely a cleansing process for the boundary layer, although a portion of the material lifted into the free troposphere could be entrained back to the surface in subsequent convection. Aircraft observations frequently have documented the occurrence of relatively high O_3 concentrations above lower concentration surface layers (e.g., Westberg et al., 1976). This is a clear indication that O_3 is preserved essentially in layers above the surface and can be transported over relatively long distances, even when continual replenishment through precursor reactions is not a factor, such as at night.

3.3.1.4 Stratospheric-Tropospheric Ozone Exchange

The fact that O_3 is formed in the stratosphere, mixed downward, and incorporated into the troposphere, where it forms a more or less uniformly mixed background concentration, has been known in various degrees of detail for many years (Junge, 1963). The mechanisms by which stratospheric air is mixed into the troposphere have been examined by a number of authors, as documented previously by the U.S. Environmental Protection Agency (EPA) (U.S. Environmental Protection Agency, 1986a, and references therein).

Although the portion of background O_3 near the surface attributed to stratospheric-tropospheric O_3 can be in the 5 to 15 ppb range for a seasonal average, this amount of O_3 by itself cannot account for peak urban O_3 values or regional episodes of elevated O_3 levels (Johnson and Viezee, 1981; Ludwig et al., 1977; Singh et al., 1980; and Viezee et al., 1979). Johnson and Viezee (1981) concluded that the O_3-rich intrusions studied sloped downward toward the south. In terms of dimensions, the average crosswind width (north to south), at an altitude of 5.5 km (ca. 18,000 ft), for six spring intrusions, averaged 226 km, and, for four fall tropopause fold systems, averaged 129 km. Ozone concentrations at 5.5 km averaged 108 ppb in the spring systems and 83 ppb in the fall systems. From this and other research described in the previous criteria document for O_3 and other photochemical oxidants (U.S. Environmental Protection Agency, 1986a), Viezee and coworkers (Viezee and Singh, 1982; Viezee et al., 1983) concluded (1) that direct ground-level impacts by stratospheric O_3 may be infrequent, occurring $<1\%$ of the time; (2) that such ground-level events are short-lived and episodic; and (3) that they are most likely to be associated on a 1- to 4-h average with O_3 concentrations in the range 60 to 100 ppb.

The monthly stratospheric-tropospheric total O_3 flux from tropospheric folding events in the Northern Hemisphere has been estimated. These fluxes, in units of 10^{35} O_3 molecules per month per tropospheric folding event, increase from 1.0 in January to 2.1 in April and May, decline to 1.0 in August, and reach a minimum of 0.5 in October and November (Viezee et al., 1983). The spring-to-fall variation resembles the seasonal variations of O_3 near ground-level often observed at more remote sites (e.g., Logan, 1985). Four of the 10 episodes in which ground-level O_3 has been attributed to stratospheric O_3 transport occurred during March, but several such episodes during summer months have been reported (see below).

Using the ^{7}Be-to-O_3 ratio as an indicator of O_3 of stratospheric origin and SO_4^{2-} concentrations as a tracer for anthropogenic sources, Altshuller (1987) estimated stratospheric contributions of O_3 in the range 0 to 40 ppb (0 to 95% of observed O_3) at ground level at Whiteface Mountain, NY, for July 1975 and mid-June to mid-July 1977. Monthly average stratospheric contributions were estimated at 5 to 10 ppb. Extant ^{7}Be and O_3 data for a number of lower-elevation rural locations in the western, midwestern, and southeastern United States also were examined, and stratospheric or upper tropospheric contributions of 6 to 8 ppb were calculated. The author concluded that the calculated values for such contributions should be viewed with caution and regarded as probable upper limits because of scatter in the ^{7}Be and corresponding O_3 data that hindered definition of the ^{7}Be-to-O_3 ratio. Altshuller also concluded that removal and dilution processes result in the loss of most stratospheric O_3 before it reaches ground level. In other work performed in England, O_3 of stratospheric origin was estimated to contribute 10 to 15 ppb to the daily maximum hourly mean O_3 concentrations in an April through October period (Derwent and Key, 1988; United Kingdom Photochemical Oxidant Review Group, 1993).

3.3.2 Meteorological Parameters

This section focuses on analyses of data from previous and ongoing measurement programs to address two key questions: (1) are there meteorological parameters which are systematically associated with O_3 levels? and (2) are relationships between O_3 and meteorological parameters sufficiently strong such that meteorological fluctuations can be filtered from the data to allow examination of longer term trends?

The meteorological factors that theoretically could influence surface O_3 levels include ultraviolet radiation, temperature, wind speed, atmospheric mixing and transport, and surface scavenging. The following examines the theoretical basis for each of these factors and identifies to what degree empirical evidence supports the hypotheses.

3.3.2.1 Sunlight

Ultraviolet radiation plays a key role in initiating the photochemical processes leading to O_3 formation. Sunlight intensity (specifically the UV portion of sunlight) varies with season and latitude, but the latter effect is strong only during winter months. The importance of photolysis to the formation of O_3 provides a direct link between O_3 and time of year. However, during the summer, the maximum UV intensity is fairly constant throughout the contiguous United States, and only the duration of the solar day varies to a small degree with latitude.

The effects of light intensity on individual photolytic reaction steps and on the overall process of oxidant formation have been studied in the laboratory (Peterson, 1976;

Demerjian et al., 1980). Early studies, however, employed constant light intensities, in contrast to the diurnally varying intensities that occur in the ambient atmosphere. The diurnal variation of light intensity was subsequently studied as a factor in photochemical oxidant formation (e.g., Jeffries et al., 1975, 1976). Such studies showed that the effect of this factor varies with initial reactant concentrations. Most important was the observation that similar NMOC/NO_x systems showed different oxidant-forming potential depending on whether studies of these systems were conducted using constant or diurnal light. This led to incorporation of the effects of diurnal or variable light into photochemical models (Tilden and Seinfeld, 1982).

There is little empirical evidence in the literature, however, linking day-to-day variations in observed UV radiation levels with variations in O_3 levels. Samson and Shi (1988) illustrated that the number of O_3 concentrations exceeding 120 ppb did not track well with potential solar radiation, as shown in Figure 3-8. Although variations in day-to-day concentrations could well be influenced by cloud cover or attenuated by haze, the seasonal peak in O_3 concentrations usually lags the peak in potential solar radiation that occurs at the Summer Solstice on or about June 23.

3.3.2.2 Temperature

There is an association between tropospheric O_3 concentration and tropospheric temperature that has been demonstrated from measurements in outdoor smog chambers and from measurements in ambient air. A linear relationship between maximum O_3 and temperature was obtained in the smog chambers with little scatter around the regression line (Kelly and Gunst, 1990). Numerous ambient studies done over more than a decade have reported that successive occurrences or episodes of high temperatures characterize seasonally high O_3 years (Clark and Karl, 1982; Kelly et al., 1986). The relationship has been observed for the South Coast Air Basin of California (Kuntasal and Chang, 1987), in New England (Wolff and Lioy, 1978; Atwater, 1984; Wackter and Bayly, 1988), and elsewhere.

Figures 3-9 and 3-10 show the daily maximum O_3 concentrations versus maximum daily temperature for summer months (May to October), 1988 to 1990, for Atlanta and New York City, NY, and for Detroit, MI, and Phoenix, AZ, respectively. There appears to be an upper-bound on O_3 concentrations that increases with temperature. Likewise, Figure 3-11 shows that a similar qualitative relationship exists between O_3 and temperature even at a number of rural locations.

The notable trend in these plots is the apparent upper-bound to O_3 concentrations as a function of temperature. It is clear that, at a given temperature, there is a wide range of possible O_3 concentrations because other factors (e.g., cloudiness, precipitation, wind speed) can reduce the O_3 production. The upper bound presumably represents the maximum O_3 concentration achieved under the most favorable conditions. These plots, based on ambient air measurements, show wide scatter in O_3 concentration with temperature because of the contributions to variations from several of these factors that do not influence the results from smog chamber studies (Kelly and Gunst, 1990). Table 3-4 lists the results of a statistical regression performed on the paired O_3-temperature data used in Figures 3-9 and 3-10 with separate slopes listed for temperatures above and below 30 °C. Results show that, for $T > 30$ °C, the O_3-temperature relationship is statistically significant at all sites. The rate of increase for $T > 30$ °C is 3 to 5 ppb/°C at eastern United States rural sites and ranges from 4 to 9 ppb/°C at the three eastern U.S. urban sites (New York, Detroit, and Atlanta). At two western sites, Williston, ND, and Billings, MT, there is a much weaker

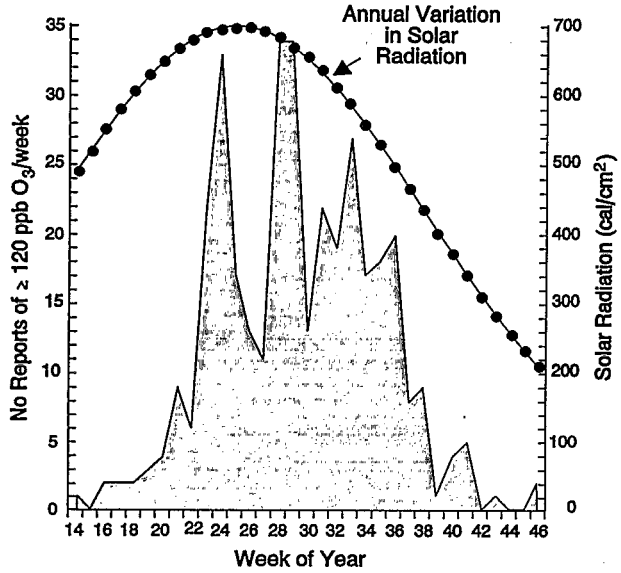


Figure 3-8. The number of reports of ozone concentrations ≥120 ppb at the 17 cities studied in Samson and Shi (1988). (1 April = Week 14, 1 May = Week 18, 1 June = Week 22, 1 July = Week 27, 1 August = Week 31, 1 September = Week 35, 1 October = Week 40, 1 November = Week 44). A representation of the annual variation in solar radiation reaching the earth's surface at 40°N latitude (units = cal cm^{-2}) is shown.

Source: Samson and Shi (1988).

dependence on temperature, possibly reflecting the lower level of anthropogenic activity. At a third western site, Medford, OR, the O_3-temperature relationship is comparable to that at rural eastern sites.

Relationships between peak O_3 and temperature also have been recorded by Wunderli and Gehrig (1991) for three locations in Switzerland. At two sites near Zurich, peak O_3 increased 3 to 5 ppb/°C for diurnal average temperatures between 10 and 25 °C, and little change in peak O_3 occurred for temperatures below 10 °C. At the third site, a high-altitude location removed from anthropogenic influence, much less variation of O_3 with temperature was observed.

The hypotheses for this correlation of O_3 with temperature include, but are not necessarily limited to:

1. Reduction in photolysis rates under meterological conditions associated with low temperatures;
2. Reduction in H_2O concentrations at low temperatures;
3. Thermal decomposition of PAN and its homologues;
4. Increased anthropogenic emissions of reactive hydrocarbons or NO_x, or both;
5. Increased natural emissions of reactive hydrocarbons; and
6. Relationships between high temperatures and stagnant circulation patterns.

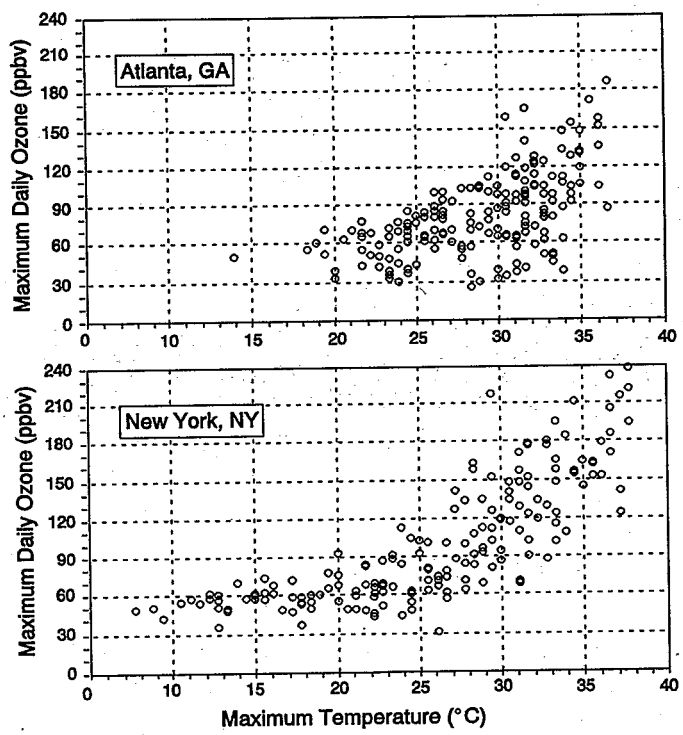


Figure 3-9. A scatter plot of maximum daily ozone concentration in Atlanta, GA, and New York, NY, versus maximum daily temperature.

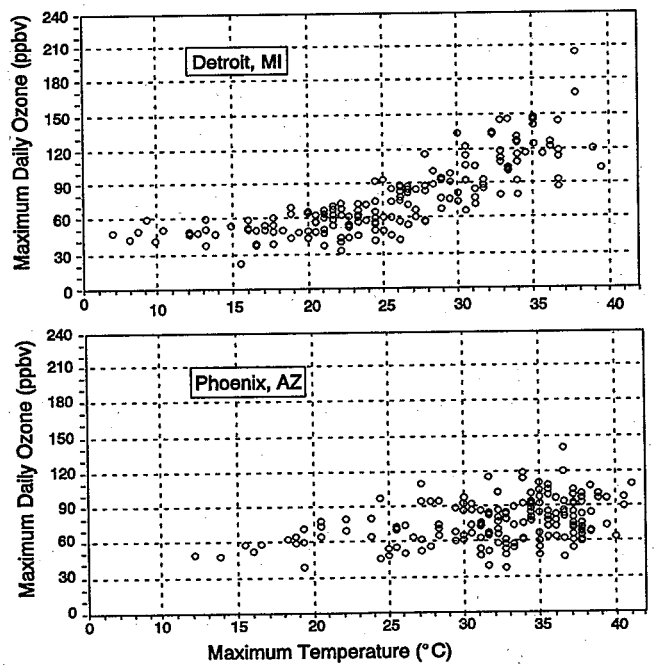


Figure 3-10. A scatter plot of maximum daily ozone concentration in Detroit, MI, and Phoenix, AZ, versus maximum daily temperature.

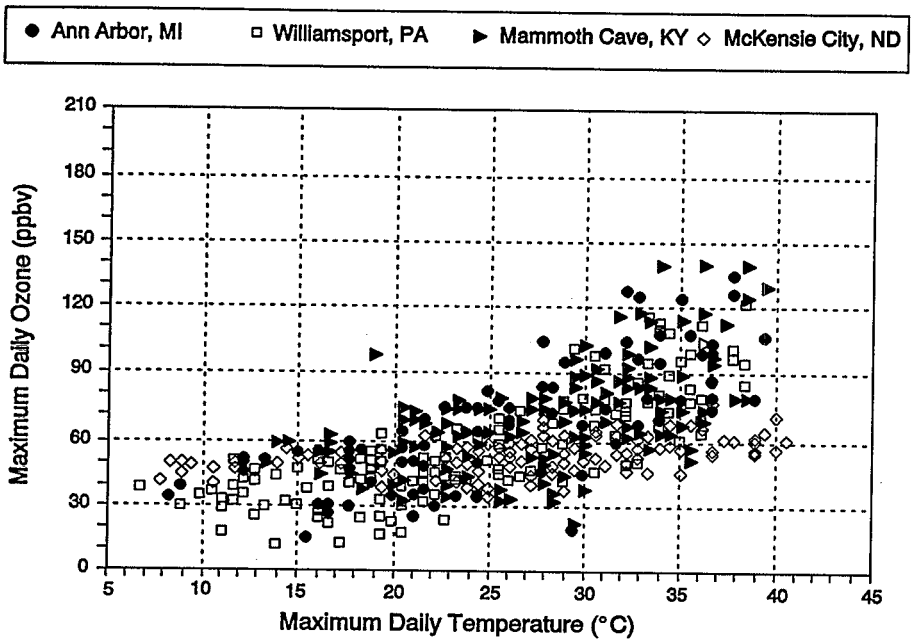


Figure 3-11. ***A scatter plot of maximum ozone concentration versus maximum daily temperature for four nonurban sites. The relationship with temperature is still apparent, although the slope is reduced from that of the urban areas.***

Table 3-4. Rates of Increase of Peak Ozone with Diurnal Maximum Temperature (ppb/°C) for Temperature <300 K (27 °C) and Temperature >300 K, Based on Measurements for April 1 to September 30, 1988[a]

Location		T < 300 K		T > 300 K	
		$\Delta O_3/\Delta T$	T-Statistic	$\Delta O_3/\Delta T$	T-Statistic
Urbanized Regions					
	NY-NJ-CT	1.5	−5.2	8.8	−7.4
	Detroit	1.4	−6.4	4.4	−6.3
	Atlanta	3.2	−4.2	7.1	−5.9
	Phoenix	—	—	1.4	−4.1
	Southern California	11.3	−8.9	—	—
Nonurban Sites					
	Williamsport, PA	1.2	−5.0	4.0	−7.4
	Saline, MI	0.8	−3.5	3.1	−4.9
	Mammoth Cave, KY	0.1	−0.3	4.4	−7.3
	Kentucky, cleanest site 3	0.3	−0.7	3.4	−6.6
	Williston, ND	0.2	−1.0	0.8	−3.7
	Billings, MT	0.1	−0.5	0.7	−2.2
	Medford, OR	0.5	−2.6	3.3	−13.7

[a]See Appendix A for abbreviations and acronyms.

The relationship with temperature is well known, but not yet reproduced by air quality models. Although it has been argued that this striking relationship with temperature is an indirect result of the stagnant synoptic meteorological conditions that lead to higher O_3 levels, the correlation is not strong with other parameters of stagnation, notably wind speed, which is discussed later.

Reduction in Photolysis Rates

It is possible that, on a seasonal scale, the correlation between temperature and O_3 may be an indirect correlation with UV radiation variability. This is insufficient, however, to explain the day-to-day correlation between the two variables.

Changes in photolysis rates and in H_2O concentrations are related in that both are linked to the supply of OH radicals, which determines the rate of O_3 production in clean atmospheres and contributes to O_3 production in polluted atmospheres. A reduction in either photolysis rates or H_2O would reduce the source of OH radicals. Calculations by Sillman and Samson (1995) showed that the difference between summer and fall photolysis rates (at 40° N latitude) has a significant impact on the rate of O_3 production in urban photochemical simulations, roughly equal to the impact of PAN thermal decomposition (discussed below). However the impact of photolysis rates and of water vapor was much lower in simulations for polluted rural environments. In the simulations by Sillman et al. (1993), O_3 production in urban environments was limited largely by the supply of OH radicals to react with hydrocarbons; whereas in rural environments the limiting factor was the source of NO_x. Consequently, photolysis rates and H_2O had less impact on O_3 production in rural environments.

Thermal Decomposition of Peroxyacetyl Nitrate

Temperature-dependent photochemical rate constants provide a link between O_3 and temperature (Sillman et al., 1990a; Cardelino and Chameides, 1990). The reason for the decline in O_3 in rural areas when the PAN decomposition rate decreases is that PAN represents a sink for NO_x in rural environments. When the rate of PAN decomposition is decreased, NO_x drops sharply, whereas OH and HO_2 remain largely unaffected. Consequently, the rate of the important HO_2 + NO reaction (see Section 3.2) shows a substantial decrease.

The photochemical response in an urban environment is fundamentally different, although the final result, a decrease in O_3 with temperature, is similar. The impact of PAN in urban environments is attributable to its role as a sink for odd hydrogen rather than to its effect on NO_x (Cardelino and Chameides, 1990). Sillman et al. (1990a) have shown that the well-known division of O_3 photochemistry into NO_x- and VOC-sensitive regimes is associated with the relative magnitude of odd-hydrogen sinks.

Sillman and Samson (1995) found that the thermal decomposition of PAN was enough to explain an increase of 1 to 2 ppb peak O_3/°C increase in temperature in rural locations in the eastern United States, based on photochemical simulations. This increase represents a significant fraction of the observed increase in peak O_3 with a rise in temperature (3 to 5 ppb/°C, Table 3-4).

Increased Anthropogenic Emissions

Emission rates for anthropogenic hydrocarbons (VOCs) also can increase with temperature (U.S Environmental Protection Agency, 1989; Stump et al., 1992). Increased

anthropogenic VOC emissions might be expected to cause increased rates of O_3 production in urban areas where O_3 is sensitive to VOC, but would be less likely to have impact on rural areas where biogenic VOC emissions can predominate. However, O_3 in rural areas is NO_x dependent. The NO_x-sensitive rural areas also would show increased O_3 production with a rise in temperature as biogenic NO_x emissions increase with temperature.

Increased Natural Emissions

Emissions of biogenic hydrocarbons increase sharply with a rise in temperature (Lamb et al., 1987). In ambient temperatures from 25 to 35 °C, the rate of natural hydrocarbon emissions from isoprene-emitting deciduous trees increased by about a factor of 4. From coniferous trees, the increase was on the order of 1.5.

Recently, Jacob et al. (1993) found that the photochemistry of O_3 production in a polluted rural environment (Blue Ridge Mountains, VA) is significantly different in September and October, when natural emissions from deciduous forests have ceased. The difference in chemistry between summer and fall leaf production also may have an impact on the O_3-temperature correlation.

Correlation with Stagnation

Recently, Jacob et al. (1993b) found that model-simulated O_3 formation in the rural United States shows a tendency to increase with a rise in temperature, based solely on the difference in atmospheric circulation between relatively warm and relatively cool days. The model-simulated O_3-temperature correlation was less than observed but large enough to represent a significant component of the observed correlation. However, the temperature-meteorology correlation identified by Jacob et al. (1993b) was based on simulated meteorology from a General Circulation Model rather than on direct observations.

3.3.2.3 Wind Speed

Ozone is expected to be influenced by wind speed because lower wind speeds should lead to reduced ventilation and the potential for greater buildup of O_3 and its precursors. Abnormally high temperatures are frequently associated with high barometric pressure, stagnant circulation, and suppressed vertical mixing resulting from subsidence (Mukammal et al., 1982), all of which may contribute to elevated O_3 levels. However, in reality this relationship varies from one part of the country to another. Figure 3-12 shows the frequency of 24-h trajectory transport distances to Southern cities on days with resulting concentrations of $O_3 \geq 120$ ppb (Samson and Shi, 1988). The frequency for Southern cities is biased toward lower wind speeds. The same bias was shown for all 17 cities in the study. A similar plot for cities in the northeastern United States (Figure 3-13) shows an opposite pattern, in which the bias is toward higher wind speeds than normal. It is unclear how much meteorological information is needed in order to perform accurate urban-area O_3 simulations using advanced photochemical models. To understand the significance of variations between upper-air wind measurements during the Southern Oxidant Study (SOS), 1992, Atlanta, an intensive, intercomparison test of the precision of upper-air measurements was conducted. Collocated measurements were made at an SOS measurement site, using a boundary-layer lidar, a wind profiler, and a rawinsonde balloon. There was generally good agreement between the profiler and rawinsonde, although some large outliers existed. Figure 3-14 illustrates that the root-mean-square difference (RMSD) varied with altitude. The RMSD reached a minimum near 1,200 m above ground level (AGL) of about 2 m/s, rising to over

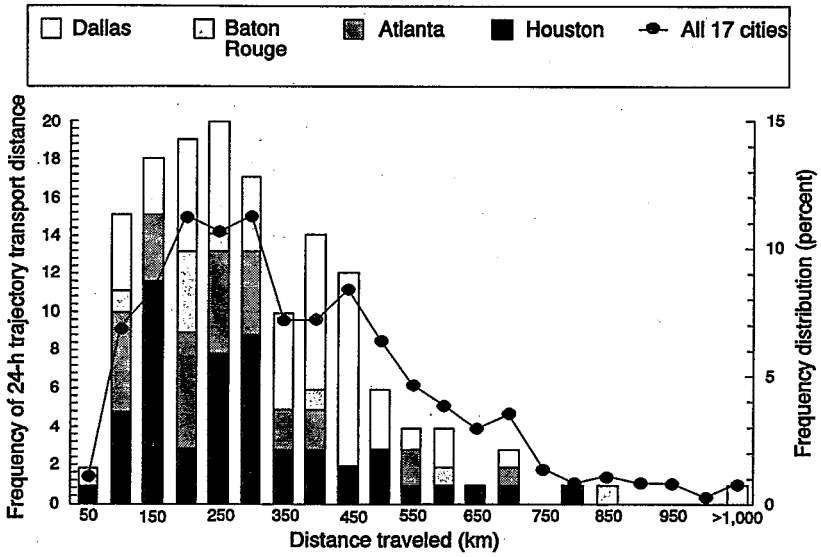


Figure 3-12. The frequency of 24-h trajectory transport distance en route to city when ozone was ≥120 ppb in four Southern U.S. cities, compared with the percent frequency distribution for all 17 cities (scale on right) of a nationwide study, 1983 to 1985.

Source: Samson and Shi (1988).

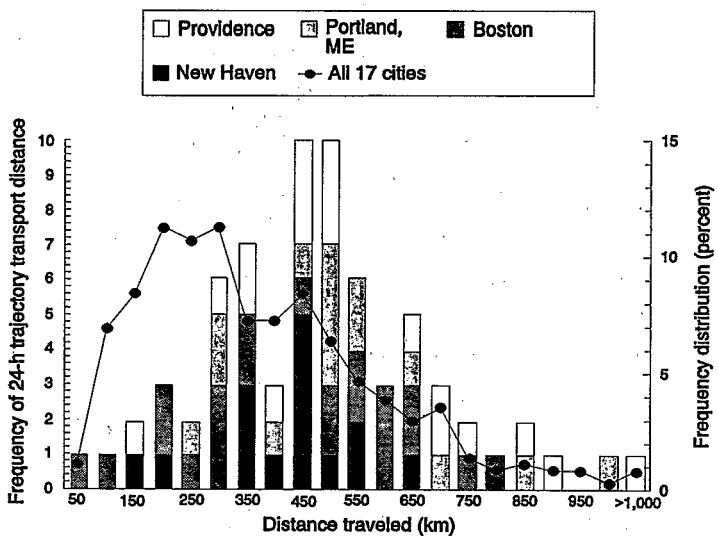


Figure 3-13. The frequency of 24-h trajectory transport distance en route to city when ozone was ≥120 ppb in four New England cities, compared with the percent frequency distribution for all 17 cities (scale on right) of a nationwide study, 1983 to 1985.

Source: Samson and Shi (1988).

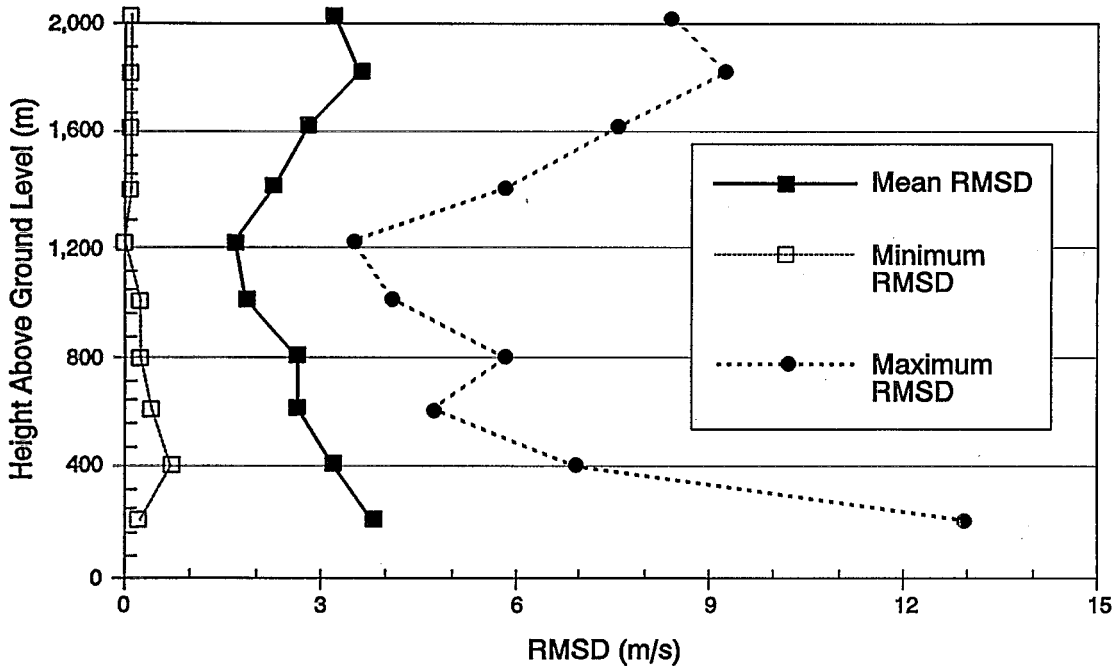


Figure 3-14. The root-mean-square difference (RMSD) between CLASS observations and profiler observations as a function of height above ground level.

3 m/s near the surface and above 1,200 m AGL. Figure 3-15 illustrates the RMSD for the lidar comparison with CLASS observations. There is slightly greater RMSD at all heights than for the profiler-rawinsonde comparison, with a relative minimum observed at about 1,200 m.

Although the measurements were significantly correlated, the results illustrate that there was still considerable disagreement between methods. The profiler had better precision than the lidar had, although the differences were negligible if the first four runs were excluded from the data set. The profiler obtained values biased slightly higher than the CLASS system (+0.2 m/s), whereas the lidar system was biased low (−0.3 m/s or −0.5 m/s). The statistical comparisons of both the profiler and the boundary-layer lidar with the rawinsonde system suggest that variations in wind speed at a particular level must be larger than about 3 m/s to be considered significant.

3.3.2.4 Air Mass Characteristics

In meteorology, an "air mass" is a region of air, usually of multistate dimensions, that exhibits similar temperature, humidity, or stability characteristics. Air masses are created when air becomes stagnant over a "source region" and subsequently takes on the characteristics of the source region. Similarly, when dealing with air pollution meteorology, it is possible to identify a "chemical air mass" as a region of air that has become stagnant over an emissions source area. Air that is stagnant over, say, the center of Canada will exhibit relatively cold, dry conditions and will be relatively devoid of pollutants. Air that

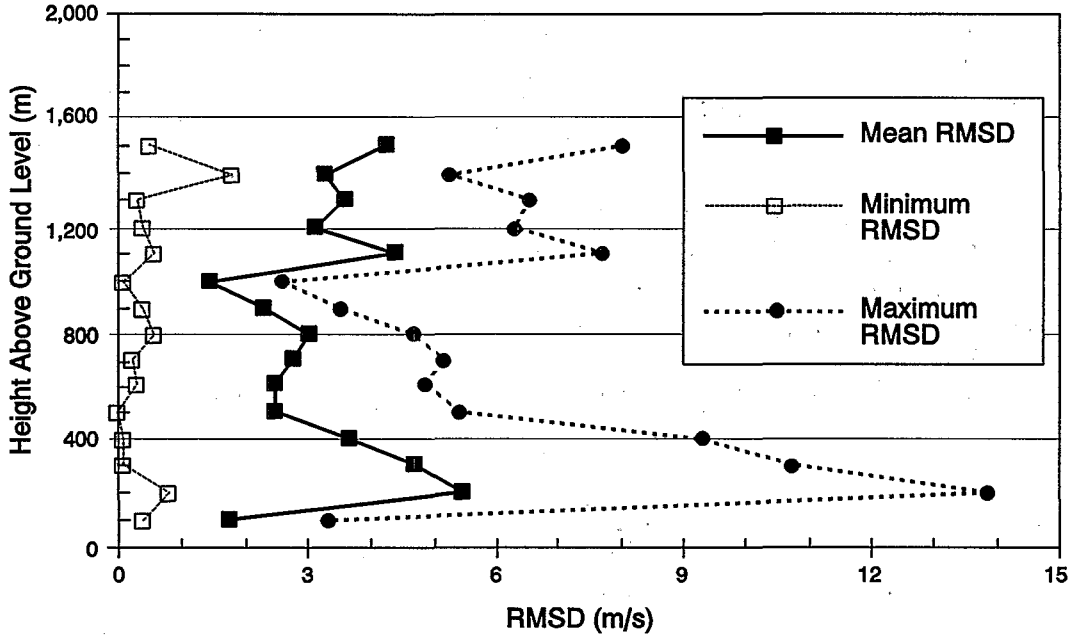


Figure 3-15. The root-mean-square difference (RMSD) between CLASS observations and lidar observations as a function of height above ground level.

resides over the industrial regions of the midwestern United States will exhibit low visibility and, often, high O_3 levels on a regional scale. Meteorological processes play an important role in determining the amount of "accumulation" of O_3 and its precursors that occurs under such stagnant conditions.

Episodes of high O_3 concentrations in urban areas often are associated with high concentrations of O_3 in the surroundings. This accumulated O_3 forms under the same atmospheric conditions that lead to high O_3 levels in urban areas and exacerbates the urban problem by supplying relatively high O_3 and precursor concentrations to the urban area from upwind. The transport of O_3 and its precursors beyond the urban scale ($\leq$50 km) to neighboring rural and urban areas has been well documented (e.g., Wolff et al., 1977a,c; Wolff and Lioy, 1978; Clark and Clarke, 1984; Sexton, 1982; Wolff et al., 1982; Altshuller, 1988). A summary of most of these reports was given in the 1986 O_3 criteria document (U.S. Environmental Protection Agency, 1986a) and will not be reiterated here. The phenomena of high nonurban O_3 levels was illustrated by Stasiuk and Coffey (1974) for transport within New York State; by Ripperton et al. (1977) for sites in the Middle Atlantic States; and by Samson and Ragland (1977) for the midwestern United States.

These areas of O_3 accumulation are characterized by synoptic-scale subsidence of air in the free troposphere, resulting in development of an elevated inversion layer; relatively low wind speeds associated with the weak horizontal pressure gradient around a surface high pressure system; lack of cloudiness; and high temperatures.

On occasion, O_3 at levels greater than 120 ppb can occur in rural areas far removed from urban or industrial sources. Ozone levels at the summit of Whiteface Mountain exceeded this value during the summer of 1988 when O_3 accumulated across a

wide expanse of the eastern United States at levels ≥120 ppb. Nonetheless, even when the regional accumulation is at a level below the current O_3 NAAQS, the increment needed to bring the level above the NAAQS in an urban area is not large.

The identification and understanding of the transport of photochemical O_3 and other oxidants and their precursors by weather systems represent a significant advance in comprehending photochemical air pollution and the potential extent of its effects. Considerable progress has been made in the development of long-range photochemical modeling techniques so that the likely impact of synoptic systems can be anticipated. Such tools are very much in the research stage, however, because the local impact of O_3 and other oxidants results from a complex interaction of distant and local precursor sources, urban plumes, mixing processes, atmospheric chemical reactions, and general meteorology.

3.3.3 Normalization of Trends

The degree to which meteorological factors can be "normalized" out of the O_3 concentration and "trends" data depends in large part on the strength of the relationships between O_3 and meteorological components. As part of the SOS Atlanta intensive field campaign, an attempt was made to model statistically the O_3 levels in Atlanta to build a predictive tool for forecasting days of specialized measurement. Figure 3-16 shows the fit of the data used to create the model to the model simulations. Figure 3-17 shows the fit obtained from independent data collected in 1992.

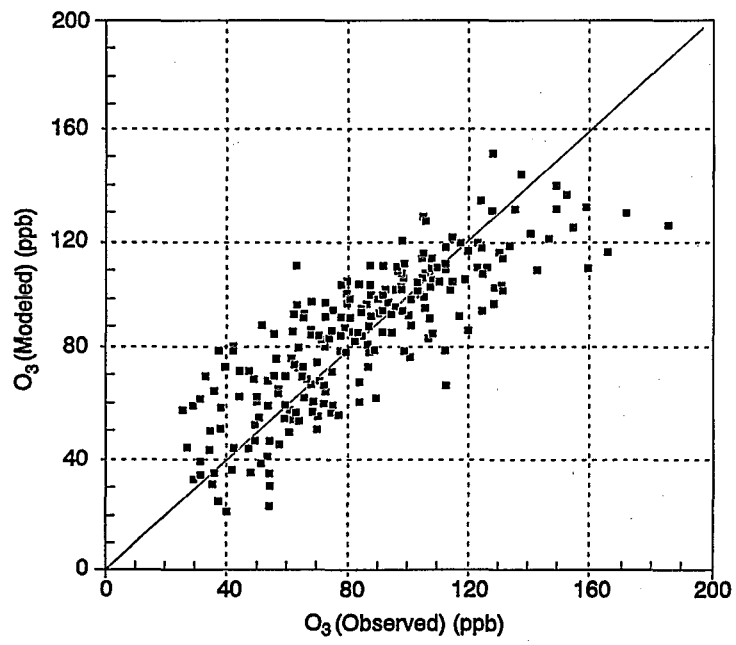


Figure 3-16. Model of ozone (O_3) levels using regression techniques. The use of wind speed, temperature, and previous-day ozone provided a means to forecast O_3 levels.

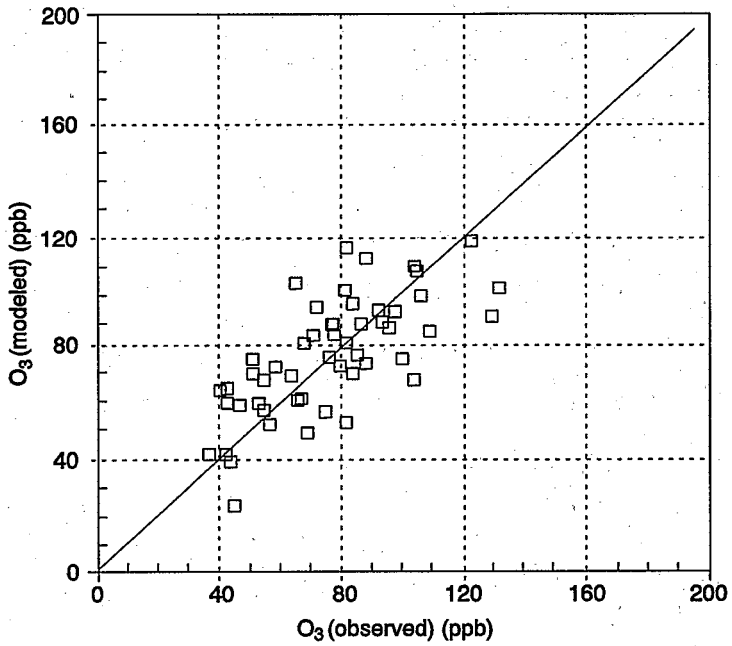


Figure 3-17. Simulated versus observed ozone levels using regression techniques on an independent data set obtained in the summer of 1992 in Atlanta, GA.

This model was used successfully to predict next-day O_3 levels in Atlanta. Ozone levels in a number of American cities should be analyzed using regression tools such as this in order to normalize meteorological variability. Through such analyses, it is possible that trends, if any, represented as systematic deviations from the model, may become observable. A summary of other techniques for removing meteorological variability is contained in the recent monograph from the National Research Council (1991). Table 3-5 lists a sample of studies aimed at evaluation of O_3 trends.

3.4 Precursors of Ozone and Other Oxidants

3.4.1 Sources and Emissions of Precursors

3.4.1.1 Introduction

As described elsewhere in this chapter, O_3 is formed in the atmosphere through a series of chemical reactions that involve VOCs and NO_x. Control of O_3 depends on reducing emissions of VOCs or NO_x or both. Thus, it is important to understand the sources and source strengths of these precursor species in order to devise the most appropriate oxidant control strategies. In the following sections, anthropogenic and biogenic NO_x and VOC sources will be described, and the best estimates of their current emission levels and trends will be provided. Confidence levels for the assigned source strengths will be discussed.

Both English and metric units have been utilized in emission inventories. Thousands or millions of short tons are the common scales in the English system. The metric unit most

Table 3-5. Recent Studies Examining Trends in Ozone Data After Removal of Variability Associated with Meteorological Factors

Study	Variables	Approach
Jones et al. (1989)	Surface temperature	Compared number of days with ozone concentrations above 120 ppb to days with temperature above 30 °C.
Pollock et al. (1988)	Surface temperature	Compared number of days with ozone concentrations above 105 ppb to days with temperature above 30 °C.
Kuntasal and Chang (1987)	850-mb temperature	Regression of ozone versus temperature for Southern California.
Wakim (1989)	Surface temperature	Regression of ozone versus temperature for Houston, New York, and Washington, DC.
Chock et al. (1982)	Surface temperature, wind speed, relative humidity, sky cover, wind direction, dew point temperature, sea level pressure, precipitation.	Regression versus a variety of meteorological parameters.
Kumar and Chock (1984)	Surface temperature, wind speed, relative humidity, sky cover, wind direction, dew point temperature, sea level pressure, precipitation.	Regression versus a variety of meteorological parameters.
Korsog and Wolff (1991)	Surface temperature, wind speed, relative humidity, sky cover, wind direction, dew point temperature, sea level pressure, precipitation.	Regression versus a variety of meteorological parameters.

Source: National Research Council (1991).

often employed is millions of metric tons, which is equivalent to teragrams (Tg). To convert English tons to teragrams, multiply English tons by 0.907×10^{-6}. For consistency, teragrams have been employed throughout the ensuing discussion.

3.4.1.2 Nitrogen Oxides

Manmade Emission Sources

Anthropogenic NO_x sources are associated with combustion processes. The primary pollutant is NO, which is formed from nitrogen and oxygen atoms that are produced at high combustion temperatures when air is present. In addition, NO_x is formed from nitrogen contained in the combustion fuel. Major NO_x source categories include transportation, stationary source fuel combustion, industrial processes, solid waste disposal,

and some miscellaneous combustion-related activities. Table 3-6 provides a more detailed summary of each of these source categories. The transportation category includes gasoline- and diesel-powered motor vehicles, aircraft, railcars, vessels, and off-highway vehicles. Electric utilities, industrial and commercial/institutional boilers, industrial furnaces, and space heaters comprise the stationary source fuel combustion category. Industrial processes include petroleum refining and paper, glass, steel, cement, and chemical production. The incineration and open burning of waste leads to emissions of NO_x in the solid waste disposal category. The miscellaneous sources category includes prescribed forest slash burning, agricultural burning, coal refuse burning, and structure fires. It should be noted at this point that, even though NO is the pollutant emitted, NO_x emission inventories are quantified relative to NO_2 (mol wt = 46). Nitrogen dioxide is a secondary pollutant produced via oxidation of NO in the atmosphere.

Quantifying NO_x emissions in all of these categories generally requires multiplying an emission factor and an activity level. Nitrogen oxides emission factors are obtained from *Compilation of Air Pollution Factors*, AP-42 (U.S. Environmental Protection Agency, 1985), and from the current mobile source emission factor model (e.g., MOBILE5) recommended by EPA. Activity levels are derived from information sources that provide consumption levels. This takes the form of fuel type and amount consumed for stationary sources and, for transportation sources, the number of vehicle miles traveled (VMT). Point-source emissions are tallied at the individual plant level. These plant-by-plant NO_x emissions are first summed at the state level, and then state totals are added to arrive at the national emissions total. Data on VMT are published for three road categories: (1) highways, (2) rural roads, and (3) urban streets.

Table 3-7 provides a summary of NO_x emissions from the various categories mentioned previously (U.S. Environmental Protection Agency, 1993b). The 1991 total is 21.39 Tg of NO_x emissions in the United States. About half of the emissions (10.69 Tg) is associated with the stationary source fuel combustion category. Transportation-related activities are the second largest source, accounting for about 45% of the national total. The remaining 7% of emissions are divided among the industrial processes, solid waste disposal, and miscellaneous sources categories. The two largest single NO_x emission sources are electric power generation and highway vehicles. Local NO_x source apportionment may differ substantially from these national figures.

Because of the dominance of the electric utility and transportation sources, the geographical distribution of NO_x emissions is related to areas with a high density of power-generating stations and urban regions with high traffic densities. Figure 3-18 shows the location of the 50 largest electric power generating sources of NO_x in the United States. The majority of these power plants are concentrated in the upper Mississippi-Ohio River corridor. Because of this congregation of large point sources, 69% of U.S. NO_x emissions occur within U.S. Environmental Protection Agency Regions III, IV, V, and VI (Figure 3-19). It is interesting to compare the annual NO_x emissions from a large electrical generating plant with the yearly transportation-related emissions in a major metropolitan region. The largest utility plants currently release between 0.06 and 0.09 Tg of NO_x annually, which compares to approximately 0.12 Tg of NO_x emitted by transportation sources in the Atlanta urban area (U.S. Environmental Protection Agency, 1993b).

Table 3-6. Source Categories Used to Inventory Nitrogen Oxides Emissions

Transportation	Stationary Source Fuel Combustion	Industrial Processes	Solid Waste Disposal	Miscellaneous
Highway vehicles	Coal	Pulp mills	Incineration	Forestries
Gasoline-powered	Electric utilities	Organic chemicals	Open burning	Other burning
Passenger cars	Industrial	Ammonia		
Light trucks - 1	Commercial/Institutional	Nitric acid		
Light trucks - 2	Residential	Petroleum refining		
Heavy-duty vehicles		Glass		
Motorcycles		Cement		
		Lime		
		Iron and steel		
Diesel-powered	Fuel oil			
Passenger cars	Electric utilities			
Light trucks	Industrial			
Heavy-duty vehicles	Commercial/Institutional			
	Residential			
Aircraft	Natural gas			
Railcars	Electric utilities			
Vessels	Industrial			
Farm machinery	Commercial/Institutional			
Construction machinery	Residential			
Industrial machinery				
Other off-highway vehicles				
	Wood			
	Industrial			
	Residential			
	Other Fuels			
	Industrial			
	Residential			

Source: U.S. Environmental Protection Agency (1992a).

Table 3-7. 1991 Emission Estimates for Manmade Sources of Nitrogen Oxides in the United States

Source Category	Emissions (Tg)	
Transportation		9.71
Highway vehicles	7.20	
Off-highway vehicles	2.51	
Stationary fuel combustion		10.69
Electric utilities	6.74	
Industrial	3.27	
Other	0.68	
Industrial processes		0.80
Solid waste disposal		0.07
Miscellaneous		0.12
Forest burning		
Other burning		
Miscellaneous organic solvents		
Total of all sources		21.39

Source: U.S. Environmental Protection Agency (1993b).

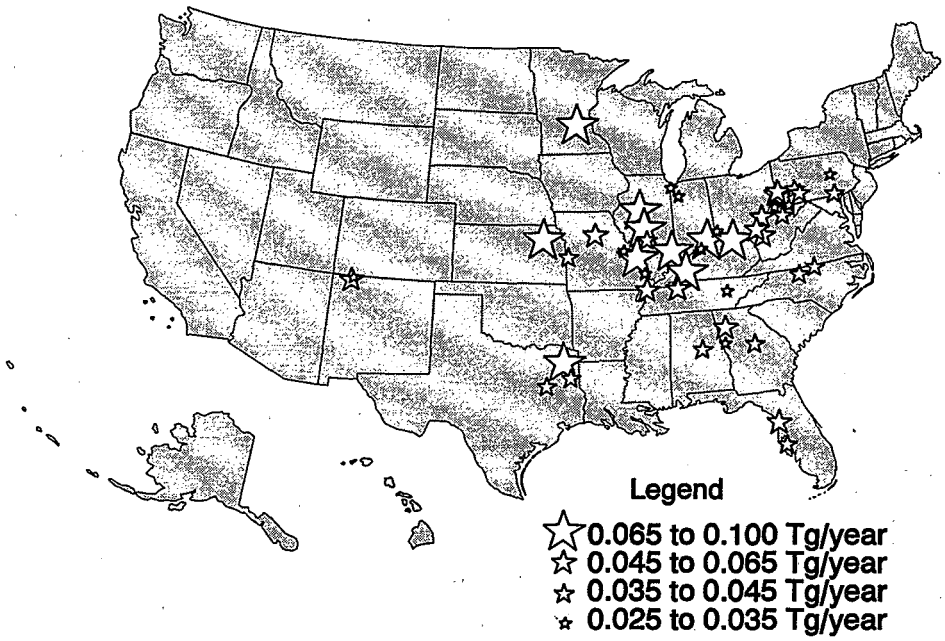


Figure 3-18. The 50 largest sources of nitrogen oxides (power plants) in the United States.

Source: U.S. Environmental Protection Agency (1992a).

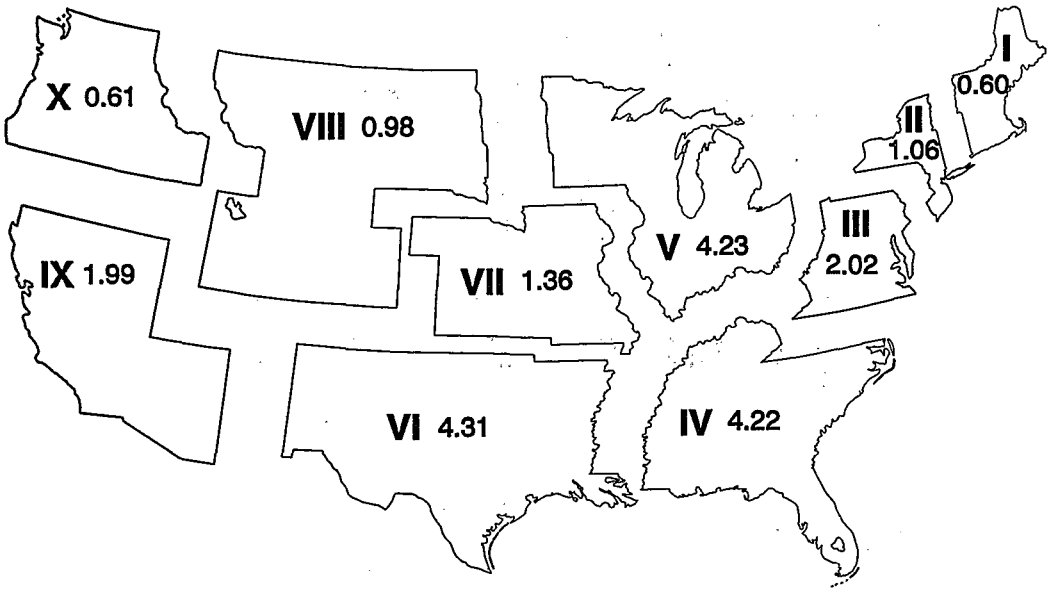


Figure 3-19. Nitrogen oxides emissions (Tg/year) from manmade sources in the 10 U.S. Environmental Protection Agency regions of the United States, 1991.

Source: U.S. Environmental Protection Agency (1993b).

Seasonal variations are available for the 1993 NO_x emissions from 14 source categories in the United States (U.S. Environmental Protection Agency, 1994). Very little seasonal variation occurred for categories contributing approximately three-quarters of the total annual NO_x emissions, including the categories of highway vehicles, electric utilities, and industrial combustion sources. For off-highway sources (comprising 20% of the total annual NO_x emissions), 29% of the off-highway NO_x emissions occur in the summer and 21% in the winter. In contrast, the category of other combustion sources (comprising 5% of the total annual NO_x emissions) emit 47% of the NO_x emissions in the winter and only 8% in the summer. An earlier inventory for 1985 (U.S. Environmental Protection Agency, 1989), which considered only total point and area anthropogenic NO_x emissions, indicated that very little variation occurred in NO_x emissions among the winter, spring, summer, and fall seasons. The contributions of these NO_x categories also were shown to vary seasonally by region of the United States.

Trends in Nitrogen Oxides Emissions

Estimates of NO_x emissions have been made back to 1900, when approximately 2.3 Tg were emitted into the atmosphere in the United States (U.S. Environmental Protection Agency, 1992a). Figure 3-20 summarizes the growth in NO_x emissions at 10-year intervals since the 1940s. Emissions grew rapidly until the 1970s and then leveled off at about

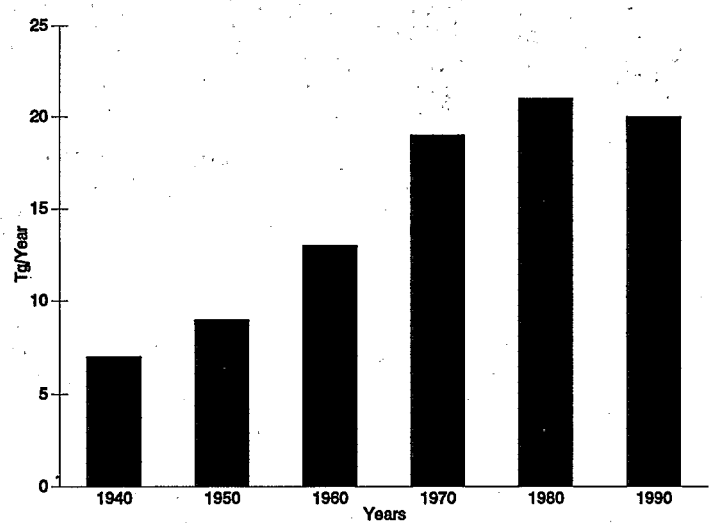


Figure 3-20. Changes in nitrogen oxides emissions from manmade sources in the United States, 10-year intervals, 1940 through 1990.

Source: U.S. Environmental Protection Agency (1992a).

20 Tg/year. Currently, more than 90% of the national NO_x emissions result from transportation activities and stationary fuel combustion. Figure 3-21 illustrates the growth in each of these categories over the last 50 years. Transportation-related NO_x emissions grew steadily until the 1980s and then exhibited a moderate decrease. However, the recent trends in transportation-related NO_x emissions shown in Table 3-8 indicate no trend between 1987 and 1991. Emissions of NO_x from fuel combustion sources have increased continually from 1940 to the present time.

Recent trends in the major NO_x emission categories are shown in Table 3-8. Between 1987 and 1991, transportation-related NO_x emissions have remained essentially constant, whereas the stationary source NO_x emissions have increased about 10%.

Transportation and stationary source fuel combustion will likely show downward trends in their NO_x emissions during the next 20 years. This will result from the provisions of the Clean Air Act, which was passed in 1990. Emission limits for electric utility boilers have been prescribed to reduce acidic deposition, automobile tailpipe emission standards will be tightened, and current technology-based applications will be required for industrial boilers (non-utility) in O_3 nonattainment areas. In addition, the average grams of NO_x per mile from passenger cars is expected to decrease because of new on-board diagnostic systems and expanded inspection and maintenance requirements.

As a result of new emission limits and revised performance standards, NO_x emissions from electric utilities are expected to decrease by 16% by the year 2000. Control requirements in the industrial non-utility sector are expected to reduce NO_x emissions by 10% between 1990 and 2000. Projections based on VMT and emission factors from the MOBILE model suggest nearly a 50% decrease in NO_x emissions from highway vehicles manufactured from 1990 to 2000 (U.S. Environmental Protection Agency, 1992a).

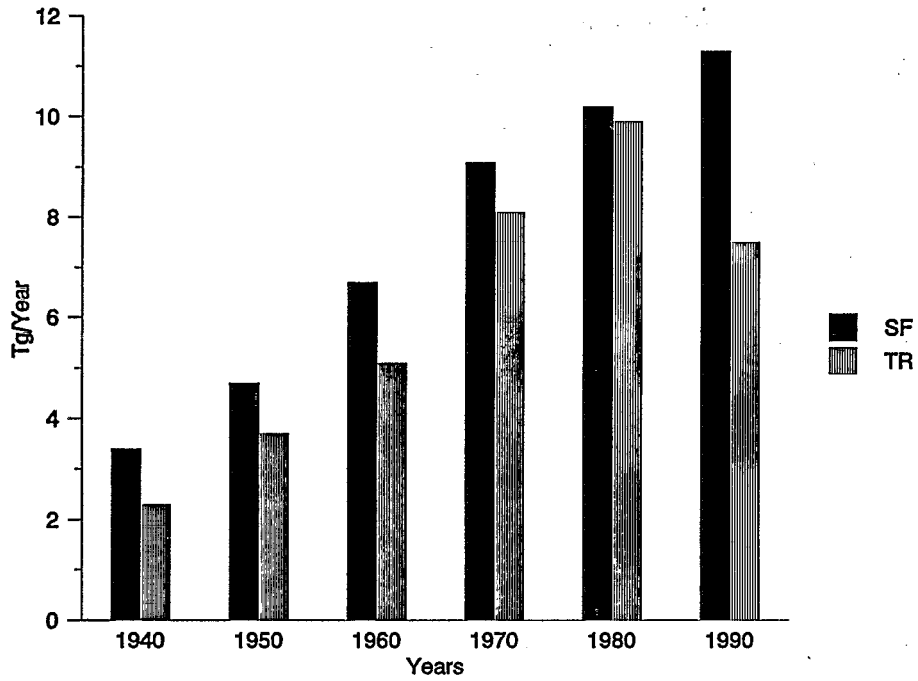


Figure 3-21. Changes in nitrogen oxides emissions from stationary source fuel combustion (SF) and transportation (TR) from 1940 through 1990.[a]

[a]The values for 1990 do not agree with those in Table 3-8 because different models were employed for deriving the short- and long-term trends.

Source: U.S. Environmental Protection Agency (1992a).

Table 3-8. Recent Trends in Nitrogen Oxides Emissions for Major Manmade Source Categories (Tg)

Year	Transportation	Stationary Source Fuel Combustion
1991	9.7	11.0
1990[a]	9.9	10.7
1989	9.7	10.7
1988	9.9	10.6
1987	9.7	10.1

[a]The values for 1990 do not agree with those in Figure 3-21 because different models were employed for deriving the short- and long-term trends.

Source: U.S. Environmental Protection Agency (1993b).

Uncertainty of Anthropogenic Nitrogen Oxides Emission Estimates

Because a large proportion of the U.S NO_x emissions are derived from distinct point sources, it generally is believed that published estimates are very reliable. For example, the National Acid Precipitation Assessment Program (NAPAP) NO_x inventory for U.S. emissions in 1985 (18.6 Tg) was assigned a 90% relative confidence interval in the range of 6 to 11% (Placet et al., 1991). This confidence level was based on judgments used to assign uncertainty to component inputs of emission models and on statistical assumptions used to aggregate uncertainty values.

Sources of error are associated with both the emission factors and the activity levels utilized in the inventorying process. Emission factors provide quantitative estimates of the average rate of emissions from many sources. Consequently, these factors are best applied to a large number of sources over relatively long time periods. In other words, an NO_x emission estimate for a single point source on a particular day in 1990 may be highly inaccurate; but the emission value for this same source for the entire year of 1990 may be very good. It appears that the emission factors assigned to the transportation sectors may be the most uncertain. This results from the emission factors having been derived from mobile source models that require multiple inputs. This type of model requires information on temperatures, vehicle speeds, gasoline volatility, and several other parameters.

Recent attempts to validate NO_x emission factors or inventories have involved comparing ambient NO_x concentrations with values predicted using emissions-based models. These generally have taken one of two forms: (1) comparisons between NO_x concentrations measured in a tunnel and those predicted from emission factors, activity levels, and dilution factors in the tunnel; or (2) whole-city integration procedures in which ambient NO_x concentrations are compared to ambient NO_x levels that have been predicted using a model such as the Urban Airshed Model (UAM). The latter approach has been applied in the South Coast Air Basin (Fujita et al., 1992). It was reported that measured and predicted NO_x concentrations agreed within 20% for a 2-day period in August 1987. Likewise, the results from tunnel studies (Pierson et al., 1990; Robinson et al., 1996) have shown reasonably good agreement between predicted and measured NO_x concentrations. It is important to keep in mind that ambient NO_x levels predicted using a modeling method cannot be assigned true value status. There could be as much or more uncertainty in the model outputs as there is in the emission inputs that are being tested. The fact, however, that an emissions-based model predicts ambient concentrations that are close to those measured tends to lend credence to the NO_x emission estimates. No systematic study of the effect of these uncertainties on model predictions has been published, but a limited summary of sensitivity analyses appears in Seinfeld (1988).

In addition, NO_x inventory validation has involved comparing annual emission estimates reported by different groups. Table 3-9 shows several annual U.S. NO_x emission estimates. In 1982, the estimates vary by less than 12% and this decreases to about 9% in the 1985 comparison.

Natural Emission Sources

Natural sources of NO_x include lightning, soils, wildfires, stratospheric intrusion, and the oceans. Of these, lightning and soils are the major contributors. The convention is to include emissions from all soils in the biogenic or natural category even though cultivated soil emissions are in a sense anthropogenic; cultivated soils also appear to produce higher emissions than those from undisturbed forest and prairie soils, as discussed later. Although

Table 3-9. Comparison of Estimates of Nitrogen Oxides Emissions from Manmade Sources in the United States[a]

	Emissions/year (Tg)	
Inventory	1982	1985
NAPAP	—	18.6
EPA	19.6	19.8
MSCET	18.8	18.2
EPRI	20.7	—

[a]See Appendix A for abbreviations and acronyms.

Source: U.S. Environmental Protection Agency (1993a).

NO_x emitted from large wildfires can be significant on a regional scale, this source is overall considered to be of minor importance for the United States. Injection of NO_x into the upper troposphere via subsidence from the stratosphere is estimated at less than 0.1 Tg/year for all of North America. Because of the relatively short lifetime of NO_x (1 to 3 days) and small NO_x emissions from sea water, transport of NO_x from oceans is thought to be a negligible source in the United States.

Lightning. Lightning produces high enough temperatures to allow N_2 and O_2 to be converted to NO. Two methods have been employed to estimate the NO_x source strength from lightning:

(1) Multiply the frequency of lightning flashes by the energy dissipated per flash and the NO production per unit of energy dissipated; or

(2) Relate NO_x production to NO_3^- deposition in remote areas where lightning-produced NO_x is thought to be the dominant NO_3^- precursor.

Method 1 yields an annual NO_x production of approximately 1.2 Tg for North America (Placet et al., 1991). The deposition-based estimate (Method 2) gives a somewhat larger value of 1.7 Tg/year (Placet et al., 1991). The NAPAP inventory included lightning-produced NO_x on a gridded 10° × 10° latitude-longitude scale. Most of the continental United States fits within 30° to 50° N latitude and 80° to 120° W longitude. The estimated annual lightning-produced NO_x for this region (continental United States) is about 1.0 Tg. Roughly 60% (0.6 Tg) of this NO_x is generated over the southern tier of states (30° to 40° N latitude; 80° to 120° W longitude).

Soils. Both nitrifying and denitrifying organisms in the soil can produce NO_x. The relative importance of these two pathways is probably highly variable from biome to biome. Nitric oxide is the principal NO_x species emitted from soils, with emission rates depending mainly on fertilization levels and soil temperature. Several reports have noted a large increase in NO_x emissions from agricultural soils treated with NO_3^--containing fertilizers (Johansson and Granat, 1984; Kaplan et al., 1988; Johansson, 1984). Measurements of soil NO_x emissions have established that the relationship with temperature is exponential,

consisting of approximately a twofold increase for each 10 °C rise in temperature (Williams et al., 1992; Valente and Thornton, 1993).

Inventorying soil NO_x emissions is difficult because of the large temporal and spatial variability in emissions. The existing inventories have been developed using emission algorithms that are functions of soil temperature and land-use type. Two broad, land-use categories—natural and agricultural—have been assigned. The natural soils are broken down into biome types, and the agricultural soils subdivided according to fertilizer applications. The highest biogenic NO emissions are in corn-growing regions of the midwest (Nebraska, Iowa, and Illinois) during summer months. Of the total U.S. biogenic emissions of NO from soils, 85% occur during the spring and summer months.

Table 3-10 provides a summary of the annual soil NO_x emissions from the 10 U.S. Environmental Protection Agency regions. Approximately 60% of this NO_x is emitted in Regions V, VII, and VIII (see Figure 3-19), which contain the central U.S. corn belt. The total estimate for U.S. soil emissions is 1.2 Tg.

Table 3-10. Annual Nitrogen Oxides Emissions from Soils by U.S. Environmental Protection Agency Region[a]

U.S. Environmental Protection Agency Region	NO_x Emissions (Tg)
I, II, and III	0.05
IV	0.11
V	0.26
VI	0.18
VII	0.27
VIII	0.21
IX	0.04
X	0.01
Total	1.20[b]

[a]See Appendix A for abbreviations and acronyms.
[b]Values do not sum to total due to independent rounding.

Source: Placet et al. (1991).

Uncertainty in Estimates of Natural Nitrogen Oxides Emissions

As previously indicated, inventorying NO_x produced from lightning requires multiplying the number of flashes by average energy factors. No attempt has been made to assign confidence limits to these variables. A measure of the uncertainty associated with lightning-produced NO_x is provided, however, by comparing emission estimates generated independently. Two estimates of the amount of lightning-generated, summertime NO_x in the southeastern United States (2.4 and 8.5×10^{-2} Tg) varied by approximately a factor of 4 (Placet et al., 1991).

Sources of uncertainty when inventorying NO_x emissions from soils include: land-use assignments, soil temperature, and emission algorithm development. Confidence levels assigned to categories 1 and 2 are about ±50%. The emission algorithm is developed from field measurements of NO_x emission rates versus temperature for various land-use categories. Measurement accuracy is approximately ±30%. However, because of the natural variability of NO_x emissions within a specific soil category, uncertainty in the exponential relationship that relates emission rate to temperature is estimated to be in the range of a factor of 2 to 4.

Comparison of Emissions from Manmade and Natural Sources

On an annual basis, natural sources (lightning and soils) contribute approximately 2.2 Tg of NO_x to the troposphere over the United States. This compares to the 1990 anthropogenic emission estimate of 19.4 Tg. Annual NO_x emissions from soils (1.2 Tg) are about 6% of the manmade emissions in the United States. This percentage increases to about 14% when the comparison includes only the summer months of July, August, and September. Even larger biogenic contributions can occur in certain regions of the United States. For example, it is estimated that biogenic NO_x emissions from soils account for about 19% of summertime NO_x emissions in Tennessee (Valente and Thornton, 1993) and actually exceed emissions from manmade sources during the summer months in the states of Nebraska and South Dakota (Williams et al., 1992).

3.4.1.3 Volatile Organic Compounds

Manmade Emission Sources

Volatile organic compounds are emitted into the atmosphere by evaporative and combustion processes. Many hundreds of different organic species are released from a large number of source types. The species commonly associated with atmospheric O_3 production contain from 2 to about 12 carbon atoms. They can be true hydrocarbons, which possess only carbon and hydrogen atoms (e.g., alkanes, alkenes, aromatics), or substituted hydrocarbons that contain a functional group such as alcohol, ether, carbonyl, ester, or halogens. The emissions of methane have been ignored because of their largely natural origin and the fact that the importance of methane is limited primarily to global scale processes. In addition, the atmospheric oxidation rate of methane is very slow compared to the higher molecular weight organics.

In 1991, the total U.S. emissions of VOCs was estimated to be 21.0 Tg (U.S. Environmental Protection Agency, 1993b). The two largest source categories were industrial processes (10.0 Tg) and transportation (7.9 Tg). Lesser contributions were attributed to waste disposal and recycling (2.0 Tg), stationary source fuel combustion (0.7 Tg), and miscellaneous area sources (0.5 Tg). Table 3-11 provides a more detailed breakdown of VOC source contributions. Within the industrial category, solvent utilization, petroleum product storage and transfer, and chemical manufacturing are the major contributors. Volatile organic compounds released from highway vehicles account for almost 75% of the transportation-related emissions.

Speciated hydrocarbon emissions from manmade sources were reported in the 1985 NAPAP Emissions Inventory. Emissions of each main hydrocarbon family exceeded 1 Tg. Alkanes comprised about 33%, aromatics 19%, and alkenes 11% of anthropogenic VOC emissions in the 1985 inventory (Placet et al., 1991). None of the major oxygenated

Table 3-11. Estimated 1991 Emissions of Volatile Organic Compounds from Manmade Sources in the United States

Source Category	Emissions (Tg)	
Transportation		7.87
Highway vehicles	6.00	
Off-highway vehicles	1.87	
Stationary fuel combustion		0.68
Electric utilities	0.03	
Industrial	0.26	
Other	0.39	
Industrial processes		9.97
Chemical manufacture	1.61	
Petroleum and related industries	0.68	
Solvent utilization	5.50	
Petroleum product storage and transport	1.69	
Other	0.49	
Waste disposal and recycling		2.01
Miscellaneous		0.51
Total all sources		21.04

Source: U.S. Environmental Protection Agency (1993b).

hydrocarbon groups (e.g., carbonyls, organic acids, phenols) listed in the speciated inventory exceeded 1 Tg. The carbonyl group, which included formaldehyde, higher aldehydes, acetone, and higher ketones, was the largest contributor of oxygenated hydrocarbons at 0.73 Tg.

Seasonal variations are available for the 1993 anthropogenic VOC emissions from 14 source categories in the United States (U.S. Environmental Protection Agency, 1994). Very few seasonal variations occur for categories contributing approximately 85% of the total annual VOC emissions. The only category of VOC emissions that showed significant seasonal variation was off-highway sources, which comprises 13% of the total annual VOC emissions. These off-highway sources contribute 31% of their VOC emissions in summer and 19% in winter. An earlier inventory for 1985 (U.S. Environmental Protection Agency, 1989), which considered total point and area anthropogenic VOC emissions, indicated that very little variation in VOC emissions occurred between the winter, spring, summer, and fall seasons. The contribution of these VOC emissions also were shown to vary seasonally by region of the United States.

Trends in Emissions

Emissions of nonmethane VOCs peaked in the early 1970s and have decreased continually since then. Emissions of VOCs increased from 15.5 Tg in 1940 to 27.4 Tg in

1970 and now are estimated to be back down to approximately the same level as in 1940 (U.S. Environmental Protection Agency, 1992a). Figure 3-22 illustrates these changes at 10-year intervals from 1940 to 1990. Until 1970, highway vehicles were the major source of VOC emissions. As automobiles have been equipped with more and better emission controls, however, emissions from the transportation sector have dropped below those from industrial processes, the category that is now the leading contributor of VOC emissions to the atmosphere. Transportation, industrial processes, and the miscellaneous burning and solvent-use categories have accounted for 83 to 93% of VOC emissions over the past 50 years. Figure 3-23 shows the emission trends for these three categories. The transportation-related emissions of VOCs are currently estimated to be at about the same level as in 1940. Industrial process VOC emissions nearly tripled between 1940 and 1980, followed by a small decline in more recent years. The miscellaneous category exhibited a decrease in emissions from 4.5 Tg in 1940 to a 1990 level estimated at 2.8 Tg/year.

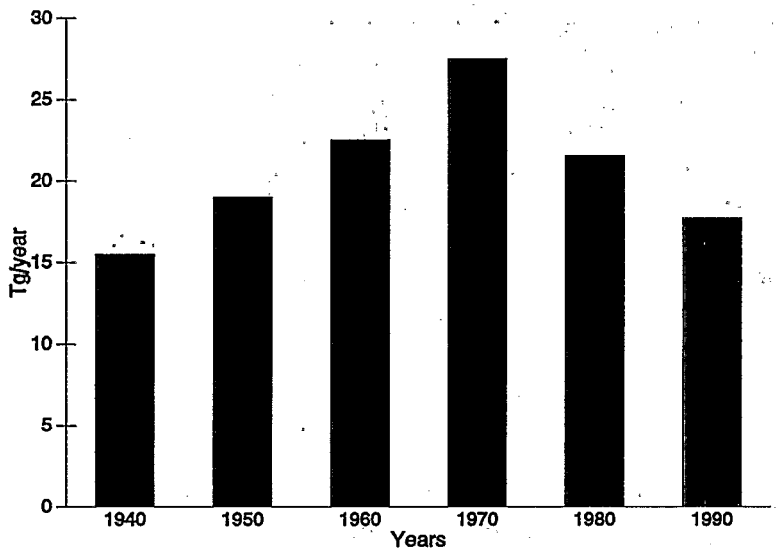


Figure 3-22. Changes in emissions of volatile organic compounds from major manmade sources in the United States, 10-year intervals, 1940 through 1990.

Source: U.S. Environmental Protection Agency (1992a).

Trends for the dominant VOC emissions categories from 1987 through 1991 are shown in Table 3-12. Projections for the year 2000 forecast a 62% reduction in VOC emissions from highway vehicles compared to 1990 levels. The major reduction in the transportation area will contribute to a predicted overall 25% decrease in total national VOC emissions between 1990 and 2000 (U.S. Environmental Protection Agency, 1992a).

Uncertainty in Estimates of Emissions from Manmade Sources

It has proven difficult to determine the accuracy of VOC emission estimates. Within an area source such as an oil refinery, emission factors and activity levels are

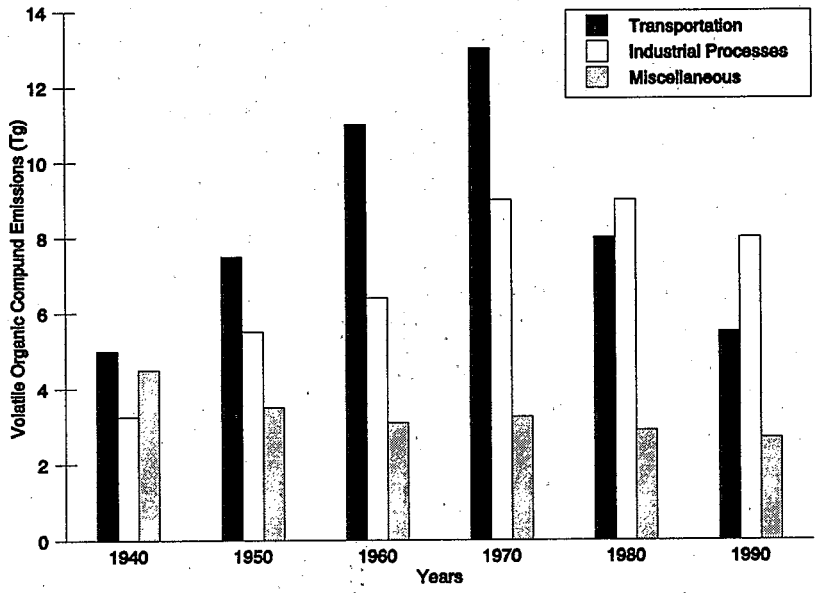


Figure 3-23. Changes in emissions of volatile organic compounds from major manmade sources, 1940 through 1990.[a]

[a]The values for 1990 do not agree with those in Table 3-12 because different models were employed for deriving the short- and long-term trends.

Source: U.S. Environmental Protection Agency (1992a).

Table 3-12. Recent Trends in Emissions of Volatile Organic Compounds from Major Categories of Manmade Sources (Tg)

Year	Transportation	Industrial Processes	Waste Disposal and Recycling
1991	7.87	7.86	2.01
1990[a]	8.07	9.96	2.05
1989	8.26	9.92	2.08
1988	9.15	10.00	2.10
1987	9.29	9.65	2.05

[a]The values for 1990 do not agree with those in Figure 3-23 because different models were employed for deriving the short- and long-term trends.

Source: U.S. Environmental Protection Agency (1993b).

assigned for thousands of individual sources (e.g., valves, flanges, meters, processes), and emission estimates for each of these sources are summed to produce the emissions total. Because it would be impractical to determine an emission factor for each of these sources within a refinery individually, average emission factors for the various source categories are utilized. This can lead to substantial error if emissions from the individual sources deviate from the assigned average factor. Even more troublesome are area sources that include a large evaporative emissions component. These sources are dependent on environmental factors such as temperature, which add to the difficulty in establishing reliable emission estimates. Such sources fall into a miscellaneous solvent evaporation category that includes emissions from processes such as dry cleaning, degreasing, printing, automobile body repair, furniture manufacture, and motor vehicle manufacture.

Assigning accurate VOC emission estimates to the mobile source category has proven troublesome, as well. Models are used that incorporate numerous input parameters, each of which has some degree of uncertainty. For example, activity models are employed to characterize the mobile source fleet. This includes the number of vehicles in various categories (e.g., gasoline-fueled, diesel-fueled, catalyst-equipped, non-catalyst-equipped, etc.), miles accumulated per year for each type of vehicle, and ages of the vehicles. Vehicle registration statistics are employed for category assignment. Errors can arise because registration data are not always up to date, and unregistered vehicles are completely omitted. Military vehicles, foreign-owned automobiles, and old "junkers" that are on the highways but not registered are included in the inventorying process. The activity models assume that vehicles of the same age accumulate mileage at the same rate. This is most likely not correct; there is a need to assess the uncertainty in this assumption through a systematic collection of vehicle type, age, and mileage accumulation statistics.

Experiments carried out in tunnels have looked at the relationship between measured VOC emission factors and those derived from automotive emission models. In a study designed to verify automotive emission inventories for the South Coast Air Basin, measurements in the Van Nuys Tunnel indicated that automotive VOC emissions were a factor of 4 larger than predicted using emission models (Pierson et al., 1990). Results from two tunnel studies conducted in 1992 (Robinson et al., 1996) show much better agreement between VOC measurements and model predictions than those obtained in the Van Nuys Tunnel. Comparisons were made for the Tuscarora Tunnel on the Pennsylvania Turnpike in south-central Pennsylvania and at the Fort McHenry Tunnel under Baltimore Harbor. For Tuscarora, MOBILE4.1 gives 131% of the average measured VOC values and MOBILE5 gives 216% of the average measured VOCs. For Fort McHenry, MOBILE4.1 gives 53% of the average measured VOC values and MOBILE5 gives 81% of the average measured VOCs. Somewhat better agreement also was obtained from the Van Nuys Tunnel data when the updated California emission model EMFACTEP was used (Robinson et al., 1996). At the Cassier Tunnel in Vancouver, Canada, agreement within $\pm 30\%$ was obtained between the VOC measurements and the Canadian version of the MOBILE models (Gertler et al., 1994). The 1993 results from the Caldecott Tunnel in the San Francisco, CA, area show deviations between VOC measurements and model predictions similar to those obtained in the Van Nuys Tunnel, possibly because of similar urban/local fleets. Differences in test results between the newer tunnel studies and those obtained in the early Van Nuys Tunnel study are likely due to the better condition of the vehicles in the newer studies and the lack of power enrichment or other transients because of the steady-speed driving. However, it is important to appreciate that results from tunnel measurements do not necessarily predict equivalency of

VOC measurements with model predictions under highly transient urban/local driving and fleet conditions.

Recent developments in remote sensing have permitted more accurate measurement of hydrocarbon exhaust emissions from on-road vehicles (Stedman et al., 1991). These studies have demonstrated a highly skewed distribution, with the majority of VOC emissions coming from about 20% of the automobiles. Emission factors developed from laboratory dynamometer testing most likely do not properly account for the high-emitting vehicle contribution (Pitchford and Johnson, 1993). In many cases, these high emitters are older cars that are poorly maintained. In order to reduce this source of uncertainty, it may be necessary to reassess the life spans assigned to vehicles. Vehicles manufactured more than 25 years prior to 1993 are not included in the inventory. However, these older vehicles are likely to be high emitters, and, if they are underrepresented in the model, emissions will be underestimated. Activity models provide data in terms of national averages. This can contribute to inaccuracies in emissions estimates if a particular region varies from the national average in terms of vehicle types, age, or VMT.

Ambient measurements of VOCs and NO_x have been employed in order to better define uncertainty levels in VOC inventories. Some of the earliest work was carried out in the Atlanta area in the 1980s. Using a simple model and measured ambient VOC and NO_x concentrations, it was shown that ambient NO_x levels were consistent with the urban NO_x emission estimates. However, measured ambient VOC concentrations were as much as a factor of 6 greater than predicted (Westberg and Lamb, 1985). Improvements in mobile source emission models have resulted in somewhat higher emission estimates, so that the discrepancy between model estimates and ambient data has been reduced to about a factor of 2.5 (Fujita et al., 1992; Cadle et al., 1993). It is clear that the relationship between emission inventories and ambient concentrations of NO_x and VOCs warrants further study. In addition to improving the mobile source emission inventories, it will be necessary to place uncertainty bounds on stationary source inventories. Whether stationary source emissions of VOCs are underpredicted using current emission inventory methodology is not known (Finlayson-Pitts and Pitts, Jr., 1993).

Biogenic Emissions

Vegetation emits significant quantities of reactive VOCs into the atmosphere. Many of these biogenic VOCs may contribute to O_3 production in urban (Chameides et al., 1988) and rural (Trainer et al., 1987) environments. The VOC emissions of primary interest are isoprene and the monoterpenes (e.g., α-pinene, β-pinene, myrcene, limonene, etc.), which are hydrocarbons. Recent field measurements have shown that a variety of oxygenated organics also are emitted from plants (Winer et al., 1992). A thorough discussion of biogenic emissions and their implication for atmospheric chemistry has been published recently by Fehsenfeld et al. (1992), who reviewed the techniques used to measure VOC emissions from vegetation, laboratory emissions studies that have been used to relate emission rates to temperature and light intensity, development of emission models, and the use of emission models in the preparation of emission inventories.

Since the late 1970s, a number of regional and national biogenic emission inventories have been reported (Zimmerman, 1979; Winer et al., 1983; Lamb et al., 1985, 1987, 1993). These inventories are based on algorithms that relate VOC emissions from a particular vegetation class to ambient temperature, land-use, and, in the case of isoprene, photosynthetically active radiation. Most biogenic VOC emissions from vegetation increase

exponentially with temperature. Isoprene emissions are light-dependent, being minimal at night and increasing with solar intensity during the day. Deciduous vegetation is the dominant source of isoprene; whereas coniferous trees emit primarily monoterpenes. Other things being equal, isoprene is emitted at a much higher rate than the monoterpenes. For example, in a southern forest of mixed pine and hardwoods, the isoprene emission rate from an oak tree is about 10 times larger than the flux of α-pinene from an adjacent loblolly pine during the midday period.

The most recent biogenic VOC emissions estimate for the United States totals 29 Tg/year (Lamb et al., 1993). This estimate includes 5.9 Tg isoprene, 4.4 Tg α-pinene, 6.5 Tg other monoterpenes, and 12.3 Tg other VOCs. Table 3-13 provides a summary of the contributions from the various vegetation categories based on an inventory of monthly statewide data for eight land-cover types. In preparing this inventory, algorithms were developed that related VOC emissions to temperature and light for each of the biomass categories shown in the table. On a national scale, coniferous forests are the largest vegetative contributor because of their extensive land coverage. The category "Other VOCs" is the dominant biogenic hydrocarbon contributor to the national total. From the standpoint of inventory accuracy, this is somewhat unfortunate because the identities of most of the "Other VOCs" are uncertain. This classification has carried over from the extensive field-measurement program conducted by Zimmerman (1979) and coworkers in the mid-1970s. The category "Other VOCs", includes peaks that showed up in sample chromatograms at retention times that could not be matched to known hydrocarbons. It is likely that if the Zimmerman study were repeated today, most of the species making up this "Other VOCs" category could be identified. Recent field studies have made use of GC/MS techniques that were not available to Zimmerman in the 1970s.

Biogenic emissions, because of their dependence on temperature and vegetational growth, vary by season. In addition, the southern tier of states is expected to produce more biogenic emissions than those in the north because of higher average temperatures. Table 3-14 shows a spatial and temporal breakdown of U.S. biogenic emissions. Summertime emissions comprise 16.7 of the 29.1 Tg (or 57%) of the annual totals in all regions. The EPA Regions IV and VI in the southeastern and southcentral United States, respectively, have the highest summertime and annual biogenic VOC emission rates. Region IV contributes 16% of the summertime and 18% of the annual biogenic VOC emissions in the United States, whereas Region VI contributes 21% of the summertime and 23% of the annual biogenic emissions in the United States. Compared to Regions IV and VI, regions to the north have more rapid increases in biogenic VOC emissions in the spring and more rapid decreases in biogenic VOC emissions in the fall.

Uncertainty in Estimates of Biogenic Emissions

Sources of error in the biogenic inventorying process arise from uncertainties in emission measurements, determination of biomass densities, land-use characterization, and measurement of light intensity and temperature. Within each of these categories, the error is relatively small. However, when emission measurements are combined with temperature or light intensity, or both, into a single algorithm, the uncertainty increases greatly. This results from the fact that temperature and light are only surrogates for the real physiological processes that control biogenic emissions. Emission rate and ambient temperature can be highly correlated for data collected from one tree branch over a 24-h period; but, when these data are combined with measurements from other branches and other trees the correlation is

Table 3-13. Annual Biogenic Hydrocarbon Emission Inventory for the United States (Tg)[a]

	Land Use								
Compound	Oak Forests	Other Deciduous Forests	Coniferous Forests	Scrub-lands	Grass-lands	Crop-lands	Inland Waters	Urban Areas	U.S. Total
Isoprene	2.31	1.01	0.61	1.17	0.49	0.2	0.02	0.08	5.9
α-pinene	0.19	0.23	2.07	0.78	0.13	0.85	0.06	0.04	4.4
Other terpenes	0.41	0.44	3.08	1.41	0.24	0.81	0.06	0.06	6.5
Other VOCs	1.12	0.88	2.72	2.49	0.45	4.51	0.07	0.08	12.3
Total	4.03	2.56	8.48	5.85	1.31	6.37	0.21	0.26	29.1
Percent of Total	13.9	8.8	29.2	20.1	4.5	21.9	0.7	0.9	

[a]See Appendix A for abbreviations and acronyms.

Source: Lamb et al. (1993).

Table 3-14. Annual Biogenic Hydrocarbon Emission Inventory by Month and by U.S. Environmental Protection Agency Region for United States Emissions (Tg)

	U.S. Environmental Protection Agency Region									
Month	III	IV	V	VI	VII	VIII	IX	X	Total	Percent of Total
1	0.018	0.092	0.004	0.084	0.007	0.022	0.060	0.043	0.3	1.1
2	0.017	0.139	0.004	0.123	0.007	0.020	0.054	0.039	0.4	1.4
3	0.071	0.428	0.067	0.519	0.078	0.108	0.113	0.102	1.5	5.1
4	0.169	0.460	0.189	0.567	0.211	0.303	0.320	0.202	2.4	8.3
5	0.206	0.475	0.240	0.586	0.226	0.362	0.331	0.208	2.6	9.1
6	0.427	0.874	0.550	1.146	0.508	0.809	0.710	0.424	5.5	18.7
7	0.441	0.903	0.568	1.184	0.524	0.836	0.734	0.438	5.6	19.3
8	0.439	0.903	0.568	1.184	0.524	0.820	0.734	0.438	5.6	19.3
9	0.123	0.461	0.137	0.561	0.136	0.280	0.357	0.212	2.3	7.8
10	0.069	0.286	0.066	0.394	0.026	0.290	0.369	0.219	1.7	5.9
11	0.066	0.162	0.063	0.174	0.025	0.110	0.130	0.109	0.8	2.9
12	0.018	0.080	0.004	0.073	0.007	0.022	0.060	0.043	0.3	1.1
Total	2.1	5.3	2.5	6.6	2.3	4.0	4.0	2.5	29.1	

Source: Lamb et al. (1993).

not nearly as good. The uncertainty associated with the algorithms used to generate the U.S. inventory described previously is estimated to be a factor of 3 (Lamb et al., 1987). Because other sources of error in the inventorying process are much smaller, a factor of 3 is the current best estimate of the overall uncertainty associated with biogenic VOC inventories. However, this may be a lower limit if it is shown that oxygenated species are emitted in significant quantities by vegetation. Emission measurement methods employed in the past have not been adequate for quantifying polar, oxygenated organics.

Comparison of Manmade and Biogenic Emissions

The most recent anthropogenic and biogenic VOC emissions estimates for the United States indicate that natural emissions (29 Tg) exceed manmade emissions (23 Tg). During the summer months in the United States, anthropogenic emissions constitute 25% of the annual anthropogenic VOC emissions, whereas biogenic emissions constitute 57% of the annual biogenic emissions. On a teragram basis, anthropogenic VOC emissions during the summer contribute 0.25×23 Tg = 5.75 Tg, whereas summer biogenic VOC emissions contribute 0.57×29 Tg = 16.5 Tg. Therefore, on a national basis, the ratio of biogenic to anthropogenic VOC emissions is approximately 2.9 for the United States. However, this ratio varies with region in the summer months. These calculations depend on the assumption that regional summertime anthropogenic VOC emissions are one-quarter of the annual VOC emissions. With this assumption, the ratios of biogenic to anthropogenic VOC emissions for three selected regions are as follows: 2.2 for Region IV, 1.6 for Region V, and 3.2 for Region VI (Lamb et al., 1993; U.S. Environmental Protection Agency, 1994). However, in a recent National Research Council review (1991), it was concluded that emissions from manmade sources are currently underestimated by a significant amount (60 to 80%). Because uncertainty in both biogenic and anthropogenic VOC emission inventories is large, it is not possible to establish whether the contribution of emissions from natural or manmade sources of VOCs is larger.

3.4.1.4 Relationship of Summertime Precursor Emissions and Ozone Production

Peak O_3 levels are recorded in most regions of the country during the months of June, July, and August. From the foregoing discussion, it is obvious that natural emissions of NO_x and VOCs peak during this same time frame. Biogenic emissions are very dependent on temperature; and, as ambient temperatures rise during the summer months, NO_x and VOC emissions reach a maximum. Figure 3-24 clearly demonstrates this for biogenic VOC emissions, and a plot of monthly biogenic NO_x emissions would show a similar pattern. Well over 50% of biogenic NO_x and VOC emissions occur during the period of maximum photochemical activity.

Seasonal changes in anthropogenic emissions of NO_x are believed to be relatively small. The transportation sector produces slightly less NO_x during the warmer months, but there is probably a small increase from the stationary source category because of higher summertime power demands. Because these are the major U.S. sources of NO_x and changes in seasonal emissions tend to offset each other, there is no reason to expect that NO_x emissions will vary significantly by season on the national level. Evaporative emissions of VOCs are enhanced during the warm summer months. Because evaporation is an important component of anthropogenic VOC emissions, there is a summertime increase. In 1993, U.S. VOC emissions during June, July, and August were estimated to exceed annual monthly average VOC emissions by about 17% (U.S. Environmental Protection Agency, 1994). The

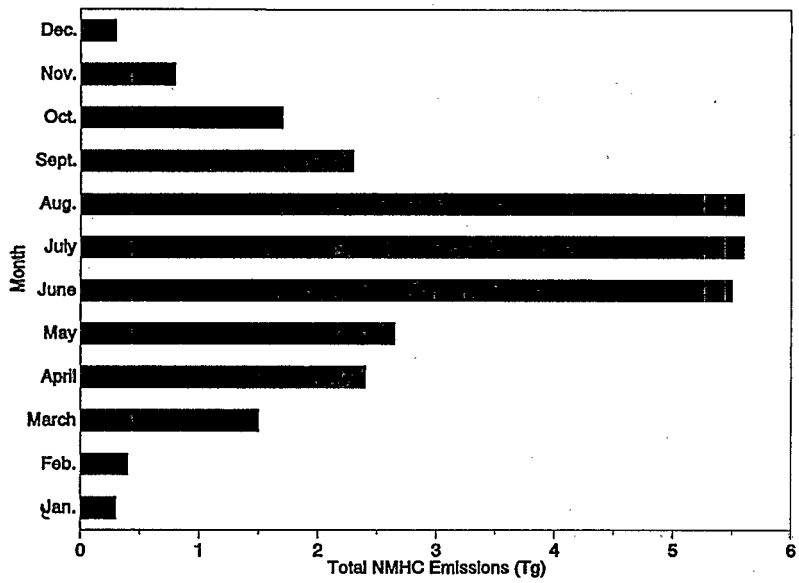


Figure 3-24. Estimated biogenic emissions of volatile organic compounds in the United States as a function of season.

Source: Fehsenfeld et al. (1992).

summertime anthropogenic VOC effect should be somewhat larger for southern regions. This is a very small change, however, relative to the uncertainty associated with VOC emission estimates. In an earlier discussion of the NAPAP inventory, VOC emissions from manmade sources were considered to be almost independent of season.

Increases in O_3 precursor emissions during the peak O_3 season will have a tendency to enhance O_3 production. Ozone production in rural areas is usually NO_x-limited (Fehsenfeld et al., 1992). Thus, enhanced summertime emissions of NO_x from soils and lightning will add NO_x to the atmosphere in rural regions, which in turn will lead to the production of more O_3. Larger summertime emissions of VOCs will enhance O_3 production in urban areas. Biogenic VOC sources in the vicinity of urban areas can contribute significant quantities of reactive hydrocarbons to the urban O_3 precursor mix (Cardelino and Chameides, 1990).

3.4.2 Concentrations of Precursor Substances in Ambient Air

The volatile organic compounds, excluding CH_4, often are referred to as NMOCs. The class of NMOCs most frequently analyzed in air are the nonmethane hydrocarbons (NMHCs). The NMHC measurements often provide an acceptable approximation of the NMOCs. The NMHCs and the NO_x within urban areas tend to have morning concentration peaks. These result from vehicular traffic in combination with limited mixing depths. Later in the morning into the afternoon hours, concentrations of NMHCs and NO_x decrease, but to varying extents (Purdue et al., 1992), because of chemical reactions and increases in mixing depths and consequent increases in dilution volumes. Photochemical atmospheric reactions also can rapidly convert NO to NO_2, and hydrocarbons to carbonyls,

PANs, and other products (Sections 3.2.4, 3.4.2.1, and 4.9). Late afternoon and early evening peaks might be expected in NMHC and NO_x concentrations because of increased vehicular traffic at urban locations, but such increases often are not discernible (Purdue et al., 1992). This effect probably results from the presence of substantial mixing depths in the warmer months that persist through these hours in many urban locations.

Because of the emphasis on early morning inputs of NMOCs and NO_x for models such as the Empirical Kinetics Modeling Approach (EKMA), most of the measurements available emphasize the 6 a.m. to 9 a.m. period. The variations in the concentrations of NMOCs and NO_x, their ratios, and the composition of NMOCs are important factors in the generation of O_3 and other photochemical products.

3.4.2.1 Nonmethane Organic Compounds

In earlier measurements based on GC analyses made during a number of different studies in urban areas between 1969 and 1983, the mean 6 a.m. to 9 a.m. NMHC concentrations were reported to range from 0.324 to 3.388 ppm carbon (ppmC) (U.S. Environmental Protection Agency, 1986a). The highest NMHC concentrations were those measured at sites in Los Angeles.

A program for analysis of NMOCs and NO_x in the months of June through September was conducted in a considerable number of U.S. cities during the 1980s. The results obtained from measurements made during the 6 a.m. to 9 a.m. period at sites in 22 cities in 1984 and 19 cities in 1985 have been subjected to statistical analysis and interpretation (Baugues, 1986). The total NMOC measurements throughout the June through September periods in these cities were obtained by the cryogenic preconcentration-direct flame ionization detection method (PDFID) (McElroy et al., 1986). In addition, during about 15% of the 6 a.m. to 9 a.m. periods, canister samples were collected for subsequent GC analysis (Seila et al., 1989). In 1984, the lowest median NMOC value obtained was 0.39 ppm C from measurements in Charlotte, NC, whereas the highest median NMOC value obtained was 1.27 ppmC from measurements in Memphis, TN. In 1985, the lowest median NMOC value obtained was 0.38 ppmC from measurements in Boston, MA, whereas the highest median NMOC value obtained was 1.63 ppmC in Beaumont, TX. The overall median values from all urban sites were approximately 0.72 ppmC in 1984 and 0.60 ppmC in 1985 (Baugues, 1986). The GC analyses made on samples collected in 1984, 1985, and 1986 have been reported (Seila et al., 1989). The more abundant individual hydrocarbons include C_2-C_6 alkanes, C_2-C_5 alkenes, C_6-C_9 aromatics, and acetylene. Based on the 48 most abundant concentrations, the overall median concentrations by class of hydrocarbon (NMHCs) were as follows: paraffins, 0.266 ppmC, 60% of total; aromatics, 0.166 ppmC, 26% of total; olefins, 0.047 ppmC, 11% of total; and acetylene, 0.013 ppmC, 3% of total (Seila et al., 1989). Additional individual NMHCs (totaling about 0.100 ppmC) were detected at concentrations $\leq$0.002 ppm C each. Most of these compounds were identified by class but not by structure.

Detailed hydrocarbon analyses for C_2-C_{10} NMHCs were obtained during the 17 intensive days of the Southern California Air Quality Study (SCAQS) in 1987 (Lonneman et al., 1989; Rasmussen, 1989; Stockberger et al., 1989). The average percentage ambient composition from eight Southern California sites during 11 intensive sampling days of the summer of 1987 by class of NMHCs were as follows: paraffins, 53.4; aromatics, 27.2; olefins, 12.1; carbonyls, 7.7 (Main and Lurmann, 1993).

In Atlanta, during the summer of 1990, hydrocarbon concentrations were measured at six sites with automated GCs. Results were reported on 54 hydrocarbons, with 24-h average concentrations ranging from 0.186 to 0.397 ppmC (Purdue et al., 1992).

A comparison of NMHC measurements made by GC analyses over a period of years in Los Angeles and in the New York City area has been reported (Lonneman and Seila, 1993). In the Los Angeles area, the NMHC concentrations averaged 2.81 ppmC in 1968, compared to 1.02 ppmC in 1987. In the New York City area, the NMHC concentrations averaged about 1.1 ppmC in 1969, compared to 0.62 ppmC from 1986 to 1988. In both the Los Angeles and New York areas, there were significant decreases in NMHC concentrations as well as compositional changes in NMHCs during these years, with increases observed in the percentage of paraffin hydrocarbons and decreases in the percentage of aromatic hydrocarbons and acetylene (Lonneman and Seila, 1993).

Aldehydes and ketones occur in urban air as O_3-oxidant precursors from emissions such as vehicular exhaust and as products of reactions of OH radicals with NMHCs, reactions of alkenes with O_3, and, at night, reactions with NO_3 radicals. Early morning aldehyde concentrations have been predicted to result to a greater extent from atmospheric reactions of alkenes than from emission of vehicular exhaust (Altshuller, 1993). During the day, aldehydes and ketones are rapidly produced from reactions of OH radicals with aliphatic and aromatic hydrocarbons and of alkenes with O_3. Carbonyl concentrations tend to increase through the daytime hours (Grosjean, 1982, 1988; Grosjean et al., 1993b).

Measurements of ambient air concentrations of carbonyls indicate the total loading of aldehydes and ketones from all processes. Ambient urban air concentrations of HCHO and total aldehydes were tabulated for the 1960 to 1981 period (Altshuller, 1983a). Subsequent studies by 2,4-dinitrophenylhydrazine high-performance liquid chromatography (DNPH-HPLC) techniques (Section 3.5.2.3) have shown consistently that HCHO and acetaldehyde are the most abundant aldehydes; however, a number of other carbonyls (including propanal, acrolein, acetone, butanal, crotonaldehyde, methyl ethyl ketone, pentanal, hexanal, benzaldehyde, and tolualdehyde) also have been measured (Fung, 1989; Grosjean 1982, 1988, 1991; Kalabokas et al., 1988; Zweidinger et al., 1988). The ratios of HCHO to acetaldehyde concentrations (in parts per billion volume) can vary from less than 0.5 in cities in Brazil, where there is high use of ethanol fuels, up to 4.0 to 5.0 at a few urban sites (Grosjean et al., 1993). However, at most urban sites, the ratios of HCHO to acetaldehyde concentrations occur in the 1.0 to 3.0 range.

A compilation of the maximum, average range of HCHO concentrations from many studies in Southern California carried out between 1960 and 1989 is available (Grosjean, 1991). A downward trend in HCHO concentrations occurs, probably because of decreased production from precursor alkenes and decreased emission in vehicular exhaust (Sigsby et al., 1987; Dodge, 1990). For example, the maximum HCHO concentrations decreased from above 100 ppbv in the 1960s to the 10- to 30-ppbv range during the last decade (Grosjean, 1991). In other U.S. cities in the early 1980s, the maximum HCHO concentrations ranged from 5 to 45 ppb (Salas and Singh, 1986).

Several studies have reported concurrent morning hydrocarbon and carbonyl concentrations in downtown Los Angeles (Grosjean and Fung, 1984); Raleigh, NC (Zweidinger et al., 1988); and Atlanta (Shreffler, 1992; Grosjean et al., 1993b). The average percentage of carbonyls relative to total NMHCs were reported as follows: Los Angeles, 3%; Raleigh, 2%; and Atlanta, $\geq$2% (formaldehyde + acetaldehyde concentrations) at two different sampling sites. In SCAQS, carbonyls were measured at eight

sites in the summer and five in the fall of 1987 (Fung, 1989; Fujita et al., 1992). The average percentage of C_1 to C_6 carbonyls relative to NMHCs was 7.6% in the summer and 3.7% in the fall.

Compilations of NMHC concentrations of nonurban and remote locations are available (U.S. Environmental Protection Agency, 1986a; Altshuller, 1989a). Total NMHC concentrations reported ranged from less than 0.01 to 0.14 ppmC. At remote locations over the Pacific, NMHC concentrations generally were less than 0.01 ppmC. Over both continental and oceanic locations there can be contributions from biogenic sources of NMHCs.

Interest in the contribution of biogenic hydrocarbons has existed for many years, and earlier work has been reviewed (Altshuller, 1983b). Photochemical modeling in the United States predicts significant effects of biogenic hydrocarbons on O_3 production (Chameides et al., 1988; Roselle et al., 1991). Similar modeling of the effect of biogenic hydrocarbons on O_3 production within urban plumes over southeastern England predicted a 2- to 8-ppb increase in plume and background O_3 concentrations (MacKenzie et al., 1991). Because of lower emissions of biogenic and lower overall NMOC/NO_x ratios, O_3 production over southeastern England is predicted to be limited by the availability of anthropogenic hydrocarbons.

Compilations of results of earlier measurements of isoprene and terpene concentrations are available (Altshuller, 1983b; U.S. Environmental Protection Agency, 1986a). Average concentrations of isoprene ranged from 0.001 to 0.020 ppmC and terpenes from 0.001 to 0.030 ppmC. When concurrent measurements of biogenic and anthropogenic NMHCs were available, the biogenic NMHCs usually constituted much less than 10% of the total NMHCs (Altshuller, 1983b).

Among more recent studies are two investigations of terpene and isoprene emissions in the central valley of California and in Louisiana (Arey et al., 1991b; Khalil and Rasmussen, 1992). Both studies reported a large number of individual terpenes, measured by using enclosure methods. When ambient air measurements were made, most of the terpenes measured in the enclosures were not detectable (Khalil and Rasmussen, 1992). In ambient air, isoprene was the predominate hydrocarbon, accounting on average for 70% of the biogenic species and 36% of NMOCs. It has been concluded that the bag enclosure method can lead to large overestimates in biogenic emissions (Khalil and Rasmussen, 1992).

In two other recent studies in deciduous forests, the isoprene oxidation products were measured as well as isoprene itself (Pierotti et al., 1990; Martin et al., 1991). Both studies report the ambient concentrations of methacrolein and methyl vinyl ketone. In an investigation in a central Pennsylvania deciduous forest in the summer of 1988, average midday concentrations of isoprene were in the 0.005- to 0.010-ppmC range; whereas the corresponding concentrations of methacrolein and methyl vinyl ketone were in the 0.001- to 0.002-ppmC range (Martin et al., 1991). In the study conducted in California forests with samples collected between noon and 4:00 p.m. in late spring and summer, the upper quartile of isoprene concentrations was within the 0.010- to 0.025-ppmC range, whereas methacrolein concentrations were within the 0.001- to 0.003-ppmC range, and methyl vinyl ketone concentrations were within the 0.0005- to 0.0015-ppmC range (Pierotti et al., 1991).

Higher-molecular-weight semivolatile carbonyls have been measured in a number of rural-remote areas (Jüttner, 1986; Yokouchi et al., 1990; Nondek et al., 1992; Ciccioli et al., 1993). The compounds identified include C_5-C_{12} aliphatic aldehydes, aliphatic ketones, and aromatic aldehydes. Comparisons of the measurement of these carbonyls

relative to aromatic hydrocarbons in two studies indicated higher carbonyl concentrations and much lower aromatic hydrocarbon concentrations in the rural-remote sites compared to the urban areas (Yokouchi et al., 1990; Ciccioli et al., 1993). Widely varying natural sources have been associated with these carbonyls, including emissions from forest species (Nordek et al., 1992) and short vegetation (Ciccioli et al., 1993) and as secondary products of natural emissions of terpenes (Ciccioli et al., 1993) or oleic acid (Yokouchi et al., 1990). Among other oxygenates reported to be of natural origin are higher-molecular-weight alcohols (Jüttner, 1986; Nordek et al., 1992; Goldan et al., 1993). These oxygenates contribute to the "Other VOCs" category in the biogenic emissions inventory (Section 3.4.1.3).

In an urban-scale study in Atlanta during the summer of 1990 (as part of the Southern Oxidant Study), isoprene concentrations rose in late morning and into the afternoon, with early evening peaks observed at residential and rural-residential sites (Purdue et al., 1992). A similar diurnal profile for isoprene was observed at a Pennsylvania forest site (Martin et al., 1991). The median concentration at the sampling sites in Atlanta early in the evening ranged from 0.006 to 0.020 ppmC. The isoprene as a percentage of total NMHCs in the early evening ranged among the sites from 2 to 12% (Shreffler, 1992).

3.4.2.2 Nitrogen Oxides

Measurements of NO_x were obtained with continuous NO_x analyzers at sites in 22 and 19 U.S. cities during the months of June through September of 1984 and 1985, respectively. These results have been evaluated and the 6 a.m. to 9 a.m. values tabulated (Baugues, 1986). In 1984, the lowest median NO_x concentration of 0.010 ppm was obtained from measurements in West Orange, TX; whereas the highest median NO_x concentration of 0.088 ppm was obtained from measurements in Memphis. In 1985, the lowest median NO_x concentration of 0.005 ppm was obtained from measurements in West Orange, whereas the highest median NO_x concentration of 0.100 ppm was obtained from measurements in Cleveland, OH. The median NO_x concentration values for sites in most of these cities in 1984 and 1985 ranged between 0.02 and 0.08 ppm. Because of high vehicular emission rates and shallow mixing depths, the median 6 a.m. to 9 a.m. concentration values in many of these cities exceeded the annual average NO_x values of 0.02 to 0.03 ppm in U.S. metropolitan areas between 1980 and 1989 (U.S. Environmental Protection Agency, 1991a). In the 1990 Atlanta study, the average summer NO_x concentration values at the six study sites ranged from 0.011 to 0.026 ppm (Purdue et al., 1992).

At nonurban sites, NO_x concentrations have been reported as mean 24-h seasonal or annual NO_x values. The available results have been compiled for work reported through 1983 (Altshuller, 1986). The average seasonal or annual NO_x concentrations ranged from less than 0.005 to 0.015 ppm. At remote sites in the earlier investigations, monthly average NO_x concentrations were less than 0.001 ppm. In more recent work, the statistics on NO_x concentrations have been reported for several relatively remote U.S. sites (Fehsenfeld et al., 1988). The 24-h average NO_x concentrations and the range in the central 90% of values were as follows: Point Arena, CA, spring 1985, 0.0004 ppm, 0.0007 to 0.001 ppm; Niwot Ridge, CO, summer 1985, 0.0005 ppm, 0.0001 to 0.002 ppm; and Scotia, PA, summer 1986, 0.002 ppm, 0.0007 to 0.009 ppm. It should be noted that each of these sites can be subject to anthropogenic influences, thus accounting for the higher NO_x values. For example, at Niwot Ridge, CO, with upslope flow from the Denver-Boulder, CO, urban area, higher NO_x concentrations are measured. Nitrogen oxide concentrations at or below 0.0001 ppm occur at other remote surface locations (Fehsenfeld et al., 1988).

3.4.2.3 Ratios of Concentrations of Nonmethane Organic Compounds to Nitrogen Oxides

The ratios of 6 a.m. to 9 a.m. NMOC/NO_x have been obtained from the measurements in the U.S. cities discussed above (Baugues, 1986). In 1984, the lowest median NMOC/NO_x ratio of 9.1 was obtained in Cincinnati, OH, and the highest median NMOC/NO_x ratio of 37.7 was obtained in Texas City, TX. In 1985, the lowest median NMOC/NO_x ratio of 6.5 was obtained in Philadelphia, PA, whereas the highest median NMOC/NO_x ratio of 53.2 was obtained in Beaumont, TX. The range in daily 6 a.m. to 9 a.m. NMOC/NO_x ratios within a given city is large, with 10th percentile to 90th percentile NMOC/NO_x ratios varying usually by factors of 2 to 4 and, at several sites, by factors of 5 to 10 (Baugues, 1986). There appears to be a tendency for higher NMOC/NO_x ratios in the cities included in the southeastern (9) and southwestern (15) United States than in the northeastern (7) and midwestern United States (7) (Altshuller, 1989b). The NMOC-to-NO_x ratios at rural sites tend to be higher than the mean NMOC-to-NO_x ratios in urban locations, with mean values at several rural sites ranging between 20 and 40 (Altshuller, 1989b).

In SCAQS, the ambient NMOC (NMHCs + carbonyl)/NO_x ratios averaged 8.8 in the summer and 6.9 in the fall of 1987 (Fujita et al., 1992). However, the 6 intensive days in the fall between November 11 and December 11 were not characterized by elevated O_3 concentrations (Zeldin, 1993). These ambient ratios were 2 to 2.5 times higher than the corresponding emission inventory ratios. Discrepancies as large or larger have been discussed previously for urban and rural NMHC/NO_x ambient-to-emission ratios in the eastern United States (Altshuller, 1989b).

A trend analysis of NMHC/NO_x ratios in the South Coast Air Basin is available for the 1976 to 1990 period (Fujita et al., 1992). The ratios were consistently higher in the summer than in the fall. These ratios started decreasing slowly during the 1980s, from maximum ratios of about 12 in the summer and 9 in the fall to 8.5 in the summer and 7 in the fall by 1990. The ambient-to-emission inventory ratios over this period ranged from as high as 3.4 in the summer to 1.7 in the winter (Fujita, 1993).

Interest in the 6 a.m. to 9 a.m. NMOC/NO_x ratios is associated with their use in the EKMA type of trajectory model (Section 3.6.1.2). The analysis at 10 eastern and midwestern sites of upper-quartile O_3 days relative to other O_3 days indicated a significant difference ($p \leq 0.10$) by the two-sample Wilcoxon Rank Sum test at four of the 10 sites with NMOC/NO_x ratios (Wolff and Korsog, 1992). However, the correlation of NMOC/NO_x ratios with maximum 1-h O_3 concentrations was very weak. It was concluded that the use of the 6 a.m. to 9 a.m. NMOC/NO_x ratio in EKMA will not provide sufficient information to distinguish among NMOC, NO_x, or combined VOC-NO_x strategies as optimum strategies for urban areas.

A compilation of VOC/NO_x ratios between 1981 and 1988 in 10 cities in the northeastern and midwestern United States presents ratios ranging from 5.8 to 11.5, but generally below 10 (Wolff, 1993). Trends between 1986 and 1991 in VOC/NO_x ratios in four of these cities; New York; Newark, NJ; Philadelphia; and Washington, DC, show downward trends towards VOC/NO_x ratios between 4 and 6 (Zalewsky et al., 1993; Wolff, 1993). For Philadelphia, and the other sites, the downward trend in VOC/NO_x is associated with decreasing VOC concentrations with little change in NO_x (Zalewsky et al., 1993; Wolff, 1993). It has been pointed out that, in the National Academy of Science report (National Research Council, 1991), hydrocarbon control is considered more effective for VOC/NO_x ratios of about 10 or less, whereas NO_x control is considered more effective for VOC/NO_x

ratios of 20 or more. Based on these results, it may be concluded that, in many cities in the northeastern and midwestern United States, continued VOC control, rather than NO_x control, will be more effective in reducing O_3 (Wolff, 1993). It also has been concluded by Wolff (1993) that models with greater spatial resolution than the Regional Oxidant Model (ROM), such as the UAM, are more applicable than ROM for determining appropriate O_3 control strategies in urban areas.

3.4.3 Source Apportionment and Reconciliation

3.4.3.1 Source Apportionment

Source apportionment refers to determining the quantitative contributions of sources to ambient air pollutant concentrations. In principle, it includes two fundamentally different approaches: (1) source-oriented and (2) receptor-oriented. In the source-oriented approach, a mathematical dispersion model is applied to an emissions inventory and meteorological data to produce an estimate of ambient pollutant concentrations that can be expected at a specified point in space and time. In contrast, the receptor-oriented approach depends on simultaneous ambient concentration measurements of a variety of pollutant species and a knowledge of the relative amounts of the species (source profiles) that are present in the emissions of the sources that are potential contributors. A mathematical receptor model operates on the source profile and ambient species concentration information to deconvolute the ambient concentrations into their source contributions, without the need of emissions inventory or meteorological information. Indeed, the desire to avoid the latter two kinds of information, whose acquisition is often problematical, has been an important motivation in the development of the receptor-oriented approach.

Although source apportionment, in its general sense, embraces both approaches, in recent years, it has come to be regarded as synonymous with the receptor-oriented approach (receptor modeling). The equivalence of source apportionment and receptor modeling is assumed in the following discussion. The most recent review of the field of receptor modeling has been given by Gordon (1988).

Because tropospheric O_3 is a secondary pollutant, the natural role of receptor modeling is in determining the quantitative source contributions of the VOC precursors of O_3. Historically, receptor modeling was first developed in the 1970s for the apportionment of ambient aerosol, and aerosol applications since then have been more extensive than VOC applications. The aerosol and VOC areas of receptor modeling application have more similarities than differences, however, so that much of the mathematical apparatus that has been developed for aerosol problems is readily adaptable to VOCs.

For reasons that will become apparent, the separation of emissions sources into anthropogenic and biogenic classes is a natural division for VOC receptor modeling and is used in the following.

Manmade Sources of Volatile Organic Compounds

A principal approach for receptor modeling of anthropogenic VOC sources is that of "mass balance". In this approach, a particular linear combination of source profiles is sought that best approximates (in a linear least-squares sense) the profile of VOC species concentrations measured in an ambient sample. Here a VOC source profile is defined as the set of numbers giving the fractional amounts (abundances) of individual species in the emissions from the source. The profile may be normalized to the sum of the abundances of

all VOC species emitted by the source or to a sum over some arbitrary subset of species. For the linear combination of profiles that gives the best fit, the coefficients are the source strengths (in the same units as the measured ambient concentrations) associated with each of the included source profiles.

Early efforts to use various versions of the mass balance approach include Ehrenfeld (1974), Mayrsohn and Crabtree (1976), and Mayrsohn et al. (1977) in Los Angeles and Nelson et al. (1983) in Sydney, Australia.

Of these studies, the work of Mayrsohn et al. (1977) is the most comprehensive: 900 samples from eight sites collected during June to September, 1974. The average results were automotive exhaust, 53%; whole gasoline evaporation, 12%; gasoline headspace vapor, 10%; commercial natural gas, 5%; geogenic natural gas, 19%; liquefied natural gas, 1%. The percentages are for NMHCs through C_{10} (i.e., not all of the total VOCs).

Together, the estimates for the first three vehicle-related sources account for 75% of the ambient NMHCs, which is the approximate percentage estimated in the other studies listed. Geogenic natural gas is obviously not anthropogenic but is included here for completeness. Its strength of 19% is striking; however, it seems unlikely that a contribution this large would be typical of other locales lacking a petroleum-related geology. In any case, accounting for the urban atmospheric concentrations of ethane and propane (the main NMHC constituents of natural gas) has remained an unsatisfactorily resolved problem, so the 19% result for geogenic natural gas has to be regarded skeptically.

Although dated, these early studies are of more than just historical interest. In one respect, they are superior to more recent studies in their recognition of two distinctly different kinds of gasoline evaporation: (1) headspace vapor, which represents the partial evaporation of gasoline in situations such as storage tank evaporation or vehicle diurnal evaporation, characterized by an enrichment of high-volatility species; and (2) whole gasoline emissions, which can arise from spillage, leakage, and vehicle hot-soak emissions, and has a composition resembling liquid gasoline itself. The implications of gasoline evaporation are discussed below.

In the mid-1980s, a useful degree of standardization was incorporated into the mass balance approach by the introduction of EPA's chemical mass balance (CMB) software. The current version, CMB7 (Watson et al., 1990), embodies a comprehensive treatment of error (including uncertainty in both ambient data and source profiles) and many diagnostics (including profile collinearity) and has been used frequently in recent VOC receptor modeling studies.

Recent studies include Wadden et al. (1986) in Tokyo, Japan; O'Shea and Scheff (1988) in Chicago, IL; Aronian et al. (1989) in Chicago; Sweet and Vermette (1992) in Chicago and East St. Louis, IL; Harley et al. (1992) in Los Angeles; Kenski et al. (1993) in Chicago, Beaumont, Detroit, Atlanta, and Washington, DC; Spicer et al. (1993) in Columbus, OH; and Lewis et al. (1993) in Atlanta.

The source categories covered by these studies taken together include vehicle exhaust, gasoline evaporation (whole gasoline and headspace vapor), industrial emissions (refineries, coke ovens, and chemical plants), architectural coatings, dry cleaning, wastewater treatment, auto painting, industrial solvents/degreasers, graphic arts (printing), and natural gas. Each study gives estimates for the percentage contributions to measured ambient VOCs (or related quantity) for a selected subset of these source categories. The one exception is the work of Sweet and Vermette (1992) that estimates the percentage source contributions to

individual species, rather than to total VOCs. Such species apportionment is always available from the CMB calculations, but often is not reported explicitly.

Usually, the source profiles used were generic; that is, from compilations of source measurements taken elsewhere (U.S. Environmental Protection Agency, 1993d). The work of Lewis et al. (1993) is unique in the use of profiles extracted from the ambient air data themselves.

Generally, for these urban-based studies, vehicle exhaust is found to be the dominant contributor to ambient VOCs. Exceptions are the Tokyo results of Wadden et al. (1986) that show an unreasonably small average contribution of 7% and the Beaumont results of Kenski et al. (1993), 14%. For all the rest, the average vehicle exhaust results fall in the range of 45 $\pm$ 15%.

The results for gasoline evaporation contribution estimates are much less satisfactory. This is because the recent studies, with the exceptions of Harley et al. (1992) and Lewis et al. (1993), included a gasoline headspace vapor profile but not a whole gasoline profile in their calculations. The latter two studies suggest that this omission is a serious error. For example, Harley et al. (1992) found a remarkably large whole gasoline contribution (nearly the same as that of vehicle exhaust), and Lewis et al. (1993) find a whole gasoline contribution that is about 20% that of vehicle exhaust. Both, however, find a whole gasoline contribution about four times greater than the headspace contribution. Because vehicle exhaust and whole gasoline profiles are quite similar (except for the very light species that are absent in gasoline but present in exhaust as combustion products), excluding the whole gasoline profile will tend to overestimate the exhaust contribution. Although this error may not greatly affect the total mobile-source-related emissions estimate, it is misleading with regard to implied control strategies.

Beyond the ubiquitous vehicle-related contributions, other anthropogenic source contribution estimates tend to be smaller or locale-specific.

Biogenic Sources of Volatile Organic Compounds

The possible role of biogenic VOC emissions in O_3 formation is being considered much more seriously now (Chameides et al., 1988) than was the case a decade ago. Because of the severe experimental problems in accurately measuring biogenic emissions directly, receptor modeling approaches are of considerable interest. Compared with anthropogenic sources, however, the application of receptor modeling methodology to biogenic sources has been very limited. The principal reason is that it has not been possible to find VOC species that are simultaneously distinctive components of biogenic emissions, emitted in an approximately fixed proportion to the total VOC biogenic emissions, and relatively unreactive. Without these conditions, the construction of a credible stable biogenic source profile is not possible, and, consequently, the CMB approach is unusable.

In this situation, a crude form of receptor modeling has been used in which the ambient concentration of a VOC species, whose only source is thought to be biogenic, is divided by the estimated abundance of the species in the total VOC biogenic emissions. Typical candidates include isoprene (deciduous emission) and the terpenes α- and β-pinene (coniferous emission), δ-caranene, and limonene. Because these are all highly reactive, any such estimate can be regarded only as a lower limit of the contribution that biogenic emissions make to total ambient VOC, if the loss resulting from atmospheric transformation is not taken into account. As an example, Lewis et al. (1993) used isoprene, the most prominent biogenic species measured in downtown Atlanta during the summer of 1990, to

infer a lower limit of 2% (24-h average) for the biogenic percentage of total ambient VOC at that location. Isoprene emissions have a strong diurnal dependence. Lower limits for biogenic emissions at other hours, inferred from average isoprene concentrations, were 1% at 8:00 a.m., 5% at noon, 6% at 4:00 p.m., and 2% at 9:00 p.m.

The recent review article by Fehsenfeld et al. (1992) lists other prominent biogenic species and calls attention to the newly recognized importance of alcohols, such as methanol (CH_3OH), as biogenic primary emissions. Goldan et al. (1993) reported the C_5 alcohol, 2-methyl-3-buten-2-ol to be the most abundant VOC of biogenic origin present in a predominantly lodgepole pine forest in Colorado. Ciccioli et al. (1993) present data from sites in Germany and Italy showing substantial contributions from various aldehydes and argue that their dominant source is biogenic primary emissions, rather than photochemical oxidation products.

A more sophisticated form of biogenic receptor modeling involves the radiocarbon isotope ^{14}C. The approach depends on the fact that ^{14}C constitutes a nearly fixed fraction (approximately 10^{-12}) of all carbon present throughout the biosphere. In contrast, the ^{14}C in dead organic material older than 40,000 years, certainly the case for fossil fuels, has been reduced by at least 99% through radioactive decay. This leads to a simple estimate of the biogenic fraction of a carbon-containing sample given by f_s/f_0 , where f_s is the ^{14}C fraction in the sample, and f_0 is the ^{14}C fraction in living material. Besides its conceptual simplicity, the approach is appealing for VOC apportionment because ^{14}C retains its identity in the reaction products that may result from atmospheric transformation of reactive VOC. The method appears to be reliable for particulate phase organics (Lewis et al., 1988, 1991) but is still under development for VOC applications (Klouda et al., 1993).

3.4.3.2 Source Reconciliation

Source reconciliation refers to the comparison of measured ambient VOC concentrations with emissions inventory estimates of VOC source emission rates for the purpose of validating the inventories. Because concentrations and emission rates are specified in different units, the comparisons are done in terms of percentages: the percentage contribution of a source to ambient total VOCs as estimated by receptor modeling versus the emission rate of the source as a percentage of the total VOC emission rate of the inventory.

Nearly all the receptor modeling studies listed above have included such a percentage comparison. Typically, the agreement is quite good for vehicle exhaust, generally the dominant VOC source in urban airsheds. Gasoline evaporation comparisons are much less consistent, at least partly for the reasons already indicated. Typically, there is at least qualitative agreement for the other anthropogenic sources: they are small in the inventory, and the receptor-estimated contributions are small. An interesting exception is refinery emissions in Chicago (Scheff and Wadden, 1993), for which the receptor estimate was 7%, five times greater than the inventory estimate. Another is the significant (5 to 20%) natural gas/propane contribution estimated in Los Angeles, Columbus, and Atlanta but not reflected in their inventories. The few biogenic source estimates provided by receptor modeling are generally smaller than those given in emissions inventories, at least partly because of the previously referred to reactivity problem. Credible ^{14}C measurements on VOC samples would be extremely helpful in validating the magnitude of the biogenic component of emissions inventories.

Lewis et al. (1993) has noted that comparisons based on percentages are quite insensitive for dominant source components, and the comparisons are more dependent on

how "total VOC" is defined than is often appreciated (the definition varies for the studies listed). Thus, unfortunately, the generally good agreement (receptor versus inventory estimates) found for vehicle exhaust does not translate into a definitive judgment on the current concern that this source component may be significantly underestimated in existing inventories. For example, if the emission rate of vehicle exhaust in a typical inventory were arbitrarily doubled, the resulting change in the percentage of this component in the inventory is well within the range of what can be produced in the receptor estimate by merely choosing a different definition of total VOC from plausible alternatives. Such alternatives relate to questions such as which subset of hydrocarbons are summed? and whether unidentified chromatographic components are included in the sum? In the future, this situation can be improved by more consistency in the total VOC definition and by transforming the receptor modeling results from a concentration-based representation to an emission-rate one. This unavoidably involves introducing some limited meteorological information (Lewis and Conner, 1991).

3.5 Analytical Methods for Oxidants and Their Precursors

3.5.1 Sampling and Analysis of Ozone and Other Oxidants

3.5.1.1 Ozone

Introduction

The measurement of O_3 in the atmosphere has been a subject of research for decades because of the importance of this compound in atmospheric chemistry and because of its potential and demonstrated effects on human health and welfare.

Because of the importance of O_3 in the air of populated regions, widespread O_3 monitoring networks have been operated for many years, and the development of measurement and calibration approaches for O_3 has been reviewed extensively (e.g., U.S. Environmental Protection Agency, 1986a). This section focuses on the measurement of O_3 in the ambient atmosphere at ground level and summarizes the current state of ambient O_3 measurement and calibration. No attempt is made here to cover the full history of development of these methods because that has been documented elsewhere (e.g., U.S. Environmental Protection Agency, 1978, 1986a). Instead, this section concentrates on those methods currently used and on new developments and novel approaches to O_3 measurement.

Although no method is totally specific for O_3, current methods for O_3 must be distinguished from earlier methods that measured "total oxidants". The wet chemical methods used earlier for total oxidants have been replaced for essentially all ambient measurements by two more specific instrumental methods based on the principles of chemiluminescence and UV absorption spectrometry. These two approaches are described below. In addition, recent developments in spectroscopic measurements, in other chemical approaches, and in passive sampling devices for O_3 are described.

Chemiluminescence Methods

Gas-Phase Chemiluminescence. The most common chemiluminescence method for O_3 is direct gas-phase reaction of O_3 with an olefin to produce electronically excited products, which decay with the emission of light. This approach was first used nearly 30 years ago for chemical analysis by Nederbragt (Nederbragt et al., 1965), and development of a portable monitor (Warren and Babcock, 1970) and application to atmospheric

measurements (Stevens and Hodgeson, 1970) followed soon after. Typically, an O_3 monitor based on this approach functions by mixing a constant flow of about 1 L/min of sample air with a small constant flow ($\approx$50 cm^3/min) of ethylene. Mixing occurs in a small inert reaction chamber fitted with a sealed window through which light can pass to the photocathode of a photomultiplier tube. Electronically excited formaldehyde molecules, generated by a small fraction of the O_3-ethylene reactions, produce a broad band of emission centered at 430 nm. The emission intensity is linearly proportional to the O_3 concentration over the range of 0.001 ppm to at least 1 ppm. Calibration of the monitor with a known O_3 source provides the relationship between monitor response and O_3 concentration. Detection limits of 0.005 ppm and a response time of less than 30 s are easily attained, and are typical of currently available commercial instruments.

Although no interference has been found from common atmospheric pollutants, a positive interference from atmospheric water vapor has been reported (California Air Resources Board, 1976; Kleindienst et al., 1993, and references therein) and has recently been confirmed (Kleindienst et al., 1993; Hudgens et al., 1994). The recent results indicate a positive interference of about 3% per percent H_2O by volume at 25 °C, based on tests at O_3 concentrations of 0.085 to 0.32 ppm, and at H_2O concentrations of 1 to 3% (i.e., dew point temperatures of 9 to 24 °C). It has been estimated that the interference of water in ethylene chemiluminescent measurements at 30 °C and 60% relative humidity could be as high as 13 ppbv of O_3, or 11% of the O_3 reading at 120 ppbv (Kleindienst et al., 1993). Calibration with known O_3 concentrations in air of temperature and humidity, similar to that of the sample air, can minimize this source of error.

A separate potential problem with the ethylene chemiluminescent method is leakage of the pure ethylene reagent gas. Because O_3 and hydrocarbon measurements are often co-located for monitoring purposes, leakage of ethylene could cause difficulty in obtaining valid measurements of total nonmethane hydrocarbons (TNMHC) in ambient air.

The measurement principle set forth by EPA for compliance monitoring for O_3 is the chemiluminescence method using C_2H_4 (Federal Register, 1971). Methods of testing and the required performance specifications that commercial O_3 monitors must meet to be designated a reference or equivalent method are documented (Federal Register, 1975). A monitor may be designated a reference method if it employs gas-phase chemiluminescence with C_2H_4 as the measuring principle and achieves the required performance specifications. An equivalent method must show a consistent relationship with the reference method and must meet the required performance specifications. Table 3-15 shows those specifications for O_3 monitors. Note that ethylene chemiluminescence monitors typically have response times far superior to those required in Table 3-15.

The list of commercial O_3 monitors designated as reference or equivalent methods by EPA is shown in Table 3-16 (updated as of August 1, 1994). Details on three monitors not described in the 1986 EPA criteria document for O_3 and other oxidants are presented in Table 3-17. All of the reference methods are C_2H_4 chemiluminescence instruments, as required by the definition of a reference method. The equivalent methods are based on either gas-solid chemiluminescence or UV-absorption measurements. Those methods are described below. A gas-liquid chemiluminescence analyzer for O_3, which may be submitted for EPA equivalency in the near future, also is described below.

Gas-Solid Chemiluminescence. The reaction of O_3 with Rhodamine-B adsorbed on activated silica gel produces chemiluminescence in the red region of the visible spectrum. This was the first chemiluminescence method ever developed for ambient O_3 measurement

Table 3-15. Performance Specifications for Automated Methods of Ozone Analysis

Performance Parameter	Units	Specification
Range	ppm	0 to 0.5
Noise	ppm	0.005
Lower detectable limit	ppm	0.01
Interference equivalent		
Each interference	ppm	±0.02
Total interference	ppm	0.06
Zero drift, 12 and 24 h	ppm	±0.02
Span drift, 24 h		
20% of upper range limit	%	±20.0
80% of upper range limit	%	±5.0
Lag time	min	20
Rise time	min	15
Fall time	min	15
Precision		
20% of upper range limit	ppm	0.01
80% of upper range limit	ppm	0.01

Source: Federal Register (1975); Code of Federal Regulations (1994).

(Regener, 1960, 1964). The emitted light intensity is linearly related to the O_3 concentration, and the detection limit can be as low as 0.001 ppm. No direct interferences from other gas-phase pollutants are known; however, decay of the sensitivity because of surface aging can occur (Hodgeson et al., 1970). Addition of gallic acid to the surface stabilizes the response characteristics, apparently by allowing direct reaction of O_3 with the gallic acid, rather than with the Rhodamine-B (Bersis and Vassiliou, 1966). A commercial analyzer (Phillips Model PW9771) based on this approach has been designated an equivalent method for ambient O_3 (see Table 3-16), but gas-solid chemiluminescence currently is used rarely for ambient measurements.

Gas-Liquid Chemiluminescence. A recently developed commercial monitor uses the chemiluminescent reaction of O_3 with the dye eosin-Y in solution (Topham et al., 1992). The monitor functions by exposing a fabric wick, wetted with the eosin-Y solution, to a flow of sample air within view of a red-sensitive photomultiplier tube. The monitor, designated the LOZ-3, is compact, portable, and requires no reagent gases. The LOZ-3 provides very fast response: a lag time of 2 s, a rise time of 3 s, and a fall time of 2 s, all relative to a step change of 400 ppbv O_3, are reported (Topham et al., 1992). Instrument noise at zero and at 382 ppbv ozone is 0.05 ppbv or less, calculated as the standard deviation of 25 successive 2-min averages. The precision of the LOZ-3 is reported to be 0.80 ppbv at 100 ppbv O_3 and 1.87 ppbv at 400 ppbv O_3, both calculated as one standard deviation of six repeated measurements at these levels (Topham et al., 1992). The instrument provides linear response up to 200 ppbv, with a gradually decreasing slope of the response curve above that

Table 3-16. Reference and Equivalent Methods for Ozone Designated by the U.S. Environmental Protection Agency[a]

Principal Method	Designation Number	Method Code
Reference Methods		
(Ethylene Chemiluminescence)		
Beckman 950A	RFOA-0577-020	020
Bendix 8002	RFOA-0176-007	007
CSI 2000	RFOA-0279-036	036
McMillan 1100-1	RFOA-1076-014	514
McMillan 1100-2	RFOA-1076-015	515
McMillan 1100-3	RFOA-1076-016	016
Meloy OA325-2R	RFOA-1075-003	003
Meloy OA350-2R	RFOA-1075-004	004
Monitor Labs 8410E	RFOA-1176-017	017
Equivalent Methods		
(UV Absorption)		
Advanced Pollution Instrument 400	EQOA-0992-087	087
Dasibi 1003-AH,-PC,-RS	EQOA-0577-019	019
Dasibi 1008-AH,-PC,-RS	EAOA-0383-056	056
Environics 300	EQOA-0990-078	078
Lear-Siegler ML9810	EQOA-0193-091	091
Monitor Labs 8810	EQOA-0881-053	053
PCI Ozone Corporation LC-12	EQOA-0382-055	055
Thermo Electron 49	EQOA-0880-047	047
Equivalent Methods (Gas/Solid CL)		
Philips PW9771	EQOA-0777-023	023

[a]As of August 1, 1994; see Appendix A for abbreviations and acronyms.

level. Temperature and pressure sensitivity are corrected by internal circuitry (Topham et al., 1992). An initial large positive interference from SO_2 that becomes smaller and negative as the eosin solution ages is reported, and a positive interference from CO_2 is also present. Topham et al. (1992) report that a pretreatment technique applied to the eosin reagent solution minimizes both of these interferences. Several of the performance characteristics of the LOZ-3 are impressive, but verification of the reported interference levels and the effectiveness of temperature and pressure corrections appears to be needed.

Table 3-17. List of Designated Reference and Equivalent Methods for Ozone[a]

Designation Number	Identification	Source	Manual or Auto	Ref. or Equiv.	Federal Register Vol.	Federal Register Page	Federal Register Notice Date
EQOA-0990-078	"Environics Series 300 Computerized Ozone Analyzer," operated on the 0-0.5 ppm range, with the following parameters entered into the analyzer's computer system: Absorption Coefficient = 308 ± 4 Flue Time = 3 Integration Factor = 1 Offset Adjustment = 0.025 ppm Ozone Average Time = 4 Signal Average = 0 Temperature/Pressure Correction = On and with or without the RS-232 Serial Data Interface	Environics, Inc. 165 River Road West Willington, CT 06279	Auto	Equiv.	55	38386	September 18, 1990
EQOA-0992-087	"Advanced Pollution Instrumentation, Inc. Model 400 Ozone Analyzer," operated on any full-scale range between 0-0.1 ppm[b] and 0-1 ppm, at any temperature in the range of 5 to 40 °C, with the dynamic zero and span adjustment features set OFF, with a 5-μm Teflon® filter element installed in the rear-panel filter assembly, and with or without any of the following options: Internal Zero/Span (IZS) Rack Mount with Slides RS-232 with Status Outputs Zero/Span Valves	Advanced Pollution Instrumentation, Inc. 8815 Production Avenue San Diego, CA 92121-2219	Auto	Equiv.	57	44565	September 28, 1992

Table 3-17 (cont'd). List of Designated Reference and Equivalent Methods for Ozone[a]

Designation Number	Identification	Source	Manual or Auto	Ref. or Equiv.	Vol.	Federal Register Page	Federal Register Notice Date
EQOA-0193-091	"Lear Siegler Measurement Controls Corporation Model ML9810 Ozone Analyzer," operated on any full-scale range between 0-0.050 ppm[b] and 0-1.0 ppm, with auto-ranging *enabled* or *disabled*, at any temperature in the range of 15 to 35 °C, with a 5-μm Teflon® filter element installed in the filter assembly behind the secondary panel, the service switch on the second panel set to the *In* position; with the following menu choices selected: Calibration: *Manual* or *Timed*; Diagnostic Mode: *Operated*; Filter Type: *Kalman*; Pres/Temp/Flow Comp: *On*; Span Comp: *Disabled*; with the 50-pin I/O board installed on the rear panel configured at any of the following output range settings: Voltage, 0.1, 1, 5, and 10V; Current, 0-20, 2-20, and 4-20 mA; and with or without any of the following options: Valve Assembly for External Zero/Span (EZS) Rack Mount Assembly Internal Floppy Disk Drive	Lear Siegler Measurement Controls Corp. 74 Inverness Drive East Englewood, CO 80112-5189	Auto	Equiv.	58	6964	February 3, 1993

[a]Designated since publication of the 1986 EPA criteria document for ozone and other photochemical oxidants (U.S. Environmental Protection Agency, 1986a).
[b]Users should be aware that designation of this analyzer for operation on any full-scale range less than 0.5 ppm is based on meeting the same absolute performance specifications required for the 0- to 0.5-ppm range. Thus, designation of any full-scale range lower than the 0- to 0.5-ppm range does not imply commensurably better performance than that obtained on the 0- to 0.5-ppm range.

This method is undergoing testing and is likely to be submitted for EPA certification as an equivalent method.

Ultraviolet Photometry

This method is based on the fact that O_3 has a reasonably strong absorption band with a maximum near 254 nm, coinciding with the strong emission line of a low-pressure mercury lamp. The molar absorption coefficient at the mercury line is well known, the accepted value being 134 ($\pm$2) $M^{-1}cm^{-1}$ in base 10 units at 0 °C and 1 atmosphere pressure (Hampson et al., 1973). Ultraviolet absorption has frequently been used to measure O_3 in laboratory chemical and kinetics studies. Ultraviolet photometry also was used for some of the first atmospheric O_3 measurements, but the early instruments suffered from poor precision because of the small absorbances being measured (U.S. Department of Health, Education, and Welfare, 1970).

Modern digital electronics have now solved the precision problems resulting from measurement of small absorbances, and several commercial O_3 monitors now employ UV photometry. Several instruments based on this principle have been designated by EPA as equivalent methods for ambient O_3 (Tables 3-16 and 3-17). Ultraviolet photometry is now the predominant method for assessing compliance with the NAAQS for O_3. The commercial monitors use pathlengths of 1 m or less, and operate in a sequential single-beam mode. Transmission of 254-nm light through the sample air is averaged over a short period of time (as short as a few seconds) and is compared to a subsequent transmission measurement on the same air stream from which O_3 has been selectively removed by a manganese dioxide (MnO_2) scrubber. The electronic comparison of the two signals can be converted directly into a digital readout of the O_3 concentration. The method is in principle absolute, because the absorption coefficient and pathlength are accurately known, and the measured absorbance can be converted directly to a concentration.

Commercial UV photometers for ambient O_3 measurements have detection limits of approximately 0.005 ppm. Time response depends on the averaging time used, but is typically <1 min. Long-term precision can be within $\pm 5\%$. The method has the advantage of requiring no gas supplies, and commercial instruments are compact and reasonably portable. Sample air flow control is not critical, within the limitations of the MnO_2 scrubber. Because the measurement is absolute, UV photometry also is used to assay O_3 calibration standards as in Section 3.5.1.1. Ambient air monitors using UV photometry are generally calibrated with standard O_3 mixtures to account for losses of O_3 in sampling lines.

A potential disadvantage of UV photometry is that any atmospheric constituent that absorbs 254-nm light and is removed fully or partially by the MnO_2 scrubber will be a positive interference in O_3 measurements. Potential interferents include aromatic hydrocarbons, mercury vapor, and SO_2. A recent study (Kleindienst et al., 1993) demonstrated that toluene and possibly aromatic reaction products, such as benzaldehyde, produce positive interferences in UV photometric O_3 measurements. This result was found using photochemically reactive mixtures of toluene and NO_x, at concentrations at a factor of 2 to 5 higher than those expected in polluted urban air. Consideration of the relative absorption coefficients of O_3 and the aromatics indicated that, at higher humidities, toluene can cause an interference of 0.1 ppbv O_3 per ppbv of toluene, whereas benzaldehyde may cause an interference as high as 5 ppbv O_3 per ppbv benzaldehyde (Kleindienst et al., 1993). This interference may be humidity dependent. In earlier work at very low humidities, no

interference was observed with toluene, and only a very small interference was observed with benzaldehyde (Grosjean and Harrison, 1985b). However, even at very low humidities, these investigators observed significant interferences from styrene, cresols, and nitrocresols. Evaluation of aromatic interference is limited by a lack of appropriate absorption spectra in the 250-nm range and by a lack of ambient measurements of most of the aromatic photochemical reaction products. The use of C_2H_4 chemiluminescence monitors in areas where aromatic concentrations are substantial has been suggested (Kleindienst et al., 1993).

The same study found no consistent effect of ambient water vapor on measured O_3 concentrations using UV photometry, in contrast to the effect noted using C_2H_4 chemiluminescence (Kleindienst et al., 1993). However, short-term disturbances in UV photometric O_3 readings were observed when the humidity of the sample air was changed substantially within a few seconds. This finding corroborates the observations of Meyer et al. (1991a) in an earlier study that indicated microscopic irregularities in the UV cell windows as the cause of such disturbances. This effect should be absent in UV photometric measurements of ambient O_3 at the ground but could be important in other applications, such as measuring vertical O_3 profiles from an aircraft (Kleindienst et al., 1993).

A different approach to evaluating potential interferences in O_3 measurements was taken by Leston and Ollison (1993). These investigators examined ambient O_3 data from instruments of different measurement principles co-located at monitoring sites. The focus of their study was the O_3 "design value", the fourth highest daily maximum hourly value from a monitoring station within an urban area, which is established in the 1990 Clean Air Act Amendments (CAAA) (U.S. Congress, 1990) as the basis for classification of the area relative to attainment of the NAAQS for O_3. Leston and Ollison (1993) examined hourly O_3 concentration data from co-located UV and C_2H_4 chemiluminescence instruments, from 1989 and 1990 at a site in Madison, CT, and from shorter periods at sites in East Hartford, CT, and Mobile, AL. They also examined 11 winter days of simultaneous O_3 data from UV and Luminox LOZ-3 instruments, from Long Beach, CA. Leston and Ollison (1993) reported positive biases in the UV data of 20 to 40 ppbv O_3 during "hot, humid, hazy conditions typical of design value days." They proposed that most O_3 data and all design values are biased high by known and suspected interferences, and that those interferences are exacerbated by water vapor. Leston and Ollison (1993) argue that the interference in UV measurements from benzene derivatives (e.g., styrene, cresols, benzaldehyde, nitro-aromatics) is poorly accounted for. For example, of these compounds, only styrene is measured in the Photochemical Aerometric Monitoring Stations (PAMS) VOC monitoring network (Leston and Ollison, 1993).

An experimental study by Hudgens et al. (1994) attempted to address the issues raised by Leston and Ollison (1993) by evaluating several aspects of both UV and chemiluminescence O_3 measurements. This study confirmed the positive interference of water vapor in the chemiluminescence method as 3% per percent water by volume (Kleindienst et al., 1993) and also confirmed that no comparable interference exists with the UV method. However, Hudgens et al. (1994) also showed that some UV instruments give noisier response when operated under conditions in which condensation of moisture may occur in the sampling lines, as in an air conditioned enclosure during hot, humid weather. Hudgens et al. (1994) also tested several aromatic hydrocarbons for both absorbance at 254 nm and behavior in the O_3 scrubber of UV instruments. Both positive and negative potential interferences were found; the former by adsorption of UV-absorbing aromatics in the scrubber, and the latter by release of those stored compounds on an increase in sample

humidity. Transient O_3 breakthrough also was said to occur under humid conditions (Hudgens et al., 1994). The combined effects of adsorbed material and sample humidity may contribute to the anomalous behavior reported for a few scrubbers from field instruments, but that behavior could not be reproduced with new scrubbers, even after continuous sampling of a smog chamber mixture for up to 13 weeks (Hudgens et al., 1994). The aromatic compounds present at highest concentrations in ambient air (e.g., benzene, toluene, xylenes, benzaldehyde) are relatively weak UV absorbers and are not efficiently removed by the O_3 scrubber. As a result, those compounds are not significant interferences in the UV method (Hudgens et al., 1994). However, less common aromatics (e.g., styrene, nitrotoluene) were found to absorb 254-nm light as effectively as does O_3 and to be efficiently adsorbed in the O_3 scrubber (Hudgens et al., 1994). The importance of such compounds as interferents in the UV method will depend on their ambient concentrations.

Interferences of the magnitude suggested by Leston and Ollison (1993) clearly would have serious implications for monitoring of ambient O_3. It is difficult to estimate whether interferences in the UV method could be as high as suggested, in part, because data are lacking on the ambient levels of potential interferents. Many potential interferents are photochemically reactive, and it is questionable whether such compounds could co-exist with ozone in sufficient quantities to constitute a significant interference. The results of Hudgens et al. (1994) also suggest that periodic replacement of the O_3 scrubber may minimize any interferences in the UV method. In any case, full evaluation of interferences in UV and ethylene chemiluminescence methods may require simultaneous measurements of O_3, humidity, temperature, and speciated organic compounds, and perhaps of other meteorological parameters and potential interferents.

Spectroscopic Methods for Ozone

Spectroscopic methods have the potential to provide direct, sensitive, and specific measurements representative of broad areas, rather than of single monitoring sites. This potential has led to investigation of spectroscopic approaches, primarily differential optical absorption spectrometry (DOAS), for O_3 measurement. Differential optical absorption spectrometry measures the absorption through an atmospheric path (typically 0.5 to 1.5 km) of two closely spaced wavelengths of light from an artificial source. One wavelength is chosen to match an absorption line of the compound of interest, and the other is close to but off that line, and is used to account for atmospheric effects. Platt and Perner (1980) reported measurements of several atmospheric species, including O_3, by DOAS, and various investigators have applied the technique since then (Stevens et al., 1993, and references therein). Stevens et al. (1993) described testing of a commercial DOAS instrument in North Carolina in the fall of 1989. Ozone was measured using wavelengths between 260 and 290 nm, over a 557-m path. A detection limit for O_3 of 1.5 ppbv was reported, based on a 1-min averaging time (Stevens et al., 1993). Comparison of DOAS results to those from a UV absorption instrument showed (DOAS O_3) = 0.90 × (UV O_3) − 2.5 ppbv, with a correlation coefficient (r^2) of 0.89, at ozone levels up to 50 ppbv. The sensitivity, multiple analytical capability, stability, and speed of response of the DOAS method are attractive, although further intercomparisons and interference tests are recommended (Stevens et al., 1993).

Personal and Passive Samplers for Ozone

A passive sampler is one that depends on diffusion of the analyte in air to a collecting or indicating medium. In general, passive samplers are not adequate for compliance-monitoring purposes because of limitations in specificity and averaging time. However, passive sampling devices (PSDs) for O_3 are of value as a means of obtaining personal human exposure data for O_3 and as a means of obtaining long-term O_3 measurements in areas where the use of instrumental methods is not feasible. Estimation of long-term population exposure and ecological monitoring for vegetation effects of O_3 in remote areas are examples of the latter application. Passive sampling devices have the advantages of simplicity, small size, and low cost, but also may present disadvantages, such as poor precision, loss of effectiveness during use or storage, and interference from other atmospheric constituents. New designs for PSDs have been implemented to overcome some of these limitations and to make them more useful for short-term ambient and indoor studies, personal exposure assessments, and validation of exposure models. Passive samplers for measuring O_3 at ambient concentrations are now commercially available.

The Ogawa PSD for O_3 (Ogawa, Inc., Pompano Beach, FL) contains 0.1 mL of a solution of $NaNO_2$ and $NaCO_3$ in glycerine on glass fiber filter paper. The nitrite ion reacts with O_3 to form nitrate. Following exposure, the PSDs are analyzed by extraction of the nitrate with deionized water, followed by ion chromatographic (IC) analysis. In a comparative ambient O_3 study over 24 weeks, this PSD demonstrated agreement within about 10% with the weekly real-time measurements taken by a UV O_3 monitor (Mulik et al., 1991). Extension of these measurements to a full year produced similar results (Mulik et al., 1991). The standard deviation of weekly average measurements by three collocated PSD samplers ranged from about ± 1 to ± 6 ppb, at weekly average O_3 levels of 12 to 45 ppb (Mulik et al. 1991). The Ogawa PSD also was used in a study of personal exposure to indoor and outdoor O_3, showing a correlation of $r = 0.91$, and relative errors of 15% (daytime) and 25% (nighttime) relative to UV photometric data (Liu et al., 1992).

Another PSD for O_3 has been developed that is based on the use of a colorant that fades when exposed to O_3 (Grosjean and Hisham, 1992; Grosjean and Williams, 1992). The plastic, badge-type PSD contains a diffusion barrier and a colorant-coated filter as the O_3 trap. The colorant used is indigo carmine (5,5'-disulfonate sodium salt of indigo, λ max = 608 nm). With a plastic grid or Teflon® filter as the diffusion barrier, detection limits of 30 ppb · day and 120 ppb · day, respectively, are achieved. Interferences from NO_2, HCHO, and PAN are 15, 4, and 16%, respectively, of the ambient interferent concentrations. For sampling ambient O_3 in most locations, these interferences are probably negligible (Grosjean and Hisham, 1992). Following sampling, the color change is measured by reflectance spectroscopy and no chemical analysis is required. The reported shelf life is 3 mo prior to O_3 exposure and 12 mo after O_3 exposure (Grosjean and Hisham, 1992).

Field tests of the indigo carmine PSD were conducted at five forest locations in California in the summer of 1990 (Grosjean and Williams, 1992). During these tests, ambient O_3 ranged up to 250 ppbv; 3-day average O_3 values at the sites ranged from 40 to 88 ppbv. The precision of the measurements was $\pm 12\%$ based on 42 sets of collocated samplers, over sampling durations of 3 to 30 days. The color change in the PSD was highly correlated ($r = 0.99$) with O_3 dose as measured by UV photometry. No effect of ambient temperature or humidity variations was observed, and the total interference caused by other pollutants (NO_2, PAN, aldehydes) was less than 5%.

A third PSD for O_3 also has been developed recently; it is based on color formation from the reaction of O_3 with an aromatic amine (Kirollos and Attar, 1991). The ChromoSense™ direct-read passive dosimeter is a credit-card-sized device that changes color proportionally to the integrated dose of exposure of the specific toxic material for which it was designed (U.S. Patent 4,772,560). The dosimeter consists of an outer polyester pouch that encloses a polymeric plate with a sorbent and membrane. A filtering layer is coated on the membrane to reduce the sensitivity of the detection process to NO_2. The chromophoric layer, consisting of an aromatic amine that can react with O_3 and form color, is encapsulated so as to create a very high surface area. A polymeric barrier separates the chromophore from a UV-absorbing layer to reduce their interaction. The UV absorber (in a polymeric matrix) helps stabilize the chromophore toward intense light exposure when the device is used outdoors. The transparent polymeric plate keeps the wafer flat and allows uninterrupted optical viewing of the color of the reference and the sample area. An electronic reading device measures color on both the exposed (sample) and unexposed (reference) areas, and displays a digital reading that is proportional to the log of the O_3 dose. Visible color is formed at doses as low as 20 ppb·h. No interference from NO_2 is observed at NO_2 concentrations up to 350 ppb, and only a small effect of ambient humidity has been reported (Kirollos and Attar, 1991). No data on precision have yet been reported.

A popularization of a PSD for O_3 has been achieved in the form of the EcoBadge®, which employs color formation by reaction of O_3 with an undisclosed reagent in a filter paper (Vistanomics, Inc., Glendale, CA). The EcoBadge is available through several scientific equipment catalogs, primarily as a tool for classroom instruction on environmental issues. The badge is said to indicate both a 1-h and an 8-h average O_3 concentration. Comparison of color development to a standard chart indicates O_3 concentrations up to about 350 ppbv, with a limit of resolution of about 20 ppbv or more. The badge is stated to be unaffected by air velocity, humidity, or temperature, with only a slight interference from NO_2. Test data apparently have not been published. The EcoBadge has been used in middle and high school programs promoting science and math and is included in the curriculum of the Global Thinking Project (1993), an international telecommunications and education network.

Calibration Methods for Ozone

Because it is an unstable molecule and cannot be stored, O_3 must be generated at the time of use to produce calibration mixtures. Electrical discharges in air or oxygen readily produce O_3, but at concentrations far too high for calibration of ambient monitors. Radiochemical methods are expensive and require the use of radioactive sources, with associated safety requirements. For calibration purposes, low levels of O_3 nearly always are generated by photolysis of oxygen at wavelengths <200 nm. Placing a mercury lamp near a quartz tube through which air is flowing produces small amounts of O_3 in the airstream. Commercial O_3 sources based on this approach typically adjust the lamp current to control the amount of light transmitted, and thus the O_3 produced.

Once a stable, low concentration of O_3 has been produced from a photolytic generator, that O_3 output must be established by measurement with an absolute reference method. The original reference calibration procedure promulgated by EPA in 1971 (Federal Register, 1971) was an iodometric procedure, employing 1% aqueous neutral buffered potassium iodide (NBKI). A large number of studies conducted in the 1970s revealed several deficiencies with potassium iodide (KI) methods, the most notable of which were poor

precision or interlaboratory comparability and a positive bias of NBKI measurements relative to simultaneous absolute UV absorption measurements.

Following investigations of problems with the NBKI method, EPA evaluated four potential reference calibration procedures and selected UV photometry on the basis of superior accuracy and precision and simplicity of use (Rehme et al., 1981). In 1979, UV photometry was designated the reference calibration procedure by EPA (Federal Register, 1979a).

The measurement principle of UV O_3 photometers used as reference standards is identical to that of O_3 photometers used for ambient measurements (see Section 3.5.1.1). A laboratory photometer used as a reference standard will typically contain a long-path cell (1 to 5 m) and employ sophisticated digital techniques for making effective double-beam measurements of small absorbances at low O_3 concentrations.

A primary reference standard is a UV photometer that meets the requirements set forth in the 1979 revision designating UV photometry as the reference method (Federal Register, 1979a). Commercially available O_3 photometers that meet those requirements may function as primary standards. The EPA and the National Institute of Standards and Technology (NIST, formerly National Bureau of Standards [NBS]) have established a nationwide network of standard reference photometers (SRPs) that are used to verify local primary standards and transfer standards. A secondary or transfer standard is a device or method that can be calibrated against the primary standard and then moved to another location for calibration of O_3 monitors. Commercial UV photometers for O_3 often are used as secondary or transfer standards, as are commercial photolytic ozone generators and apparatus for the gas-phase titration of O_3 with NO.

The latter method, gas-phase titration (GPT) of O_3 with NO ($NO + O_3 \rightarrow NO_2 + O_2$), is a direct and absolute means of determining O_3, provided NO is in excess so that no side reactions occur. Under such conditions, GPT has the advantage that measurement of the NO or O_3 consumed or the NO_2 produced gives a simultaneous measurement of the other two species. All three modes have been used, and this method is often used for calibration of NO/NO_x analyzers. Gas-phase titration has been compared to UV photometry in several studies. The most detailed study is that of Fried and Hodgeson (1982), who used an NBS primary standard UV photometer, highly accurate flow measurements, photoacoustic detection of NO_2, and NBS (now NIST) Standard Reference Materials as sources of NO and NO_2. That study showed that decreases in O_3 as measured by the UV method averaged 3.6% lower than the corresponding decrease in NO and increase in NO_2 measured independently. Because of uncertainty about the origin of the small bias relative to UV photometry, GPT is used as a transfer standard but not as a primary reference standard.

3.5.1.2 Peroxyacetyl Nitrate and Its Homologues

During laboratory organic photooxidation studies, Stephens et al. (1956a,b) determined the presence of a number of alkyl nitrates and an unidentified species called "Compound X". The presence of Compound X in the atmosphere of Los Angeles was confirmed by Scott et al. (1957). In later work (Stephens et al., 1961), its structure was determined and Compound X was named peroxyacetyl nitrate, or PAN. Since the discovery of PAN, much effort has been directed toward its atmospheric measurement. In the following subsections PAN measurement and calibration techniques are described. The discussion on measurement techniques includes a summary description and identifies limits of

detection, specificity (interferences), reproducibility, and accuracy of each method. The relative merits of each method also are presented. The subsection on calibration techniques includes those methods most often employed during ambient air measurement studies.

Measurement Methods

Two methods generally have been employed to make atmospheric measurements of PAN. These methods are infrared spectroscopy (IR) and GC. Infrared spectroscopy permits the sampling and analysis to be conducted in real time. Because PAN is very reactive in the gas phase and exhibits surface adsorptive effects, the minimal contact time offered by IR makes this method very attractive. However, IR instrumentation is expensive and complex and requires a good deal of space. On the other hand, GC is inexpensive and requires minimal space and operator training. A GC can be set up to automatically sample and analyze air for PAN and PANs. Application of these methods for obtaining ambient concentrations of PAN and other organic nitrates recently has been reviewed by Roberts (1990).

Infrared Spectroscopy. Conventional long-path infrared spectroscopy and FTIR have been used to detect and measure atmospheric PAN. Sensitivity is enhanced by the use of FTIR. The most frequently used IR bands have been assigned, and the absorptivities reported in the literature (Stephens, 1964; Bruckmann and Willner, 1983; Holdren and Spicer, 1984; Niki et al., 1985; Tsalkani and Toupance, 1989) permit the quantitative analysis of PAN without calibration standards. Tuazon et al. (1978) have described an FTIR system operable at pathlengths up to 2 km for ambient measurements of PAN and other trace constituents. This system employed an eight-mirror multiple reflection cell with a 22.5-m base path. The spectral windows available at pathlengths of 1 km were 760 to 1,300, 2,000 to 2,230 cm^{-1}. Thus, PAN could be detected by the bands at 793 and 1,162 cm^{-1}. The 793-cm^{-1} band is characteristic of peroxynitrates, whereas the 1,162-cm^{-1} band is reportedly caused by PAN only (Stephens, 1969; Hanst et al., 1982). Tuazon et al. (1981a,b) reported ambient measurements with this system during a smog episode in Claremont, CA, in 1978. Maximum daily PAN concentrations ranged from 6 to 37 ppb over a 5-day episode. A detection limit for PAN was 3 ppb at a pathlength of $\approx$1 km. Hanst et al. (1982) modified the FTIR system used by Tuazon by changing it from an eight-mirror to a three-mirror cell configuration and by considerably reducing the cell volume. A detection limit for PAN was increased to 1 ppb at a similar pathlength.

The limited sensitivity ($\approx$1 ppb) and the complexity of the above FTIR systems generally have limited their field use to urban areas such as Los Angeles. More recently, cryogenic sampling and matrix-isolation FTIR have been used to measure PAN in 15-L integrated samples of ambient air. The matrix isolation technique has a theoretical level of detection of $\approx$50 ppt (Griffith and Schuster, 1987).

Gas Chromatography-Electron Capture Detection. Peroxyacetyl nitrate is normally measured by using a GC coupled to an electron capture detector (GC/ECD). The method was originally described by Darley et al. (1963) and subsequently has been refined and employed by scientists over the years. Key features of the method remain unchanged. The column and detector temperatures are kept relatively low ($\approx$50 and 100 °C, respectively) to minimize PAN thermal decomposition. Short columns of either glass or Teflon® generally are used (1 to 5 ft in length). Finally, column packing normally includes a Carbowax stationary phase coated onto a deactivated solid support. Using packed columns, detection limits of 10 ppt have been reported using direct sampling with a 20-mL sample

loop (Vierkorn-Rudolph et al., 1985). Detection limits were further extended to 1 to 5 ppt using cryogenic enrichment of samples (Vierkorn-Rudolph et al., 1985; Singh and Salas, 1983). These studies have found only slight overall losses of PAN (10 to 20%) associated with cryosampling, provided samples are warmed only to room temperature during desorption.

Recently, improved precision and sensitivity have been reported using fused-silica capillary columns instead of packed columns (Helmig et al., 1989; Roberts et al., 1989). Signal-to-noise enhancement of 20 has been claimed (Roberts et al., 1989).

Gas Chromatography-Alternate Detection. As noted earlier, PAN is readily reduced to NO in the gas phase. To separate PAN, NO, and NO_2, Meyrahn et al. (1987) coupled a GC with a molybdenum converter and used a chemiluminescent analyzer to measure PAN as NO. Using a 10-mL sample loop, a detection limit of 10 ppb was reported.

A luminol-based detector also has shown sensitivity to PAN. Burkhardt et al. (1988) used GC and a commercially available luminol-based instrument (i.e., Scintrex LMA-3 Luminox) to detect both NO_2 and PAN. Using a sampling interval of 40 s, linear response was claimed from 0.2 to 170 ppb NO_2 and from 1 to 65 ppb PAN. Although the PAN calibration was nonlinear below 1 ppb, a detection level of 0.12 ppb was reported. Drummond et al. (1989) slightly modified the above approach by converting the PAN from the GC column to NO_2 and measuring the resulting NO_2 with a luminol-based instrument.

Peroxyacetyl Nitrate Stability

Peroxyacetyl nitrate is an unstable gas and is subject to surface-related decomposition as well as thermal instability. Peroxyacetyl nitrate exists in a temperature-sensitive equilibrium with the peroxyacetyl radical and NO_2 (Cox and Roffey, 1977). Increased temperature favors the peroxyacetyl radical and NO_2 at the expense of PAN. Added NO_2 should force the equilibrium toward PAN and enhance its stability. In the presence of NO, peroxyacetyl radicals react rapidly to form NO_2 and acetoxy radicals, which decompose in O_2 to radicals that also convert NO to NO_2. As a result, the presence of NO acts to reduce PAN stability and enhance its decay rate (Lonneman et al., 1982). Stephens (1969) reported that appreciable PAN loss in a metal sampling valve was traced to decomposition on a silver-soldered joint. Meyrahn et al. (1987) reported that PAN decayed according to first-order kinetics at a rate of 2 to 4%/h in glass vessels, and they suggested first-order decay as the basis for a proposed method of in-field PAN calibration. In contrast, Holdren and Spicer (1984) found that without NO_2 added, 20 ppb PAN decayed in Tedlar bags according to first-order kinetics at a rate of 40%/h. The addition of 100 ppb NO_2 acted to stabilize the PAN (20 ppb) in the Tedlar bags.

A humidity-related difference in GC/ECD response has been reported (Holdren and Rasmussen, 1976). Low responses observed at humidities below 30% and PAN concentrations of 10 and 100 ppb, but not 1,000 ppb, were attributed to sample-column interactions. A humidity effect was alluded to by Nieboer and Van Ham (1976) but details were not given. No humidity effect was observed by Lonneman (1977). Watanabe and Stephens (1978) conducted experiments at 140 ppb and did not conclude that the reduced response was from faults in the detector or the instrument. They concluded that there was no column-related effect, and they observed surface-related sorption by PAN at 140 ppb in dry acid-washed glass flasks. They recommended that moist air be used to prepare PAN calibration mixtures to avoid potential surface-mediated effects.

Another surface-related effect has been reported for PAN analyses of remote marine air (Singh and Viezee, 1988). Peroxyacetyl nitrate concentrations were found to increase by 20 to 170 ppt, an average factor of 3, when the sample was stored in a glass vessel for 1 to 2 min prior to analysis. This effect remains to be explained.

Preparation and Calibration

Because PAN is unstable, the preparation of reliable calibration standards is difficult. The more promising methods are described here. The original method used the photolysis of ethyl nitrite in pure oxygen (Stephens, 1969). When pure PAN is desired, the reaction mixture must be purified, usually by chromatography, to remove the major by-products, acetaldehyde and methyl and ethyl nitrates (Stephens et al., 1965). For GC calibration, purification is unnecessary; the PAN concentration in the reactant matrix is established from the IR absorption spectrum and subsequently diluted to the parts-per-billion working range needed for calibration purposes (Stephens and Price, 1973).

Static mixtures of molecular chlorine, acetaldehyde, and NO_2 in the ratio of 2:4:4 can be photolyzed in the presence of a slight excess NO_2 to give a near-stoichiometric yield of PAN (Gay et al., 1976). This method was adapted by Singh and Salas (1983) and later by Grosjean et al. (1984), using photolytic reactors to provide continuous PAN calibration units at concentrations between 2 and 400 ppb. In the former approach, the PAN concentration is established by measuring the change in acetaldehyde concentration across the reactor. In the latter approach, the PAN concentration is established by measuring the acetate in an alkaline bubbler where PAN is hydrolyzed.

A static technique involving the photolysis of acetone in the presence of NO_2 and air at 250 nm has been reported to produce a constant concentration of PAN (Meyrahn et al., 1987; Warneck and Zerbach, 1992). A Penray mercury lamp is inserted into a mixture of 10 ppm NO_2 and 1% acetone and irradiated for 3 min to yield 8.9 $\pm$ 0.3 ppm PAN.

Peroxyacetyl nitrate can be synthesized in the condensed phase by the nitration of peracetic acid in C_6H_{14} (Helmig et al., 1989), heptane (Nielsen et al., 1982), C_8H_{18} (Holdren and Spicer, 1984), or *n*-tridecane (Gaffney et al., 1984). Purification of PAN in the liquid phase is needed using the first two methods. The resulting PAN-organic solution can be stored at −20 to −80 °C with losses of less than 3.6%/mo and can be injected directly into a vessel containing air to produce a calibration mixture. The PAN concentration is normally established by FTIR analysis of the solution or the resulting PAN-air mixture.

Peroxyacetyl nitrate readily disassociates to NO, and chemiluminescence NO_x analyzers have near-quantitative response to PAN. Thus, under some circumstances, chemiluminescent NO_x response can be used for PAN calibration. One method uses the difference in NO_x signal measured upstream and downstream of an alkaline bubbler (Grosjean and Harrison, 1985a). Joos et al. (1986) coupled a chemiluminescence NO_x analyzer with a GC system to permit calibration of the ECD response by reference to the chemiluminescence NO_x analyzer that has been calibrated by traditional methods.

As noted previously, NO in the presence of PAN is converted to NO_2. Approximately four molecules of NO can react per molecule of PAN. Lonneman et al. (1982) devised a PAN calibration procedure based on the reaction of PAN with NO in the presence of benzaldehyde, which is added to control unwanted radical chemistry and to improve precision. Using this approach and an initial NO-to-PAN ratio between 10 and 20 to 1, the change in NO concentration is monitored with a chemiluminescence NO analyzer, the change in PAN GC-ECD response is monitored, and the resulting ratio (i.e.,

$\Delta NO/\Delta PAN$) is divided by the stoichiometric factor of 4.7 to arrive at a calibration factor for the ECD.

Peroxyacetyl nitrate and *n*-propyl nitrate (NPN) have similar ECD responses. Serial dilution of the more stable compound, NPN, has been used for field operations (Vierkorn-Rudolph et al., 1985). This approach is not recommended for primary calibration, however, because it does not permit verification of quantitative delivery of PAN to the detector (Stephens and Price, 1973).

3.5.1.3 Gaseous Hydrogen Peroxide

Although O_3 has long been considered to be the primary oxidant affecting air quality, atmospheric chemists recently have identified H_2O_2, a photochemical reaction product, as another oxidant that also may play a significant role in diminishing air quality. In order to assess the role of atmospheric H_2O_2, good measurement methods are needed. Early measurements in the 1970s reported H_2O_2 concentrations ranging from 10 to 180 ppb (Gay and Bufalini, 1972 a,b; Kok et al., 1978 a,b). However, these measurements are in error because of artifact formation of H_2O_2 from reactions of absorbed gaseous O_3 (Zika and Saltzman, 1982; Heikes et al., 1982; Heikes, 1984). Modeling results also indicate that H_2O_2 atmospheric concentrations should be on the order of 1 ppb (Chameides and Tan, 1981; Logan et al., 1981).

In the following section, the discussion focuses on those sampling and analytical methods most frequently used within the last decade to determine atmospheric levels of H_2O_2. The measurement techniques are described and limits of detection, specificity (interferences), reproducibility, and accuracy are discussed.

Measurement Methods

In situ measurement methods that have been employed for determining gaseous H_2O_2 include both FTIR and tunable diode laser absorption spectrometry (TDLAS). Four methods involving sample collection via wet chemical means and subsequent analysis via chemiluminescent or fluorescent detection also have been used frequently: (1) luminol, (2) peroxyoxalate, (3) enzyme-catalyzed (peroxidase), and (4) benzoic acid-fenton reagent methods. Application of most of these methods for obtaining ambient concentrations of H_2O_2 recently has been reviewed by Sakugawa et al. (1990) and Gunz and Hoffmann (1990).

In Situ Methods. Fourier transform infrared spectroscopy was employed in the early 1980s for atmospheric measurements (Tuazon et al., 1980; Hanst et al., 1982). Even though the FTIR is very specific for H_2O_2, it saw limited use because of the high detection level of $\approx$50 ppb when using a 1-km path length. The TDLAS also has very high specificity for H_2O_2 and was subsequently evaluated and shown to have a much improved detection limit of 0.1 ppb when using scan-averaging times of several minutes (Slemr et al., 1986; MacKay and Schiff, 1987a; Schiff et al., 1987).

Wet Chemical Methods. Numerous wet chemical techniques for measuring H_2O_2 have been reported. However, discussion in this section is limited to the four approaches most frequently used by researchers.

Luminol Method. Hydrogen peroxide concentrations in the atmosphere have been determined by the chemiluminescent response obtained from the catalyzed oxidation of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) by H_2O_2. Copper^{2+} (Armstrong and Humphreys, 1965; Kok et al., 1978 a,b; Das et al., 1982) and hemin, a blood component (Yoshizumi et al., 1984), have been reported as catalysts for the luminol-based H_2O_2

oxidation. Method sensitivity of ≈ 0.01 ppb has been achieved. Interference from O_3, SO_2, metal ions, and high pH have been reported along with ways to mitigate these effects (Heikes et al., 1982; Zika and Saltzman, 1982; Ibusuki, 1983; Lazrus et al., 1985; Aoyanagi and Mitsushima, 1985; Hoshino and Hinze, 1987).

Peroxyoxalate Method. The peroxyoxalate chemiluminescence method also has been employed by a number of researchers (Rauhut et al., 1967; Scott et al., 1980; Klockow and Jacob, 1986). Hydrogen peroxide reacts with *bis*(2,4,5-trichloro-6-phenyl)-oxalate to form a high-energy dioxetanedione (Stauff and Jaeschke, 1972). The chemiluminescence is transmitted to the fluorophore, perylene, which emits light on return to the ground state. Method sensitivity of ≈ 0.01 ppb is achieved, and no interferences are observed from O_3 and metal ions. A signal depression has been reported for trace levels of nitrite ($>10^{-5}$ M), sulfite ($>10^{-4}$ M), and formaldehyde ($>10^{-3}$ M) (Klockow and Jackob, 1986).

Enzyme-Catalyzed Method (Peroxidase). This general method involves three components: (1) a substrate that is oxidizable; (2) the enzyme, horseradish peroxidase (HRP); and (3) H_2O_2. The production or decay of the fluorescence intensity of the substrate or reaction product is measured as it is oxidized by H_2O_2, catalyzed by HRP. Some of the more widely used chromogenic substrates have been scopoletin (6-methoxy-7-hydroxy-1,2-benzopyrone) (Andreae, 1955; Perschke and Broda, 1961), 3-(*p*-hydroxphenyl)propionic acid (HPPA) (Zaitsu and Okhura, 1980), leuco crystal violet (LCV) (Mottola et al., 1970), and *p*-hydroxyphenylacetic acid (POPHA) (Guilbault et al., 1968).

Of the chromogens used, POPHA is one of the better indicating substrates. Hydrogen peroxide oxidizes the peroxidase and is itself reduced by electron transfer from POPHA. The POPHA radicals form a dimer that is highly fluorescent. Because the chemical reaction is sensitive to both H_2O_2 and organic peroxides, a dual-channel system with a H_2O_2 removal step (use of catalase) is used to distinguish H_2O_2 from organic peroxides (Lazrus et al., 1985; Wei and Weihan, 1987; Dasgupta and Hwang, 1985; Kok et al., 1986).

The peroxidase-POPHA-fluorescence technique has been used by several groups to measure gas-phase H_2O_2 concentrations (Lazrus et al., 1986; Tanner et al., 1986; Heikes et al., 1987; Van Valin et al., 1987; Dasgupta et al., 1988; Olszyna et al., 1988; Meagher et al., 1990). Method detection levels range from 0.01 to 0.1 ppb. However, artifact formation does occur as a result of the reaction of dissolved O_3 in the collection devices (Staehelin and Hoigne, 1982; Heikes, 1984; Gay et al., 1988). To overcome the O_3 interference, researchers have used NO to eliminate O_3 (Tanner, 1985; Tanner et al., 1986; Shen et al., 1988).

Fenton Reagent-Isomeric Hydroxybenzoic Acids Method. This technique involves the formation of aqueous OH radicals from the reaction of Fenton reagent (Fe^{2+}) complex) with gaseous H_2O_2. The OH radicals, in turn, react with benzoic acid (hydroxyl radical scavenger) to form isomeric hydroxybenzoic acids (OHBA). The OHBA fluoresces weakly at the pH necessary to carry out the above reactions. Fluorescence is enhanced by adding NaOH to the product stream (Lee et al., 1990) or by using a low pH Al^{3+} fluorescence enhancing reagent (Lee et al., 1993).

Comparison of Methods

The above techniques have been shown to measure H_2O_2 in the atmosphere with detection levels of ≈ 0.1 ppb. Kleindienst et al. (1988) compared several of these techniques using three sources of H_2O_2: (1) zero air in the presence and absence of common

interferences, (2) steady-state irradiations of hydrocarbon-NO_x mixtures, and (3) ambient air. The measurements were conducted simultaneously from a common manifold. For pure samples in zero air, agreement within 23% was achieved among methods over a concentration range of 0.06 to 128 ppb. A negative SO_2 interference was caused with the luminol technique. During the irradiation experiment, significant concentrations of organic peroxides were generated, and the agreement among techniques for H_2O_2 was very poor. For ambient measurements, the methods agreed reasonably well with an average deviation of 30% from the mean values.

Atmospheric intercomparison studies also have been conducted as part of the Carbon Species Methods Comparison Study (Calif, 1986). The results of the study indicated that the wet chemical methods still suffer from sampling artifacts and interferences from other atmospheric constituents (Dasgupta et al., 1990; MacKay et al., 1990; Kok et al., 1990; Sakugawa et al., 1990; Tanner and Shen, 1990). Lee et al. (1991) showed that substantial loss of airborne H_2O_2 can occur when air is drawn through Teflon® tubing of inlet sampling devices. In addition to reducing the H_2O_2 in incoming ambient air, this line loss also compromises the use of aqueous standards to calibrate a gas-phase monitoring system. More recently, Lee et al. (1994) have demonstrated a surfaceless inlet system to eliminate line loss problems. It is clear from the above studies that further comparisons of techniques are needed to resolve questions of errors and to provide improved measurement techniques.

Calibration Methods

The most frequently used method for generating aqueous standards is simply the serial dilution of commercial grade 30% H_2O_2/water. The dilute solutions of H_2O_2 as low as 10^{-4} have been found to be stable for several weeks if kept in the dark (Armstrong and Humphreys, 1965). The stock H_2O_2 solution is standardized by iodometry (Allen et al., 1952; Hochanadel, 1952; Cohen et al., 1967) or, more recently, by using a standardized permanganate solution (Lee et al., 1991).

Gaseous H_2O_2 standards are not as easily prepared, and stability problems require the use of standard mixtures immediately. One method makes use of the injection of microliter quantities of 30% H_2O_2 solution into a metered stream of air that flows into a Teflon® bag. The amount of H_2O_2 in the gas phase is determined by the iodometric titration method (Cohen and Purcell, 1967). Gas-phase H_2O_2 standards also have been generated by equilibrating N_2 with an aqueous H_2O_2 solution of known concentration that is maintained at constant temperature. Equilibrium vapor pressures and corresponding gas-phase concentrations are calculated using Henry's law constant (Lee et al., 1991).

3.5.2 Sampling and Analysis of Volatile Organic Compounds

3.5.2.1 Introduction

The term *volatile organic compounds* generally refers to gaseous organic compounds that have a vapor pressure greater than 0.15 mm and, generally, have a carbon content ranging from C_1 through C_{12}. As discussed in Sections 3.2 and 3.4, VOCs are emitted from a variety of sources and play a critical role in the photochemical formation of O_3 in the atmosphere.

The U.S. Environmental Protection Agency revised the ambient air quality surveillance regulations in Title 40, Part 58, of the Code of Federal Regulations to include, among other activities, the monitoring of VOCs. The revisions require states to establish

VOC air monitoring stations in nonattainment areas as part of their existing State Implementation Plan (SIP) monitoring networks. Authority for requiring the enhanced monitoring is provided for in Title I, Section 182, of the CAAA of 1990 (U.S. Congress, 1990). Several states have begun acquiring VOC data on 55 O_3 precursors at these PAMS, using methodology discussed in an EPA technical assistance document (U.S. Environmental Protection Agency, 1991c).

The term *nonmethane organic compounds* also is used frequently and refers to a subset of VOCs, because it excludes the compound CH_4. Numerous sampling, analytical, and calibration methods have been employed to determine NMOCs in ambient air. Some of the analytical methods utilize detection techniques that are highly selective and sensitive to specific functional groups or atoms of a compound (e.g., formyl group of aldehydes, halogen), whereas others respond in a more universal manner (i.e., to the number of carbon atoms present in the organic molecule). In this overview of the most pertinent measurement methods, NMOCs have been arranged into three major classifications: (1) NMHCs, (2) carbonyl species, and (3) polar volatile organic compounds (PVOCs). Measurement and calibration procedures are discussed for each classification.

3.5.2.2 Nonmethane Hydrocarbons

Nonmethane hydrocarbons constitute the major portion of NMOC in ambient air. Traditionally, NMHCs have been measured by methods that employ a flame ionization detector (FID) as the sensing element. This detector was originally developed for GC and employs a sensitive electrometer that measures a change in ion intensity resulting from the combustion of air containing organic compounds. Ion formation is essentially proportional to the number of carbon atoms present in the organic molecule (Sevcik, 1975). Thus, aliphatic, aromatic, alkenic, and acetylenic compounds all respond similarly to give relative responses of 1.00 ± 0.10 for each carbon atom present in the molecule (e.g., 1 ppm hexane = 6 ppmC; 1 ppm benzene = 6 ppmC; 1 ppm propane = 3 ppmC). Carbon atoms bound to oxygen, nitrogen, or halogens give reduced relative responses (Dietz, 1967). Consequently, the FID, which is primarily used as a hydrocarbon measuring method, more correctly should be viewed as an organic carbon analyzer.

In the following sections, discussion focuses on the various methods utilizing this detector to measure total nonmethane organics. Methods in which no compound speciation is obtained are covered first. Methods for determining individual organic compounds then are discussed.

Nonspeciation Measurement Methods

The original EPA reference method for NMOC, which was promulgated in 1971, involves GC separation of CH_4 from the remaining organics in an air sample (Federal Register, 1971). A second sample is injected directly to the FID without CH_4 separation. Subtraction of the first value from the second produces a nonmethane organic concentration.

A number of studies of commercial analyzers employing the Federal Reference Method have been reported (Reckner, 1974; McElroy and Thompson, 1975; Harrison et al., 1977; Sexton et al., 1982). These studies indicated overall poor performance of the commercial instruments when either calibration or ambient mixtures containing NMOC concentrations <1 ppmC were used. The major problems associated with using these NMOC instruments have been reported in an EPA technical assistance document (Sexton et al., 1981). The technical assistance document also suggests ways to reduce the effects of

existing problems. Other nonspeciation approaches to the measurement of nonmethane organics also have been investigated. These approaches have been discussed in the 1986 EPA air quality criteria document (U.S. Environmental Protection Agency, 1986a). Again, these approaches also are subject to the same shortcomings as the EPA reference method (i.e., poor performance below 1 ppmC of NMHC).

More recently, a method has been developed for measuring NMOC directly and involves the cryogenic preconcentration of nonmethane organic compounds and the measurement of the revolatilized NMOCs using FID (Cox et al., 1982; Jayanty et al., 1982). This methodology has been formalized and is referred to as Method TO-12 and is published in a compendium of methods for air toxics (Winberry et al., 1988). The EPA recommends this methodology for measuring total NMOC and has incorporated it into the *Technical Assistance Document for Sampling and Analysis of Ozone Precursors* (U.S. Environmental Protection Agency, 1991c).

A brief summary of the method is as follows. A whole air sample is drawn through a glass bead trap that is cooled to approximately −185 °C using liquid argon. The cryogenic trap collects and concentrates the NMOC, while allowing the CH_4, nitrogen, oxygen, etc., to pass through the trap without retention. After a known volume of air has been drawn through the trap, carrier gas is diverted to the trap first to remove residual air and CH_4. When the residual gases have been flushed from the trap, the cryogen is removed and the temperature of the trap is ramped to approximately 100 °C. The revolatilized compounds pass directly to a FID (no analytical column). The corresponding signal is integrated over time (several minutes) to obtain a total FID response from the NMOC species. Water vapor, which also is preconcentrated, causes a positive shift in the FID signal. The effect of this shift is minimized by optimizing the peak integration parameters.

The sensitivity and precision of Method TO-12 are proportional to the sample volume. However, ice formation in the trap limits sampling volumes to $\approx$500 cc. The detection level is 0.02 ppmC (with a signal-to-noise ratio [S/N] of 3), and the precision at 1 ppmC and above has been determined to be $\leq 5\%$. The instrument response has been shown to be linear over a range of 0 to 10 ppmC. Propane gas certified by NIST is normally used as the calibrant. Accuracy at the method quantitation level (S/N = 10) is $\pm 20\%$.

Speciation Measurement Methods

The primary measurement technique utilized for NMOC speciation is GC. Coupled with FID, this analytical method permits the separation and identification of many of the organic species present in ambient air.

Separation of compounds is accomplished by means of both packed and capillary GC columns. If high resolution is not required and large sample volumes are to be injected, packed columns are employed. The traditional packed column may contain either a solid polymeric adsorbent (gas-solid chromatography) or an inert support, coated with a liquid (gas-liquid chromatography). Packed columns containing an adsorbent substrate normally are required to separate C_2 and C_3 compounds. The second type of column can be a support-coated or wall-coated open tubular capillary column. The latter column has been used widely for environmental analysis because of its superior resolution and broader applicability. The wall-coated capillary column consists of a liquid stationary phase coated or bonded (cross-linked) to the specially treated glass or fused-silica tubing. Fused-silica tubing is most commonly used because of its physical durability and flexibility. When a complex mixture is

introduced into a GC column, the carrier gas (mobile phase) moves the sample through the packed or coated capillary column (stationary phase). The chromatographic process occurs as a result of repeated sorption-desorption of the sample components (solute) as they move along the stationary phase. Separation occurs as a result of the different affinities that the solute components have for the stationary phase.

As described in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986a), the GC-FID technique has been used by numerous researchers to obtain ambient NMOC data. Singh (1980) drew on the cumulative experience of these researchers to prepare a guidance document for state and local air pollution agencies interested in obtaining speciation data. In general, most researchers have employed two gas chromatographic units to carry out analyses of NMOC species in ambient air. The more volatile VOCs (C_2 through C_5) generally are measured on one unit using packed-column technology, whereas the other GC separates the less volatile organics using a capillary column. In typical chromatograms of urban air, all major peaks are identified and, on a mass basis, represent from 65 to 90% of the measurable nonmethane organic burden.

Identification of GC peaks is based on matching retention times of unknown compounds with those of standard mixtures. Subsequent verification of the individual species is normally accomplished with gas chromatographic-mass spectrometric (GC/MS) techniques. Compound-specific detection systems, such as electron capture, flame photometry, and spectroscopic techniques, also have been employed to confirm peak identifications. The peak matching process is far from being a trivial task. Ambient air chromatograms are often very complex (>200 peaks/run) and require a good deal of manual labor to assure that the peak matching process is being carried out correctly by the resident peak identification/ quantification software. Efforts to improve on the accuracy of peak assignment and diminish the labor hours normally associated with the objective recently have been reported. Silvestre et al. (1988) developed an off-line spreadsheet program that is menu-driven and used to identify and edit a chromatogram containing 200 peaks within 15 min. The accuracy of peak assignment was typically better than 95%. Mason et al. (1992) developed a novel algorithm that is embedded within the Harwell MatchFinder software package and demonstrated the potential of the algorithm for enhancing peak identification in complex chromatograms. The authors indicate that the software could be used to batch-process large volumes of chromatographic data. A commercial software package from Meta Four Software, Inc., was recently employed during the Atlanta Ozone Precursor Monitoring Study to batch process chromatographic data from over 6,000 GC runs (Purdue et al., 1992). This software was also used to validate peak identities from two GC databases and was shown to improve peak identities from the originally processed data by 10 to 20% (Holdren et al., 1993).

Because the organic components of the ambient atmosphere are present at parts-per-billion levels or lower, sample preconcentration is necessary to provide sufficient material for the GC-FID system. The two primary techniques utilized for this purpose are the use of solid adsorbents and cryogenic collection. The more commonly used sorbent materials are divided into three categories: (1) organic polymeric adsorbents, (2) inorganic adsorbents, and (3) carbon adsorbents. Primary organic polymeric adsorbents used for NMOC analyses include the materials Tenax®-GC and XAD-2®. These materials have a low retention of water vapor, and, hence, large volumes of air can be collected. These materials do not, however, efficiently capture highly volatile compounds such as C_2 to C_5 hydrocarbons, nor certain polar compounds such as CH_3OH and C_3H_6O. Primary inorganic adsorbents are silica gel, alumina, and molecular sieves. These materials are more polar

than the organic polymeric adsorbents and are thus more efficient for the collection of the more volatile and polar compounds. Unfortunately, water also is collected efficiently, which in many instances leads to rapid deactivation of the adsorbent. Carbon adsorbents are less polar than the inorganic adsorbents and, as a result, water adsorption by carbon adsorbents is a less significant problem. The carbon-based materials also tend to exhibit much stronger adsorption properties than organic polymeric adsorbents; thus, lighter-molecular-weight species are more easily retained. These same adsorption effects result, however, in irreversible adsorption of many compounds. Furthermore, the very high thermal desorption temperatures required (350 to 400 °C) limit the use of carbon adsorbents and also may lead to degradation of labile compounds. The commonly available classes of carbon adsorbents include various conventional activated carbons, carbon molecular sieves (Spherocarb®, Carbosphere®, Carbosieve®), and carbonaceous polymeric adsorbents (Ambersorb® XE-340, XE-347, SE-348).

Although a number of researchers have employed solid adsorbents for the characterization of selected organic species in air, only a few attempts have been made to identify and quantitate the range of organic compounds from C_2 and above. Westberg et al. (1982) evaluated several carbon and organic polymeric adsorbents and found that Tenax®-GC exhibited good collection and recovery efficiencies for $\geq C_6$ organics; the remaining adsorbents tested (XAD-4®, XE-340®) were found unacceptable for the lighter organic fraction. The XAD-4® retained $\geq C_2$ organic gases, but it was impossible to desorb these species completely without partially decomposing the XAD-4®. Good collection and recovery efficiencies were provided by XE-340® only for organics of C_4 and above. Ogle et al. (1982) used a combination of adsorbents in series and designed an automated GC-FID system for analyzing C_2 through C_{10} hydrocarbons. Tenax-GC® was utilized for C_6 and above; whereas Carbosieve S® trapped C_3 through C_5 organics. Silica gel followed these adsorbents and effectively removed water vapor while passing the C_2 hydrocarbons onto a molecular-sieve, 5A adsorbent. More recently, Levaggi et al. (1992) used a combination of adsorbents in series for analyzing C_2 through C_{10} hydrocarbons. Tenax GR, Carbotrap, and Carbosieve S-III were evaluated. At room temperature collection, excellent recovery efficiencies were obtained for all species except acetylene (breakthrough begins after 220 cc). Smith et al. (1991b) evaluated a commercially available GC system (Chrompack, Inc.) and found that a Carbotrap C, Carbopack B, and Carbosieve S-III combination was effective for all C_2 and above species, if the trap temperature was maintained at −30 °C during collection (600 cc). The above researchers also caution that artifact peaks do occur during thermal desorption and recommend closely screening the resulting data.

The preferred method for obtaining NMOC data is cryogenic preconcentration (Singh, 1980). Sample preconcentration is accomplished by directing air through a packed trap immersed in either liquid oxygen (B.P. −183 °C) or liquid argon (B.P. −186 °C). For the detection of about 1 ppbC of an individual compound, a 250-cc air sample normally is processed. The collection trap generally is filled with deactivated 60/80 mesh glass beads (Westberg et al., 1974), although coated chromatographic supports also have been used (Lonneman et al., 1974). Both of the above cryogens are sufficiently warm to allow air to pass completely through the trap, yet cold enough to collect trace organics efficiently. The use of cryogenic preconcentration for collection of VOCs, in general, was automated to allow sequential hourly updates of GC data (McClenny et al., 1984), leading to the initial configuration of what are now referred to as automated GCs ("auto GCs") for O_3 precursor monitoring. The cryogenic collection procedure also condenses water vapor. An air volume

of 250 cc at 50% relative humidity and 25 °C contains approximately 2.5 mg of water that appears as ice in the collection trap. The collected ice at times will plug the trap and stop the sample flow; furthermore, water transferred to the capillary column during the thermal desorption step occasionally causes plugging and other deleterious column effects.
To circumvent water condensation problems, Pleil et al. (1987) have characterized the use of a Nafion® tube drying device to remove water vapor selectively during the sample collection step. Although hydrocarbon species are not affected, polar organics are partially removed when the drying device is used. Burns et al. (1983) also showed that partial loss or rearrangement of monoterpenes, or both (e.g., α-pinene, limonene), occurs when the Nafion® tube is used to reduce water vapor.

The EPA has recently provided technical guidance for measuring VOCs that is based on the above studies as well as emerging and developing technology (U.S. Environmental Protection Agency, 1991c). Guidance for the use of auto GC sampling and analysis for VOCs has been derived from experience gained from application of this technology during an O_3 precursor study conducted by EPA in Atlanta during the summer of 1990 (Purdue et al., 1992). For that study, an auto GC system developed and manufactured by Chrompack, Inc., and modified for O_3 precursor monitoring (McClenny et al., 1991b) was used to obtain hourly VOC measurements. The GC system was equipped with a preconcentration adsorption trap, a cryofocusing secondary trap, and a single analytical column. The study was focused on the identification and quantitation of 55 O_3 precursor compounds, and resulted in accounting for 65 to 80% of the total NMOC mass. Sample volumes of 600 cc were used and a detection level of 0.1 ppb C was reported. External auditing indicated accuracy of $\pm 30\%$ at challenge concentrations of 2 ppbC (17-component audit mixture).

The study also revealed several weaknesses. First of all, excessive amounts of liquid cryogen were consumed in carrying out the measurements. The inferior quality of the cryogen containers and poor delivery schedules resulted in reduced data capture. Secondly, because of the single-column approach, numerous target species either co-eluted or were poorly resolved. Thirdly, several significant artifact peaks co-eluted with the target species and, therefore, biased the reported concentrations of those species, as well as the total NMOC (by summation of peaks). Additionally, Shreffler (1993) reported results from analyses of canister samples collocated with the automated field GC systems. In general, good agreement between the systems was found when comparing the sum of the 55 identified O_3 precursors. However, regression analysis indicated that the average total NMOC concentrations found from the analyses of canister samples were 50% higher than those measured with the field GC systems.

Based on these operational deficiencies, EPA has challenged commercial GC instrument makers with improving the current state of the art. One result has been the evolution of systems that require no liquid cryogen for operation, yet provide sufficient gas chromatographic resolution of target species (McClenny, 1993; Holdren et al., 1993).
A recent comparison study of auto GCs at Research Triangle Park with five participating vendors has indicated that the newer auto GC designs use cryogens more efficiently (Purdue, 1993).

In addition to direct sampling via preconcentration with sorbents and cryogenic techniques, collection of whole air samples is frequently used to obtain NMOC data. Rigid devices such as syringes, glass bulbs, or metal containers and nonrigid devices such as Tedlar® and Teflon® plastic bags are often utilized during sampling. The primary purpose of

whole-air collection is to store an air sample temporarily until subsequent laboratory analysis is performed. The major problem with this approach is assuring the integrity of the sample contents prior to analysis. The advantages and disadvantages of the whole air collection devices were summarized in the 1986 air quality criteria document (U.S. Environmental Protection Agency, 1986a).

The canister-based method is the preferred means for collecting VOCs and is described as part of the "EPA Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air" (Compendium Method TO-14). McClenny et al. (1991a) recently reviewed the canister-based method and discussed basic facts about the canisters, described canister cleaning procedures, contrasted the canister collection system versus solid adsorbents, and discussed the storage stability of VOCs in canisters. Although storage stability studies have indicated that many target VOCs can be stored with integrity over time periods of at least 7 days, there are still many VOCs for which there are no stability data (Pate et al., 1992; Oliver et al., 1986; Holdren and Smith, 1987; Westberg et al., 1982, 1984; Gholson et al., 1990). Coutant (1993) has developed a computer-based model for predicting adsorption behavior and vapor-phase losses in multicomponent systems, based on the potential for physical adsorption as well as the potential for dissolution in condensed water for canister samples collected at high humidities. At present, the database for the model contains relevant physicochemical data for 78 compounds (including water), and provisions for inclusion of up to 120 additional compounds are incorporated in the software.

Calibration Methods

Calibration procedures for NMOC instrumentation require the generation of dilute mixtures at concentrations expected to be found in ambient air. Methods for generating such mixtures are classified as static or dynamic systems.

As described in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986a), static systems generally are preferred for quantitating NMOCs. The most commonly used static system is a compressed-gas cylinder containing the appropriate concentration of the compound of interest. These cylinder gases also may be diluted with hydrocarbon-free air to provide multi-point calibrations. Cylinders of calibration gases and hydrocarbon-free air are available commercially. Also, some standard gases such as propane and benzene, as well as a 17-component ppb mixture, are available from NIST as certified standard reference materials (SRMs). Commercial mixtures generally are referenced against these NIST standards. In its recent technical assistance document for sampling and analysis of O_3 precursors, EPA recommended propane (or benzene)-in-air standards for calibration (U.S. Environmental Protection Agency, 1991c). Some commercially available propane cylinders have been found to contain other hydrocarbons (Cox et al., 1982), so that all calibration data should be referenced to NIST standards.

Because of the uniform carbon response of a GC-FID system ($\pm 10\%$) to hydrocarbons (Dietz, 1967), a common response factor is assigned to both identified and unknown compounds obtained from the speciation systems. If these compounds are oxygenated species, an underestimation of the actual concentrations will be reported. Dynamic calibration systems are employed when better accuracy is needed for these oxygenated hydrocarbon species. Dynamic systems normally are employed to generate in situ concentrations of the individual compound of concern and include devices such as permeation and diffusion tubes and syringe delivery systems.

3.5.2.3 Carbonyl Species

Historically, the major problem in measuring concentrations of carbonyls in ambient air has been to find an appropriate monitoring technique that is sensitive to low concentrations and specific for the various homologues. Early techniques for measuring HCHO, the most abundant aldehyde, were subject to some interferences and lacked sensitivity at low parts-per-billion concentrations (Altshuller and Leng, 1963; Altshuller et al., 1961; Altshuller and McPherson, 1963). However, spectroscopic methods such as FTIR and DOAS also lack sensitivity for HCHO in the low parts-per-billion concentration range. The 1986 air quality criteria document described two methods frequently used: (1) the chromotropic acid (CA) method for HCHO and (2) the 3-methyl-2-benzothiazolone hydrazone (MBTH) technique for total aldehydes (U.S. Environmental Protection Agency, 1986a). However, spectroscopic methods, on-line colorimetric methods, and the HPLC method employing DNPH derivatization are the preferred methods currently used for measuring atmospheric levels of carbonyl species.

Spectroscopic Methods

Three spectroscopic methods have been used to make measurements for atmospheric levels of HCHO and were recently intercompared at an urban site in California (Lawson et al., 1990). The FTIR method used gold-coated 30-cm-diameter mirrors and a total optical path of 1,150 m. The 2,781.0-cm^{-1} "Q-branch" adsorption peak was used to measure HCHO. The limit of detection was 3 ppb, and the measurement errors were within ± 3 ppb. The DOAS method was operated at an 800-m pathlength, and an absorption peak at 339 nm was used to measure HCHO; NO_2 and HNO_2 spectral features were subtracted. The limit of detection was 4.5 ppb, and the experimental error was $\pm 30\%$. A TDLAS method was operated at a pathlength of 150 m. Laser diodes were mounted in a closed-cycle helium cryocooler with a stabilizing heater circuit for constant temperature control. Radiation from the diode was collected and focused into the sampling by reflective optics. Formaldehyde absorption was measured at 1,740 cm^{-1}. The limit of detection was 0.1 ppb and the measurement errors were within $\pm 20\%$. Additional information on FTIR and DOAS has been reported by Winer et al. (1987), Atkinson et al. (1988), and Biermann et al. (1988). A more complete description of TDLAS is given by MacKay and Schiff (1987a).

On-Line Fluorescence Method

A wet chemical method based on the derivatization of HCHO in aqueous solution to form a fluorescent product was developed by Kelly et al. (1990) and recently tested (Kelly and Fortune, 1994). The detection of fluorescent product was made more sensitive by using intense 254 nm light from a mercury lamp for excitation. This procedure allowed the use of a simple and efficient glass coil scrubber for collection of gaseous HCHO. A detection limit of 0.2 ppb was obtained with a response time of 1 min. The instrument is portable and highly selective for HCHO.

High-Performance Liquid Chromatography-2,4-Dinitrophenylhydrazine Method

The preferred and most current method for measuring aldehydes in ambient air is one involving derivatization of the aldehydes concurrent with sample collection, followed by analysis using HPLC. This method takes advantage of the reaction of carbonyl compounds with DNPH to form a 2,4-dinitrophenylhydrazone:

$$RR'C{=}O + NH_2NHC_6H_3(NO_2)_2 \rightarrow H_2O + RR'C{=}NNHC_6H_3(NO_2)_2. \quad (3\text{-}82)$$

Because DNPH is a weak nucleophile, the reaction is carried out in the presence of acid in order to increase protonation of the carbonyl.

In this method, atmospheric sampling initially was conducted with micro-impingers containing an organic solvent and an aqueous, acidified DNPH reagent (Papa and Turner, 1972; Katz, 1976; Smith and Drummond, 1979; Fung and Grosjean, 1981). After sampling was completed, the hydrazone derivatives were extracted, and the extract was washed with deionized water to remove the remaining acid and unreacted DNPH reagent. The organic layer was then evaporated to dryness, subsequently dissolved in a small volume of solvent, and analyzed by reversed-phase liquid chromatographic techniques employing a UV detection system (360 nm).

An improved procedure subsequently was reported that is much simpler than the above aqueous impinger method (Lipari and Swarin, 1982; Kuntz et al., 1980; Tanner and Meng, 1984). This scheme utilizes a midget impinger containing a C_2H_3N solution of DNPH and an acid catalyst. After sampling, an aliquot of the original collection solution is injected directly into the liquid chromatograph. This approach eliminates the extraction step and several sample-handling procedures associated with the DNPH-aqueous solution and provides much better recovery efficiencies. This method has been formalized by EPA as Compendium Method TO-5 (Winberry et al., 1988). The TO-5 Method has been further modified to include the use of a DNPH-impregnated solid adsorbent, rather than DNPH impinger solutions, as the collection medium. This modification and associated sampling conditions are referred to as EPA Method TO-11. The methodology can be used easily for long-term (1 to 24 h) sampling of ambient air. Sampling rates of 500 to 1,200 cc/min can be achieved and detection levels of 1 ppbv can be attained with sampled volumes of 100 L. The method currently calls for the use of SepPak® silica gel material as the sorbent material. However, researchers have noted that O_3 present in ambient air reacted more easily with carbonyl compounds collected on DNPH-coated silica gel cartridges than on DNPH-coated C_{18}-bonded silica material. To eliminate this negative bias, these researchers used an O_3 scrubber (Arnts and Tejada, 1989). Smith et al. (1989) noted that artifact peaks occurred when O_3 was bubbled through impingers containing DNPH solution. These artifacts were identified as DNPH-O_3 reaction products and were shown to cause positive interferences unless they were chromatographically resolved from the HCHO-hydrazone derivative. Although the TO-11 Method has been included in EPA's *Technical Assistance Document for Sampling and Analysis of Ozone Precursors* (U.S. Environmental Protection Agency, 1991c), a KI-coated denuder tube also has been recommended to remove O_3 upstream of the DNPH-coated cartridges.

Although both the Sep-Pak® C_{18} and silica gel cartridges have been used by researchers (either with or without O_3 scrubbers), there is still considerable uncertainty as to which type of cartridge gives the most reliable carbonyl results. Experiments are currently underway at several laboratories to investigate the effect of O_3 on the performance of DNPH-coated cartridges. However, results have not yet been reported in the literature.

Calibration of Carbonyl Measurements

Because they are reactive compounds, it is extremely difficult to make stable calibration mixtures of carbonyl species in pressurized gas cylinders. Although gas-phase

standards are available commercially, the vendors do not guarantee long-term stability and accuracy.

Formaldehyde standards generally are prepared by one of several methods. The first method utilizes dilute commercial formalin (37% HCHO, w/w). Calibration is accomplished by the direct spiking into sampling impingers of the diluted mixture or by evaporation into known test volumes, followed by impinger collection. Formaldehyde also can be prepared by heating known amounts of paraformaldehyde, passing the effluent gases through a methanol-liquid nitrogen slush trap to remove impurities, and collecting the remaining HCHO. Paraformaldehyde permeation tubes also have been used (Tanner and Meng, 1984).

For the higher molecular weight carbonyl species, liquid solutions can be evaporated, or pure vapor can be generated in dynamic gas-flow systems (permeation tubes, diffusion tubes, syringe delivery systems, etc.). These test atmospheres are then passed through the appropriate collection system and analyzed. A comparison of these data, with the direct spiking of liquid carbonyl species into the particular collection system, provides a measure of the overall collection efficiency.

3.5.2.4 Polar Volatile Organic Compounds

The VOCs discussed earlier in this chapter (Section 3.5.2.2) have included aliphatic, aromatic, alkenic, and acetylenic hydrocarbons. These compounds are relatively nonpolar, nonreactive species, and measurement methods have been easily applied in determining ambient concentrations.

Recently, attention also has been directed toward the more reactive oxygen- and nitrogen-containing organic compounds, in part by the inclusion of many of these compounds on a list of 189 hazardous air pollutants specified in the 1990 CAAA (U.S. Congress, 1990). Many of these compounds are emitted directly from a variety of industrial processes, mobile sources, and consumer products, and some also are formed in the atmosphere by photochemical oxidation of hydrocarbons. However, as indicated earlier in this document, very few ambient data exist for these species. These compounds have been referred to collectively as PVOCs, although it is their reactivity and water solubility, more than simple polarity, that make their measurement difficult with existing methodology.

Two approaches have been utilized in developing analytical methods for PVOCs. One approach has incorporated the use of cryogenic trapping techniques similar to those discussed earlier for the nonpolar hydrocarbon species; the second approach has utilized adsorbent material for sample preconcentration. To be effective for sensitive parts-per-billion measurement of PVOCs, both approaches require some type of water management system to mitigate the adverse effects that water has on the chromatography and detector sensitivity and reliability. Several researchers have reported the use of cryogenic trapping with two-dimensional chromatography to selectively remove water vapor from the analytical process (Pierotti, 1990; Cardin and Lin, 1991). Although this column "heart cutting" technique has been successful for selected compounds, additional studies are needed to determine its potential use for the wide range of PVOCs. Ogle et al. (1992) developed a novel water management system based on the condensation of moisture from the saturated carrier gas stream during thermal desorption of a cryogenic trap. The moisture management system was found to be effective for reducing the amount of water delivered to the column during laboratory analyses of spiked mixtures. However, the system has not yet been extended to field monitoring. Gordon and Miller (1989) have used cryogenic trapping and

GC/MS spectrometry techniques to demonstrate the potential of chemical ionization (CI) within an ion trap to detect PVOCs. The water vapor present in the sample served as the CI reagent gas and appeared to be an effective reagent gas; however, deleterious chromatography results were also encountered. The authors concluded that further laboratory work is needed before this methodology can be applied to ambient air monitoring. Martin et al. (1991) also reported the use of cryogenic trapping with a GC-FID system to measure ambient levels of isoprene and two of its oxidation products, methacrolein and methylvinyl ketone (detection level of 0.5 ppb). The water vapor was selectively removed by using a potassium carbonate (K_2CO_3) trap ahead of the cryogenic trap. Frequent replacement of the K_2CO_3 trap was required.

The use of solid adsorbents for sample preconcentration of PVOCs has been reported by Kelly et al. (1993). The analytical method was used extensively at two field sites that formerly were used in EPA's Toxic Air Monitoring Study (TAMS). The analytical method consisted of GC separation of PVOCs with quantification by a ion-trap mass spectrometer. A two-stage adsorbent trap containing Carbopack B and Carbosieve S-III (Supelco catalog number 2-0321) was used to separate water vapor from the PVOCs. The optimum room temperature trapping and drying procedure consisted of a 320-cc sample (100 cc/min) followed by a dry nitrogen purge of 1,300 cc (100 cc/min). The trap was then backflushed and thermally desorbed with helium at 220 °C. A 5-min, 260 °C trap bakeout followed each collection-analysis cycle. The target list contained 14 PVOCs, including alcohols, ethers, esters, and nitrile species. Individual detection limits ranged from 0.2 to 1 ppb.

3.5.3 Sampling and Analysis of Nitrogen Oxides

3.5.3.1 Introduction

The measurement of NO_x in ambient air is of interest because of the role that certain of those compounds play as precursors to O_3 and because NO_2 has been shown to impact health effects. Most of the NO_x emitted from combustion sources are NO and NO_2. Collectively these two compounds are called NO_x. They contribute to O_3 formation by means of reactions discussed in Section 3.2. As a result, measurement of NO_x is important in efforts to understand and control O_3 and NO_2 in ambient air.

The atmospheric photochemistry that produces O_3 also results in conversion of NO and NO_2 to products such as HNO_3, nitrous acid (HNO_2), organic nitrates such as PAN ($CH_3C(O)O_2NO_2$), and other species. The total of all of these labile nitrogen species in air, NO_x included, is termed NO_y. Such compounds may be labile via photolysis (e.g., HNO_2) or thermal decomposition (e.g., PAN), and may be toxic, irritating, or acidic. The organic nitrates can occur in the atmosphere as reservoirs for NO_2. However, in general, they do not play the same critical role that NO_2 and NO play as O_3 precursors. For that reason, this section focuses on measurement methods for NO and NO_2, as the primary O_3 precursors of NO_x. Nitrogen oxides other than NO_x may be important, however, as interferents in efforts to measure NO and NO_2. These non-NO_x species are considered in this section in that regard.

Measurements of NO_x may involve measurements of NO, of NO_2, or of the sum of NO_x. Nitrogen dioxide, but not NO, is a criteria air pollutant, and, thus, reference and equivalent methods are specified for NO_2 measurements. In this section, the current state of measurement methods for NO and NO_2 will be summarized separately. Such methods in

some cases rely on measurements of total NO_x, or at least an approximation of NO_x. This discussion focuses on current methods and on promising new technologies, but no attempt is made here to cover the extensive history of the development of these methods. More detailed discussions of such methods may be found elsewhere (U.S. Environmental Protection Agency, 1993c; National Aeronautics and Space Administration, 1983). Wet chemical methods are no longer commonly used and are not discussed here; a review of such methods is given by Purdue and Hauser (1980).

3.5.3.2 Measurement of Nitric Oxide

Gas-Phase Chemiluminescence Methods

By far the most common method of NO measurement is gas-phase CL with O_3. In this method, excess O_3 is added to air containing NO in a darkened, internally reflective chamber monitored by a photomultiplier tube. A small portion of the NO reactions with O_3 produce electronically excited NO_2 molecules that decay by emission of light of wavelengths longer than 600 nm. The emitted light is detected by a red-sensitive photomultiplier tube, through an optical filter that prevents passage of wavelengths shorter than 600 nm. This optical filtering minimizes interference from CL produced by O_3 reactions with other species (e.g., hydrocarbons). The excited NO_2 is readily quenched in air, so that, in typical instruments, air and O_3 are mixed at reduced pressure (i.e., at least 20 in. of Hg vacuum). The intensity of the emitted light is linearly proportional to the NO content of the sample air over several orders of magnitude in concentration.

Commercial CL instruments for continuous measurement of NO are available from several manufacturers. The chemiluminescence approach is also an EPA-designated measurement principle for measuring ambient NO_2; it requires a means of converting NO_2 to NO for detection. The complexities of this conversion are discussed in Section 3.5.3.3, on NO_2 methods. The commercial NO monitors typically are claimed to have detection limits of a few parts per billion by volume in air, with response time of a few minutes. Field evaluations of several commercial instruments have indicated that minimum levels of detection for NO_2 are 5 to 13 ppbv (Michie et al., 1983; Holland and McElroy, 1986). However, more recent evaluations have indicated better performance. Rickman et al. (1989) reported detection limits of 0.5 to 1 ppbv, and precision of ± 0.3 ppbv, from laboratory and field evaluations of two commercial instruments operated on their 50 ppbv full-scale ranges. Commercial NO analyzers are portable and quite reliable and now are commonly used in ambient air monitoring networks.

Commercial NO analyzers may not have sensitivity sufficient for surface measurements in urban, rural, or remote areas, or for airborne measurements. As a result, several investigators have devised modifications to commercial instruments to improve their sensitivity and response time (Delany et al., 1982; Tanner et al., 1983; Dickerson et al., 1984; Kelly et al., 1986). Those modifications include operating at low pressure and high sample flow rate; using a larger, more reflective reaction chamber that promotes mixing of the reactants close to the photomultiplier tube; increasing the O_3 supply; for example, by use of oxygen in the O_3 source; cooling of the photomultiplier to reduce noise; adopting photon-counting techniques for light detection; and adding a prereactor to obtain a more stable and appropriate background signal. Commercial instruments modified in these ways are generally reported to have detection limits of 0.1 ppbv or less, with response times of 30 s or less.

Research-grade NO instruments specially designed for ultra-high sensitivity also have been built for use in remote ground-level or airborne applications (e.g., Ridley and Howlett, 1974; Kley and McFarland, 1980; Kelly et al., 1980; Helas et al., 1981; Drummond et al., 1985; Torres, 1985; Kondo et al., 1987; Parrish et al., 1990). These instruments typically have detection limits of 10 ppt (i.e., 0.01 ppbv) or less, with response times from a few seconds to 1 min.

A number of studies indicate that the CL method is essentially specific for NO. Operation at reduced pressure prevents interference resulting from quenching by water vapor (Michie et al., 1983; Drummond et al., 1985). In air sampling, no significant interferences have been found in NO detection from sulfur-, chlorine-, and nitrogen-containing species (Joshi and Bufalini, 1978; Sickles and Wright, 1979; Grosjean and Harrison, 1985b; Fahey et al., 1985). However, H_2S and possibly other sulfur-containing compounds from seawater have been reported to give false NO signals (Zafiriou and True, 1986). This effect should not be important for ambient air measurements. Fahey et al. (1985) and Drummond et al. (1985) also reported no significant NO interference from a variety of other nitrogen-containing species, including NO_2, HNO_3, PAN, N_2O_5, NH_3, HCN, N_2O, and HO_2NO_2; as well as no interference from CH_4, propylene, and H_2O_2.

Several ambient air intercomparisons have been done of CL NO instruments (Walega et al., 1984; Hoell et al., 1987; Fehsenfeld et al., 1987; Gregory et al., 1990a). These studies have focused on high-sensitivity research instruments, rather than the commercial instruments used for widespread ambient air measurements. These studies have shown excellent agreement among the CL NO instruments, even at NO levels in the low ppt range (Hoell et al., 1987; Gregory et al., 1990a). These results support the validity of the CL approach for NO. Good agreement also has been found between CL measurements and spectroscopic NO measurements in these studies (see Section 3.5.3.2).

Spectroscopic Methods for Nitric Oxide

Direct spectroscopic methods for NO include two-photon laser-induced fluorescence (TPLIF), TDLAS, and two-tone frequency-modulated spectroscopy (TTFMS). The primary characteristics of these methods are their very high sensitivity and selectivity for NO. For example, a detection limit of 10 ppt has been quoted for TPLIF with a 30-s integration time, with no significant interferences from atmospheric species (Davis et al., 1987). An accuracy of $\pm 16\%$ as a 90% confidence limit has been calculated for NO measurement by TPLIF from an aircraft (Davis et al., 1987). The TDLAS method is similarly highly selective for NO and achieves a detection limit of 0.5 ppbv (Schiff et al., 1983). The response time of the TDLAS instrument is about 1 min for NO, and is limited by stabilization of concentrations with the large surface area of the multi-pass White cell. The newest method is TTFMS, which appears in laboratory studies to be very sensitive, fast, and selective. With a 100-m path length in a 20-torr multiple-pass cell, and a 1-min averaging time, the detection limit of NO is estimated to be 4 ppt (Hansen, 1989).

Spectroscopic methods have compared well with the CL method for NO in ambient measurements. Walega et al. (1984) reported good agreement between CL and TDLAS results for NO in laboratory air, in ambient air, and in downtown Los Angeles air. Gregory et al. (1990a) reported comparisons of TPLIF and CL NO methods in airborne measurements. Agreement at levels below 20 ppt was within the expected accuracy and precision of the instruments (i.e., within 15 to 20 ppt).

The major drawbacks of these spectroscopic methods are their complexity, size, and cost. Although possessing remarkable characteristics, these methods are restricted to research applications. The TTFMS approach, in fact, is still in the laboratory development stage.

Passive Samplers

At present, no passive sampler exists that directly measures NO. Instead, passive samplers developed for NO_2 have been adapted for NO measurement, using an oxidizing material that converts NO to NO_2. Palmes tubes (Palmes and Tomczyk, 1979) have been adapted for NO measurement by using two tubes in parallel. One tube collects NO_2 on a triethanolamine (TEA)-coated grid, whereas the other collects NO_2 on a TEA grid, plus NO oxidized by a chromic acid-coated surface. The grids are then extracted and analyzed for NO_2^- ion. Nitric oxide is determined by difference between the two results, after accounting for the different diffusivities of NO and NO_2. The sampling rates depend on temperature and air velocity. The tubes cannot be used for periods longer than 24 h and are intended for use at ppm NO levels important in the workplace (e.g., 2 to 200 ppm · h). Applicability to ambient NO levels has not been demonstrated.

A more sensitive passive sampler for NO has been reported (Yanagisawa and Nishimura, 1982) that uses the same TEA chemistry, with CrO_3 as the NO oxidizer. A detection limit of 70 ppbv-h has been reported. As with any currently available passive sampler, the disadvantages of the method are the potential for interferences, relatively poor precision, and low sensitivity for ambient air measurements.

3.5.3.3 Measurements for Nitrogen Dioxide

Gas-Phase Chemiluminescence Methods

In 1976, the gas-phase CL approach described above for NO detection was designated as the measurement principle on which EPA reference methods for ambient NO_2 must be based. The CL method thus filled the vacancy left by withdrawal of the Jacobs-Hochheiser method, because of technical problems, in 1973. To be designated as a reference method, an NO_2 detection method must use the CL approach and be calibrated by the specified methods (gas-phase titration of NO with O_3, or use of an NO_2 permeation device). In addition the instrument must meet the performance specifications shown in Table 3-18. An equivalent method, either manual or automated, must meet certain requirements for comparability with a reference method when measuring simultaneously in a real atmosphere. Those comparability requirements are shown in Table 3-19. An automated equivalent method must also meet the performance requirements shown in Table 3-18.

The selection of the O_3-CL method as the reference measurement principle for ambient NO_2 was the result of comparison tests of CL and wet chemical methods. Chemiluminescence analyzers were found superior to the wet chemical methods in response time, zero and span drift, and overall operation, although agreement among all the methods tested was good, at the NO_2 spike levels provided (Purdue and Hauser, 1980). Table 3-20 lists the methods currently designated (as of August 1, 1994) by EPA as reference and equivalent methods for ambient NO_2. Three wet chemical methods are shown as equivalent methods, but these rarely are used for ambient air measurements.

The O_3 CL method does not measure NO_2 directly, because the CL is produced by reaction of NO with O_3. As a result, NO_2 must first be reduced to NO for detection. In principle, such a reduction should readily result in measurement of NO + NO_2 (i.e.,

Table 3-18. Performance Specifications for Nitrogen Dioxide Automated Methods[a]

Performance Parameter	Units	NO_2
Range	ppm	0-0.5
Noise		
0% Upper range limit	ppm	0.005
80% Upper range limit	ppm	0.005
Lower detectable limit	ppm	0.01
Interference equivalent		
Each interferant (SO_2,NO,NH_3,H_2O)	ppm	±0.02
Total interferant	ppm	≤0.04
Zero drift, 12 and 24 hours	ppm	±0.02
Span drift, 24 hours		
20% Upper range limit	%	±20.0
80% Upper range limit	%	±5.0
Lag time	min	20
Rise time	min	15
Fall time	min	15
Precision		
20% Upper range limit	ppm	0.02
80% Upper range limit	ppm	0.03

[a]See Appendix A for abbreviations and acronyms.

Source: Code of Federal Regulations (1987), Ambient Air Monitoring Reference and Equivalent Methods, C.F.R. Title 40, Part 53.

Table 3-19. Comparability Test Specifications for Nitrogen Dioxide

Nitrogen Dioxide Concentration Range (ppm)		Maximum Discrepancy Specification (ppm)
Low	0.02 to 0.08	0.02
Medium	0.10 to 0.20	0.02
High	0.25 to 0.35	0.03

NO_x), and allow indirect measurement of NO_2 by difference between NO and NO_x responses, measured either sequentially, or simultaneously by separate detectors. In practice, however, selective measurement of NO_x by this approach has proven very difficult.

Several methods have been employed to convert NO_2 to NO, including catalytic reduction with heated molybdenum or stainless steel, reaction with CO over a gold catalyst surface, reaction with ferrous sulfate ($FeSO_4$) at room temperature, reaction with carbon at 200 °C, and photolysis of NO_2 at wavelengths of about 320 to 400 nm (Kelly et al., 1986). It has been found in many separate investigations that the heated converters reduce NO_2 to

Table 3-20. Reference and Equivalent Methods for Nitrogen Dioxide Designated by U.S. Environmental Protection Agency[a]

Method	Designation Number	Method Code
Reference Methods (Continuous CL Analyzers)		
Advanced Pollution Instrumentation 200	RFNA-0691-082	082
Beckman 952A	RFNA-0179-034	034
Bendix 8101-B	RFNA-0479-038	038
Bendix 8101-C	RFNA-0777-022	022
CSI 1600	RFNA-0977-025	025
Dasibi 2108	RFNA-1192-089	089
Lear Siegler ML9841	RFNA-1292-090	090
Meloy NA53OR	RFNA-1078-031	031
Monitor Labs 8440E	RFNA-0677-021	021
Monitor Labs 8840	RFNA-0280-042	042
Monitor Labs 8841	RFNA-0991-083	083
Philips PW9762/02	RFNA-0879-040	040
Thermo Electron 14B/E	RFNA-0179-035	035
Thermo Electron 14D/E	RFNA-0279-037	037
Thermo Environmental 42	RFNA-1289-074	074
Equivalent Methods (Wet Chemical)		
Sodium arsenite	EQN-1277-026	084
Sodium arsenite/Technicon II	EQN-1277-027	084
TGS-ANSA[b]	EQN-1277-028	098

[a]As of August 1, 1994.
[b]Triethanolamine-guaiacol-sulfite with 8-amino-1-naphthalene-sulfonic acid ammonium salt.

NO effectively, but also reduce other NO_y species as well (e.g., Winer et al., 1974; Cox, 1974; Joseph and Spicer, 1978; Grosjean and Harrison, 1985b; Fahey et al., 1985). Efficiencies of conversion near 100% are reported in these studies for NO_2 and for NO_y species such as HNO_3, HNO_2, PAN, and organic nitrates. This finding is particularly important for widespread monitoring networks that use commercial instruments, because such instruments without exception use heated catalytic converters (typically molybdenum). Thus, such instruments measure not NO and NO_x, but more nearly NO and total NO_y. Although NO_x is the predominant NO_y species during early morning hours, other NO_y species constitute a substantial percent of the NO_y later in the day, especially in rural areas. The NO_2 value inferred from such measurements may be significantly in error (see below), and may in turn affect the results of models of ambient O_3. The completeness of the measured NO_y value is also questionable because, for example, HNO_3 is readily lost to surfaces, and, in ambient sampling, may be removed within the sampling system before reaching the heated converter.

Other conversion methods for NO_2 have been tried in an effort to achieve higher selectivity. Ferrous sulfate has been used for ambient NO_2 measurements using high-sensitivity research grade CL instruments (e.g., Kelly et al., 1980; Helas et al., 1981; Dickerson et al., 1984). This material is an efficient reducer of NO_2, but also has been found to convert a portion of PAN, and possibly a portion of HNO_2 and organic nitrates (Fehsenfeld et al., 1987). Memory effects and reduction in efficiency can occur because of humidity effects (Fehsenfeld et al., 1987). As a result of these characteristics, use of $FeSO_4$ has given high readings in comparison with spectroscopic instruments and the photolytic NO_2 converter, and its use likely results in overestimating ambient NO_x by a significant amount (Fehsenfeld et al., 1987; Ridley et al., 1988a; Gregory et al., 1990b). Ferrous sulfate has never been used in commercial NO_x instruments and is no longer used in research measurements.

The most specific method for converting NO_2 to NO is photolysis (Kley and McFarland, 1980). In the most common approach, ambient NO_2 is photolyzed to NO by light of 350 to 410 nm from a xenon arc lamp. The method does not produce NO from the major potential interferents present in air (i.e., HNO_3, PAN, and organic nitrates), but less abundant NO_y species such as HNO_2 or HO_2NO_2 may interfere. A detailed description of steps to minimize such interferences is given by Ridley et al. (1988b). As currently used, the photolytic converter appears to be essentially specific for NO_2. However, it does not provide complete conversion of NO_2. Conversion efficiencies are 50 to 60% with a new lamp but may decline to 20% over the course of several weeks (Parrish et al., 1990). Thus, the conversion efficiency must be calibrated repeatedly. This approach has not been implemented with commercial NO detectors but has been implemented with research-grade CL NO instruments for studies of NO_x and NO_y chemistry at a variety of locations (e.g., Buhr et al., 1990; Parrish et al., 1990; Trainer et al., 1991; Parrish et al., 1992, 1993). The photolytic method compares well with other techniques, including spectroscopic methods, even at NO_2 levels as low as 0.05 ppbv (Gregory et al., 1990b). Further improvement of the photolytic converter approach is continuing. Bradshaw et al. (1994) reported on plans to minimize wall effects in the photolytic converter and to use a metal halogen lamp in place of the xenon arc lamp. The metal halogen lamp emits strongly in the proper wavelength region and is much less expensive than the xenon arc lamp, allowing more frequent replacement of the lamp and consequently higher long-term photolytic efficiency.

As noted above, the commercial CL analyzers used for most ambient air NO and NO_x measurements actually measure NO and NO_y. The magnitude of the resulting overestimation of NO_2, determined by difference, obviously depends on the portion of NO_y that is NO_x. The smaller the portion of NO_y that is NO_x, the greater will be the error in the NO_2 determined by difference. In rural/remote areas, where NO_x has undergone extensive conversion to other products during transport from a source region, NO_x may contribute a small fraction of NO_y. In urban areas, close to sources, NO_x may comprise nearly all of NO_y. For example, in measurements at Point Arena, Parrish et al. (1992) report NO_x/NO_y ratios averaging 0.3 in air of marine origin and 0.75 in air subject to continental influence. Buhr et al. (1990) and Parrish et al. (1993) reported measurements at rural sites in eastern North America that indicate NO_x/NO_y ratios ranging from about 0.25 to 0.75, varying with the time of day, with the lowest ratios occurring during daytime, photochemically active periods. Clearly, although the commercial CL instruments are designated as reference methods for NO_2, the great majority of existing ambient air data for NO_2 or NO_x are biased

high, due to the inclusion of some portion of other NO_y species. The magnitude of this bias may not be large in urban areas, but, in any case, it is essentially unknown at this time.

Luminol Chemiluminescence Method

This approach is based on the CL reaction of gaseous NO_2 with the surface of an aqueous solution of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione). Emission occurs primarily between 380 and 520 nm. In commercial instruments, luminol solution flows down a fabric wick that lies vertically on a clear window viewed by a photomultiplier tube. Nitrogen dioxide in sample air passing over the wick produces light, the intensity of which is proportional to the NO_2 concentration. Commercial instruments using this approach are compact, light, and relatively inexpensive and can provide detection limits as low as 0.01 ppbv, with response times below 30 s. The instrument has the advantage of detecting NO_2 directly. However, several difficulties have had to be dealt with in developing the method.

Original reports of the approach (Maeda et al., 1980) indicated positive interferences from O_3 and SO_2 and a negative one from CO_2. Reformulation of the luminol reagent solution has minimized, although not fully eliminated, those interferences (Wendel et al., 1983; Schiff et al., 1986). Reported effects include a slight negative response from NO, and sensitivity to PAN, HNO_2, and O_3 (Wendel et al., 1983; Schiff et al., 1986; Rickman et al., 1989; Kelly et al., 1990; Spicer et al., 1991). Response to NO_2 may be nonlinear at low concentrations (Kelly et al., 1990), although recent reformulation of the reagent apparently has reduced this behavior (Busness, 1992). Evaluation of the luminol NO_2 monitor indicates that great care must be taken in using and calibrating the instrument in order to achieve good precision and accuracy in ambient measurements (Kelly et al., 1990). The monitor has been used widely as a research tool, but has not been used widely in ambient air monitoring nor has it been designated an equivalent method for NO_2.

An O_3 scrubber is available to eliminate the O_3 interference noted above, but it also was found to remove a portion of the NO_2 (Kelly et al., 1990). The luminol approach also has been modified to measure NO, by using a CrO_3 converter that oxidizes NO to NO_2 for detection. Thus NO is detected by difference. This method has the potential for measurement of total NO_x; however, evaluations of the CrO_3 converter are still underway at several laboratories. Given the known interferences in the luminol approach, careful evaluation of this method must be completed before it gains acceptance as an NO measurement method.

An adaptation of the commercial luminol NO_2 detector has been reported to provide measurements of total NO_y, NO_2, and NO_x (Drummond et al., 1992). This adaptation, called the LNC-3M, uses a commercial luminol instrument for NO_2 detection, with a CrO_3 converter for NO_x detection. The NO_x measurement must be corrected for the few percent of the ambient NO_2 that is lost in the CrO_3 converter (Drummond et al., 1992). The NO_y measurement is achieved using a stainless steel converter maintained at 400 °C. Tests indicate that this converter provides a more complete conversion of alkyl nitrates, and consequently a more complete measurement of NO_y, than is provided by either the heated molybdenum converters used in commercial O_3 CL NO_x detectors or the gold converters with CO addition used in research instruments (Drummond et al., 1992). The LNC-3M adds a small amount of NO_2 to the sample to eliminate the nonlinearity at low concentrations, and uses a zeroing scrubber that greatly reduces the interference from PAN. However, this scrubber must be replaced weekly when it is in continuous use (Drummond et al., 1992).

Spectroscopic Methods

Several spectroscopic approaches to NO_2 detection have been developed; TDLAS, TTFMS, DOAS, and differential absorption lidar (DIAL) are absorption methods that have been used. The TDLAS method is probably the most commonly used spectroscopic NO_2 method. It can provide high selectivity for NO_2, with a detection limit of 0.1 ppbv, accuracy of $\pm 15\%$, and a response time on the order of 1 min because of the White cell (Mackay and Schiff, 1987b). The DOAS method is an open-path, long-pathlength system. The detection limit for NO_2 with a 0.8-km pathlength and 12-min averaging time has been reported as 4 ppbv, with measurement accuracy reported as $\pm 10\%$ (Biermann et al., 1988). However, recent improvements have resulted in a commercial DOAS instrument capable of an NO_2 detection limit of 0.6 ppbv, based on a 557-m path and a 1-min averaging time (Stevens et al., 1993). The detection limit for NO_2 by the DIAL technique has been reported as 10 ppbv with a 6-km pathlength (Staehr et al., 1985). The novel TTFMS method noted above for NO is reported to have an NO_2 detection limit of 0.3 ppt, but is not fully proven for ambient measurements.

Fluorescence methods also have been used for NO_2, including photofragmentation TPLIF (PF/TPLIF) (Davis, 1988). This method uses two cells in which NO is measured by TPLIF. In one of the cells, an excimer laser emitting at 353 nm photolyzes NO_2 to NO for detection. Thus NO_2 is ultimately measured, by difference, as NO, but the NO is formed directly by photolysis of NO_2. With a 2-min integration time, an NO_2 detection limit of 12 ppt is reported. The method is highly selective for NO_2, because an interferant would have to photolyze to produce NO. Several potential atmospheric species have been ruled out in this regard (Davis, 1988).

The drawbacks of most of these methods are, as noted earlier, complexity, size, and cost. At present, these factors outweigh the obvious advantages of the sensitivity and selectivity of these spectroscopic methods and largely have restricted the use of these NO_2 methods to specific research applications or as reference methods in intercomparisons. In such intercomparisons, absorption measurements have been used most commonly. The TDLAS method has been used in ground-level comparisons with O_3 CL and luminol instruments to provide specific NO_2 measurements (Walega et al., 1984; Sickles et al., 1990; Fehsenfeld et al., 1990) and in an airborne comparison with PF/TPLIF and O_3 CL instruments (Gregory et al., 1990b). A finding of these studies was that the TDLAS consistently read higher than other established methods at very low NO_2 levels (i.e., <0.4 ppbv) (Fehsenfeld et al., 1990; Gregory et al., 1990b).

The spectroscopic NO_2 method most fully developed beyond the research stage is the DOAS technique. Stevens et al. (1993) report testing of a commercial DOAS instrument in North Carolina over 17 days in the fall of 1989. The DOAS measured NO_2 using wavelengths between 400 and 460 nm and achieved a detection limit of 0.6 ppbv, as noted above. Simultaneous measurements of O_2, SO_2, HCHO, and HNO_2 also were provided by the DOAS instrument. Comparison of the DOAS NO_2 results to those from a commercial CL detector showed (DOAS NO_2) = $1.14 \times$ (CL NO_2) + 2.7 ppbv, with an r^2 of 0.93, at NO_2 levels up to 50 ppbv (Stevens et al., 1993). The sensitivity, stability, response time, and multicomponent capability are the primary advantages of the DOAS approach. Further intercomparisons and interference tests are recommended (Stevens et al., 1993).

Passive Samplers

Passive samplers are attractive, inexpensive, and simple means to obtain long-term or personal exposure data for NO_2 or NO_x. The simplest passive sampler for NO_2 is the nitration plate, which is essentially an open dish containing filter paper impregnated with TEA. Nitrogen dioxide diffuses to the paper and is extracted later as NO_2^- for analysis. No diffusion barrier exists in this approach or in a similar approach using a candle-shaped absorber (Kosmus, 1985). Consequently, results are very much subject to ambient conditions and give, at best, a qualitative indication of NO_2 or NO_x.

Addition of a diffusion barrier to the nitration plate concept has led to badge-type passive samplers for NO_2 (e.g., Mulik and Williams, 1986, 1987; Mulik et al., 1989, 1991). In general, such devices use perforated screens, plates, or filters as diffusion barriers on the chemically reactive material, which may be exposed on one or both sides, depending on the application. Extraction of the sorbent then allows measurement of the NO_2 collected, typically as NO_2^- ion. Such a device using TEA as the active material gave very good agreement relative to a CL analyzer in laboratory tests with NO_2 at 10 to 250 ppbv (Mulik and Williams, 1987). However, interferences from PAN and HNO_2 (the latter in both outdoor and indoor air) are expected (Sickles and Michie, 1987). Comparison of ambient NO_2 results in the 5 to 25 $\mu g/m^3$ range (i.e., about 2.5 to 12.5 ppbv) from the passive device to those from TDLAS showed good agreement on average values, but a correlation coefficient (r) of only 0.47 on daily values (Mulik et al., 1989).

Badge-type personal samplers for NO_2 also have been developed by Yanagisawa and Nishimura (1982) (YN) and by Cadoff and Hodgeson (1983) (CH). Triethanolamine is used as the active collecting medium in both samplers, and both use colorimetry as the analytical method for detection of NO_2^-. The samplers differ in that the YN device uses TEA-coated on a cellulose filter, with a Teflon® filter as a diffusion barrier; whereas the CH sampler uses TEA-coated on a glass fiber filter, with a polycarbonate filter as a diffusion barrier. Detection limits are reported to be 0.07 ppm·h (Yanagisawa and Nishimura, 1982) and 0.06 ppm·h (Cadoff and Hodgeson, 1983) for the YN and CH samplers, respectively. Interferences from PAN and HNO_2 are expected (Sickles and Michie, 1987); likewise, the devices are sensitive to the speed of ambient air movement.

Palmes tubes have been developed for NO_2 measurement and adapted to NO measurement as described above. The device has been used for workplace and personal exposure monitoring (Wallace and Ott, 1982) and for ambient air measurements (U.S. Environmental Protection Agency, 1993c). A detection limit of 0.03 ppm·h can be achieved if IC is used to determine the extracted NO_2^- (Mulik and Williams, 1986). Adsorption of NO_2 to the tube walls may raise this limit considerably (Miller, 1988), but this effect can be counteracted by use of stainless steel tubes. The device is sensitive to temperature and wind speed, and PAN and HNO_2 are likely interferences (Sickles and Michie, 1987). In a comparison with two commercially produced NO_2 passive samplers, the Palmes tube showed reasonable accuracy and precision at loadings of 1 to 80 ppm·h. However, the commercial devices were designed for use at relatively high loadings; therefore, this comparison does not support the use of Palmes tubes for ambient air monitoring.

3.5.3.4 Calibration Methods

Calibration of NO measurement methods is done using standard cylinders of NO in nitrogen. Typical NO concentrations in such cylinders are 1 to 50 ppmv. Dilution of such standards with clean air using mass flow controllers can accurately provide NO

concentrations in the ambient (i.e., 1 to 100 ppbv) range for calibration. Nitric oxide standards are available as SRMs from NIST and as commercially available Certified Reference Standards. Commercially available certified NO standards have been shown to be stable and accurate for the specified concentrations.

Standard cylinders of NO_2 in nitrogen or air are sometimes used for NO_2 calibration. These standards are commercially available and are readily diluted to parts-per-billion-volume levels in the same manner as for NO standards. However, instability of the NO_2 levels in such standards has been reported, and caution must be used in relying on NO_2 standards as the primary means of calibration.

Two calibration methods for NO_2 are specified in the Code of Federal Regulations (1987) for calibration of ambient NO_2 measurements: (1) permeation tubes and (2) gas-phase titration.

An NO_2 permeation tube is an inert enclosure, generally of Teflon®, glass and Teflon®, or stainless steel and Teflon®, that contains liquid NO_2. As long as liquid NO_2 is present, NO_2 will permeate through the Teflon® at a rate that depends on the temperature of the tube. Maintaining the permeation tube at a constant temperature (i.e., ± 0.1 °C) results in permeation of NO_2 at a constant rate. Dilution of the emitted NO_2 with a flow of dry air or N_2 results in known low NO_2 concentrations for calibration. Nitrogen dioxide permeation tubes are supplied as SRMs by NIST, and tubes are commercially available with a wide range of permeation rates. Permeation tubes are small, simple, reliable, and relatively inexpensive, although constant temperature ovens and dilution systems are required to obtain good results. Nitrogen dioxide permeation tubes are susceptible to moisture, and changes in permeation rate or emission of other species (HNO_3, HNO_2, NO) may occur if they are not kept dry. As with NO_2 cylinder standards, the NO_2 permeation tube requires care as a calibration method for NO_2.

Gas-phase titration uses the rapid reaction of NO with O_3 to produce NO_2 with 1:1 stoichiometry. In practice, excess NO generated from a standard cylinder containing 50 to 100 ppmv NO is reacted with O_3 from a stable source. The resultant decrease in NO concentration, usually measured on the NO channel of a CL NO_x analyzer, equals the concentration of NO_2 generated. Varying amounts of NO_2 can be produced by varying the amount of O_3.

3.6 Ozone Air Quality Models

To plan control strategies to achieve compliance with the NAAQS for O_3 at some future date, it is necessary to predict how O_3 concentrations change in response to prescribed changes in source emissions of precursor species (NO_x and VOCs). This assessment requires an air quality model, which in the case of O_3 prediction is often called a photochemical air quality model. The model, in effect, is used to determine the emission reductions needed to achieve the O_3 air quality standard. For at least a decade, EPA has offered guidelines on the selection of air quality modeling techniques for use in SIP revisions, new source reviews, and studies aimed at the prevention of significant deterioration of air quality.

It is worth noting the interrelated nature of O_3 and other air quality issues. Ozone, PM_{10}, visibility, and acid deposition are all connected as a result of similar sources and complex chemical mechanisms. Consequently, strategies for O_3 abatement that involve

reductions of VOC and NO_x emissions also will impact particulate matter, visibility, and acid deposition.

Ozone air quality models provide the ability to address "what if" questions, such as, what if emissions of VOCs or NO_x are reduced? The model can be used as an experiment that cannot be run in the atmosphere. Sensitivity questions can be asked, such as, how important is emissions change A relative to emissions change B? or what is the effect of an X% uncertainty in a particular chemical reaction rate constant on the predicted O_3 levels?

Models are the ultimate integrators of our knowledge of the comprehensive chemistry and physics of the atmosphere. As such, they are an indispensable tool for understanding the complex interactions of transport, transformation, and removal in the atmosphere. Models are useful in the design of field measurement programs and are essential in the interpretation of data from such programs.

Models can be verified by the demonstration of agreement between observations and predictions, but confirmation is inherently partial. Verification of mathematical models of natural systems is always incomplete because complete information on the natural system is never available. Furthermore, model results always include some degree of nonuniqueness because model inputs and parameters are never precisely known. Ozone air quality model applications are most reliable in the domain and conditions where model predictions have been evaluated by extensive, valid data and the comparisons of observations and predictions fall within accepted guidelines.

Historically, the primary measure of model performance has been degree of agreement between observed and predicted O_3 concentrations during simulated episodes, although it now is recognized that comparisons of observations and predictions for other compounds, such as organics and NO_y components, are also important in assessing model performance.

The purpose of Section 3.6 is to review briefly the main elements of O_3 air quality models, to describe several of the current models, to discuss the performance evaluation of these models, and to present examples of the use of the models for determining VOC and NO_x control strategies.

3.6.1 Definitions, Description, and Uses

Air quality models are mathematical descriptions of the atmospheric transport, diffusion, removal, and chemical reactions of pollutants. These models operate on sets of input data that characterize the emissions, topography, and meteorology of a region and produce outputs that describe air quality in that region. Mathematical models for photochemical air pollution first were developed in the early 1970s and have been improved, applied, and evaluated since that time. Much of the history of the field is described in reviews by Tesche (1983), Seinfeld (1988), and Roth et al. (1990).

Photochemical air quality models include treatments of the important physical and chemical processes that contribute to O_3 formation in and downwind of urban areas. In particular, such models contain a representation of the following phenomena (Roth et al., 1990):

- Precursor emissions. The spatial and temporal characteristics of reactive hydrocarbon, CO, and NO_x emissions sources must be supplied as inputs to the model. Hydrocarbon emissions generally are apportioned into groups (e.g.,

alkanes, alkenes, aromatics, etc.) according to the speciation requirements of the chemical kinetic mechanism embedded in the model.

- Pollutant transport. Once the O_3 precursors are emitted into the atmosphere, they are transported by the wind. When O_3 is formed, it also is subject to transport by the wind. Grid-based models require the preparation of three-dimensional, time-varying fields of wind speed and direction. These values must be specified for each grid cell. Cloud venting and mixing processes that are important on the regional scale also can be included in the pollutant transport description.
- Turbulent diffusion. Ozone and its precursors also are subject to turbulence-related dispersion processes that take place on a subgrid scale. These turbulent diffusion effects usually are represented in grid-based models by the so-called gradient transport hypothesis, where the pollutant flux is assumed to be proportional to the spatial gradient in the concentration field. The turbulent diffusivities employed in the model are dependent on atmospheric stability and other meteorological variables.
- Chemical reactions. Ozone results from chemical transformations involving reactive organics and NO_x (See Section 3.2). A chemical kinetics mechanism representing the important reactions that occur in the atmosphere is employed to estimate the net rate of change of each pollutant simulated by the model. Description of chemical reactions requires actinic flux, cloud cover, temperature, and relative humidity.
- Removal processes. Pollutants are removed from the atmosphere via interactions with surfaces at the ground, so-called "dry deposition", and by precipitation, called "wet deposition".

Guidelines issued by EPA (U.S. Environmental Protection Agency, 1986b) identify two kinds of photochemical model: (1) the grid-based UAM is the recommended model for modeling O_3 over urban areas, and (2) the trajectory model EKMA is identified as an acceptable approach. The 1990 CAAA (U.S. Congress, 1990) mandate that three-dimensional, or grid-based, air quality models, such as UAM, be used in SIPs for O_3 nonattainment areas designated as extreme, severe, serious, or multistate moderate (U.S. Environmental Protection Agency, 1991b).

3.6.1.1 Grid-Based Models

The basis for grid-based air quality models is the atmospheric diffusion equation that expresses the conservation of mass of each pollutant in a turbulent fluid in which chemical reactions occur (Seinfeld, 1986). The region to be modeled is bounded on the bottom by the ground, on the top by some height that characterizes the maximum extent of vertical mixing, and on the sides by east-west and north-south boundaries. The choice of the size of the modeling domain will depend on the spatial extent of the O_3 problem, including the distribution of emissions in the region, the meteorological conditions, and, to some extent, the computational resources available. This space then is subdivided into a three-dimensional array of grid cells. The horizontal dimensions of each cell are usually a few kilometers for urban applications up to tens of kilometers for regional applications. Some older grid-based models assumed only a single, well-mixed vertical cell extending from the ground to the inversion base; current models subdivide the region into layers. Vertical dimensions can vary, depending on the number of vertical layers and the vertical extent of

the region being modeled. Increasing the vertical resolution in the computation should be accompanied by increased vertical resolution of the physical parameters used. A compromise generally must be reached between the better vertical resolution afforded by the use of more vertical layers and the associated increase in computing time. Although aerometric data, such as the vertical temperature profile, which are needed to define the vertical structure of the atmosphere, are generally lacking, it is still important to use enough vertical layers so that vertical transport processes are represented accurately.

There are practical and theoretical limits to the minimum horizontal grid cell size. Increasing the number of cells increases computing and data acquisition effort and costs. In addition, the choice of the dimension of a grid cell implies that the input data information about winds, turbulence, and emissions, for example, are resolved to that scale. The spatial resolution of the concentrations predicted by a grid-based model corresponds to the size of the grid cell. Thus, effects that have spatial scales smaller than those of the grid cell cannot be resolved. Such effects include the depletion of O_3 by reaction with NO near strong sources of NO_x like roadways and power plants. Ozone predictions are sensitive to the choice of grid cell size. The use of a larger grid tends to smooth out VOC and NO_x precursor concentrations, affecting the computed chemical production of O_3. Multigrid models, in which a region with a finer grid resolution is embedded within a larger grid, are an approach to obtain a better resolution of O_3 formation processes in regions of intense source emissions (Odman and Russell, 1991).

Jang (1992) has examined the sensitivity of O_3 predictions to model grid resolution in regional air quality models. A high-resolution version of the Regional Acid Deposition Model (RADM) was used to simulate O_3 formation over the northeastern United States at different grid resolutions. The high-resolution version of RADM, with horizontal grid cell sizes of 20, 40, and 80 km, was operated within the 80-km RADM domain. Coarser grid sizes were found to result in lower resolved emission intensities of NO_x and VOCs. Because of the smearing effect of the large grid sizes, the coarser grid model tended to underpredict the O_3 highs in the areas downwind of cities and overpredict the O_3 lows in the intense NO_x emissions areas. It was found that the impact of model grid resolution on the chemistry of NO_x is more important than that on the chemistry of VOCs, and that model grid resolution has no significant impact on the total amount of odd oxygen ($O_x = O_3 + NO_2$) produced in the models but has great impact on the interactions of chemistry and transport processes that control the balance of O_x. As a result, the coarser grid model tends to predict higher O_3 and lower NO_2 than does the finer grid model, and the coarser grid tends to transport O_x more in the form of O_3, whereas the finer grid model tends to transport the O_x more in the form of NO_2.

Uncertainties arise in photochemical modeling from the basic model components (chemical mechanism and numerical techniques in solving the governing equations) and from inputs to the simulations that reflect the particular episode (boundary and initial conditions, emission inventory, wind field, and mixing depth). Sensitivity studies aim to determine the range of uncertainty in model predictions corresponding to ranges of uncertainty in the basic model components and input quantities. Such studies are valuable in pinpointing those quantities to which model predictions are most sensitive and, therefore, in directing future efforts in reducing the uncertainty in key parameters. These studies are also valuable in assessing the sensitivity of future air quality changes to uncertainties in the base case episode. It is not possible to state general, widely applicable levels of uncertainty for photochemical model inputs and parameters. These will depend on the particular region being modeled,

and, in the case of meteorological and emissions inputs, may even depend on the time of day during the simulation. All model application exercises should include, to the extent possible, an analysis of the uncertainties in model inputs and parameters.

Several grid-based photochemical air quality models have been developed to simulate O_3 production in urban areas or in larger regions. They differ primarily in their treatment of specific atmospheric processes, such as chemistry, and in the numerical procedures used to solve the governing system of equations. These models will be reviewed in Section 3.6.3.

3.6.1.2 Trajectory Models

In the trajectory model approach, a hypothetical air parcel moves through the area of interest along a path calculated from wind trajectories. Emissions are injected into the air parcel and undergo vertical mixing and chemical transformations. The data requirements for trajectory models include: (1) initial concentrations of all relevant pollutants and species; (2) rates of emissions of VOC and NO_x precursors into the parcel along its trajectory; (3) meteorological characteristics, such as wind speed and direction, needed to define the path of the air parcel through the region; (4) mixing depth; and (5) solar ultraviolet radiation.

The key assumption inherent in the trajectory model is that a hypothetical air parcel maintains its integrity along the trajectory. Almost certainly, the parcel assumption fails at night, when flows drift and the atmosphere stratifies; for hilly or mountainous terrain; and under convergence conditions. Thus, the trajectory model concept does not apply in many areas and under a variety of conditions (Liu and Seinfeld, 1975).

Trajectory models provide a dynamic description of atmospheric source-receptor relationships that is simpler and less expensive to derive than that obtained from grid models. Trajectory models are designed to study the photochemical production of O_3 in the presence of sources and vertical diffusion of pollutants; otherwise the meteorological processes are highly simplified.

A simple trajectory model is used in EKMA (Dodge, 1977a). This modeling approach relates the maximum level of O_3 observed downwind of an urban area to the levels of VOCs and NO_x observed in the urban area. It is based on the use of a simple, one-cell moving box model. As the box moves downwind, it encounters emissions of organics and NO_x that are assumed to be uniformly mixed within the box. The height of the box is allowed to expand to account for the breakup of the nocturnal inversion layer. As the height of the box increases, pollutants above the inversion layer are transported into the box. The model is first used to generate a series of constant O_3 lines (or isopleths) as depicted in Figure 3-25. The isopleths show the downwind, peak 1-h O_3 levels as a function of the concentrations of VOCs and NO_x for a hypothetical urban area. These isopleths were generated by carrying out a large number of model simulations in which the initial concentrations and anthropogenic emissions of VOCs and NO_x were varied systematically, whereas all other model inputs were held constant. When it was first conceived, EKMA employed a very simple, highly empirical chemical mechanism and the isopleths generated were for a hypothetical situation in Los Angeles. As understanding of the chemical processes responsible for O_3 formation increased, the EKMA model was updated to include more complete representations of atmospheric chemistry. Although EKMA has employed the CBM-IV mechanism, the same mechanism that is currently being used in several grid-based models, the most recent version allows the input of any mechanism. The EKMA method is

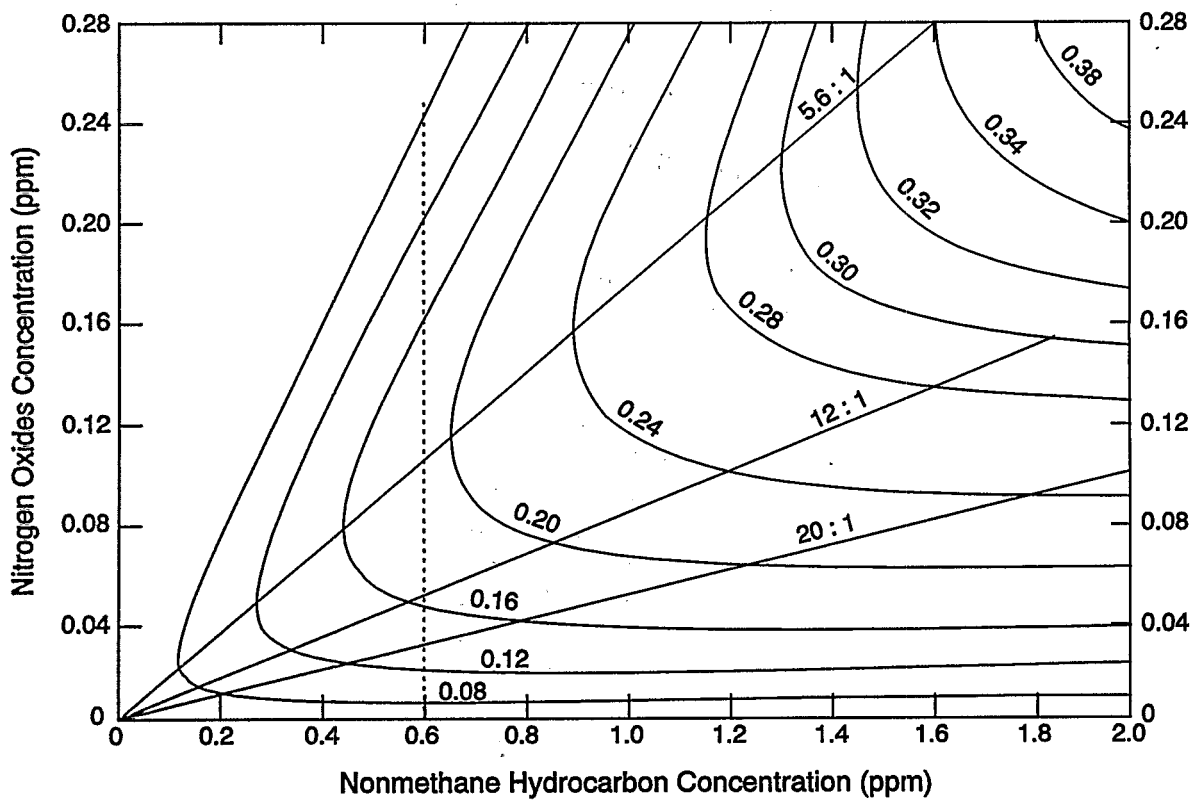


Figure 3-25. Example of Empirical Kinetic Modeling Approach diagram for high-oxidant urban area.

Source: Derived from U.S. Environmental Protection Agency (1986a).

now used to generate city-specific isopleth diagrams using information on emissions, transport, and dilution that are appropriate to the particular city being modeled.

City-specific O_3 isopleths can be used to estimate the reduction in NMHC or NO_x levels needed to achieve the NAAQS for O_3 in a specific urban area. The first step is to determine the early-morning NMHC/NO_x ratio for the urban area in question and the maximum 1-h downwind O_3 concentration. Both the NMHC/NO_x ratio and the peak O_3 concentration are obtained from air monitoring data. These two values define a point on the isopleth surface and, from this point, the percentage reductions in NMHC or NO_x, or both, needed to achieve the O_3 NAAQS can be determined.

As examination of Figure 3-25 reveals, for an NMHC concentration of 0.6 ppmC, for example, increasing NO_x leads to increased O_3 until NMHC/NO_x ratios of about 5:1 to 6:1 are reached; further NO_x increases, leading to lower NMHC/NO_x ratios, inhibit O_3 formation. Thus, in this example, there is a "critical" ratio (in the range of 5:1 to 6:1) at which the NO_x effect on O_3 changes direction. Besides this "critical" ratio, an "equal control" NMHC/NO_x ratio also exists, above which the reduction of NO_x is more beneficial in terms of O_3 reduction than an equal percentage reduction in NMHC. This ratio, for the

isopleths shown in Figure 3-25, is roughly 8:1 to 9:1 for low levels of control and as high as 20:1 for the levels of control needed to reduce O_3 to 0.12 ppm. Thus, for this particular case (Figure 3-25), the chemical mechanism modeling evidence suggests that NO_x control will increase the peak downwind O_3 concentration at NMHC/NO_x ratios of between 5:1 and 6:1 or lower; both NO_x control and NMHC control will be beneficial at somewhat higher ratios, with control of NMHC being more effective; and, for ratios above 20:1, NO_x control is relatively more effective in reducing O_3 to attain the O_3 NAAQS.

The EKMA-based method for determining control strategies has some limitations, the most serious of which is that predicted emissions reductions are critically dependent on the initial NMHC/NO_x ratio used in the calculations. This ratio cannot be determined with any certainty because it is expected to be quite variable in time and space in an urban area. Another limitation is that trajectory models have limited spatial and temporal scopes of application. They are generally 1-day models, simulating only one cell at a time. Another problem with the use of morning NMHC/NO_x ratios is the failure to account for photochemical evolution as urban emissions are carried downwind. As demonstrated in simulations by Milford et al. (1989) and in smog chamber studies by Johnson and Quigley (1989), an urban plume that is in the VOC-controlling regime (low NMHC/NO_x ratio) near city center can move increasingly into the NO_x-controlling regime (high NMHC/NO_x ratio) as the air parcels age and move downwind. This progression occurs because NO_x is photochemically removed from an aging plume more rapidly than VOCs, causing the VOC/NO_x ratio to increase. As demonstrated by Milford et al. (1989), the implication of this evolution is that different locations in a large urban area can show very different O_3 sensitivities to VOC and NO_x changes. Because of this and other drawbacks, the 1990 CAAA (U.S. Congress, 1990) require that grid-based models be used in most O_3 nonattainment areas.

3.6.2 Model Components

3.6.2.1 Emissions Inventory

The spatial and temporal characteristics of VOC and NO_x emissions must be supplied as inputs to a photochemical air quality model. Emissions from area and point sources are injected into ground-level grid cells, and emissions from large point sources are injected into upper level cells. Total VOC emissions generally are apportioned into groups of chemically similar species (e.g., alkanes, alkenes, aromatics, etc.) according to the requirements of the chemical mechanism. This apportionment may be accomplished using actual emission sampling and analysis or be based on studies of similar emission sources. Recognition of potential undercounting in existing inventories has spurred efforts to improve the accuracy of emissions inventories. In fact, at present, the emissions inventory is the most rapidly changing component of photochemical models. It has been recognized that both mobile and stationary source components have been highly uncertain and that there is significant ongoing effort to improve the accuracy of emissions inventories.

Some emissions terminology is as follows (Tesche, 1992):

- Emissions data—the primary information used as input to emissions models.
- Emissions model—the integrated collection of calculational procedures, or algorithms, properly encoded for computer-based computation.
- Emissions estimates—the output of emissions models; used as input to photochemical models.

- Emissions inventory—the aggregated set of emissions estimate files.
- Emissions model evaluation—the testing of a model's ability to produce accurate emissions estimates over a range of source activity and physicochemical and meteorological conditions.

Emissions input requirements for the UAM, for example, include:

- Spatial allocation of precursor emissions (VOCs, NO_x, CO):
  - Actual location of individual point sources;
  - Spatial allocation by gridding surrogates;
  - Assignment of surrogates to other categories.
- Stack parameters for point sources:
  - Temperature, height, diameter, and exit velocity.
- Speciation of VOC emissions for CBM-IV mechanism:
  - Region-specific speciation profiles;
  - EPA default speciation profiles.
- Temporal allocation of precursor emissions:
  - Operating schedules for individual point sources;
  - Assignment of diurnal profiles for area and mobile sources.

The emissions inventory component of modeling is moving in the direction of the use of emissions models rather than inventories. Emissions models are being developed for the Lake Michigan Oxidant Study (LMOS), the San Joaquin Valley Air Quality Study (SJVAQS), and the Atmospheric Utility Signatures, Predictions, and Experiments (AUSPEX), designated as the SJVAQS/AUSPEX Regional Model Adaptation Project (SARMAP). The consistency of existing inventories was improved in 1990 when EPA released the Emissions Preprocessor System (EPS) as a component of the UAM (U.S. Environmental Protection Agency, 1992b). The EPS was updated in 1992 to EPS Version 2 (EPS2). It is an emissions model that considers spatial and temporal disaggregation factors, speciation data, and meteorological data to convert daily emissions estimates for each point source and for area source categories and mobile source emissions factors computed by the EPA MOBILE5 model into hourly, gridded speciated estimates that are needed by a photochemical grid model.

A step beyond the EPS is the Emissions Modeling System (EMS)[1] (Tesche, 1992). The EMS utilizes emissions estimation and information processing methods to provide gridded, temporally resolved, and chemically speciated base-year emissions estimates for all relevant source categories; to provide flexibility in forecasts of future-year emissions rates; and to provide modular code design for use in module updating and replacement. The EMS provides for easy substitution of alternative assumptions, theories, or input parameters (e.g., emissions factors, activity levels, spatial distributions) and facilitates sensitivity and uncertainty testing.

As a result of a variety of independent studies, it recently has been determined that urban VOC emissions inventories, particularly motor vehicle emissions, have been significantly understated. These studies include tunnel studies (Pierson et al., 1990) and comparisons of ambient and emission inventory VOC/NO_x ratios (Fujita et al., 1992).

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[1]The EMS has been renamed the GMEP (Geocoded Model of Emissions and Projections).

3.6.2.2 Meteorological Input to Air Quality Models

Grid-based air quality models require, as input, the three-dimensional wind field for the episode being simulated. This input is supplied by a so-called meteorological module. Meteorological modules for constructing wind fields for air quality models fall into one of four categories (Tesche, 1987; Kessler, 1988):

(1) Objective analysis procedures that interpolate observed surface and aloft wind speed and direction data throughout the modeling domain;
(2) Diagnostic methods in which the mass continuity equation is solved to determine the wind field;
(3) Dynamic, or prognostic, methods based on numerical solution of the governing equations for mass, momentum, energy, and moisture conservation, along with the thermodynamic state equations on a three-dimensional, finite-difference mesh; or
(4) Hybrid methods that embody elements from both diagnostic and prognostic approaches.

Objective Analysis

Objective wind-field analysis involves the interpolation and extrapolation of wind speed and direction measurements (collected at a number of unequally spaced monitoring stations) to grid points throughout the region (Goodin et al., 1980). For flat terrain settings away from complex mesoscale forcings, this class of techniques may provide an adequate method for estimating the wind field, provided that appropriate weighting and smoothing functions are used (Haltiner, 1971). For complex terrain or coastal/lake environments, however, it is tenuous to interpolate between and extrapolate from surface observational sites except with an unusually dense monitoring network. In most cases, the routinely available rawinsonde network sounding data are even more severely limited because of the large distances (300 to 500 km) between sites and because soundings are made only every 12 h. The limitations of even the best available data sets are most severe above the surface layer, where upper level observations are less frequent and more expensive to obtain. It will remain economically unfeasible to obtain sufficiently dense atmospheric observations to allow any direct objective analysis scheme to provide the required detail and accuracy necessary for use in advanced, high-resolution photochemical models.

Diagnostic Modeling

In diagnostic wind modeling, the kinematic details of the flow are estimated by solving the mass conservation equation. Dynamic interactions such as turbulence production and dissipation and the effects of pressure gradients are parameterized. Various diagnostic wind models have been developed, many employing the concepts introduced by Sherman (1978) and Yocke (1981).

In recent years, attempts have been made to combine the best features of objective analysis and pure diagnostic wind modeling. The current release of EPA's UAM-IV includes the Diagnostic Wind Model (DWM) as the suggested wind-field generator for this urban-scale photochemical model. The DWM (U.S. Environmental Protection Agency, 1990c) is representative of this class of hybrid objective-diagnostic models. The DWM combines the features of the Complex Terrain Wind Model (CTWM) (Yocke, 1981) and the objective wind interpolation code developed at the California Institute of Technology (Goodin et al., 1980). In the DWM, a two-step procedure normally is followed. First, a "domain-scale"

wind is estimated from available surface and upper-air synoptic data. This initial field consists of a single wind vector (e.g., horizontal homogeneity) for each elevation. The domain-scale wind is adjusted using procedures derived from the CTWM for the kinematic effects of terrain such as lifting, blocking, and flow acceleration. Thermodynamically generated influences such as mountain-valley winds are parameterized. This first step produces a horizontally varying field of wind speed and direction for each vertical layer within the DWM modeling domain. Typically, 10 to 12 vertical layers are used. In the second step, available hourly surface and upper air measurements are combined objectively with the step 1 hourly diagnostic flow fields to produce a resultant wind field that matches the observations at the monitoring points and obeys the general constraints of topography in regions where data are absent. The DWM contains a number of user-specified options whereby different final flow fields may be produced, depending on selection of various smoothing and weighting parameters. The final output of the DWM is a set of hourly averaged horizontal wind fields for each model layer.

Diagnostic models may invoke scaling algorithms that propagate the influence of the surface-flow field into upper levels according to the local height of the inversion and the Pasquill-Gifford-Turner stability category for the hour. Once the winds are created by DWM, they must be "mapped" onto the photochemical model's vertical grid structure. This function is normally accomplished in a two-step process. First, the DWM winds are interpolated onto the photochemical model grid using simple linear interpolation. Second, the three-dimensional divergence is computed in each grid cell and an iterative scheme is used to minimize this divergence to a user-specified level. Typically, the output consists of "nondivergent" x- and y-direction wind components for direct input to the photochemical model.

Among the advantages of the diagnostic modeling approach are its intuitive appeal and modest computing requirements. The method generally reproduces the observed wind values at the monitoring locations and provides some information on terrain-induced airflows in regions where local observations are absent. In addition, diagnostic model parameters for a particular locale based on site-specific field measurements may be calibrated. However, there are several disadvantages. Diagnostic models cannot represent complex mesoscale circulations, unless these features are well represented by surface and aloft observations. Often the vertical velocities produced by a diagnostic model are unrealistic and, in regions of complex terrain, local horizontal flow velocities often may be an order of magnitude too high (Tesche et al., 1987). Because the diagnostic model is not time-dependent, there is no inherent dynamic consistency in the winds from one hour to the next. That is, calculation of the flow field at 1200 hours, for example, is not influenced by the results of the 1100-hour winds. This is a particular problem in applications involving important flow regimes, such as land-sea breezes, mountain-valley winds, eddy circulations, and nocturnal valley jets, that take several hours to develop and whose three-dimensional character is poorly characterized by even the most intensive sampling networks. Finally, the inadequacy of the upper-air synoptic data causes significant difficulty in the validation of the model wind fields.

Prognostic Modeling

In prognostic meteorological modeling, atmospheric fields are computed based on numerical solutions of the coupled, nonlinear conservation equations of mass, momentum, energy, and moisture. Derivations of these equations are presented extensively in the literature (Haltiner, 1971; Pielke, 1984; Seinfeld, 1986; Cotton and Anthes, 1989). Many

prognostic models have been developed for computing mesoscale wind fields, as shown in the recent survey by Pielke (1989), and they have been applied to a variety of problems, including the study of land-sea and land-lake circulations. Available prognostic models range from relatively simple one-dimensional representations to complex three-dimensional codes.

Prognostic wind models are attractive because they explicitly address the various physical processes governing atmospheric flows. Consequently, they have the potential for describing a number of wind regimes that are particularly relevant to air pollution modeling, such as flow reversal, daytime upslope flows, wind shear, and other mesoscale thermally induced circulations. Drawbacks of prognostic models include the need to gather detailed data for model performance testing and the large computational costs. Indeed, prognostic models may require as much or more computer time than regional-scale photochemical models. More intensive data sets are needed to evaluate prognostic models than for diagnostic models, but this is not necessarily a disadvantage. Rather, it provides the modeler and decision-maker with a far better basis for judging the adequacy of the model than can be achieved with objective or diagnostic models.

Summaries of prognostic models available for use in air quality modeling are presented extensively in the literature (e.g., Pielke, 1989; Benjamin and Seaman, 1985; McNally, 1990; Stauffer et al., 1985; Stauffer and Seaman, 1990; Ulrickson, 1988; Wang and Warner, 1988; and Yamada et al., 1989). From these reviews, two models stand out as representing the present state-of-science in applications-oriented prognostic modeling. These are the Mesoscale Model Versions 4 and 5 (MM4/MM5) developed by Pennsylvania State University and the National Center for Atmospheric Research (NCAR) (Anthes and Warner, 1978; Anthes et al., 1987; Zhang et al., 1986; Seaman, 1990; Stauffer and Seaman, 1990), and the Coast and Lake Regional Atmospheric Modeling System (CAL-RAMS) (Tripoli and Cotton, 1982; Pielke, 1974, 1984, 1989; Lyons et al., 1991).

Three ongoing regional O_3 modeling programs in the United States are using prognostic models to drive regional O_3 models. These include LMOS; SARMAP; and a regional O_3 modeling program in Southeast Michigan, Northern Ohio, and Southwest Ontario. Part of EPA's long-range plan (in the Office of Research and Development [ORD]) for model development is to construct a "third" generation modeling framework referred to as MODELS 3 (Dennis and Novak, 1992). This modeling system will consolidate all of the agency's three-dimensional models. The current plan calls for meteorological inputs to the MODELS 3 system to be supplied by prognostic models. The MM4 model (the hydrostatic version of MM5) is presently being examined by EPA for this purpose.

Activities are currently underway in LMOS to supply prognostic model fields to EPA's ROM for use in simulating regional O_3 distributions in four multiple-day O_3 episodes extensively monitored during the 1991 field program in the midwest. The EPA will be utilizing ROM2.2 (version 2.2) with fields obtained from CAL-RAMS (Lyons et al., 1991) to examine whether prognostic model output gives improved regional O_3 estimates (Guinnup and Possiel, 1991).

The SARMAP program is the modeling and data analysis component of a multiyear collaboration between two projects, SJVAQS and AUSPEX. The major near-term objective of SARMAP is to understand the processes that lead to high O_3 concentrations in the San Joaquin Valley of California. An overview of the regional meteorological and air quality modeling approach of SARMAP is described by Tesche (1993). For SARMAP, the MM5 model was chosen as the "platform" prognostic meteorological model because of its broad application history; its demonstrated reliability on large domains, requiring spatially

and temporally varying boundary conditions; and its capability for four-dimensional data assimilation (FDDA)—needed for longer-range simulations (see Section 3.6.2.2). All of these attributes are crucial to the success of mesoscale meteorological modeling.

Prognostic models are believed to provide a dynamically consistent, physically realistic, three-dimensional representation of the wind and other meteorological variables at scales of motion not resolvable by available observations. However, the meteorological fields generated by a prognostic model do not always agree with observational data. Numerical approximations, physical parameterizations, and initialization problems are among the potential sources of error growth in model forecasts that can cause model solutions to deviate from actual atmospheric behavior. Methods that have been devised over the past 20 years to mitigate these problems are described below.

"Post-processing" refers to methods whereby output fields from prognostic models are selectively adjusted through a series of objective techniques with the aim of improving the realism of the resultant fields. Examples of this procedure (sometimes referred to as objective combination) are given by Cassmassi et al. (1991) in the Los Angeles Basin, Kessler and Douglas (1989) in the South Central Coast Air Basin, and Moore et al. (1987) in the San Joaquin Valley.

Ideally, a prognostic model should be initialized with spatially varying, three-dimensional fields (i.e., wind, temperature, moisture) that represent the state of the atmosphere at the initial simulation time. A prognostic model that is initialized with such fields, however, can generate large nonmeteorological "waves" when the initial conditions do not contain a dynamic balance consistent with the model formulation (Hoke and Anthes, 1976; Errico and Bates, 1988). The objective of an initialization procedure is to bring the initial conditions into dynamic balance so that the model can integrate forward with a minimum of noise and a maximum of accuracy (Haltiner and Williams, 1980). Dynamic initialization makes use of a model's inherent adjustment mechanism to bring the wind and temperature into balance prior to the initial simulation time. In this technique, a "presimulation" integration of the model equations produces a set of dynamically balanced initial conditions. By allowing the simulation to begin with a balanced initial state, this technique reduces the generation of meteorological noise and thus improves the quality of the simulation.

Four-Dimensional Data-Assimilation Techniques

Four-dimensional data assimilation refers to a class of procedures in which observational data are used to enhance the quality of meteorological model predictions (Harms et al., 1992). The most common use of FDDA today in applications-oriented models is known as Newtonian relaxation, or simply as "nudging". With this method, model estimates at a particular time interval are adjusted toward the observations by adding artificial tendency terms to the governing prognostic equations. The objective of this method is to improve prognostic model estimates through the use of valid, representative observational data. As an example of this procedure, a linear term is added to the momentum equations to "nudge" the dynamic calculation towards the observed state at each time step in regions where data are available. The FDDA procedures may be thought of as the joint use of a dynamic meteorological model in conjunction with observed data (or analysis fields based on these data) in such a manner that the prognostic equations provide temporal continuity and dynamic coupling of the hourly fields of monitored data (Seaman, 1990).

A recent example of the use of FDDA in regional-scale applications with the MM4/RADM model is given by Stauffer and Seaman (1990). Attempts to apply FDDA in support of urban-scale photochemical grid modeling are described by Tesche et al. (1990b) and McNally (1990) for the San Diego Air Basin and by Stauffer et al. (1993) for the Grand Canyon region of Arizona. Currently, FDDA is being used in the CAL-RAMS simulations in the LMOS program (Lyons et al., 1991) and in the MM5 simulations for SARMAP (Seaman, 1992).

3.6.2.3 Chemical Mechanisms

A chemical kinetic mechanism (a set of chemical reactions), representing the important reactions that occur in the atmosphere, is used in an air quality model to estimate the net rate of formation of each pollutant simulated as a function of time.

Various grid models employ different chemical mechanisms. Because so many VOCs participate in atmospheric chemical reactions, chemical mechanisms that explicitly treat each individual VOC component are too lengthy to be incorporated into three-dimensional atmospheric models. "Lumped" mechanisms are therefore used (e.g., Lurmann et al., 1986; Gery et al., 1989; Carter, 1990; Stockwell et al., 1990). These lumped mechanisms are highly condensed and do not have the ability to follow explicit chemistry because of this lumping. Lumped-molecule mechanisms group VOCs by chemical classes (alkanes, alkenes, aromatics, etc.). Lumped-structure mechanisms group VOCs according to carbon structures within molecules. In both cases, either a generalized (hypothetical) or surrogate (actual) species represents all species within a class. Organic product and radical chemistry is limited to a few generic compounds to represent all products; thus, chemistry after the first oxidation step is overly uniform. Some mechanisms do not conserve carbon and nitrogen mass. Some molecules do not easily "fit" the classes used in the reduced mechanisms. Because different chemical mechanisms follow different approaches to lumping, and because the developers of the mechanisms made different assumptions about how to represent chemical processes that are not well understood, models can produce somewhat different results under similar conditions (Dodge, 1989).

No single chemical mechanism is currently considered "best". Both UAM-IV and ROM utilize the carbon-bond mechanism (CBM-IV), which, along with the SAPRC (Statewide Air Pollution Research Center, University of California, Riverside) and RADM mechanisms, is considered to represent the state of the science (Tesche et al., 1993; National Research Council, 1991). Agreement among mechanisms is better for O_3 than for other secondary pollutants (Dodge, 1989, 1990; National Research Council, 1991), raising concern that the mechanisms may suffer from compensating errors. These mechanisms are at least 5 years old and often are tested on much older smog chamber data.

The chemical mechanisms used in existing photochemical O_3 models contain uncertainties that may limit the accuracy of the model predictions. The reactions that are included in these mechanisms generally fall into one of three categories.

(1) Reactions for which the magnitude of their rate constants and their product distribution is well known. These include mostly the inorganic reactions and those for the simple carbonyls.

(2) Reactions with known rate constants and known products but with uncertain product yields. These are mostly organic reactions, and the actual product yields assumed may vary among mechanisms.

(3) Reactions with known rate constants but unknown products. Each mechanism assumes its own set of products for reactions in this class. This class includes aromatic oxidation reactions.

Most inorganic gas-phase processes are understood. Regarding classes of VOCs the following general comments can be made:

- The *n*-alkanes comprise approximately one-half of the major carbon emissions in urban areas. Reaction rates are relatively slow. The only important reaction is with the hydroxyl radical. For alkanes C_4 or below, the chemistry is well understood and the reaction rates are slow. For C_5 and higher alkanes the situation is more complex because few reaction products have been found.
- Branched-chain alkanes have rates of reaction that are highly dependent on structure. Rate constants have been measured for only a few of the branched alkanes, and reaction products for this class of organics are not well characterized.
- Alkenes are reactive with OH, O_3, and the NO_3 radical. Most rate constants of these reactions are known. Alkenes make up $\leq 15\%$ of the emitted carbon and constitute about 25% of the hydrocarbon reactions in urban areas. Ozone reaction products are not well characterized, and the mechanisms are poorly understood. Mechanisms for the NO_3 radical are also uncertain.
- Aromatics constitute about 15 to 20% of the carbon compounds emitted and 25% of the hydrocarbons reacting in urban areas. Aromatics have been studied frequently, but only a few reaction products have been well characterized. Aromatics act as strong NO_x sinks under low NO_x conditions.

Mechanisms used in photochemical air quality models thus have uncertainties, largely attributable to a lack of fundamental data on products and product yields. The missing information necessitates that assumptions be made. Current mechanisms provide acceptable overall simulation of O_3 generation in smog chamber experiments. Specific VOCs may, however, be simulated poorly, and products other than O_3 may not be simulated accurately. Existing mechanisms are mostly applicable to single-day, high NO_x conditions because those are the conditions of almost all smog chamber experiments. Low NO_x condition simulations are verified less thoroughly. Fundamental kinetic data are needed on the photooxidation of aromatics, higher alkanes, and higher alkenes to fill in areas of uncertainty in current mechanisms. Whereas these uncertainties are important and require continued research to remove, the uncertainties are likely not such that general conclusions about the relative roles of hydrocarbons and NO_x in O_3 formation will be changed by new data.

3.6.2.4 Deposition Processes

Species are removed from the atmosphere by interaction with ground-level surfaces, so-called dry deposition, and by absorption into airborne water droplets followed by transport of the water droplets, wet deposition. Dry deposition is an important removal process for ozone and other species on both the urban and regional scales and is included in all urban and regional scale models as a contribution to the ground-level flux of pollutants. Wet deposition is a key removal process for gaseous species on the regional scale and is included in regional scale acid deposition models. Urban-scale photochemical models generally have not included a treatment of wet deposition as O_3 episodes do not occur during periods of significant clouds or rain.

Dry Deposition

It is generally impractical to simulate, in explicit detail, the complex of multiple physical and chemical pathways that result in dry deposition to individual surface elements. Because of this, the usual practice has been to adopt simple parameterizations that consolidate the multitude of complex processes. For example, it generally is assumed that the dry deposition flux is proportional to the local pollutant concentration [at a known reference height (z_r), typically 10 m], resulting in the expression $F = -v_d C$, where F represents the dry deposition flux (the amount of pollutant depositing to a unit surface area per unit time), and C is the local pollutant concentration at the reference height. The proportionality constant, v_d, has units of length per unit time and is known as the deposition velocity.

It is customary to interpret the dry deposition process in terms of the electrical resistance analogy, where transport of material to the surface is assumed to be governed by three resistances in series: (1) the aerodynamic resistance (r_a), (2) the quasi-laminar layer resistance (r_b), and (3) the surface or canopy resistance (r_s) (Wu et al., 1992). The aerodynamic resistance characterizes the turbulent transport through the atmosphere from reference height z_r down to a thin layer of stagnant air very near the surface. The molecular-scale diffusive transport across the thin quasi-laminar sublayer near the surface is characterized by r_b. The chemical interaction between the surface and the pollutant of interest once the gas molecules have reached the surface is characterized by r_c. The total resistance (r_t) is the sum of the three individual resistances, and is, by definition, the inverse of the deposition velocity, $1/v_d = r_t = r_a + r_b + r_s$. Note that the deposition velocity is small when any one of the resistances is large. Hence, either meteorological factors or the chemical interactions on the surface can govern the rate of dry deposition.

Dry deposition velocities of HNO_3 and SO_2 are typically ≈ 2 cm s^{-1}, and those of O_3 and PAN are generally ≈ 0.5 and ≈ 1 cm s^{-1}, respectively (Dolske and Gatz, 1985; Colbeck and Harrison, 1985; Huebert and Robert, 1985; Shepson et al., 1992). With a 1 km-deep inversion or boundary layer, the time scale for dry deposition is on the order of 1 day for a deposition velocity of 1 cm s^{-1}. Dry deposition is important for those chemicals with high or fairly high deposition velocities and long or fairly long lifetimes (≥ 10 days) due to photolysis and chemical reaction (for example, HNO_3, SO_2, and H_2O_2, as well as O_3 and PAN).

A number of researchers have reviewed the deposition literature and provided summaries of deposition velocity data. The rank ordering of deposition velocity values among pollutant species based on several such studies is summarized as follows:

<u>McRae and Russell (1984):</u>
$HNO_3 > SO_2 > NO_2 \approx O_3 > PAN > NO$;
<u>Derwent and Hov (1988):</u>
$HNO_3 > SO_2 = O_3 > NO_2 > PAN$;
<u>McRae et al. (1982b):</u>
$O_3 > NO_2 > PAN > NO > CO$; and
<u>Chang et al. (1987):</u>
$HNO_3 > H_2O_2 > NH_3 > HCHO > O_3 = SO_2 = NO_2 = NO > RCHO$.

There is general agreement that HNO_3 is removed at the highest observed rates, which is consistent with the relative deposition rates observed by Huebert and Robert (1985). Most of the surveys are roughly consistent with the relative deposition velocity ordering seen in the experiments of Hill and Chamberlain (1976): diffusion-limited acids $> SO_2 > NO_2 \approx O_3 > PAN > NO > CO$. This suggests surface resistance values should be ordered

approximately as CO > NO > PAN > $O_3 \approx NO_2 > SO_2 > HNO_3 = 0$. However, there is still a substantial range of variability in reported deposition velocities. For example, McKeen et al. (1991) calculated dry deposition velocities for HNO_3, O_3, and PAN of 10, 0.5, and 0.3 cm s^{-1}, respectively. Note that a large deposition velocity for HNO_3 will limit the lifetime of HNO_3 relative to O_3 in photochemically aged air.

There are a significant number of other gases for which there are no surface resistance data and for which values must be estimated using expert judgment. The values should be consistent with the existing experimental values for vegetative surfaces and should preserve the apparent rank ordering among the pollutant species (discussed above). For O_3, surface resistance values by land-use type and season recommended by Sheih et al. (1986) and Wesely (1988) are appropriate. For NO, NO_2, NH_3, H_2O_2, HCHO, and CH_3CHO, the surface resistance values for each land use can be estimated from that for SO_2 (Wesely, 1988), except different proportionality factors should be used for NO and NO_2.

The treatment of dry deposition is perhaps the most primitive of the scientific modules in photochemical air quality models. Knowledge of deposition rates is limited, and uncertainties in deposition velocities are high. For travel times of one to several days, the quantities of pollutants that are predicted to be removed by dry deposition can be substantial for those species with appreciable deposition velocities. Setting all species deposition velocities to zero in a model provides an indication of the importance of dry deposition relative to other processes influencing pollutant dynamics. Further effort to describe the dynamics of deposition are needed, together with evaluation against available data that can be used to test deposition modules.

Wet Deposition

Wet deposition refers to the removal of gases and particles from the atmosphere by precipitation events, through incorporation of gases and particles into rain, cloud, and fog water followed by precipitation at the earth's surface. Removal of gases and particles during snow falls is also wet deposition. Wet removal of gases arises from equilibrium partitioning of the chemical between the gas and aqueous phases (Bidleman, 1988; Mackay, 1991). This partitioning can be defined by means of a washout ratio, Wg, with Wg = [C]rain/[C]air, where [C]rain and [C]air are the concentrations of the chemical in the aqueous and gas phases, respectively. Because Wg is the inverse of the air/water partition coefficient, K_{aw}, then Wg = RT/H, where R is the gas constant, T is the temperature, and H is the Henry's Law constant (Mackay, 1991).

Particles and particle-associated chemicals are efficiently removed from the atmosphere by precipitation events, and the washout ratios for particles, Wp, are typically in the range 10^4 to 10^6 (Eisenreich et al., 1981; Bidleman, 1988). Wet deposition is important for particles (and particle-associated chemicals) and for those gas-phase compounds with washout ratios of Wg $\geq 10^4$. Examples of such gaseous chemicals are HNO_3, H_2O_2, phenol, and cresols, all of which are highly soluble in water. Formaldehyde is present in the aqueous phase as the glycol, $H_2C(OH)_2$, and has an effective washout ratio of 7×10^3 at 298 K (Betterton and Hoffmann, 1988; Zhou and Mopper, 1990). Note that the importance of wet deposition may depend on whether the chemical is present in the gas phase or is particle-associated. For example, the gas-phase alkanes have low values of Wg and are inefficiently removed by wet deposition, whereas the particle-associated alkanes are efficiently removed by wet deposition (Bidleman, 1988), through removal of the host particles.

3.6.2.5 Boundary and Initial Conditions

When a grid-based photochemical model is applied to simulate a past pollution episode, it is necessary to specify the concentration fields of all the species computed by the model at the beginning of the simulation. These concentration fields are called the initial conditions. Throughout the simulation, it is necessary to specify the species concentrations, called the boundary conditions, in the air entering the three-dimensional geographic domain.

Three general approaches for specifying boundary conditions for urban-scale applications can be identified: (1) use the output from a regional-scale photochemical model, (2) use objective or interpolative techniques with ambient observational data, or (3) use default regional background values and expand the area that is modeled for urban areas sufficiently isolated from significant upwind sources.

In the ideal case, observed data would provide information about the concentrations for all the predicted species at the model's boundaries. An alternative approach is to use regional models to set boundary and initial conditions. This is, in fact, preferred when changes in these conditions are to be forecast. In any event, simulation studies should use boundaries that are far enough from the major source areas of the region that concentrations approaching regional values can be used for the upwind boundary conditions. Boundary conditions at the top of the area that is being modeled should use measurements taken from aloft whenever they are available. Regional background values often are used in lieu of measurements. An emerging technique for specifying boundary conditions is the use of a nested grid, in which concentrations from a larger, coarse grid are used as boundary conditions for a smaller, nested grid with finer resolution. This technique reduces computational requirements compared to those of a single-size, fine-resolution grid.

Initial conditions are determined mainly with ambient measurements, either from routinely collected data or from special studies. Where spatial coverage with data is sparse, interpolation can be used to distribute the surface ambient measurements. Because few measurements of air quality data are made aloft, it generally is assumed that species concentrations are initially uniform in the mixed layer and above it. To ensure that the initial conditions do not dominate the performance statistics, model performance should not be assessed until the effects of the initial conditions have been swept out of the grid.

3.6.2.6 Numerical Methods

The core of a grid-based O_3 air quality model is the numerical solution of the three-dimensional atmospheric diffusion equation (McRae et al., 1982c). The central numerical schemes involve horizontal advection and simultaneous vertical mixing, advection, and chemistry. A possible source of model inaccuracy is the numerical method used to solve the governing equations. The solution of chemical kinetics is generally the most computationally intensive step in O_3 air quality models. To compute the rate of chemical reaction one essentially must solve a system of stiff nonlinear ordinary differential equations. The desirable characteristics of the integration routine are speed and stability, at a certain prescribed level of accuracy. The chemistry integration routines used in several ozone air quality models are based on the implicit, hybrid, exponential scheme developed by Young and Boris (1977). The integration is stable, efficient, and sufficiently accurate. It has been concluded from several studies that the numerical solution of the vertical/chemical portion of the model is less likely to be a source of O_3 prediction inaccuracy (Odman et al., 1992) than the horizontal advection, numerical method (McRae et al., 1982; Chock, 1985, 1991; Dabdub and Seinfeld, 1994). Although the horizontal transport computations typically

consume only a small fraction of the total computer time, it is well known that numerical diffusion and dispersion degrade the computed solution and that available methods differ greatly in their numerical performance in this regard (Chock, 1985, 1991; Dabdub and Seinfeld, 1994). Continued work on optimizing the numerical methods used in O_3 air quality models is necessary.

3.6.3 Urban and Regional Ozone Air Quality Models

Several grid-based models have been widely used to evaluate O_3 and acid deposition control strategies.

- The Urban Airshed Model, developed by Systems Applications, Inc., has been, and continues to be, applied to urban areas throughout the country. It is described in Section 3.6.3.1. The current EPA-approved version is UAM-IV. The UAM-V, which has been developed for LMOS, is a nested regional-scale model.
- The California Institute of Technology (CIT) model has been applied to California's South Coast Air Basin (McRae et al., 1982a,b; McRae and Seinfeld, 1983; Milford et al., 1989; Harley et al., 1993).
- The ROM, developed by EPA, has been applied to the northeastern and southeastern United States (Schere and Wayland, 1989a,b). It is described in Section 3.6.3.2.
- The Acid Deposition and Oxidant Model (ADOM) was developed by ENSR Consulting and Engineering for the Ontario Ministry of the Environment and Environment Canada (Venkatram et al., 1988) and the German Umweltbundesamt. Its primary application has been to acidic deposition.
- The RADM was developed by NCAR and the State University of New York for NAPAP. The primary objective of RADM applications is the calculation of changes in sulfur and nitrogen deposition over the eastern United States and southeastern Canada, resulting from changes in emissions (National Acid Precipitation Assessment Program, 1989). See Section 3.6.3.3 for a description of RADM.

A summary of the major applications of the above air quality models, including the Sulfur Transport Eulerian Model (STEM-II), is presented in Table 3-21. All of the models are based nominally on a 1-h time resolution. The horizontal spatial resolutions vary from 5 to 120 km. Typical spatial resolutions used in past model applications are summarized in Table 3-22. It is important to note that the spatial scale at which a model is applied is governed by the manner in which physical processes are treated and the spatial scale of the inputs. The regional models can have a vertical resolution on the order of 10 to 15 layers extending up to 6 to 10 km in order to treat vertical redistribution of species above the planetary boundary layer. This increased vertical resolution often comes at the expense of decreased horizontal resolution. Urban models typically have two to five layers extending up to 1,000 to 2,000 m. The treatment of meteorological fields by the six models is summarized in Table 3-23. Generally, the treatment of meteorology is separate from the air quality model itself, and models can employ wind fields prepared by different approaches as long as consistent assumptions, such as nondivergent wind field, are employed in each model. The regional models, ROM, RADM, ADOM, and STEM-II, address the vertical redistribution of pollutants resulting from the presence of cumulus clouds.

Table 3-21. Grid-Based Urban and Regional Air Pollution Models: Overview of Three-Dimensional Air Quality Models[a]

Model	Major Applications	Major References for Model Formulation	Selected References for Model Performance Evaluation and Application
UAM	Urban and nonurban areas in the United States and Europe	Reynolds et al. (1973, 1974, 1979) Tesche et al. (1992) U.S. Environmental Protection Agency (1990a,b,c; 1992b) Scheffe and Morris (1993)	Tesche et al. (1993)
CIT	Los Angeles Basin	McRae et al. (1982a)	McRae and Seinfeld (1983) Russell et al. (1988a,b) Harley et al. (1993)
ROM	Eastern United States (E of 99° W longitude)	Lamb (1983)	Schere and Wayland (1989a,b) Meyer et al. (1991b)
RADM	Eastern North America	Chang et al. (1987)	Middleton et al. (1988, 1993) Middleton and Chang (1990) Dennis et al. (1993a) Cohn and Dennis (1994)
ADOM	Eastern North America and Northern Europe	Venkatram et al. (1988)	Venkatram et al. (1988) Macdonald et al. (1993) Karamchandani and Venkatram (1992)
STEM-II	Philadelphia area, Kentucky, and northeastern United States, central Japan	Carmichael et al. (1986)	Carmichael et al. (1991) Saylor et al. (1991)

[a]See Appendix A for abbreviations and acronyms.

Table 3-22. Grid-based Urban and Regional Air Pollution Models: Treatment of Emissions and Spatial Resolution[a]

Model	Emitted Species	Point-Source Emissions	Area-Source Emissions	Vertical Resolution
UAM	SO_2, sulfate, NO, NO_2, CO, NH_3, and 8 classes of ROG and PM (4 size classes)	Released into grid cell in layer corresponding to plume rise in UAM; treated with a reactive plume model in PARIS	Grid-average with resolution ranging from 4 km × 4 km to 10 km × 10 km in past applications	Typically, 5-6 layers up to about 1.5 km
CIT	SO_2, sulfate, NO, NO_2, CO, NH_3, and 6 classes of ROG and PM (4 size classes)	Treated with a plume model with simple NO_x and O_3 chemistry	Grid-average with 5 km × 5 km resolution in past applications	Five layers up to about 1.5 km
ROM	CO, NO, NO_2, and 8 classes of ROG	Released into grid cell in layer corresponding to plume rise	Grid-average with 18.5 km × 18.5 km resolution in present applications	Three layers up to about 4 km
RADM	SO_2, sulfate, NO, NO_2, CO, NH_3, and 12 classes of ROG	Released into grid cell in layer corresponding to plume rise	Grid-average with 80 km × 80 km resolution in past applications	Fifteen layers up to about 16 km
ADOM	SO_2, sulfate, NO, NO_2, NH_3, and 8 classes of ROG and PM	Released into grid cell in layer corresponding to plume rise	Grid-average with resolution ranging from 60 km × 60 km to about 120 km × 120 km in past applications	Twelve layers up to about 10 km
STEM-II	SO_2, sulfate, NO, NO_2, NH_3, and 8 classes of ROG	Released into grid cell in layer corresponding to plume rise	Grid-average with resolution ranging from 10 km × 10 km to 56 km × 56 km in past applications	Ten to 14 layers up to about 6 km

[a]See Appendix A for abbreviations and acronyms.

Table 3-23. Grid-based Urban and Regional Air Pollution Models: Treatment of Meteorological Fields, Transport, and Dispersion[a]

Model	Meteorology	Transport	Turbulent Diffusion
UAM	Constructed through data interpolation or calculated with land-sea breeze or complex terrain wind model.	3-D wind field. Finite difference numerical technique.	Vertical turbulent diffusion function of atmospheric stability and friction velocity. Constant horizontal turbulent diffusion coefficient.
CIT	Constructed through data interpolation with diagnostic wind model.	3-D wind field. Finite element numerical technique.	Vertical turbulent diffusion function of atmospheric stability and friction velocity. Horizontal turbulent diffusion function of mixing height and convective velocity scale.
ROM	Constructed through data interpolation.	3-D wind field with vertical transport through cumulus clouds. Finite difference numerical technique.	Vertical turbulent diffusion function of atmospheric stability. Horizontal turbulent diffusion function of atmospheric stability, convective cloud cover and velocity scale, and the depths of the boundary layer and clouds.
RADM	Calculated with Community Climate Model (CCM) and MM4.	3-D wind field with vertical transport through cumulus clouds. Finite difference numerical technique.	Vertical turbulent diffusion function of atmospheric stability and wind shear. No horizontal turbulent diffusion.
ADOM	Constructed through data interpolation in combination with prognostic planetary boundary-layer model.	3-D wind field with vertical transport through cumulus clouds. Cubic spline numerical technique.	Vertical turbulent diffusion calculated from planetary boundary layer model. No horizontal turbulent diffusion.
STEM-II	Calculated with dynamic wind model (MASS) or constructed through data interpolation.	3-D wind field with vertical transport through clouds. Finite element numerical technique.	Vertical turbulent diffusion function of atmospheric stability and surface roughness. Horizontal turbulent diffusion proportional to vertical turbulent diffusion.

[a]See Appendix A for abbreviations and acronyms.

Table 3-24 summarizes the gas-phase chemical mechanisms incorporated into the six models. Generally three chemical mechanisms are used in the models: (1) CBM-IV used in ROM and UAM; (2) versions of the SAPRC mechanism used in ADOM, STEM-II, and CIT; and (3) the RADM mechanism. Of the three chemical mechanisms, RADM is the largest and CBM-IV is the smallest. Aqueous-phase chemistry is currently treated only in the regional models. Cloud processes are treated in the three regional models, RADM, ADOM, and STEM-II (Table 3-25). Cumulus venting and solar attenuation are treated in ROM. Layer 3 depths also are influenced by cloud thickness. At present, only RADM, ADOM and STEM-II treat wet deposition. The treatment of dry deposition in the models also is summarized in Table 3-25.

Regional-scale modeling is an important contributor to the development of boundary conditions for urban-scale models. In recent years, regional-scale modeling has been receiving increased attention as the need for addressing interlinked air quality problems at broader scales is increasing. At the expanded spatial and temporal scales of regional-scale models, the simulation of certain dynamic processes becomes more critical. For example, in regional-scale models the treatment of biogenic VOC emissions and removal by dry and wet deposition generally require greater attention and accuracy than at the urban scale. On the other hand, the exact mechanistic details of the oxidation of some highly reactive VOCs may be somewhat less important.

More detailed descriptions now will be presented for UAM, ROM, and RADM. The UAM is described, as it is specified officially by EPA, as a grid-based model for urban-scale O_3 control strategy determination. The regional-scale O_3 model, ROM, is being used by EPA to evaluate O_3 control measures for the eastern United States and to provide boundary conditions for urban area simulations using UAM. Representative of a comprehensive state-of-the-science O_3/acid deposition model, RADM has been used to evaluate combined O_3 and acid deposition abatement strategies for the northeastern United States and Canada.

The EPA is embarking on a project to produce the next generation of photochemical models, termed MODELS 3 (Dennis et al., 1993b). This group of models will be flexible (scalable grid and domain), will be modular (modules with interchangeable data structure), will have uniform input/output across subsystems, and will contain advanced analysis and visualization features. The models will be designed to take advantage of the latest advances in computer architecture and software.

3.6.3.1 The Urban Airshed Model

The UAM is the most widely applied and broadly tested grid-based photochemical air quality model. The model is described in a number of sources, including a multi-volume series of documents issued by the U.S. Environmental Protection Agency (1990a,b,c; 1992b) and a comprehensive evaluation by Tesche et al. (1993). Current versions include provisions enabling the user to model transport and dispersion within both the mixed and inversion layers. The computer codes have been structured to allow inclusion of up to 10 vertical layers of cells and any number of cells horizontally.

The original UAM developed by Reynolds et al. (1973) simulated the dynamic behavior of six pollutants: (1) reactive and (2) unreactive hydrocarbons, (3) NO, (4) NO_2, (5) O_3, and (6) CO. Since 1977, the UAM has employed various versions of the CBM. Currently, the model utilizes the CBM-IV Mechanism (Gery et al., 1988, 1989), which treats

Table 3-24. Grid-based Urban and Regional Air Pollution Models: Treatment of Chemical Processes[a]

Model	Gas-Phase Chemistry	Aqueous- Phase Chemistry
UAM	Eighty-seven reactions among 36 species including NO_x, O_3, ROG, and SO_2 (CBM-IV) (Gery et al., 1988, 1989)	No treatment of aqueous-phase chemistry
CIT	One hundred and twelve reactions among 53 species including NO_x, O_3, ROG, and SO_2 (Lurmann et al., 1986)	No treatment of aqueous-phase chemistry
ROM	Eighty-seven reactions among 36 species including NO_x, O_3, ROG, and SO_2 (CBM-IV)	No treatment of aqueous-phase chemistry
RADM	One hundred and fifty-seven reactions among 59 species including NO_x , O_3, ROG, and SO_2 (Stockwell et al., 1990)	Forty-two equilibria and five reactions for SO_2 oxidation
ADOM	One hundred and twelve reactions among 53 species including NO_x, O_3, ROG, and SO_2 (Lurmann et al., 1986)	Fourteen equilibria and five reactions for SO_2 oxidation
STEM-II	One hundred and twelve reactions among 53 species including NO_x, O_3, ROG, and SO_2 (Lurmann et al., 1986)	Twenty-six equilibria and about 30 reactions for SO_2 and NO_x oxidation, radical chemistry, and transition metal chemistry

[a]See Appendix A for abbreviations and acronyms.

Table 3-25. Grid-based Urban and Regional Air Pollution Models: Treatment of Cloud and Deposition Processes[a]

Model	Cloud Processes	Wet Deposition	Dry Deposition
UAM	No treatment of cloud processes.	No treatment of wet deposition.	Dry deposition velocity approach; function of wind speed, friction velocity, land type, and species.
CIT	No treatment of cloud processes.	No treatment of wet deposition.	Dry deposition velocity approach; function of atmospheric stability, wind speed, land type, and species.
ROM	No treatment of cloud processes, except vertical transport treatment.	No treatment of wet deposition.	Resistance transfer approach; function of land type, wind speed, atmospheric stability, and species.
RADM	Treatment of precipitating cumulus clouds, precipitating stratus clouds, and fair-weather cumulus clouds, based on precipitation amount, temperature, and relative humidity vertical profiles. Use of cloud-averaged properties for aqueous chemistry.	Calculated from precipitation rate and cloud average chemical composition; no below-cloud scavenging.	Resistance transfer approach; function of atmospheric stability, wind speed, season, land type, insolation, surface wetness, and species.
ADOM	Treatment of cumulus clouds and stratus clouds, based on precipitation amount (for stratus clouds), temperature, and relative humidity vertical profiles. Vertical resolution for cloud chemistry.	Calculated from precipitation rate and vertically weighted cloud average chemical composition, below-cloud scavenging included.	Resistance transfer approach; function of atmospheric stability, wind speed, land type, season, insolation, and species.
STEM-II	Treatment of clouds with the Advanced Scavenging Module based on cloud-base height, precipitation rate, and surface temperature.	Calculated with the Advanced Scavenging Module. Treats cloud water, rain water, and snow; below-cloud scavenging included.	Resistance transfer approach; function of atmospheric stability, land type, wind speed, and species.

[a]See Appendix A for abbreviations and acronyms.

36 reacting species. Reactive organic compounds include alkanes, alkenes, aromatics, and aldehydes, and nitrogen-bearing species include HNO_2, HNO_3, and PAN.

Under development at the time of writing of the present document is version V of UAM (Morris et al., 1991, 1992). This version (UAM-V) contains the following features: the ability to treat two-way interactive nested grids; the use of state-of-the-science treatment of atmospheric, meteorological, and chemical processes; the treatment of subgrid-scale plume processes using a plume-in-grid algorithm; and the use of structured programming techniques to take advantage of computational speed enhancement opportunities offered by the current and next generation of computers.

3.6.3.2 The Regional Oxidant Model

The ROM was designed to simulate most of the important chemical and physical processes that are responsible for the photochemical production of O_3 over regional domains and for episodes of up to 15 days in duration. These processes include horizontal transport; atmospheric chemistry and subgrid-scale chemical processes; nighttime wind shear and turbulence associated with the low-level nocturnal jet; the effects of cumulus clouds on vertical mass transport and photochemical reaction rates; mesoscale vertical motions induced by terrain and the large-scale flow; terrain effects on advection, diffusion, and deposition; emissions of natural and anthropogenic O_3 precursors; and dry deposition. The processes are simulated mathematically in a three-dimensional Eulerian model with three vertical layers, including the boundary layer and the capping inversion or cloud layer. The ROM geographical domains are summarized in Table 3-26 and illustrated in Figure 3-26.

Meteorological data are used to model objectively both regional winds and diffusion. The three model layers of ROM are prognostic (predictive) and are free to expand and contract locally in response to changes in the physical processes occurring within the layers. During an entire simulation period, horizontal advection and diffusion and gas-phase chemistry are modeled in the three layers. Predictions from Layer 1 are used as surrogates for surface concentrations. Layers 1 and 2 model the depth of the well-mixed layer during the day. Some special features of Layer 1 include the modeling of the substantial wind shear that can exist in the lowest few hundred meters above ground in local areas where strong winds exist and the surface heat flux is weak, the thermal internal boundary layer that often exists over large lakes or near sea coasts, and deposition onto terrain features that protrude above the layer. At night, Layer 2 represents what remains of the daytime mixed layer. As stable layers form near the ground and suppress turbulent vertical mixing, a nocturnal jet forms above the stable layer and can transport aged pollutant products and reactants considerable distances. At night, emissions from tall stacks and warm cities are injected directly into Layers 1 and 2. Surface emissions are specified as a mass flux through the bottom of Layer 1. During the day, the top model layer, Layer 3, represents the synoptic-scale subsidence inversion characteristic of high O_3-concentration periods; the base of Layer 3 is typically 1 to 2 km above the ground. Relatively clean tropospheric air is assumed to exist above Layer 3 at all times, and stratospheric intrusion of O_3 is assumed to be negligible. If cumulus clouds are present, an upward flux of O_3 and precursor species is injected into the layer by penetrative convection. At night, O_3 and the remnants of other photochemical reaction products may remain in this layer and be transported long distances downwind. These processes are modeled in Layer 3.

When cumulus clouds are present in a Layer 3 cell, the upward vertical mass flux from the surface is partially diverted from injection into Layer 1 to injection directly into the

Table 3-26. Regional Oxidant Model Geographical Domains

GENERAL INFORMATION

ROM grid cells are 1/4° longitude and 1/6° latitude in size or approximately 18.5 km. Actual domain names are included in parenthesis after the general geographical description. In addition, all domains can be run independently or windowed from the "super" domain.

SUPER DOMAIN (SUPROXA) 99.00 W to 67.00 W Longitude 26.00 N to 47.00 N Latitude 128 × 126 Grid Cells (columns × rows)	SOUTHERN DOMAIN (TEXROXA) 99.00 W to 81.00 W Longitude 26.00 N to 37.67 N Latitude 72 × 70 Grid Cells (columns × rows)
NORTHEAST DOMAIN (NEROXA) 89.00 W to 67.00 W Longitude 35.00 N to 47.00 N Latitude 88 × 72 Grid Cells (columns × rows)	NORTHEAST DOMAIN (ROMNET) 85.00 W to 69.00 W Longitude 36.33 N to 45.00 N Latitude 64 × 52 Grid Cells (columns × rows)
MIDWEST DOMAIN (MIDROXA) 97.00 W to 78.00 W Longitude 35.00 N to 47.00 N Latitude 76 × 72 Grid Cells (columns × rows)	NORTHEAST DOMAIN (NEROS1) 84.00 W to 69.00 W Longitude 38.00 N to 45.00 N Latitude 60 × 42 Grid Cells (columns × rows)
SOUTHEAST DOMAIN (SEROXA) 98.75 W to 76.25 W Longitude 27.67 N to 37.67 N Latitude 90 × 60 Grid Cells (columns × rows)	SOUTHEAST DOMAIN (SEROS1) 97.00 W to 82.00 W Longitude 28.00 N to 35.00 N Latitude 60 × 42 Grid Cells (columns × rows)

cumulus cloud of Layer 3. In the atmosphere, strong thermal vertical updrafts, primarily originating near the surface in the lowest portion of the mixed layer, feed growing "fair-weather cumulus" clouds with vertical air currents that extend in one steady upward motion from the ground to well above the top of the mixed layer. These types of clouds are termed fair-weather cumulus because atmospheric conditions are such that the clouds do not grow to the extent that precipitation forms. The dynamic effects of this transport process and daytime cloud evolution can have significant effects on the chemical fate of pollutants. Within the ROM system, a submodel parameterizes the above-cloud flux process and the subsequent impact on mass fluxes among all layers of the model. In the current implementation of the chemical kinetics, liquid-phase chemistry is not included, and, thus, part of the effects from the cloud flux processes are not accounted for in the simulations. The magnitude of the mass flux proceeding directly from the surface layer to the cloud layer is modeled as being proportional to the observed amount of cumulus cloud coverage and inversely proportional to the observed depth of the clouds.

Horizontal transport within the ROM system is governed by hourly wind fields that are interpolated from periodic wind observations made from upper-air soundings and

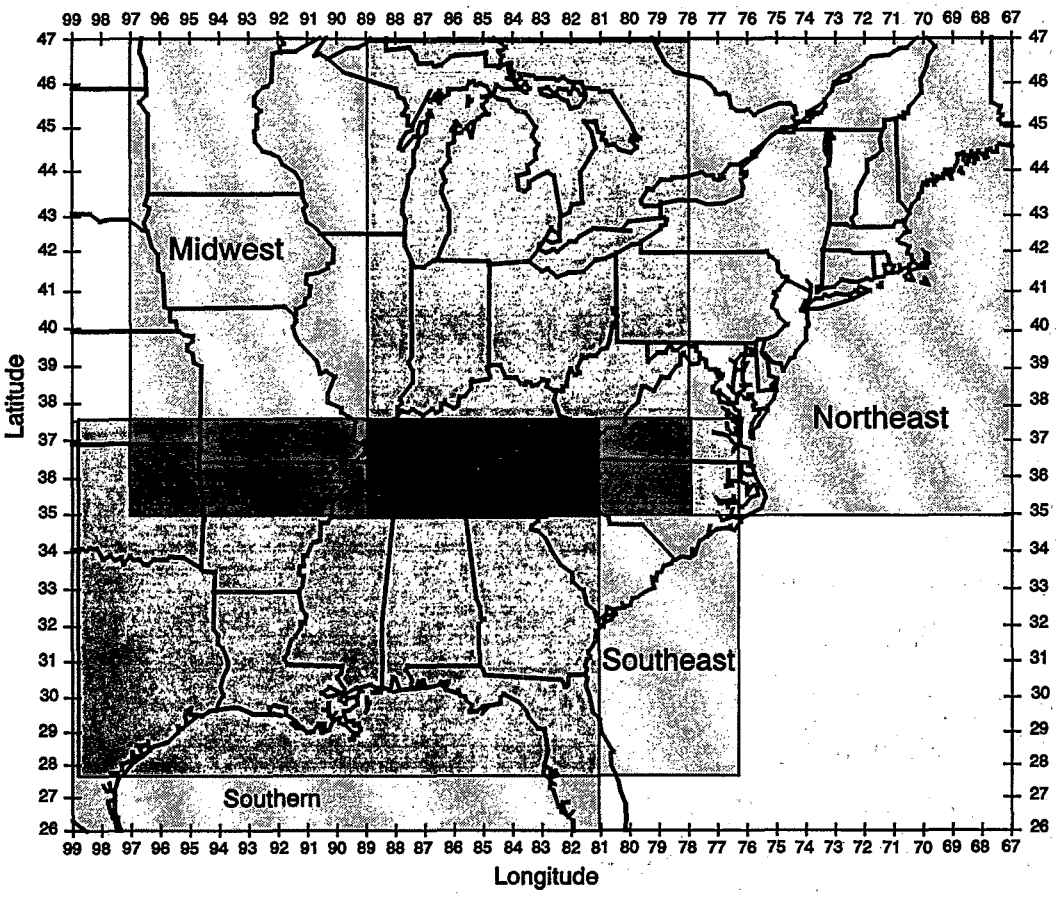

Figure 3-26. Regional oxidant model superdomain with modeling domains.

surface measurements. During the nighttime simulation period, the lowest few hundred meters of the atmosphere above the ground may become stable as a radiation inversion forms. Wind speeds increase just above the top of this layer, forming the nocturnal jet. This jet is capable of carrying O_3, other reaction products, and emissions injected aloft considerable distances downwind. This phenomenon is potentially significant in modeling regional-scale air quality and is implicitly treated by the model, where the definition of Layer 1 attempts to account for it.

The ROM system requires five types of "raw" data inputs: (1) air quality, (2) meteorology, (3) emissions, (4) land use, and (5) topography.

Air quality data required by the ROM include initial conditions and boundary conditions. The model usually is initialized 2 to 4 days before the start of the period of interest with clean tropospheric conditions for all species. This period of interest is called an "episode" and usually lasts around 15 days. Ideally, the initial condition field will have been transported out of the model domain in advance of the portion of the episode of greatest interest. Upwind lateral boundary conditions for O_3 are updated every 12 h based on measurements, except for the large superdomain, where tropospheric background values are

used. Other species concentrations at the boundaries, as well as all species at the top of the modeling domain, are set to tropospheric clean-air concentrations.

Meteorological data are assimilated by the first stage of preprocessors. These data contain regular hourly observations from U.S. National Weather Service surface stations (and from similar stations in Canada as necessary), including wind speed and direction, air temperature, dew point, atmospheric pressure, and cloud amounts and heights. Twice-daily sounding data, from the upper-air observation network also are included in the meteorological database. Upper-air meteorological parameters include atmospheric pressure, wind speed and direction, and air temperature, dew point. Finally, both buoy and Coastal Marine Automated Station data are used. The parameters that typically are reported include wind speed and direction and air and sea temperatures.

Emissions data for the primary species are input to the ROM system as well. Originally these data were provided from the 1985 emissions inventory of NAPAP, with 18.5-km spatial resolution. Most recently, the interim regional inventory has been used widely to support current applications of the ROM. It represents an update and improvement of the NAPAP inventory and is being used to support SIP modeling until state inventories are approved (U.S. Environmental Protection Agency, 1993a,b). Species included are CO, NO, NO_2, and 10 hydrocarbon reactivity categories. Natural hydrocarbons also are input, including isoprene explicitly, monoterpenes divided among the existing reactivity classes, and unidentified hydrocarbons. The chemical mechanism in ROM is the CBM-IV.

Land-use input data consist of 11 land-use categories in 1/4° longitude by 1/6° latitude grid cells. The data are more than 20 years old and represent a weakness. New land-use data slowly are being collected and released. Changes in land use over the last 20 years may change significantly the estimates of biogenic hydrocarbon emissions for large regions of the United States. Data are provided for the United States and Canada as far as 55° N. The land-use categories are (1) urban land, (2) agricultural land, (3) range land, (4) deciduous forests, (5) coniferous forests, (6) mixed-forest wetlands, (7) water, (8) barren land, (9) nonforested wetland, (10) mixed agricultural land and range land, and (11) rocky, open places occupied by low shrubs and lichens. Land-use data are used to obtain biogenic emissions estimates, as a function of the area of vegetative land cover, and for the determination of surface heat fluxes.

Topography input data consist of altitude matrices of elevations in a 7.5° × 7.5° grid. The data are obtained from the GRIDS database operated by EPA's Office of Information Resources Management. Topography data are used in the calculation of layer heights.

The ROM does have its limitations, including the large grid size, relatively crude wind fields, and highly empirical vertical mixing assumptions (Wolff, 1993).

3.6.3.3 The Regional Acid Deposition Model

The RADM initially was developed at the NCAR for EPA and subsequently was refined and improved at the State University of New York at Albany. The model is an Eulerian transport, transformation, and removal model that includes a treatment of the relevant physical and chemical processes leading to acid deposition and the formation of photochemical oxidants. As summarized in Tables 3-21 through 3-25, these processes include atmospheric transport and mixing, gas-phase and aqueous-phase chemical transformations, dry deposition, and cloud mixing and scavenging.

Chemical trace species are transported and diffused through the three-dimensional RADM grid using externally specified meteorological data. The RADM uses hourly three-dimensional fields of horizontal winds, temperature, and water vapor mixing ratio calculated by the meteorological model MM4 with FDDA. In addition, RADM requires two-dimensional, hourly fields of surface temperature, surface pressure, and precipitation rates over the model domain. Kuo et al. (1985) found that in order to calculate accurate mesoscale trajectories, at least 3-h temporal resolution is desirable, and the 12-h resolution of upper air observations is inadequate. Recent verification studies with 30 meteorological episodes by Stauffer and Seaman (1990) further support the use of MM5 data with FDDA. Using meteorology generated from a dynamically consistent meteorological model can introduce errors caused by simulation errors associated with the meteorological model. These uncertainties can be quantified through objective verification studies with observed data (Anthes et al., 1985; Stauffer and Seaman, 1990).

The RADM2 chemical mechanism has been described by Stockwell et al. (1990), Chang et al. (1991b), Carter and Lurmann (1990), and Stockwell and Lurmann (1989). For RADM2, the VOCs are aggregated into 12 classes of reactive organic species. Each category of VOC is represented by several model species that span the required range for reaction with the OH radical. Most emitted organic compounds are lumped into surrogate species of similar reactivity and molecular weight, although organic chemicals with large emissions are treated as separate model species even though their reactivities may be similar. Categories of VOCs with large reactivity differences and complicated secondary chemistries are represented by larger numbers of intermediate and stable species. During the aggregation of organic species, the principle of reactivity weighting is followed to attempt to account for differences in reactivity.

A major part of the SARMAP program described earlier is the extension of the RADM. The SARMAP is the modeling and data analysis component of a multi-year collaboration between two projects— SJVAQS and AUSPEX. In the near term, the objective of SARMAP is to produce a model that can be used to examine scenarios for control of O_3 precursor emissions as required under the CAAA for the 1994 planning cycle. The goals of the SARMAP modeling program can be summarized as follows:

- Development of a comprehensive state-of-the-science three-dimensional modeling system (consisting of emissions, meteorological, and air quality models) suitable for the simulation of O_3 concentrations, PM_{10} concentrations, visibility degradation, and acid deposition;
- Evaluation of the modeling system and its individual components against experimental data collected during the SJVAQS/AUSPEX field program; and
- Application of the model to estimate the effect of changes in emission levels on O_3 concentrations, PM_{10} concentrations, visibility degradation, and acid deposition.

The general attributes of the SARMAP modeling system are listed below.

- Integrated system of individual modules, including air quality, meteorological, emissions, and emissions projection; full compatibility of gridding system among all models.
- Ozone estimation capability; capability for efficiently incorporating modules for simulating aerosols, visibility, and acid deposition.
- Applicability at urban, subregional, and regional scales, embodying a full range of anticipated physical, chemical, and terrain characteristics.

- Capability of being driven by larger meteorological models, if desired (for generating initial and boundary conditions).
- Capability of generating as output a full complement of chemical species concentrations and meteorological parameters.
- Variable horizontal grid size.
- Variable number of vertical layers.
- Variable depth of vertical layers.
- Capability of nested grid application.
- Capability of varying the number of vertical layers with time of day. Selection of number of layers and timing of changes to be model-driven.
- Improved treatment of emissions injection aloft, including placement of plumes in the vertical, treatment of inversion penetration, proper vertical dilution of plumes, and proper treatment of chemistry.
- Inclusion of plume-in-grid capability.
- Capability for use of "computational tracers" for a variety of tests.
- Capability of simulating the O_3-VOC-NO_x system alone or in tandem with the aerosol system.
- Capability of simulating aerosols for the O_3-VOC-NO_x system.

The following modifications to the RADM2 gas-phase chemical mechanism have been made:

- Updating the rate constants, product parameters, and absorption cross sections and quantum yields for consistency with current recommendations;
- Improving the treatment of isoprene chemistry;
- Adapting the SAPRC emissions processing scheme to the RADM2 mechanism; and
- Adding extra species (acetaldehyde, PAN, and an additional aromatic) and their associated reactions and products.

The Smolarkiewicz scheme currently used in RADM will be replaced with the Bott scheme. This scheme is more accurate than the Smolarkiewicz scheme for continuous plumes and at low grid resolutions. The RADM cloud module will be replaced with the ADOM module. The RADM dry deposition module currently underestimates dry deposition velocities under stable conditions. This can result in unrealistically high O_3 concentrations at night.

3.6.4 Evaluation of Model Performance

Air quality models are evaluated by comparing their predictions with ambient observations. Because a model's demonstration of attainment of the O_3 NAAQS is based on hypothetical reductions of emissions from a base-year-episode simulation, the accuracy of the base-year simulation is necessary, but not sufficient. An adequate model should give accurate predictions of current peak O_3 concentrations and temporal and spatial O_3 patterns. It should also respond accurately to changes in VOC and NO_x emissions, to differences in VOC reactivity, and to spatial and temporal changes in emissions patterns for future years.

Model performance can be evaluated at several levels. The important sub-models, the emissions model, the meteorological model, and the chemical mechanism can be evaluated independently, and the model as a whole can be evaluated. Evaluation of emissions models can be carried out with special measurements designed to isolate the effects of emissions from a particular source category, such as tunnel studies (Pierson et al., 1990)

or on-road surveillance of motor vehicles (Lawson et al., 1990) to evaluate the accuracy of motor vehicle emissions models. Meteorological sub-models can be evaluated from the results of tracer experiments. Chemical mechanisms have traditionally been developed and evaluated on the basis of smog chamber experiments. A question that merits continued attention is how well chemical mechanisms developed with reference to smog chamber data perform when simulating the ambient atmosphere. As noted in this section, comparisons of observed and predicted concentrations for all important precursors, intermediates, and products are important in assessing the accuracy of a chemical mechanism.

Compilations of the performance of photochemical models in the South Coast Air Basin of California and in other urban areas indicate a general tendency toward the underprediction of O_3 concentrations and particularly O_3 maxima. It should be noted that different areas of the country are characterized by different controlling factors in O_3 generation, so the reasons for O_3 underprediction in one area may not be the same as in another. A case in point is the possibility of anthropogenic ROG emissions underestimation in urban areas versus biogenic ROG emissions underestimation in rural and regional areas. It is well-recognized that urban and regional photochemical models have a number of uncertain input quantities, so it is possible, by adjusting these quantities within their ranges of uncertainty, to improve O_3 predictions. This process, which is inherent in any modeling exercise because of the uncertainty associated with many of the input quantities, can lead to getting the right answer for the wrong reason. Because the modeling of an O_3 episode usually is carried out to establish a "base case" against which to evaluate the effects of VOC and NO_x emissions changes, the accuracy of the base case is vital for obtaining a valid assessment of the effects of emissions perturbations. Due to the nonlinear response of the O_3/VOC/NO_x system, conclusions drawn about the effect of VOC and NO_x emissions changes may not reflect actual atmospheric response if the base case simulation is inaccurate. For this reason, it is important to understand the reasons why the base case simulation may not agree with observations. Several more or less equivalent alternate base cases may exist due to the fact that it often is possible to vary inputs within their ranges of uncertainties to achieve comparable model performance. Unfortunately, the O_3 responses to identical VOC/NO_x controls may be rather different depending on which base case is used.

3.6.4.1 Model Performance Evaluation Procedures

Specific numerical and graphic procedures have been recommended for evaluation of the accuracy of grid-based photochemical models (Tesche et al., 1990b). The recommended methods include the calculation of peak prediction accuracy; various statistics based on concentration residuals; and time series of predicted and observed hourly concentrations. Four numerical measures appear to be most helpful in making an initial assessment of the adequacy of a photochemical simulation (Tesche et al., 1990b): (1) the paired peak prediction accuracy, (2) the unpaired peak prediction accuracy, (3) the mean normalized bias, and (4) the mean absolute normalized gross error.

Accurate matching of O_3 alone may not be sufficient to ensure that a model is performing accurately. The possibility of compensatory errors must be recognized (in which two or more sources of error interact in such a way that O_3 is predicted accurately, but for the wrong reasons). The inaccuracies offset each other in part. The modeling effort should be designed to minimize the likelihood of the presence of compensatory errors.

Evaluation of model performance for precursor and intermediate species, as well as for product species other than O_3, when ambient concentration data for these species are

available, significantly improves the chances that a flawed model will be identified. Comparisons of observed and predicted concentrations for all important precursors, intermediates, and products involved in photochemical air pollution, such as individual VOCs, NO, NO_2, PAN, O_2, H_2O_2, HNO_2, and HNO_3, are useful in model evaluation, especially with respect to the chemistry component of the model (Jeffries et al., 1992). Comparisons of predictions and observations for total organic nitrates (mainly PAN) and inorganic nitrates (HNO_3 and nitrate aerosol) can be used to test qualitatively whether the emissions inventory has the correct relative amounts of VOCs and NO_x. However, in order to include HNO_3 and nitrate aerosol in the data set for model comparisons, the model should include an adequate description of the HNO_3 depletion process associated with aerosol formation.

Adequate model performance for several reactive species increases the assurance that correct O_3 predictions are not a result of chance or fortuitous cancellation of errors introduced by various assumptions. Multispecies comparisons could be the key in discriminating among alternative modeling approaches that provide similar predictions of O_3 concentrations.

As noted above, photochemical models have the potential to produce nearly the right O_3 concentrations when performance is evaluated, but do so because two or more flaws were compensating each other. The existence of compensating errors in many modeling applications is suspected because most applications have used emission inventories whose validity is now in question (National Research Council, 1991). Underestimation of VOC emissions from motor vehicles may be responsible for the lack of agreement between inventories and ambient concentration data (Baugues, 1986; Lawson et al., 1990; Pierson et al., 1990; Fujita et al., 1992). Underestimation of emissions from other sources is also a possibility. One potentially underestimated VOC source is vegetation, which naturally emits VOCs. An underestimation of VOC emissions could be compensated for by underestimation of mixing height or wind speed, by overestimation of boundary concentrations of O_3 or precursors, or by inaccurate chemistry modules. Boundary concentrations (which can be obtained from measurements or regional models or by assuming background concentrations, often are poorly defined.

If only a routine database is available for modeling O_3 in an urban area, then there are four areas of concern that require attention (Roth, 1992).

(1) Air Quality Aloft. These data most likely will not be available. These measurements are important and are instrumental for diagnostic analysis of model simulations.

(2) Boundary Conditions. If the possibility of significant transport into the region exists, but the data are not available, the boundary conditions become a variable that allows the introduction of compensatory errors if the emissions estimates are inaccurate. An approach to circumventing this problem is to define the region in such a way that the boundaries become a much less significant issue.

(3) Ambient VOC Data. These generally are not routinely available. In their absence, evaluation of model performance is hampered.

(4) Meteorological Data Aloft. Very often, there are only surface measurements and a few soundings from which to extrapolate the needed data.

If any of these four areas is missing from the database, the performance evaluation and subsequent model application must be planned to minimize the possibility of compensatory errors.

3.6.4.2 Performance Evaluation of Ozone Air Quality Models

Urban Airshed Model

The UAM has been applied to many urban areas in the United States and Europe, and most of these studies have included some form of performance evaluation (see summary in Tesche et al., 1993, Table 6-2). Thus, there is a growing body of information concerning the accuracy of the model's predictions; UAM itself is continuing to undergo revision. Evaluations of UAM's performance have been carried out for a number of geographic areas. Evaluations conducted since 1985 have indicated mean discrepancies between predicted and measured O_3 values of 20 to 40% of the observations, when paired in space and time (Roth et al., 1990). The prediction of peaks exhibits relative errors that are smaller than the average error, with a tendency toward underprediction (Roth et al., 1990). The discrepancies between predicted and measured NO_2 in UAM applications are on the order of 30 to 50%, with no improvement over the history of modeling applications (Roth et al., 1990). Underprediction of NO_2 by UAM has been typical, generally on the order of 20 to 40% (Roth et al., 1990).

As a result of the discovery of significantly underestimated mobile source VOC emissions (in the late 1980s), this emissions underestimation is the leading cause of O_3 underprediction in urban areas.

Regional Oxidant Model

A primary role of the ROM is to estimate boundary conditions for use by UAM in evaluating hydrocarbon and NO_x reduction strategies for urban areas in the eastern United States. This is especially the case in areas where transport is a significant element (U.S. Environmental Protection Agency, 1990d). Analysis of regional O_3 abatement strategies also is a major role of the ROM (Possiel et al., 1990).

The ROM has been used in the EPA program, the Regional Ozone Modeling for Northeast Transport (ROMNET) program, to assess the effectiveness of various regional emission control strategies in lowering O_3 concentrations to nationally mandated levels for the protection of human health, forests, and crops (Meyer et al., 1991b). As part of the ROMNET program, the ROM also is being used to provide regionally consistent initial and upwind boundary conditions to smaller-scale urban models for simulations of future-year scenarios.

The most complete testing of ROM2.0 was accomplished in an evaluation with the 50-day (July 12 to August 31, 1980) Northeastern Regional Oxidant Study database (Schere and Wayland, 1989a,b). The model underestimated the highest values and overestimated the lowest. It produced an overall 2% overprediction in predicting maximum daily O_3 concentrations averaged over aggregate groups of monitoring stations. A key indicator of model performance on the regional scale is the accuracy of simulating the spatial extent and location, as well as the magnitude, of the pollutant concentrations within plumes from significant source areas. In ROM2.0 performance analyses, plumes from the major metropolitan areas of the Northeast Corridor, including Washington, DC; Baltimore, MD; New York; and Boston, could be clearly discerned in the model predictions under episodic conditions. Generally, the plumes were well characterized by the model, although there was

evidence of a westerly transport bias and underprediction of O_3 concentrations near the center of the plume. Using aircraft data, ROM2.0 was found to underpredict the regional tropospheric burden of O_3.

The evaluation of ROM2.1 (Pierce et al., 1990), unlike that of ROM2.0, was based on routinely archived data from state and local agency monitoring sites rather than on an intensive field-study period. The evaluation consisted of the comparison of observed and predicted O_3 concentrations during selected episodes (totaling 26 days) of high O_3 observed during the summer of 1985. Evaluation showed that ROM2.1 underestimated the highest values and slightly overestimated the lowest; underestimates of the upper percentiles tended to be more prevalent in the southern and western areas of the ROMNET domain (Table 3-26). The model exhibited an overall 1.4% overprediction in predicting maximum daily O_3 concentrations averaged over aggregate groups of monitoring stations, and it appears to correct for the westerly transport bias of high-O_3 plumes in the Northeast Corridor seen in ROM2.0. As with ROM2.0, model performance degraded as a function of increasingly complex mesoscale wind fields.

In a recent evaluation of ROM (Systems Applications International, 1993), ROM2.2 overestimated observed O_3 maxima by 20 to 30 ppb over the period of July 4 through 6, 1988, and predicted an episodic peak of 242 ppb on July 9, 1988, when the observed peak was 138 ppb. The ROM2.2 performance for hourly O_3 concentrations in the New York region exceeded the range of EPA acceptable performance by a factor of two 90% of the time during the July 1988 episode. The Systems Applications International (1993) report concluded that "the patchiness of the ROM2.2 predictions compared to the observations raises serious questions as to whether the model will respond correctly to emission control strategies." The major conclusions of that report were:

- Model performance downwind of New York City is "unacceptable". The model significantly overpredicts peak O_3 levels, and the predicted diurnal variation of O_3 occurs too late in the afternoon.
- Model performance for the Philadelphia and Baltimore/Washington urban plumes is "poor" with "unpaired peak estimation accuracy at the outer edge of the acceptable range."
- Elsewhere, the model seems to give good results, although it produces O_3 spatial distributions that are too "patchy" when compared to observations.
- There is a systematic westerly bias in the ROM2.2 wind fields.
- The model performance for NO_x is "extremely poor" indicating that ROM2.2 may be overestimating the VOC/NO_x ratios across the region.

3.6.4.3 Database Limitations

As previously mentioned, the use of routine air quality and meteorological data requires that a number of assumptions be made about key model inputs. Although intensive field studies are desirable during O_3 episodes to acquire the full set of data required, three key problems arise: (1) such studies are expensive and, therefore, are limited in number; (2) the time required to carry out field studies usually exceeds the time available; and (3) most field studies have not captured the worst O_3 episodes. Because EPA guidance emphasizes planning to meet worst-case conditions, field data often must be manipulated to approximate highest O_3 concentrations. Such adjustments invariably increase uncertainty in model projections.

Studies that have, or will, provide data for model evaluation include the St. Louis, MO, RAPS, conducted in 1975 and 1976; the Northeast Corridor Regional Modeling Project, conducted in 1979 and 1980; the South Central Coast Cooperative Aerometric Monitoring Program, conducted in 1985; SCAQS conducted in 1987; studies in Sacramento and San Diego, CA, in 1990; SJVAQS/AUSPEX conducted in 1990; LMOS conducted in 1990 and 1991; SOS conducted in 1991 and 1992; and a Gulf Coast study for 1993.

In most cases, field studies have not coincided with periods in which ozone concentrations have attained values as high as that on which the SIP must be based. Given the low probabilities of occurrence of the most adverse meteorological conditions and the fact that field studies typically acquire data for two or three ozone episodes, obtaining a design value concentration during the course of a field study is unlikely.

The EPA recommends that the five highest daily maximum O_3 concentrations at a design-value site, selected from the three most recent years, be modeled if EKMA is used for a SIP (U.S. Environmental Protection Agency, 1989b). Because EKMA's data requirements are minimal, it can be applied to the worst cases. In contrast, the number of episodes available for grid-based modeling is less than desirable in all areas. In addition, any available intensive databases often do not include the worst-case meteorology; intensive databases typically restrict modeling to two or three O_3 episodes having a duration of 2 to 3 days each. Moreover, the intensive databases never encompass the full range of meteorological conditions of interest (if O_3 exceedances occur in an area under different meteorological conditions, the relative effectiveness of different control strategies might vary with the different meteorological conditions). The EPA specifies procedures for episode selection for use with grid-based models (U.S. Environmental Protection Agency, 1991b).

Because the number of intensive databases is limited both in terms of episodes and regions, EPA has investigated the feasibility of applying UAM without conducting intensive field studies (Scheffe and Morris, 1990, 1991). These studies, known as the Practice for Low-cost Application in Nonattainment Regions (PLANR), were conducted for New York; Philadelphia; Atlanta; Dallas-Fort Worth, TX; and St. Louis. Of the five cities studied, St. Louis, New York, and Philadelphia had intensive databases available. Simulations were carried out using both routine and intensive databases for St. Louis and Philadelphia. Model performance using routine data was much better for St. Louis than for Philadelphia (Scheffe and Morris, 1990, 1991). Scheffe and Morris (1990, 1991) cautioned that the differing results may be complicated by the quality of the databases, but they speculate that model performance using routine databases for Philadelphia might have been poorer because of regional transport. Performance statistics for all four applications using routine data were consistent with other UAM applications (Scheffe and Morris, 1990, 1991); however, the paucity of data in the routine databases precluded any investigation of the possibility that compensating errors occurred.

Scheffe and Morris (1990, 1991) note that the PLANR lack of air quality data was addressed by extending the length of the simulations and expanding the upwind boundary, which, in effect, increased the need for accurate emissions inventories (boundary conditions could also be obtained through use of ROM). For PLANR applications, gridded emissions were created from routine county-level emission inventories by utilizing an emissions program that made use of surrogate information, such as population distribution. The PLANR study represents an interesting start on the problem of model application to areas without intensive databases; however the results were not sufficiently definitive for drawing conclusions of a broad, general nature.

3.6.5 Use of Ozone Air Quality Models for Evaluating Control Strategies

Photochemical air quality models are used for control strategy evaluation by first demonstrating that a past episode, or episodes, can be adequately simulated and then reducing hydrocarbon or NO_x emissions in the model inputs and in assessing the effects of these reductions on O_3 in the region. Ozone concentrations can be decreased by reducing either VOC or NO_x concentrations to sufficiently low levels. The effects of NO_x emissions reductions on O_3 concentrations vary because NO_x is an atypical precursor (i.e., although it is necessary for O_3 formation, fresh NO emissions remove O_3, and high concentrations of NO_x retard the rate of O_3 formation by removing radicals). Control of NO_x tends to accelerate the rate of O_3 formation; however, its effects on peak O_3 concentration depend on the location and timing of the control and on ambient concentrations of VOCs and NO_x, which vary widely in time and space, even within a single urban area during 1 day.

At a given VOC level, as the initial NO_x is increased, O_3 first increases, then peaks, and then decreases. The reduction in peak O_3 with increasing NO_x is a well-established chemical phenomenon. The peak in O_3 formation occurs at an initial VOC/NO_x ratio of about 10/1 (i.e., 10 ppbC/1 ppb). At fixed NO_x level, as VOC is increased, O_3 formation increases but then levels off. As a result of this behavior, at VOC/NO_x ratios below about 10/1, VOC reduction has been the preferred strategy for O_3 reduction. In this region NO_x reductions speed up O_3 formation and lead to higher peak O_3 values. At VOC/NO_x ratios exceeding about 10/1, both VOCs and NO_x will reduce O_3, but less than proportionally. The reason the reduction in O_3 is less than proportional is because equal reductions of VOCs and NO_x at intermediate ratios tend to keep O_3 production at its maximum. The nonlinear chemical behavior of the VOC/NO_x system, discussed earlier in this chapter, is at the heart of the controversy over the role of NO_x in O_3 control (Heuss and Wolff, 1993).

As noted in Section 3.6.1.2, the concept that a region is characterized by a single VOC/NO_x ratio is oversimplified and may actually lead to incorrect conclusions concerning the optimal approach to O_3 reduction (Milford et al., 1989). The VOC/NO_x ratio in a region is a function of location and time of day; the source-rich center city area may be characterized by a lower ratio than that in downwind, suburban areas at any given time of day. Because of the complex spatial and temporal dependence of O_3 formation, grid-based photochemical air quality models are necessary to evaluate the effect of emission reduction strategies for a region.

Moreover, location-specific studies need to be performed to ascertain whether a given area is in the VOC- or NO_x-controlled regime. Research is being conducted into the relationship between O_3 and NO_y to determine whether NO_y is a better indicator of the O_3-forming potential than the VOC/NO_x ratio (Shepson et al., 1992b; Trainer et al., 1993; Kleinman et al., 1994; Milford et al., 1994).

In most modeling applications, inputs are adjusted within their range of uncertainty to improve performance. A key test of quality of performance is to evaluate the model predictions for other episodes without adjustments, using the same procedures for establishing inputs as for the original episode.

Grid modeling applications are currently underway by or for state agencies for approximately 20 areas within the United States to support regional O_3 SIP revisions.

An immediate problem faced for almost all urban areas is that even if an adequate number of episodes exist, the episodes may not include the most adverse O_3 levels.

An inherent question in using a less adverse episode to develop control strategies is how do these strategies extrapolate to a more severe set of conditions? There is no clear answer to this question. At present, control strategies, evaluated by using grid-based models, are determined based on available episodes that have the largest amount of data, whether or not these episodes contain the highest O_3 concentration achieved. Another issue is that the form of the NAAQS for O_3 does not correspond with the output from a grid-based model. The model output does not provide a direct answer to whether an area will meet the standard in its current statistically based form.

Table 3-27 summarizes a number of recent O_3 control strategy evaluations for different areas of the United States. Some general observations can be made concerning issues that have arisen in control strategy exercises, particularly as they relate to problems associated with different areas of the country (Roth, 1992). In California, model results indicate that O_3 has been underestimated, most likely because VOC emissions from motor vehicles have been seriously underestimated. The underestimation was hidden by adjusting other model inputs within their range of uncertainty. In Atlanta, it has been estimated that approximately 60% of the VOC inventory is of biogenic origin, and the variation of anthropogenic emissions reductions required to achieve O_3 attainment within the uncertainty range of the biogenic emissions is on the order of 20%. The uncertainty range of the biogenic VOC emissions needs to be reduced to obtain tighter control strategy estimates.

The eastern United States poses special problems in regional-scale photochemical modeling. Boundary conditions typically contribute 40 to 70% of pollutant loading in many urban areas east of the Mississippi River. Regional-scale models are often either not available or not sufficiently reliable to use in estimating upwind boundary conditions. Furthermore, data are rarely available. If data are available, their use is limited to estimation of present conditions. If models are used in control strategy assessment and 40 to 70% of pollutant loading originates outside of the modeling region, major questions arise as to just how control strategies are to be determined. If uncertainties at the regional scale are significant and if regional-scale modeling is inaccurate, the limits of accuracy for urban-scale control strategy determination need to be carefully assessed.

An essential question is, given the inevitable uncertainties associated with O_3 air quality model predictions, can the effect of VOC and NO_x emissions changes on O_3 levels be unambiguously determined? The best approach to answering this question is a combination of sensitivity/uncertainty studies. Given the estimated uncertainties in model inputs and parameters for a particular application, the proposed VOC and NO_x emissions change scenarios should be examined for the full range of model inputs and parameters to determine how sensitive conclusions about the effect on O_3 levels are to the inherent uncertainties.

3.6.6 Conclusions

The 1990 CAAA (U.S. Congress, 1990) have mandated the use of photochemical grid models for demonstrating how most O_3 nonattainment areas can attain the NAAQS. Predicting O_3 is a complex problem. There are still many uncertainties in the models; nonetheless, models are useful for regulatory analysis and constitute one of the major tools for attacking the O_3 problem. These models have developed considerably in the past 10 years. However, their usefulness is constrained by having limited databases for use in model evaluation and from having to rely on hydrocarbon emissions data that may be inaccurate.

Table 3-27. Applications of Photochemical Air Quality Models to Evaluating Ozone[a]

Investigators	Region/Episode	Model Used	Strategies Evaluated
Chu et al. (1993) Chu and Cox (1993) Roselle et al. (1992) Mathur and Schere (1993)	Eastern United States; July 2-10, 1988	ROM2.2	Across-the-board NO_x/VOC reductions
Possiel et al. (1993) Possiel and Cox (1993)	Northeastern United States; July 1-12, 1988	ROM2.2	Estimate O_3 reductions per 1990 CAAA
Milford et al. (1992)	Northeastern United States; July 2-17, 1988	ROM	Analysis of effect of NO_x reductions
Rao (1987) Rao et al. (1989) Rao and Sistla (1993)	New York metropolitan area, 5 days in 1980	UAM/ROM2.1	Evaluation of 1988 SIPs and VOC/NO_x strategies
Scheffe and Morris (1990, 1991)	New York St. Louis Atlanta Dallas-Ft. Worth Philadelphia	UAM	Use of UAM for demonstrating attainment with routinely available data
Possiel et al. (1990)	Northeastern United States; July 2-17, 1988	ROM	Ozone control strategies in Northeast
Roselle and Schere (1990) Roselle et al. (1991)	Northeastern United States; July 12-18, 1980	ROM2.1	Sensitivity of O_3 in Northeast to biogenic emissions
Dunker et al. (1992a,b)	Los Angeles New York Dallas-Ft. Worth	UAM	Effects of alternate fuels and reformulated gasolines on O_3 levels
Milford et al. (1989)	South Coast Air Basin	CIT	Effects of systematic VOC and NO_x reductions
Middleton et al. (1993)	Eastern United States and southeastern Canada	RADM	2010 emissions projections

[a]See Appendix A for abbreviations and acronyms.

Primary issues and limitations associated with the use of photochemical air quality models are described below.

- High noise-to-signal ratios. Model imprecision for ozone predictions typically ranges from 25 to 40%, and inaccuracy (bias) ranges from 5 to 20%. These uncertainties are often of the same order as the percentage of reduction in the peak O_3 concentration for an area (from 160 to 120 ppb). Reasons for these inaccuracies include uncertainties in emissions inventories.
- Inadequacies of supporting databases in most geographical areas. Most areas are lacking or are deficient in data needed to estimate boundary conditions and

meteorological and air quality conditions aloft. There are few areas where speciated VOC concentrations are measured; surface NO_x data may be inaccurate. Where important data gaps exist, modeling accuracy suffers, and the prospects for reducing or eliminating the presence of compensating errors are diminished.

- Continuing need for improvements. Examples include the introduction of prognostic meteorological modeling in the mid-1980s, the discovery of underestimation of VOC emissions in the late 1980s, the inclusion of NO_x emissions from soils in 1993, and major adjustment of the emissions rates of isoprene in 1994.
- Presence of compensating errors. It appears that compensating errors have been present in many past applications, introducing the potential for bias into the estimation of the impacts of emissions control strategies.

Comparison of model predictions against ozone measurements, although necessary, is not a robust test of a model's accuracy. Ideally, one should evaluate performance against more extensive sets of species such as individual VOCs, NO_x, and NO_y. Compensating errors in input information to a model and within the model formulation can cause an O_3 model to generate correct O_3 predictions for the wrong reasons. Therefore, model evaluation indicators are needed to demonstrate the reliability of a prediction before the model can be used effectively in making control strategy decisions.

It is important to stress that, in O_3 modeling, a modeling system also is at issue, not just the air quality model itself. The modeling system includes a meteorological model, an emissions representation (where an emissions model is preferred to the traditional "inventory" approach), the air quality model, and a comprehensive supporting database. Where a problem exists, the entire modeling system must be evaluated.

Models can be used effectively in a relative sense to rank different control alternatives in terms of their effectiveness in reducing O_3 and to indicate the approximate magnitude of improvement in peak O_3 levels expected under various control strategies. To do so, there must be a sound emissions model and data and an adequate database on which to construct the modeling. Grid-based O_3 air quality modeling is superior to the available alternatives for O_3 control planning, but results can be misleading if the model is not evaluated sufficiently. The goal is to minimize the chances of incorrect use of the model.

3.7 Summary and Conclusions

3.7.1 Tropospheric Ozone Chemistry

3.7.1.1 Ozone in the Unpolluted Atmosphere

Ozone is found in the stratosphere, the "free" troposphere, and the PBL of the earth's atmosphere. In the stratosphere, O_3 is produced through cyclic reactions that are initiated by the photolysis of molecular oxygen by short-wavelength radiation from the sun and are terminated by the recombination of molecular oxygen and ground-state oxygen atoms.

In the "free" troposphere, O_3 occurs as the result of incursions from the stratosphere; upward venting from the PBL (which is the layer next to the earth, extending to altitudes of ≈ 1 to 2 km) through certain cloud processes; and photochemical formation from

precursors, notably CH_4, CO, and NO_x. These processes contribute to the background O_3 in the troposphere.

Ozone is present in the PBL as the result of downward mixing from the stratosphere and free troposphere and as the result of photochemical processes occurring within the PBL. The photochemical production of O_3 and other oxidants found at the earth's surface is the result of atmospheric physical and chemical processes involving two classes of precursor pollutants, reactive VOCs and NO_x. The formation of O_3 and other oxidants from its precursors is a complex, nonlinear function of many factors, including the intensity and spectral distribution of sunlight; atmospheric mixing and related meteorological conditions; the reactivity of the mixture of organic compounds in ambient air; the concentrations of precursor compounds in ambient air; and, within reasonable concentrations ranges, the ratio between the concentrations of reactive VOCs and NO_x.

In the free troposphere and in many relatively "clean" areas of the PBL, CH_4 is the chief organic precursor to in situ photochemical production of O_3 and related oxidants. Exceptions can include clean forested or vegetated areas emitting biogenic organics. The major tropospheric removal process for CH_4 is by reaction with OH radicals. In the complex cyclic reactions that result in oxidation of CH_4, there can be a net increase in O_3 or a net loss of O_3, depending mainly on the NO concentration.

3.7.1.2 Ozone Formation in the Polluted Troposphere

The same basic processes by which CH_4 is oxidized occur in the atmospheric oxidative degradation of other, even more reactive and more complex VOCs. The only significant initiator of the photochemical formation of O_3 in the troposphere is the photolysis of NO_2, yielding NO and a ground-state oxygen atom that reacts with molecular oxygen to form O_3. The O_3 thus formed reacts with NO, yielding O_2 and NO_2. These cyclic reactions attain equilibrium in the absence of VOCs. In the presence of VOCs, however, the equilibrium is upset, resulting, from a complex series of chain reactions, in a net increase in O_3.

The key reactive species in the troposphere is the OH radical, which is responsible for initiating the oxidative degradation reactions of almost all VOCs. As in the CH_4 oxidation cycle, the conversion of NO to NO_2 during the oxidation of VOCs is accompanied by the production of O_3 and the efficient regeneration of the OH radical. The O_3 and PANs formed in polluted atmospheres increase with the NO_2/NO concentration ratio.

At night, in the absence of photolysis of reactants, the simultaneous presence of O_3 and NO_2 results in the formation of the NO_3^- radical. The reaction with NO_3^- radicals appears to constitute a major sink for alkenes, cresols, and some other compounds, although alkyl NO_3^- chemistry is not well characterized.

Most inorganic gas-phase processes, that is, the nitrogen cycle and its interrelationships with O_3 production, are well understood; the chemistry of the VOCs in ambient air, however, is not. The chemical loss processes of gas-phase VOCs, with concomitant production of O_3, include reaction with OH, NO_3, O_3, and photolysis.

The major classes of VOCs in ambient air are alkanes, alkenes (including alkenes from biogenic sources), aromatic hydrocarbons, carbonyl compounds, alcohols, and ethers. A wide range of lifetimes in the atmosphere, from minutes to years, characterize the VOCs.

The only important reaction of alkanes is with OH radicals. For alkanes having carbon-chain lengths of four or less ($\leq C_4$), the chemistry is well understood and the reaction rates are slow. For $\geq C_5$ alkanes, the situation is more complex because few reaction

products have been found. Branched alkanes (e.g., isobutane) have rates of reaction that are highly dependent on structure. It is difficult to represent reactions of these VOCs satisfactorily in the chemical mechanisms of air quality models. Stable products of alkane photooxidation are known to include carbonyl compounds, alkyl nitrates, and δ-hydroxycarbonyls. Major uncertainties in the atmospheric chemistry of the alkanes concern the chemistry of alkyl nitrate formation; these uncertainties affect the amount of NO-to-NO_2 conversion occurring and, hence, the amounts of O_3 formed during photochemical degradation of the alkanes.

Alkenes react in ambient air with OH and NO_3^- radicals and with O_3. All three processes are important atmospheric transformation processes, and all proceed by initial addition to the $>C=C<$ bonds. Products of alkene photooxidation include carbonyl compounds, hydroxynitrates and nitratocarbonyls, and decomposition products from the energy-rich biradicals formed in alkene-O_3 reactions. Major uncertainties in the atmospheric chemistry of the alkenes concern the products and mechanisms of their reactions with O_3, especially the radical yields (which affect the O_3 formation yields).

The only tropospherically important loss process for aromatics (benzene and the alkyl-substituted benzenes) is by reaction with the OH radical, followed by H-atom abstraction or OH radical addition. Products of aromatic hydrocarbon photooxidation include phenolic compounds, aromatic aldehydes, α-dicarbonyls (e.g., glyoxal), and unsaturated carbonyl or hydroxycarbonyl compounds. Aromatics appear to act as strong NO_x sinks under low NO_x conditions. Major uncertainties in the atmospheric chemistry of aromatic hydrocarbons are mainly with regard to reaction mechanisms and products under ambient conditions (i.e., for NO_x concentration conditions that occur in urban and rural areas). These uncertainties impact on the representation of mechanisms in models.

Tropospherically important loss processes for carbonyl compounds not containing $>C=C<$ bonds are photolysis and reaction with the OH radical; those that contain such bonds can undergo the same reactions as alkenes. Photolysis is the major loss process for HCHO (the simplest aldehyde) and acetone (the simplest ketone), as well as for the dicarbonyls. Reactions with OH radicals are calculated to be the dominant gas-phase loss process for the higher aldehydes and ketones. Products formed and the importance of photolysis are major uncertainties in the chemistry of carbonyl compounds.

Alcohols and ethers in ambient air react only with the OH radical, with the reaction proceeding primarily via H-atom abstraction from the C-H bonds in these compounds.

It should be noted that the photooxidation reactions of certain higher molecular weight VOCs can lead to the formation of significant yields of organic particulates in ambient air. The chemical processes involved in the formation of O_3 and other photochemical pollutants lead to the formation of OH radicals and oxidized VOC reaction products that are of low enough volatility to be present as organic particulate matter. Hydroxyl radicals that oxidize VOCs also react with NO_2 and SO_2 to form HNO_3 and H_2SO_4, respectively, which can become incorporated into aerosols as particulate nitrate and sulfate. Controls aimed at reducing O_3 will also impact acid and secondary aerosol formation in the atmosphere.

3.7.2 Meteorological Processess Influencing Ozone Formation and Transport

3.7.2.1 Meteorological Processes

The surface energy (radiation) budget of the earth strongly influences the dynamics of the PBL and, in combination with synoptic winds, provides the forces for the vertical fluxes of heat, mass, and momentum. The redistribution of energy through the PBL creates thermodynamic conditions that influence vertical mixing. Energy balances require study so that more realistic simulations can be made of the structure of the PBL.

Day-to-day variability in O_3 concentrations depends heavily on day-to-day variations in meteorological conditions. For example, the concentration of an air pollutant depends significantly on the degree of mixing that occurs between the time a pollutant, or its precursors, is emitted and the arrival of the pollutant at the receptor. Inversion layers (layers in which temperature increases with height above ground level) are prominent determinants of the degree of atmospheric vertical mixing and, thus, the degree to which O_3 and other pollutants will be dispersed or accumulate. Ozone left in a layer aloft, as the result of reduced turbulence and mixing at the end of daylight hours, can be transported through the night, often to areas far removed from pollution sources. Downward mixing on the subsequent day can result in increases in local concentrations from the transported O_3.

Growing evidence indicates that the conventional use of mixing heights in modeling is an oversimplification of the complex processes by which pollutants are redistributed within urban areas. In addition, it is necessary to treat the turbulent structure of the atmosphere directly and to acknowledge the vertical variations in mixing.

Geography can significantly affect the dispersion of pollutants along the coast or shore of oceans and lakes. Temperature gradients between bodies of water and land masses influence the incidence of surface conditions. The thermodynamics of water bodies may play a significant role in some regional-scale episodes of high O_3 concentrations.

An "air mass" is a region of air, usually of multistate dimension, that exhibits similar temperature, humidity, and stability characteristics. Episodes of high O_3 concentrations in urban areas often are associated with high concentrations of O_3 in the surroundings.

The transport of O_3 and its precursors beyond the urban scale (≤ 50 km) to neighboring rural and urban areas has been well documented and was described in the 1986 EPA criteria document for O_3. Areas of O_3 accumulation are characterized by synoptic-scale subsidence of air in the free troposphere, resulting in development of an elevated inversion layer; relatively low wind speeds associated with a weak horizontal pressure gradient around a surface high pressure system; a lack of cloudiness; and high temperatures.

3.7.2.2 Meteorological Parameters

Ultraviolet radiation from the sun plays a key role in initiating the photochemical processes leading to O_3 formation and affects individual photolytic reaction steps. There is little empirical evidence in the literature, however, linking day-to-day variations in observed UV radiation levels with variations in O_3 levels.

An association between tropospheric O_3 concentrations and tropospheric temperature has been demonstrated. Plots of daily maximum O_3 concentrations versus maximum daily temperature for the summer months of 1988 to 1990 for four urban areas,

for example, show an apparent upper bound on O_3 concentrations that increases with temperature. A similar qualitative relationship exists at a number of rural locations.

The relationship between wind speed and O_3 buildup varies from one part of the country to another. Research done during the SOS (in the "Atlanta intensive" field study) indicates that measurements of variations in wind speed among methods at a particular level above ground must be larger than about 3 m/s to be considered statistically significant.

3.7.2.3 Normalization of Trends

Statistical techniques (e.g., regression techniques) can be used to help identify real trends in O_3 concentrations, both intra-annual and inter-annual, by normalizing meteorological variability. In the SOS, for example, regression techniques were used successfully to forecast O_3 levels to ensure that specialized measurements were made on appropriate days.

3.7.3 Precursors

3.7.3.1 Volatile Organic Compound Emissions

Hundreds of VOCs, commonly containing from 2 to about 12 carbon atoms, are emitted by evaporative and combustion processes from a large number of source types. Total U.S. VOC emissions in 1991 were estimated at 21.0 Tg. The two largest source categories were industrial processes (10.0 Tg) and transportation (7.9 Tg). Emissions of VOCs from highway vehicles accounted for almost 75% of the transportation-related emissions; studies have shown that the majority of these VOC emissions come from about 20% of the automobiles in service, many, of which are older cars that are poorly maintained.

The accuracy of VOC emission estimates is difficult to determine, both for stationary and mobile sources. Within major area sources, deviations of emission rates from individual sources from assigned average factors can result in error for the entire area source. Evaporative emissions, which depend on temperature and other environmental factors, compound the difficulties of assigning accurate emission factors. In assigning VOC emission estimates to the mobile source category, models are used that incorporate numerous input parameters (e.g., type of fuel used, type of emission controls, age of vehicle), each of which has some degree of uncertainty.

According to recent studies, vegetation emits significant quantities of VOCs into the atmosphere, chiefly monoterpenes and isoprene, but also oxygenated VOCs. The most recent biogenic VOC emissions estimate for the United States showed annual emissions of 29.1 Tg/year. Coniferous forests are the largest vegetative contributor on a national basis, because of their extensive land coverage. Summertime biogenic emissions comprise more than half of the annual totals in all regions because of their dependence on temperature and vegetational growth. Biogenic emissions are, for those reasons, expected to be higher in the southern states than in the northern.

Uncertainties in both biogenic and anthropogenic VOC emission inventories prevent establishing the relative contributions of these two categories.

3.7.3.2 Nitrogen Oxides Emissions

Anthropogenic NO_x is associated with combustion processes. The primary pollutant emitted is NO, formed at high combustion temperatures from the nitrogen and oxygen in air and from nitrogen in combustion fuel. Emissions of NO_x in 1991 in the

United States totaled 21.39 Tg. The two largest NO_x emission sources are electric power generation plants and highway vehicles. Emissions of NO_x therefore are highest in areas having a high density of electric-power-generating stations and in urban regions having high traffic densities. Between 1987 and 1991, transportation-related emissions remained essentially constant, whereas stationary source NO_x emissions increased about 10%.

Natural NO_x sources include stratospheric intrusion, oceans, lightning, soils, and wildfires. Lightning and soil emission are the only two significant natural sources of NO_x in the United States. The estimated annual lightning-produced NO_x for the continental United States is ≈ 1.0 Tg, about 60% of which is generated over the southern states. Both nitrifying and denitrifying organisms in the soil can produce NO_x, principally NO. Emission rates depend mainly on fertilization levels and soil temperature. Inventorying soil NO_x emissions is difficult because of large temporal and spatial variability, but the nationwide total has been estimated at 1.2 Tg/year, of which about 85% is emitted in spring and summer. About 60% of the total soil NO_x is emitted in the area of the country containing the central corn belt.

Combined natural sources contribute about 2.2 Tg of NO_x to the troposphere over the continental United States. Uncertainties in natural NO_x inventories are much larger than that for anthropogenic NO_x emissions. Because a large proportion of anthropogenic NO_x emissions come from distinct point sources, published annual estimates are thought to be very reliable.

3.7.3.3 Concentrations of Volatile Organic Compounds in Ambient Air

The VOCs most frequently analyzed in ambient air are NMHCs. Morning concentrations (6:00 a.m. to 9:00 a.m.) have been measured most often because of the use of morning data in EKMA and in air quality simulation models. Major field studies in 22 cities in 1984 and in 19 cities in 1985 produced NMHC measurements that showed median values ranging from 0.39 to 1.27 ppmC for 1984 and 0.38 to 1.63 ppmC in 1985. Overall median values from all urban sites were about 0.72 ppmC in 1984 and 0.60 ppmC in 1985.

Comparative data over two decades (the 1960s through the 1980s) in the Los Angeles and New York City areas showed decreases in NMHC concentrations in those areas. Concomitant compositional changes were observed over the two decades, with increases observed in the percentage of alkanes and decreases in the percentage of aromatic hydrocarbons and acetylene.

Concurrent measurements of anthropogenic and biogenic NMHCs have shown that biogenic NMHCs usually constituted much less than 10% of the total NMHCs. For example, average isoprene concentrations ranged from 0.001 to 0.020 ppmC and terpenes from 0.001 to 0.030 ppmC.

3.7.3.4 Concentrations of Nitrogen Oxides in Ambient Air

Measurements of NO_x at sites in 22 and 19 U.S. cities in 1984 and 1985, respectively, showed that median NO_x concentrations ranged from 0.02 to 0.08 ppm in most of these cities. The 6 a.m. to 9 a.m. median concentrations in many of these cities exceeded the annual average NO_x values of 0.02 to 0.03 ppm found in U.S. metropolitan areas between 1980 and 1989. Nonurban NO_x concentrations, reported as average seasonal or annual NO_x, range from <0.005 to 0.015 ppm.

Ratios of 6 a.m. to 9 a.m. NMOC to NO_x are higher in southeastern and southwestern U.S. cities than in northeastern and midwestern U.S. cities, according to data from EPA's multi-city studies conducted in 1984 and 1985. Median ratios ranged from 9.1 to 37.7 in 1984; in 1985, median ratios ranged from 6.5 to 53.2 in the cities studied. Rural NMOC/NO_x ratios tend to be higher than urban ratios. Morning (6 a.m. to 9 a.m.) NMOC/NO_x ratios are used in the EKMA-type of trajectory model. Trends from 1976 to 1990 show decreases in these ratios in the South Coast Air Basin of California. The correlation of NMOC/NO_x ratios with maximum 1-h O_3 concentrations, however, was weak in a recent analysis.

3.7.3.5 Ratios of Concentrations of Nonmethane Organic Compounds to Nitrogen Oxides

The ratios of NMOC/NO_x vary substantially between cities and within a given city. With certain exceptions, urban NMOC/NO_x ratios have been in the range of 10 and below. In contrast, ratios of NMOC/NO_x in rural areas tend to equal or exceed 20. Discrepancies have been found between ambient NMOC/NO_x ratios and emission inventory NMOC/NO_x ratios, with ambient ratios of NMOC/NO_x significantly exceeding emission ratios of NMOC/NO_x.

Trends in ratios of NMOC/NO_x have shown downward trends to well below 10 during the 1980s, both for the South Coast Air Basin and for cities in the eastern United States. Based on these low ratios, hydrocarbon control should be more effective than NO_x control within a number of cities.

3.7.3.6 Source Apportionment and Reconciliation

Source apportionment (now regarded as synonymous with receptor modeling) refers to determining the quantitative contributions of various sources of VOCs to ambient air pollutant concentrations. Source reconciliation refers to the comparison of measured ambient VOC concentrations with emissions inventory estimates of VOC source emission rates for the purpose of validating the inventories.

Early studies in Los Angeles employing a "mass balance" approach to receptor modeling showed the following estimated contributions of respective sources to ambient air concentrations of NMOCs through C_{10}: automotive exhaust, 53%; whole gasoline evaporation, 12%; gasoline headspace vapor, 10%; commercial natural gas, 5%; geogenic natural gas, 19%; and liquefied natural gas, 1%. Recent studies in eight U.S. cities showed that vehicle exhaust was the dominant contributor to ambient VOCs (except in Beaumont, where 14% was reported). Estimates of the contributions of gasoline evaporation differ in methodology; the more appropriate methods used result in estimates of large whole gasoline contributions (i.e., equal to vehicle exhaust in one study and 20% of vehicle exhaust in a second study).

The chemical mass balance approach used for estimating anthropogenic VOC contributions to ambient air cannot be used for receptor modeling of biogenic sources. A modified approach, applied to 1990 data from a downtown site in Atlanta, indicated a lower limit of 2% (24-h average) for the biogenic percentage of total ambient VOCs at that location (isoprene was used as the biogenic indicator species). The percentage varies during the 24-h period because of the diurnal (e.g., temperature, light intensity) dependence of isoprene concentrations.

Source reconciliation data have shown disparities between emission inventory estimates and receptor-estimated contributions. For biogenics, emission estimates are greater than receptor-estimated contributions. The reverse has been true for natural gas contributions estimated for Los Angeles, Columbus, and Atlanta and for refinery emissions in Chicago.

3.7.4 Analytical Methods for Oxidants and Their Precursors

3.7.4.1 Oxidants

Current methods used to measure O_3 are CL, UV absorption spectrometry, and newly developed spectroscopic and chemical approaches, including chemical approaches applied to passive sampling devices for O_3.

The CL method, designated as the reference method by EPA, involves the direct gas-phase reaction of O_3 with an alkene (C_2H_4) to produce electronically excited products, which decay with the emission of light. Detection limits of 0.005 ppm and a response time of less than 30 s are typical of currently available commercial instruments. A positive interference from atmospheric water vapor was reported in the 1970s and has recently been confirmed. Proper calibration can minimize this source of error.

Commercial UV photometers for measuring O_3 have detection limits of about 0.005 ppm, long-term precision within about $\pm$ 5%, and a response time of <1 min. Ozone has a fairly strong absorption band with a maximum near 254 nm; its molar absorption coefficient at that wavelength is well known. Because the measurement is absolute, UV photometry also is used to calibrate other O_3 methods.

A potential disadvantage of UV photometry is that atmospheric constituents that absorb 254-nm radiation (and that are removed fully or partially by the MnO_2 scrubber used in UV O_3 photometers) will be positive interferences in O_3 measurements. Interferences have been reported in two recent studies but assessment of the potential importance of such interferences (e.g., toluene, styrene, cresols, nitrocresols) is hindered by lack of absorption spectra data in the 250-nm range and by lack of ambient measurements of most of the aromatic photochemical reaction products. An interference from water also appears to occur from condensation of moisture in sampling level. Results from collocated UV and CL instruments indicated positive biases in the UV data of 20 to 40 ppb on hot, humid days.

Differential optical absorption spectrometry has been used to measure ambient O_3, but further intercomparisons with other methods and interference tests are recommended. Passive sampling devices permit acquisition of personal human exposure data and of O_3 monitoring data in areas where the use of instrumental methods is not feasible. Three PSDs are commercially available; all employ solid absorbents that react with O_3.

Calibration of O_3 measurement methods (other than PSDs) is done by UV spectrometry or by GPT of O_3 with NO. Ultraviolet photometry is the reference calibration method approved by EPA. Ozone is unstable and must be generated in situ at time of use to produce calibration mixtures.

Two methods generally have been employed to measure atmospheric PAN and its higher homologues: IR and GC using an ECD. A third method, less often used, couples GC with a molybdenum converter that reduces PAN to NO in the gas phase and subsequently measures the NO with a CL analyzer. Peroxyacetyl nitrate and the higher PANs are normally measured by GC-ECD. Detection limits have been extended to 1 to 5 ppt, using cryogenic enrichment of samples and specified desorption procedures that limit losses associated with cryosampling. Because PAN is unstable (explosive, and subject to

surface-related decomposition), the preparation of reliable calibration standards is difficult. Methods devised to generate calibration standards include photolysis of static concentrations of gases, nitration of peracetic acid in single hydrocarbons, and analysis of PAN as NO under specified conditions of the dissociation of PAN into its precursors.

Early measurements of 10 to 80 ppb H_2O_2 reported in the 1970s have been found to be in error because of artifact formation of H_2O_2 from reactions of absorbed gaseous O_3. Modeling results also indicate that lower levels of H_2O_2, on the order of 1 ppb, occur in the atmosphere.

In situ measurement methods for H_2O_2 include FTIR and TDLAS. The FTIR method is specific for H_2O_2 but has a high detection level of $\approx$50 ppb (using a 1-km path length). The TDLAS method also is specific and has a detection level of 0.1 ppb over averaging times of several minutes. Four frequently used wet chemical methods for measurement of H_2O_2 are available. All involve the oxidation of a substrate followed by instrumental detection and quantification of the resulting CL or fluorescence. Detection limits are comparable to those of FTIR and TDLAS, but interferences are common and must be obviated or minimized with specified procedures.

Calibration of methods for gaseous H_2O_2 measurement requires the immediate use of standard mixtures prepared by one of several wet chemical methods.

3.7.4.2 Volatile Organic Compounds

Increased monitoring of VOCs is required under Title I, Section 182, of the CAAA of 1990 because of the role of VOCs as precursors to the formation of O_3 and other photochemical oxidants. Volatile organic compounds are those gaseous organic compounds that have a vapor pressure greater than 0.15 mm and, generally, have a carbon content ranging from C_1 through C_{12}.

Traditionally, NMHCs have been measured by methods that employ a FID as the sensing element that measures a change in ion intensity resulting from the combustion of air containing organic compounds. The method recommended by EPA for total NMOC measurement involves the cryogenic preconcentration of nonmethane organic compounds and the measurement of the revolatilized NMOCs using FID. The main technique for speciated NMOC/NMHC measurements is cryogenic preconcentration followed by GC-FID. Systems for sampling and analysis of VOCs have been developed that require no liquid cryogen for operation, yet provide sufficient resolution of species.

Stainless steel canisters have become the containers of choice for collection of whole-air samples for NMHC/NMOC data. Calibration procedures for NMOC instrumentation require the generation, by static or dynamic systems, of dilute mixtures at concentrations expected to occur in ambient air.

Preferred methods for measuring carbonyl species (aldehydes and ketones) in ambient air are spectroscopic methods, on-line colorimetric methods, and HPLC method employing DNPH derivatization in a silica gel cartridge. The most common method in current use for measuring aldehydes in ambient air is the HPLC-DNPH method. Use of an O_3 scrubber has been recommended to prevent interference in this method by O_3 in ambient air. Carbonyl species are reactive, making preparation of stable calibration mixtures difficult; but several methods are available.

Impetus for the development of methods for measuring the more reactive oxygen- and nitrogen-containing organic compounds has come from their roles as precursors or products of photochemical oxidation and also from the inclusion of many of these compounds

on the list of hazardous air pollutants in the 1990 CAAA. Measurement of these PVOCs is difficult because of their reactivity and water solubility. Methods are still in development.

3.7.4.3 Oxides of Nitrogen

Nitric oxide and NO_2 comprise the NO_x involved as precursors to O_3 and other photochemical oxidants.

The most common method of NO measurement is the gas-phase CL reaction with O_3. The CL method is essentially specific for NO. Commercial NO monitors have detection limits of a few parts per billion by volume in ambient air. Commercial NO analyzers may not have sensitivity sufficient for surface measurements in rural or remote areas or for airborne measurements. Direct spectroscopic methods for NO exist that have very high sensitivity and selectivity for NO. Major drawbacks of these methods are their complexity, size, and cost, which restrict these methods to research applications. No PSDs exist for measurement of NO.

Chemiluminescence analyzers are the method of choice for NO_2 measurement, even though they do not measure NO_2 directly. Minimum detection levels for NO_2 have been reported to be 5 to 13 ppb, but more recent evaluations have indicated detection limits of 0.5 to 1 ppbv. Reduction of NO_2 to NO is required for measurement. In practice, selective measurement of NO_x by this approach has proved difficult. Commercial instruments that use heated catalytic converters to reduce NO_2 to NO measure not NO and NO_x, but more nearly NO and total NO_y. Thus, the NO_2 value inferred from such measurements may be significantly in error, which may in turn affect the results of modeling of ambient O_3.

Several spectroscopic approaches to NO_2 detection have been developed. As noted above for NO, however, these methods have major drawbacks that include their complexity, size, and cost, which, at present, outweigh the advantages of their sensitivity and selectivity. Passive samplers for NO_2 exist but are still in the developmental stage for ambient air monitoring.

Calibration of methods for NO measurement is done using standard cylinders of NO in nitrogen. Calibration of methods for NO_2 measurement include the use of cylinders of NO_2 in nitrogen or air, the use of permeation tubes, and gas-phase titration.

3.7.5 Ozone Air Quality Models

3.7.5.1 Definitions, Descriptions, and Uses

Photochemical air quality models are used to predict how O_3 concentrations change in response to prescribed changes in source emissions of NO_x and VOCs. They are mathematical descriptions of the atmospheric transport, diffusion, removal, and chemical reactions of pollutants. They operate on sets of input data that characterize the emissions, topography, and meteorology of a region and produce outputs that describe air quality in that region.

Two kinds of photochemical models are recommended in guidelines issued by EPA: (1) the grid-based UAM is recommended for modeling O_3 over urban areas, and (2) EKMA is identified as an acceptable approach under certain circumstances. The 1990 CAAA mandate the use of three-dimensional (grid-based) air quality models such as UAM in developing SIPs for areas designated as extreme, severe, serious, or multistate moderate.

In grid-based air quality models, the region to be modeled (the modeling domain) is subdivided into a three-dimensional array of grid cells. Pertinent atmospheric processes and chemical reactions are represented for each cell.

In trajectory models, such as EKMA, a hypothetical air parcel moves through the area of interest along a path calculated from wind trajectories. Emissions are injected into the air parcel and undergo vertical mixing and chemical transformations. Trajectory models provide a dynamic description of atmospheric source-receptor relationships that is simpler and less expensive to derive than that obtained from grid models, but meterological processes are highly simplified in trajectory models

The EKMA-based method for determining O_3 control strategies has some limitations, the most serious of which is that predicted emissions reductions are critically dependent on the initial NMHC/NO_x ratio used in the calculations. This ratio cannot be determined with any certainty because it is expected to be quite variable in time and space in an urban area. Grid-based models have their limitations as well. These are pointed out subsequently.

3.7.5.2 Model Components

Spatial and temporal characteristics of VOC and NO_x emissions are major inputs to a photochemical air quality model. Greater accuracy in emissions inventories is needed, for biogenics and for both mobile and stationary source components. Grid-based air quality models also require as input the three-dimensional wind field for the photochemical episode being simulated. This input is supplied by "meteorological modules" which fall into one of four categories: (1) objective analysis procedures; (2) diagnostic methods; (3) dynamic, or prognostic, methods; and (4) hybrid methods that embody elements from both diagnostic and prognostic approaches. Prognostic models are believed to provide a dynamically consistent, physically realistic, three-dimensional representation of the wind and other meteorological variables at scales of motion not resolvable by available observations. Outputs of prognostic models do not always agree with observational data, but methods have been devised to mitigate these problems.

A chemical kinetic mechanism (a set of chemical reactions), representing the important reactions that occur in the atmosphere, is used in an air quality model to estimate the net rate of formation of each pollutant simulated as a function of time. Chemical mechanisms that explicitly treat each individual VOC component of ambient air are too lengthy to be incorporated into three-dimensional atmospheric models. " Lumped" mechanisms are therefore used. The chemical mechanisms used in existing photochemical O_3 models contain uncertainties that may limit the accuracy of their predictions. Because of different approaches to "lumping" of reactions, models can produce somewhat different results under similar conditions. Both the UAM (UAM-IV) and EPA's ROM use the CMB-IV. The CBM-IV and the SAPRC and RADM mechanisms are considered to represent the state of the science.

Dry deposition, the removal of chemical species from the atmosphere by interaction with ground-level surfaces, is an important removal process for O_3 on both urban and regional scales; and is included in all urban- and regional-scale models. Wet deposition (the removal of gases and particles from the atmosphere by precipitation events) generally is not included in urban-scale photochemical models, because O_3 episodes do not occur during periods of significant clouds or rain.

Concentration fields of all species computed by the model must be specified at the beginning of the simulation; these concentration fields are called the initial conditions. These initial conditions are determined mainly with ambient measurements, either from routinely collected data or from special studies, but interpolation can be used to distribute the surface ambient measurements.

3.7.5.3 Evaluation of Model Performance

Air quality models are evaluated by comparing their predictions with ambient observations. An adequate model should give accurate predictions of current peak O_3 concentrations and temporal and spatial O_3 patterns. It also should respond accurately to changes in VOC and NO_x emissions, to differences in VOC reactivity, and to spatial and temporal changes in emissions patterns for future years. Likewise, multispecies comparisons could be the key in discriminating among alternative modeling approaches that provide similar predictions of O_3 concentrations. Adequate model performance for several reactive species increases the assurance that correct O_3 predictions are not a result of chance or fortuitous cancellation of errors introduced by various assumptions.

If only a routine database is available for modeling O_3 in an urban area, then several concerns require attention relative to model performance evaluation: air quality aloft, boundary conditions, ambient VOC data, and meteorological data aloft. If any of these four areas is missing from the database, the performance evaluation and subsequent model application must be adequately planned to minimize the possibility of compensatory errors.

3.7.5.4 Use of Ozone Air Quality Model for Evaluating Control Strategies

Photochemical air quality models are used for control strategy evaluation by first demonstrating that a past episode, or episodes, can be simulated adequately and then reducing hydrocarbon or NO_x emissions, or both, in the model inputs and assessing the effects of these reductions on O_3 in the region. The adequacy of control strategies based on grid-based models depends, in part, on the nature of input data for simulations and model validation, on input emissions inventory data, and on the relationship between model output and the current form of the NAAQS for O_3.

Grid-based models that have been widely used to evaluate control strategies for O_3 or acid deposition, or both, are the UAM, the CIT model, the ROM, the ADOM, and the RADM.

3.7.5.5 Conclusions

Urban air quality models are becoming readily available for application and have been applied in recent years in several urban areas. Significant progress also has been made in the development of regional models and in the integration of state-of-the-art prognostic meteorological models as drivers.

There are still many uncertainties in photochemical air quality modeling. Prime among these are emission inventories. However, models are essential for regulatory analysis and constitute one of the major tools for attacking the O_3 problem. Grid-based O_3 air quality modeling is superior to the available alternatives for O_3 control planning, but the chances of its incorrect use must be minimized.

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4

Environmental Concentrations, Patterns, and Exposure Estimates

4.1 Introduction

The effects of ozone (O_3) on humans, animals, and vegetation have received extensive examination and are discussed elsewhere in this document. As indicated in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986), most of the human and welfare effects research has focused on evaluating those impacts on health or vegetation of exposure to O_3 that simulate ambient O_3 exposures (e.g., matching the occurrence of hourly average concentrations or more prolonged times of exposure). This information on concentrations obtained from extensive monitoring in the United States can be useful both for linking anthropogenic emissions of O_3 precursors with the protection of health and welfare (i.e., determining compliance with air standards) and for augmenting exposure assessment and epidemiology studies. The major emphasis in this chapter, however, will be on characterizing and summarizing the extensive O_3-monitoring data collected under ambient conditions. Although most of the O_3 air quality data summarized were gathered for compliance and enforcement purposes, the hourly averaged O_3 information can be used for determining patterns and trends and as inputs to exposure and health assessments (e.g., U.S. Environmental Protection Agency, 1992a; Lefohn et al., 1990a). In the sections that follow, the hourly averaged ambient O_3 data have been summarized in different ways to reflect the interests of those who wish to know more about the potential for O_3 to affect humans and the environment. This chapter is not an exposure assessment for ambient O_3; rather, this chapter elucidates the features of O_3 concentration patterns and exposure possibilities.

Trend patterns for O_3 over several periods of time are described in Section 4.2. The trends for O_3 have been summarized by the U.S. Environmental Protection Agency (1994) for 1983 to 1993. In addition, trends analysis for specific regions of the United States have been performed by several investigators. In some cases, attempts have been made to adjust for meteorological variation. In Section 4.3, the hourly averaged concentration information from several monitoring networks has been characterized for urban and rural areas. The diurnal variation (Section 4.4) occurring at urban and rural locations, as well as seasonal patterns, also are described. Specific focus is provided on O_3 monitoring sites that experience low maximum hourly average concentrations because these locations form the "basis for comparison" for O_3 concentrations and exposures. In Section 4.5, the seasonal patterns of hourly average concentrations are discussed. The hourly average concentration information is used in Section 4.6 to compare the spatial variations that occur in urban areas with those in nonurban areas, as well as with those in high-elevation locations.

For comparing indoor to outdoor O_3 exposures or concentrations, information is provided in Section 4.7 on the latest data on indoor/outdoor (I/O) ratios. Section 4.8 describes efforts to estimate both human and vegetation exposure to O_3. Examples are provided on how both fixed-site monitoring information and human exposure models are used to estimate risks associated with O_3 exposure. A short discussion is provided on the importance of hourly average concentrations, which are used in human health and vegetation experiments that simulate "real world" exposures.

As indicated in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986), O_3 is the only photochemical oxidant other than nitrogen dioxide (NO_2) that is routinely monitored and for which a comprehensive aerometric database exists. Data for peroxyacetyl nitrate (PAN) and hydrogen peroxide (H_2O_2) have been obtained only as part of special research investigations. Consequently, no data on nationwide patterns of occurrence are available for these non-O_3 oxidants; nor are extensive data available on the correlations of levels and patterns of these oxidants with those of O_3. Sections 4.9 and 4.10 summarize the available data for these other oxidants. Section 4.11 describes the co-occurrence patterns of O_3 with NO_2; sulfur dioxide (SO_2); and acidic aerosols, precipitation, and cloudwater.

4.1.1 Characterizing Ambient Ozone Concentrations

It is important to distinguish among concentration, exposure, and dose when using air quality data to assess human health and vegetation effects. For this document, the following definitions apply:

1. The "concentration" of a specific air pollutant is the amount of that material per unit volume of air. Air pollution monitors measure pollutant concentrations, which may or may not provide accurate exposure estimates.
2. The term "exposure" is defined as the concentration of a pollutant encountered by the subject (animal, human, or plant) for a duration of time. Exposure implies that such an encounter leads to intake (i.e., through the respiratory tract or stomata).
3. The term "dose" is defined as that mass of pollutant delivered to an inner target. This term has numerous quantitative descriptions (e.g., micrograms of O_3 per square centimeter of lung epithelium per minute), so the context of the use of this term within the document must be considered. Human dosimetry is discussed in Chapter 8.

The dose incurred by an organism (e.g., plant, animal, or human) is a more complicated measure involving the concentration, the exposure duration, and the concurrent state of the organism's susceptibility. These distinctions become important because the concentration of an airborne contaminant that is measured in an empty room or at a stationary outdoor monitor is not in fact an exposure. A measured concentration functions as an alternative to an exposure only to the degree to which it represents concentrations actually experienced by individuals.

Concentrations of airborne contaminants for vegetation are considered to represent an exposure when a plant is subjected to them over a specified time period. As indicated in Chapter 5 (see Section 5.5), dose has been defined historically by air pollution vegetation researchers as ambient air quality concentration multiplied by time (O'Gara, 1922). However, a more rigorous definition was required. Runeckles (1974) introduced the concept of "effective dose" as the amount or concentration of pollutant that is adsorbed by vegetation,

in contrast to that which is present in the ambient air. Fowler and Cape (1982) developed this concept further and proposed that the "pollutant adsorbed dose" be defined in units of grams per square meter (of ground or leaf area) and could be obtained as the product of concentration, time, and stomatal (or canopy) conductance for the gas in question. Taylor et al. (1982) suggested internal flux (milligrams per square meter per hour) as a measure of the dose to which plants respond. In this chapter, dose will be taken to signify, for the purposes of vegetation, that amount of pollutant absorbed by the plant.

In order to characterize the specific doses responsible for affecting human health and vegetation, there has to be a linkage between exposure and actual dose. Unfortunately, it is difficult to predict this relationship, even with the available models. For example, the sensitivity of vegetation to O_3 as a function of time of day, period of growth, or edaphic conditions can determine the severity of response. For example, high O_3 concentrations may cause minimum injury or damage to plants, whereas more moderate O_3 concentrations may cause a greater degree of injury or damage (Showman, 1991). Because not enough is known to quantify the links between exposure and dosage, and routine monitoring for O_3 is summarized as hourly average concentrations (i.e., potential exposure), most of the information provided in this chapter is characterized in terms of concentration and exposure.

As indicated in Chapter 5, for many years, air pollution specialists have explored alternative mathematical approaches for summarizing ambient air quality information in biologically meaningful forms that can serve as alternatives for characterizing dose.

For vegetation, as indicated in Chapter 5 (Section 5.5), extensive research has focused on identifying indicators of concentration and duration (exposure) that are firmly founded on biological principles. Many of these indicators have been based on research results indicating that the magnitude of vegetation responses to air pollution is determined more as a function of the magnitude of the concentration than of the length of the exposure (U.S. Environmental Protection Agency, 1992b). Short-term (1- to 8-h), high O_3 concentrations (>0.1 ppm) have been identified by many researchers as being more important than long-term, low O_3 concentrations for induction of visible injury to vegetation (see Chapter 5 for further discussion).

Long-term, average concentrations were used initially as an exposure indicator to describe O_3 concentrations over time when assessing vegetation effects (Heck et al., 1982). Based on the view presented in the previous criteria document (U.S. Environmental Protection Agency, 1986) that higher concentrations of O_3 should be given more weight than lower concentrations (see Section 5.5 for further details), the following specific concerns about the use of a long-term average to summarize exposures of O_3 began appearing in the literature: the use of a long-term average failed to consider the impact of peak concentrations and of duration; a large number of hourly data sets within the commonly used 7-h window (0900 to 1559 hours), although diversely distributed and implying potentially diverse exposure potentials, were characterized by the same 7-h seasonal mean; and high hourly average concentrations (e.g., values greater than 0.1 ppm) occurred outside of a fixed 7-h window.

In summarizing the hourly average concentrations in this chapter, specific attention is given to the relevance of the exposure indicators used. For example, for human health considerations, concentration (or exposure) indicators such as the daily maximum 1-h average concentrations, as well as the number of daily maximum 4-h or 8-h average concentrations, are used to characterize information in the population-oriented locations. For vegetation, several different types of exposure indicators are used. For example, much of

the National Crop Loss Assessment Network (NCLAN) exposure information is summarized in terms of the 7-h average concentrations. However, because peak-weighted, cumulative indicators (i.e., exposure parameters that sum the products of hourly average concentrations multiplied by time over an exposure period) have shown considerable promise in relating exposure and vegetation response (see Section 5.5), several exposure indicators that use either a threshold or a sigmoidal weighting scheme are discussed in this chapter to provide insight concerning the O_3 exposures that are experienced at a select number of rural monitoring sites in the United States. The peak-weighted, cumulative exposure indicators used in this chapter are SUM06 and SUM08 (the sums of all hourly average concentrations equal to or greater than 0.06 and 0.08 ppm, respectively) and W126 (the sum of the hourly average concentrations that have been weighted according to a sigmoid function [see Lefohn and Runeckles, 1987] that theoretically is based on a hypothetical vegetation response).

The exposure indicators used for human health considerations are in concentration units (i.e., parts per million), whereas the indicators used for vegetation are in both parts per million (e.g., 7-h seasonal average concentrations) and parts per million per hour (e.g., SUM06, SUM08, W126). The magnitude of the peak-weighted, cumulative indicators at specific sites can be compared with those values experienced at areas that experience low hourly average maximum concentrations. In some cases, to provide more detailed information about the distribution patterns for a specific O_3 exposure regime, the percentile distribution of the hourly average concentrations (in parts per million) is given. For further clarification of the determination and rationale for the exposure indicators that are used for assessing human health and vegetation effects, the reader is encouraged to read Chapters 5 (Section 5.5) and 7.

4.1.2 The Identification and Use of Existing Ambient Ozone Data

Information is readily available from the database supported by a network of monitoring stations that were established to determine compliance with the National Ambient Air Quality Standards (NAAQS) for O_3. Most of the data presented in this chapter were obtained from data stored in the U.S. Environmental Protection Agency's (EPA's) computerized Aerometric Information Retrieval System (AIRS) and were collected after 1978. As pointed out in the previous criteria document for O_3 and other photochemical oxidants (U.S. Environmental Protection Agency, 1986), there was some difficulty in interpreting the O_3 data obtained at most sites across the United States prior to 1979 because of calibration problems.

In the United States, O_3 hourly average concentrations are monitored routinely through the National Air Monitoring Network, consisting of three types of sites. The National Air Monitoring Station (NAMS) sites are located in areas where the concentrations of O_3 and subsequent potential human exposures are expected to be high. Criteria for these sites have been established by regulation to meet uniform standards of siting, quality assurance, equivalent analytical methodology, sampling intervals, and instrument selection to assure consistency among the reporting agencies. For O_3, NAMS sites are located only in urban areas with populations exceeding 200,000. The other two types of sites are State and Local Air Monitoring Stations and Special Purpose Monitors, which meet the same rigid criteria for the NAMS sites but may be located in areas that do not necessarily experience high concentrations in populated areas.

For O_3, the reporting interval is 1 h, with the instruments operating continuously and producing an integrated hourly average measurement. In many cases, EPA summarizes air quality data by an O_3 "season". Table 4-1 summarizes the O_3 season for the District of Columbia and each of the states in the United States.

Table 4-1. Ozone Monitoring Season by State

State	Begin	End	State	Begin	End
Alabama	March	November	Montana	June	September
Alaska	April	October	Nebraska	April	October
Arizona	January	December	Nevada	January	December
Arkansas	March	November	New Hampshire	April	October
California	January	December	New Jersey	April	October
Colorado	March	September	New Mexico	January	December
Connecticut	April	October	New York	April	October
Delaware	April	October	North Carolina	April	October
D.C.	April	October	North Dakota	May	September
Florida	January	December	Ohio	April	October
Georgia	March	November	Oklahoma	March	November
Hawaii	January	December	Oregon	April	October
Idaho	April	October	Pennsylvania	April	October
Illinois	April	October	Rhode Island	April	October
Indiana	April	October	South Carolina	April	October
Iowa	April	October	South Dakota	June	September
Kansas	April	October	Tennessee	April	October
Kentucky	April	October	Texas[a]	January	December
Louisiana	January	December	Texas[b]	March	October
Maine	April	October	Utah	May	September
Maryland	April	October	Vermont	April	October
Massachusetts	April	October	Virginia	April	October
Michigan	April	October	Washington	April	October
Minnesota	April	October	West Virginia	April	October
Mississippi	March	November	Wisconsin	April	October
Missouri	April	October	Wyoming	April	October

[a]Air Quality Control Region (AQCR) Numbers 4, 5, 7, 10, and 11.
[b]AQCR Numbers 1, 2, 3, 6, 8, 9, and 12.

Source: Code of Federal Regulations (1991).

In this chapter, data are analyzed for the purpose of providing focus on specific issues of exposure-response relationships that are considered in the later effects chapters. The analyses proceed from a national picture of peak annual averages in Metropolitan Statistical Areas (MSAs), through national 10- and 3-year trends, to characteristic seasonal and diurnal patterns at selected stations, and then a brief examination of the incidence of episodic 1-h levels. Although there are O_3 data collected from monitoring stations not listed in AIRS, the major source of information was derived from ambient air concentrations from monitoring sites operated by the State and local air pollution agencies who report their data to AIRS. Because meteorology affects the identification of trends, methodologies that adjust for meteorology are described below.

To obtain a better understanding of the potential effect of ambient O_3 concentrations on human health and vegetation, hourly average concentration information was summarized for urban versus rural (forested and agricultural) areas in the United States. A land use characterization of "rural" does not imply that any specific location is isolated from anthropogenic influences. For example, Logan (1989) has noted that hourly average O_3 concentrations above 0.08 ppm are common in rural areas of the eastern United States in spring and summer, but are unusual in remote western sites. Consequently, for the purposes of comparing exposure regimes that may be characteristic of clean locations in the United States with those that are urban influenced (i.e., located in either urban or rural locations), this chapter characterizes data collected from those stations whose locations appear to be isolated from large-scale anthropogenic influences.

Long-term (multiyear) patterns and trends are available only from stationary ambient monitors; data on indoor concentrations are collected predominantly in selected settings during comparatively short-term studies. Data from the indoor and outdoor environments are reviewed here separately.

4.2 Trends in Ambient Ozone Concentrations

Ozone concentrations and, thus, exposure change from year to year. High O_3 levels occurred in 1983 and 1988 in some areas of the United States. These levels more than likely were attributable, in part, to hot, dry, stagnant conditions. However, O_3 levels in 1992 were the lowest of the 1983 to 1992 period (U.S. Environmental Protection Agency, 1993). These low levels may have been due to meteorological conditions that were less favorable for O_3 formation and to recently implemented control measures. Nationally, the summer of 1992 was the third coolest summer on record (U.S. Environmental Protection Agency, 1993). The U.S. Environmental Protection Agency (1993) has reported a 21% improvement in O_3 levels between 1983 and 1992, which, in part, may be attributed to relatively high O_3 levels in 1983, compared to the low O_3 exposure years from the period 1989 through 1992. However, new statistical techniques accounting for meteorological influences have been used by EPA and they appear to suggest an improvement (independent of meteorological considerations) of 10% for the 10-year period, 1983 to 1992 (U.S. Environmental Protection Agency, 1993).

The EPA summarizes trends for the NAAQS for the most current 3- and 10-year periods. In order to be included in the 10-year trend analysis in the annual *National Air Quality and Emissions Trend Report* (U.S. Environmental Protection Agency, 1993), a

station must report valid data for at least 8 of the last 10 years. A companion analysis of the most recent 3 years requires valid data in all 3 years. Analysis in the above report covers the periods 1983 to 1992 and 1990 to 1992, respectively; 509 sites met the 10-year period criteria, and 672 sites are included in the 1990 to 1992 database. The NAMS sites comprise 196 of the long-term trends sites and 222 of the sites in the 3-year database.

Figure 4-1 displays the 10-year composite average trend for the second highest daily maximum hourly average concentration during the O_3 season for the 509 trend sites and the subset of 196 NAMS sites. The 1992 composite average for the 509 trend sites is 21% lower than the 1983 average and 20% lower for the subset of 196 NAMS sites. The 1992 value is the lowest composite average of the past 10 years (U.S. Environmental Protection Agency, 1993). The 1992 composite average is significantly less than all the previous nine years, 1983 to 1991. As discussed in U.S. Environmental Protection Agency (1992a), the relatively high O_3 concentrations in 1983 and 1988 likely were attributable in part to hot, dry stagnant conditions in some areas of the country that were especially conducive to O_3 formation.

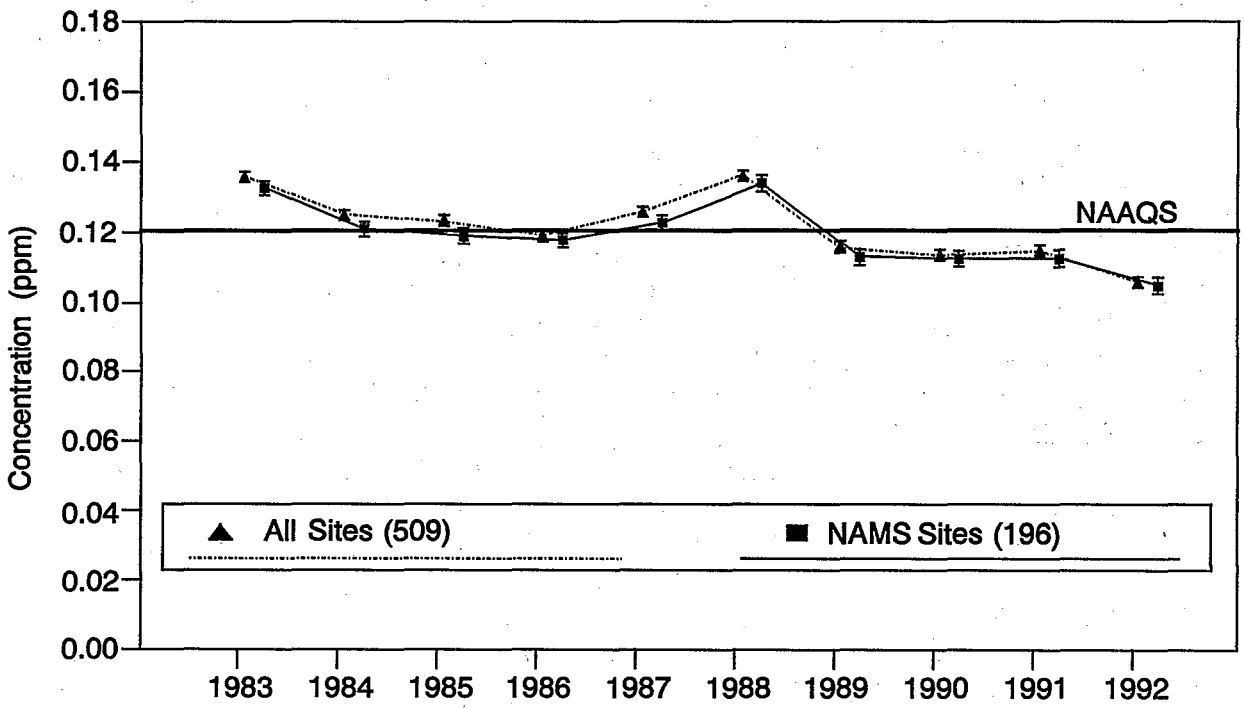


Figure 4-1. National trend in the composite average of the second highest maximum 1-h ozone concentration at both National Air Monitoring Stations (NAMS) and all sites with 95% confidence intervals, 1983 to 1992.

Source: U.S. Environmental Protection Agency (1993).

From 1991 to 1992, the composite mean of the second highest daily maximum 1-h O_3 concentrations decreased 7% at the 672 sites and 6% at the subset of 222 NAMS sites.

Also, from 1991 to 1992, the composite average of the number of estimated instances of O_3 exceeding the standard decreased by 23% at the 672 sites, and by 19% at the 222 NAMS sites. Nationwide volatile organic compound (VOC) emissions decreased 3% from 1991 to 1992 (U.S. Environmental Protection Agency, 1993).

The composite average of the second daily maximum concentrations decreased in 8 of the 10 EPA regions from 1991 to 1992, and remained unchanged in Region VII. Except for Region VII, the 1992 regional composite means are lower than the corresponding 1990 levels. Although meteorological conditions in the east during 1993 were more conducive to O_3 formation than those in 1992, the composite mean level for 1993 was the second lowest composite average for the decade (1984 to 1993) (U.S. Environmental Protection Agency, 1994).

Investigators have explored methods for investigating techniques for adjusting O_3 trends for meteorological influences (Stoeckenius and Hudischewskyj, 1990; Wakim, 1990; Shively, 1991; Korsog and Wolff, 1991; Lloyd et al., 1989; Davidson, 1993; Cox and Chu, 1993). Stoeckenius and Hudischewskyj (1990) used a classification method to group days into categories according to the magnitude of O_3 and the similarity of meteorological conditions within each defined group. Adjusted O_3 statistics for each year were computed from the meteorologically grouped data, and the yearly frequency of occurrence of each group relative to its long-term frequency was described. Wakim (1990) used standard regression analysis to quantify the effect of daily meteorology on O_3. Adjusted O_3 statistics were calculated by adding the expected O_3 statistic for a year with typical meteorology to the average of the regression residuals obtained for the adjusted year. Shively (1991) described a model in which the frequency of exceedance of various O_3 thresholds was modeled as a nonhomogeneous Poisson process where the parameter is a function of time and meteorological variables. Kolaz and Swinford (1990) categorized O_3 days as "conducive" or "nonconducive", based on selected meteorological conditions within the Chicago, IL, area. Within these categories, the meteorological intensity of days conducive to daily exceedances of the NAAQS for O_3 was calculated and used to establish long-term trends in the annual exceedance rate.

Cox and Chu (1993) modeled the daily maximum O_3 concentration using a Weibull distribution with fixed-shape and scale parameters, the logarithm of which varies as a linear function of several meteorological variables and a yearly index. The authors tested for a statistically significant trend term to determine if an underlying meteorologically adjusted trend could be detected. Overall, the measured and modeled predicted percentiles tracked closely in the northern latitudes but performed less adequately in southern coastal and desert areas. The results suggested that meteorologically adjusted upper percentiles of the distribution of daily maximum 1-h O_3 are decreasing in most urban areas over the period 1981 through 1991. The median rate of change was -1.1% per year, indicating that O_3 levels have decreased approximately 11% over this time period. The authors reported that trends estimated by ignoring the meteorological component appear to underestimate the rate of improvement in O_3 primarily because of the uneven year-to-year distribution of meteorological conditions favorable to O_3 formation.

Lefohn et al. (1993a) focused on a potentially useful method for identifying monitoring sites whose improvement in the level of O_3 concentrations may be attributed more to the implementation of abatement control strategies than meteorological changes. As has been pointed out previously, meteorology plays an important role in affecting the O_3 concentrations that are contained in the tail of the 1-h distributions, as indicated by the

successful predictive application of the exponential-tail model to distributions (California Air Resources Board, 1992). Because meteorology plays such an important role in affecting the tail of the 1-h distribution at a specific site, changes in "attainment" status are not expected to affect changes in the entire distribution pattern and, thus, the average diurnal pattern. Lefohn et al. (1993b) investigated the change in the annual average diurnal pattern as changes in O_3 levels occurred. The authors reported that, although the amplitude of the diurnal patterns changed, there was little evidence for consistent changes in the shape of the annual diurnal patterns (Figure 4-2). In a follow-up to this analysis, Lefohn et al. (1993a) reported that 25 of the 36 sites that changed compliance status across years showed no statistically significant change in the shape of the average diurnal profile (averaged by O_3 season). In addition, the authors reported that for 71% (10 of 14) of the sites in Southern California and Dallas-Fort Worth, TX, that showed improvement in O_3 levels (i.e., reductions in the number of exceedances over the years), but still remained in "nonattainment," a statistically significant change in the shape of the seasonally averaged diurnal profile occurred (e.g., Figure 4-3). Thus, the authors noted that, for the Southern California and Dallas-Fort Worth sites, changes were observed in the seasonally averaged diurnal profiles, whereas for the sites moving between attainment and nonattainment status, such a change in shape generally was not observed. Lefohn et al. (1993a) pointed out that it was possible that meteorology played a more important role in affecting attainment status than did changes in emission levels.

Historically, the long-term O_3 trends in the United States characterized by EPA have emphasized air quality statistics that are closely related to the NAAQS. A report by the National Academy of Sciences (NAS) (National Research Council, 1991) stated that the principal measure currently used to assess O_3 trends is highly sensitive to meteorological fluctuations and is not a reliable measure of progress in reducing O_3 over several years for a given area. The NAS report recommended that "more statistically robust methods be developed to assist in tracking progress in reducing ozone." The NAS report also points out that most of the trends analyses are developed from violations of standards based on lower concentration cutoffs or using percentile distributions. Because of the interest by EPA in tracking trends in the quality of the air that people breathe when outdoors, most of the above measures have some association with the existing NAAQS, in the form of either threshold violations or O_3 concentrations.

Several of the alternative examples provided in the NAS report were described previously by Curran and Frank (1991). Several of the examples mentioned in the NAS report involved threshold violations: the number of days on which the maximum O_3 concentration was above 0.12 ppm (Jones et al., 1989; Kolaz and Swinford, 1990; Wakim, 1990); the number of times during the year that the daily summary statistics exceeded 0.080 or 0.105 ppm (Stoeckenius, 1991), or the number of days in California when the O_3 concentration exceeded 0.2 ppm (Zeldin et al., 1991). Several other O_3 concentration measures are described in this report.

As an alternative to the way in which EPA historically has implemented its trends analysis, U.S. Environmental Protection Agency (1992a) used percentiles in the range of the 50th percentile (or median) to the 95th percentile. The U.S. Environmental Protection Agency (1992a) reported that the pattern for the 10-year trends (1982 to 1991), using the various alternative O_3 summary statistics, were somewhat similar. There was a tendency for the curves to become flatter in the lower percentiles. The peak years of 1983 and 1988 were still evident in the trend lines for each indicator. The increase of 8% recorded in the annual

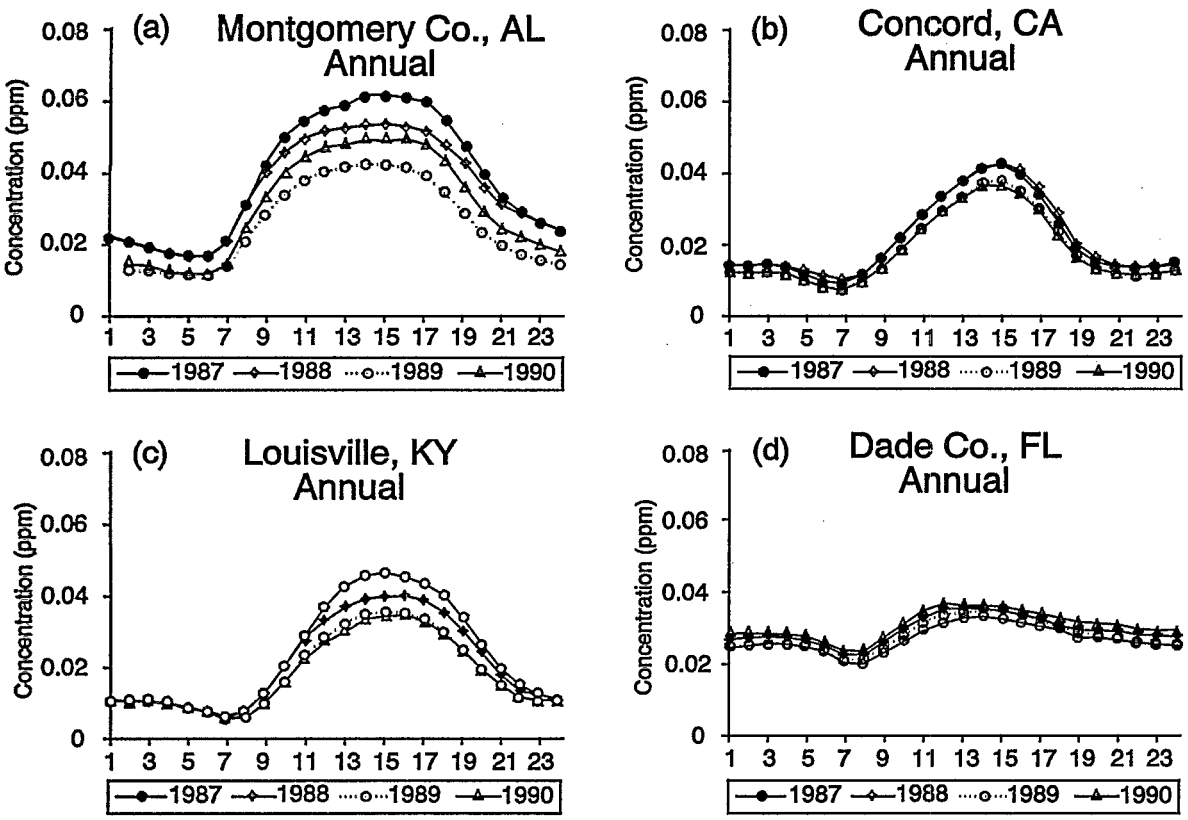


Figure 4-2. The annually averaged composite diurnal curves for the following sites that changed from nonattainment to attainment status: (a) Montgomery County, AL; (b) Concord, CA; (c) Louisville, KY; and (d) Dade County, FL; for the period 1987 to 1990. The darkened curve in each figure identified the year in which the greatest number of daily maximum 4-h average concentrations ≥0.08 ppm occurred.

Source: Lefohn et al. (1993b).

second-highest daily maximum 1-h concentration from 1987 to 1988 also was seen in the 95th and 90th percentile concentrations. The lower percentile indicators had smaller increases of 3 to 4%. The percent change between 1982 and 1991 for each of the summary statistics follows: annual daily maximum 1-h concentration, −11%; annual second daily maximum 1-h concentration, −8%; 95th percentile of the daily maximum 1-h concentrations, −5%; 90th percentile, −4%; 70th percentile, −1%; 50th percentile, or median of the daily maximum 1-h concentrations, +1%; and the annual mean of the daily maximum 1-h concentrations, −1%.

Besides EPA, additional investigators have assessed trends at several locations in the United States (e.g., Kuntasai and Chang, 1987; Gallopoulos et al., 1988; Korsog and Wolff, 1991; Lloyd et al., 1989; Rao et al., 1992; Davidson, 1993). For example, Kuntasai and Chang (1987) performed a basin-wide air quality trend analysis for the South Coast Air

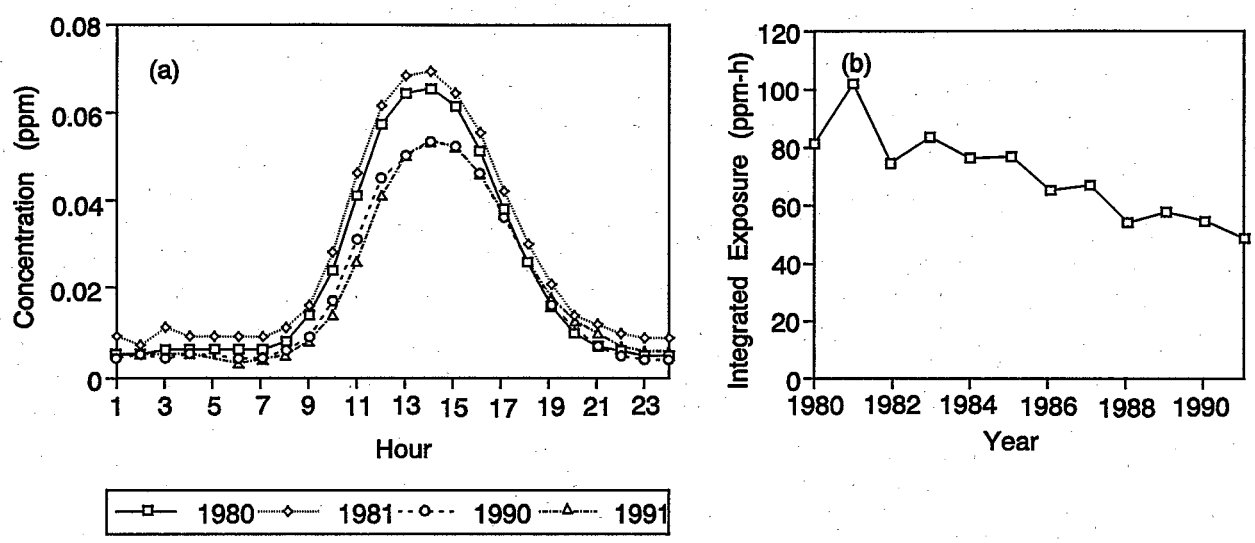


Figure 4-3. A summary of the (a) seasonal (January to December) averaged composite ozone diurnal curve and (b) integrated exposure W126 index for the Los Angeles, CA, site for the period 1980 to 1991.

Source: Lefohn et al. (1993a).

Basin of California using multistation composite daily maximum 1-h average ambient concentrations for the third quarter from 1968 to 1985. Basin-wide ambient O_3 concentrations appeared to show downward trends for the period 1970 to 1985, but because of high fluctuations, it was difficult to delineate trends for shorter periods. The meteorology-adjusted O_3 showed a more consistent downward trend than did unadjusted O_3. Korsog and Wolff (1991) examined trends from 1973 to 1983 at eight major population centers in the northeastern United States, using a robust statistical method. The 75th percentile was used by the authors in determining trends. The data were collected over a 3-mo (June through August) period. The surface temperature and upper air temperature variables were found to be the best predictors of O_3 behavior. Two regression procedures were performed to remove the variability of meteorological conditions conducive to high O_3 (i.e., O_3 concentrations >0.08 ppm). The results of the analysis showed that there had been a decrease of a few ppb on a yearly basis for the majority of the sites investigated by the authors.

Lloyd et al. (1989) investigated the improvement in O_3 air quality from 1976 to 1987 in the South Coast Air Basin. The authors reported that when the trend in total exceedance hours of a consistent set of basin air monitoring stations was considered, the improvement over the period of investigation was substantial. The authors reported that the number of station hours at or above the Stage I Episode Level (0.2 ppm, 1-h average) had decreased by about two-thirds over the period 1976 to 1987. Davidson (1993) reported on the number of days on which O_3 concentrations at one or more stations in the South Coast Air Basin exceeded the federal standard and the number of days reaching Stage I episode levels, for the months of May through October in the years 1976 to 1991. The author

reported that the number of basin days exceeding the federal standard declined at an average annual rate of 2.27 days/year over the period. In addition, the number of basin days with Stage I episodes declined at an average annual rate of 4.70 days/year over the period 1976 to 1991. Rao et al. (1992) demonstrated the use of some statistical methods for examining trends in ambient O_3 air quality downwind of major urban areas. The authors examined daily maximum 1-h O_3 concentrations measured over New Jersey, metropolitan New York City, and Connecticut for the period 1980 to 1989. The analyses indicated that although there has been an improvement in O_3 air quality downwind of New York City, there has been little change in O_3 levels upwind of New York City during this 10-year period.

Lefohn and Runeckles (1987) proposed a sigmoidal weighting function that was used in developing a cumulative integrated exposure index (W126):

$$w_i = \frac{1}{[1 + M \times \exp^{(-A \times c_i)}]}, \qquad (4\text{-}1)$$

where: w_i = weighting factor for concentration i,
M and A are positive arbitrary constants, and
c_i = concentration i.

Lefohn et al. (1988b) reported the use of the sigmoidally weighted index with constants, M and A, 4,403 and 126 ppm^{-1}, respectively. The authors referred to the index as W126. The values were subjectively determined to develop a weighting function that (1) included hourly average concentrations as low as 0.04 ppm, (2) had an inflection point near 0.065 ppm, and (3) had an equal weighting of one for hourly average concentrations at approximately 0.10 ppm and above. To determine the value of the index, the sigmoidal weighting function at c_i was multiplied by the hourly average concentration, c_i, and summed over all relevant hours. The index included the lower, less biologically effective concentrations in the integrated exposure summation. The weighting function has been used to describe the relationship between O_3 exposure and vegetation response (e.g., Lefohn et al., 1988b, 1992a).

Lefohn and Shadwick (1991), using the W126 sigmoidally weighted exposure index, assessed trends in O_3 exposures at rural sites in the United States over 5- and 10-year periods (1984 to 1988 and 1979 to 1988, respectively) for forestry and agricultural regions of the United States. Although the statistical analysis did not explore the effects on trends of the lower O_3 exposure period 1989 to 1992, the analysis did reflect the effect of the higher O_3 exposure years (1983 and 1988). The hot, dry summer of 1988 was associated with the highest O_3 exposures in both the forest and agricultural regions of the eastern United States. To compare the exposure index values across years, a correction for missing data was applied for each pollutant. The corrections were determined for each site on a monthly basis. The Kendall's K statistic (Mann-Kendall test) was used to identify linear trends. Estimates of the rate of change (slope) for the index were calculated. Table 4-2 summarizes the results of the analysis. For sites distributed by forestry regions, there were more positive than negative slope estimates for the 5-year analysis of sites in the southern, midwestern, and Mid-Atlantic regions. For the 10-year analysis, the above was true except for the Mid-Atlantic seasonal analysis, where there was one positive and one negative significant trend. In the southern region, 38% of the sites showed significant trends. For the sites in

Table 4-2. Summary by Forestry and Agricultural Regions for Ozone Trends Using the W126 Exposure Parameter Accumulated on a Seasonal Basis[a]

	Forestry						
	5-Year Trends				10-Year Trends		
			Significant			Significant	
Region	Not Significant[b]		–	+	Not Significant	–	+
South	53	(16)	0	14	13	1	7
Midwest	38	(1)	0	7	20	1	6
West	10	(0)	0	3	4	2	1
Pacific Northwest	4	(2)	0	0	2	0	0
Plains	3	(0)	0	0	2	0	0
Northeast	14	(0)	1	0	7	1	1
Mid-Atlantic	12	(0)	0	3	4	1	1
Rocky Mountains	5	(2)	0	1	2	0	1
All	139	(21)	1	28	54	6	17

	Agricultural						
	5-Year Trends				10-Year Trends		
			Significant			Significant	
Region	Not Significant[b]		–	+	Not Significant	–	+
Pacific	14	(2)	0	3	6	2	1
Mountain	5	(2)	0	1	2	0	1
Northern Plains	3	(0)	0	0	2	0	0
Lake States	10	(0)	0	1	5	0	1
Corn Belt	20	(1)	0	3	11	1	2
Northeast	26	(0)	1	3	11	2	2
Appalachian	27	(9)	0	14	8	0	8
Southeast	16	(5)	0	1	4	1	0
Delta State	9	(0)	0	2	4	0	1
Southeastern Plains	9	(2)	0	0	1	0	1
All	139	(21)	1	28	54	6	17

[a]See Appendix A for abbreviations and acronyms.
[b]Numbers in parentheses in the "Not Significant" column under "5-Year Trends" are the number of sites with exactly 3 years of data.

Source: Lefohn and Shadwick (1991).

the northeastern region, few sites showed a significant trend. There were considerably fewer sites in the remaining regions than in the four forestry regions above. Hence, for these regions, no significance was assigned to the differences in the number of negative and positive slope estimates in the tables. Similar to the results reported for the forestry regions, most of the sites in the agricultural regions showed no O_3 trends. However, in the Appalachian agricultural region, as many as 50% of the sites showed a pronounced indication of a trend. A predominance of positive significant trends for both the 5- and 10-year analyses was observed. In the other agricultural regions, there were approximately an equal number of positive and negative significant 5- and 10-year trends. The O_3 results produced patterns that were not pronounced enough to draw more than tentative conclusions for the 10-year analysis. For the 5-year analysis, there was still not a strong indication of an O_3 trend. However, when significant trends were observed, they were almost always positive. This can be attributed to eastern O_3 levels that were generally higher in 1988 than in previous years.

4.3 Surface Ozone Concentrations

4.3.1 Introduction

Ozone is measured at levels above the minimum detectable level at all monitoring locations in the world (Lefohn et al., 1990a). As dicussed earlier in Chapter 3, the concept of a "natural" background of O_3 is complex. Concentrations of background O_3 can vary with temperature, wind speed and direction, vertical motion, geographic location including latitude and altitude, and season of the year. This background O_3 can be attributed the following sources: (1) downward transport of stratospheric O_3 through the free troposphere to near ground level; (2) in situ O_3 production from methane emitted from swamps and wetlands reacting with natural NO_x emitted from soils, lighting strikes, and from downward transport of NO from the stratosphere into the troposphere; and (3) in situ production of O_3 from the reactions of biogenic VOCs with natural NO_x (National Research Council, 1991). A fourth source to be considered is the O_3 production resulting from long range transport of O_3 from distant pollutant sources (see Chapter 3).

The occasional occurrence of stratospheric injection of O_3, at specific times and in certain locations, is accepted and may be responsible for some of the rare occurrences of elevated levels that have been observed at some high- and low-elevation remote sites. A summer season average contribution of approximately 5 to 10 ppb for surface-level O_3 concentration from stratospheric intrusion has been estimated (Altshuller, 1989).

For purposes of comparing how O_3 levels have changed over time, it would be interesting to know how current levels compare to previous, historical natural background levels. However, estimations of background O_3 concentrations are difficult to make. The definition of background and the use of O_3 measurements are subject to much uncertainty. It is difficult, if not impossible, to determine whether any geographic location on earth is free from human influence (Finlayson-Pitts and Pitts, 1986). The natural precursor emissions can be responsible for the production of the O_3 concentrations observed at remote sites (Chameides et al., 1988; Zimmerman, 1979; Trainer et al., 1987). Citing indirect evidence for the possible importance of natural emissions, Lindsay et al. (1989) have emphasized that additional research is required to assess the role that natural hydrocarbons might play in urban and regional O_3 episodes.

It is possible for urban emissions, as well as O_3 produced from urban area emissions, to be transported to more rural downwind locations. This can result in elevated O_3 concentrations at considerable distances from urban centers (Wolff et al., 1977; Husar et al., 1977; Wight et al., 1978; Vukovich et al., 1977; Wolff and Lioy, 1980; Pratt et al. 1983; Logan, 1985; Altshuller, 1986; U.S. Environmental Protection Agency, 1986; Kelly et al., 1986; Pinkerton and Lefohn, 1986; Lefohn et al., 1987a; Logan, 1989; Lefohn and Lucier, 1991; Taylor and Hanson, 1992). For example, on over 40% of the 98 days that the maximum 1-h O_3 concentrations exceeded 0.12 ppm, the highest value was measured downwind of St. Louis at one of the rural sites, which was located approximately 50 km from downtown St. Louis (Altshuller, 1986). Urban O_3 concentration values often are depressed because of titration by NO_x (Stasiuk and Coffey, 1974). Reagan (1984) and Lefohn et al. (1987a) have observed this phenomenon where O_3 concentrations at center-city sites were lower than some rural locations. Because of the absence of chemical scavenging, O_3 tends to persist longer in nonurban than in urban areas (U.S. Environmental Protection Agency, 1986; Coffey et al., 1977; Wolff et al., 1977; Isaksen et al., 1978).

The distribution of O_3 or its precursors at a rural site near an urban source is affected by wind direction (i.e., whether the rural site is located up- or downwind from the source) (Kelly et al., 1986; Lindsay and Chameides, 1988). Thus, it may be difficult to apply land-use designations to the generalization of exposure regimes that may be experienced in urban versus rural areas. Because of this, it is difficult to identify a set of unique O_3 distribution patterns that adequately describe the hourly average concentrations experienced at monitoring sites in rural locations (Lefohn et al., 1991).

4.3.2 Urban Area Concentrations

Figure 4-4 shows the highest second daily maximum 1-h average O_3 concentrations in 1991 across the United States. The highest second daily maximum 1-h O_3 concentrations by MSA for the years 1989 to 1991 are summarized in Table 4-3. The highest O_3 concentrations are observed in Southern California, but high levels of O_3 also occur in the Texas Gulf Coast, the Northeast Corridor, and other heavily populated regions of the United States, but with a much lower frequency.

Lefohn (1992a) reported that, for many urban sites that experience high second daily maximum 1-h average values (i.e, >0.125 ppm), most are associated with only a few episodes. Monitoring sites in polluted regions tend to experience frequent hourly average O_3 concentrations at or near minimum detectable levels. The percentile summary information for some of these sites shows that, although some of the highest hourly average concentrations occur at these locations, their occurrence is infrequent (Table 4-4). For example, O_3 monitoring sites at Delmar, CA; Stratford and Madison, CT; Baton Rouge, LA; Bayonne, NJ; New York City and Babylon, NY; Harris County, TX; and Bayside, WI, exhibit maximum hourly average concentrations above 0.125 ppm; however, only 1% of the hourly average concentrations generally exceed 0.100 ppm. Although for human health considerations, the occurrence of a second daily maximum hourly average concentration >0.125 ppm is important, Table 4-4 illustrates that, for most of the sites listed (except for several sites in California), such high hourly average concentrations occur less than 1% of the time and are associated with occasional episodes.

As indicated in Section 4.1, interest has been expressed in characterizing O_3 exposure regimes for sites experiencing daily maximum 8-h concentrations above specific

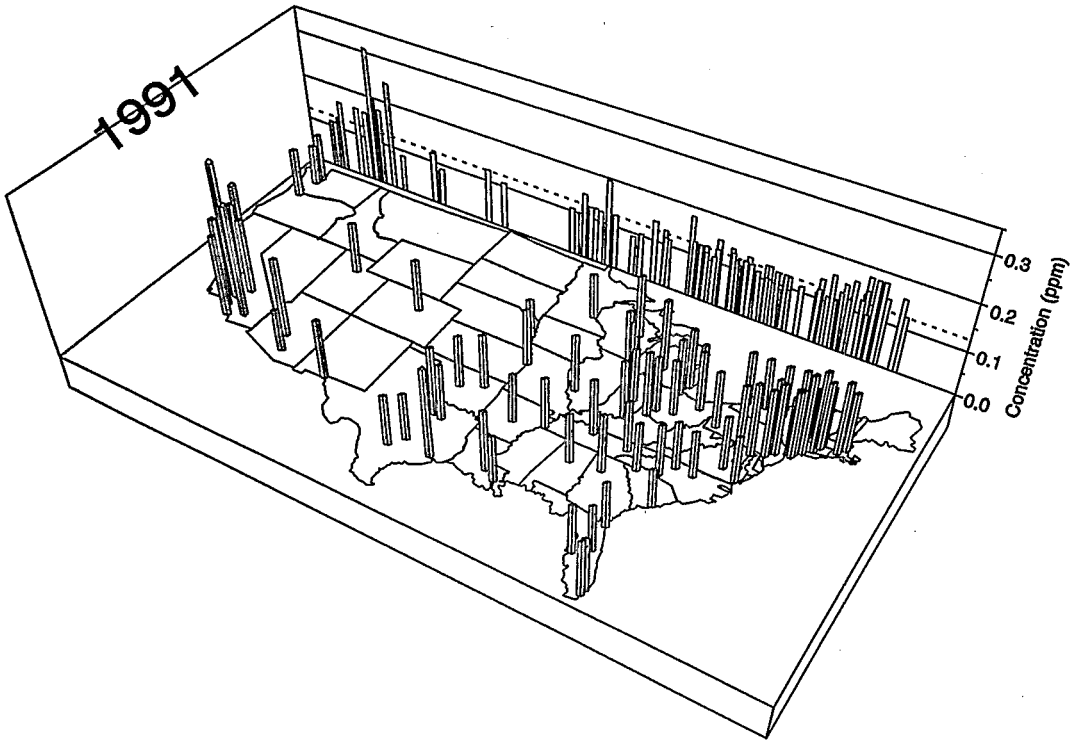


Figure 4-4. United States map of the highest second daily maximum 1-h average ozone concentration by Metropolitan Statistical Area, 1991.

Source: U.S. Environmental Protection Agency (1992a).

thresholds (e.g., 0.08 or 0.10 ppm). Table 4-5 summarizes the highest second daily maximum 8-h average O_3 concentrations by MSA for the years 1989 to 1991. The data have been reported for the O_3 season as summarized in Table 4-1. In some cases, high concentrations occur in the fall and winter periods as well as in the summertime. Analyses documented the occurrence, at some sites, of multihour periods within a day of O_3 at levels of potential health effects. Although most of these analyses were made using monitoring data collected from sites in or near nonattainment areas, the analysis of Berglund et al. (1988) showed that at five sites, two in New York state, two in rural California, and one in rural Oklahoma, an alternative O_3 standard of an 8-h average of 0.10 ppm would be exceeded even though the existing 1-h standard would not be. Berglund et al. (1988) described the occurrence at these five sites (none of which was in or near a nonattainment area) of O_3 concentrations showing only moderate peaks but exhibiting multihour levels above 0.10 ppm. Lefohn et al. (1993b) identified those areas in the United States for the period 1987 to 1989 where more than one occurrence of an 8-h daily maximum average concentration of 0.08 ppm was experienced, but an hourly average concentration equal to or greater than 0.12 ppm never occurred.

A follow-up to the points made above is whether an improvement in O_3 levels may produce distributions of 1-h O_3 that result in a broader diurnal profile than those seen in

Table 4-3. The Highest Second Daily Maximum One-Hour Ozone Concentration (ppm) by Metropolitan Statistical Area (MSA) for the Years 1989 to 1991

MSA	1989	1990	1991	MSA	1989	1990	1991
Akron, OH	0.14	0.11	0.13	Decatur, IL	0.09	0.09	0.10
Albany-Schenectady-Troy, NY	0.10	0.11	0.10	Denver, CO	0.11	0.11	0.11
Albuquerque, NM	0.10	0.10	0.09	Des Moines, IA	0.08	0.07	0.07
Allentown-Bethlehem, PA-NJ	0.10	0.11	0.12	Detroit, MI	0.14	0.12	0.13
Altoona, PA	0.10	0.10	0.11	Duluth, MN-WI	0.06		
Anaheim-Santa Ana, CA	0.24	0.21	0.20	Eau Claire, WI		0.06	
Anderson, IN	0.10			El Paso, TX	0.14	0.14	0.13
Anderson, SC			0.09	Elmira, NY	0.09	0.10	0.10
Ann Arbor, MI	0.10	0.09	0.11	Erie, PA	0.12	0.10	0.11
Appleton-Oshkosh-Neenah, WI	0.10	0.08	0.09	Eugene-Springfield, OR	0.08	0.09	0.09
Asheville, NC	0.08	0.09	0.08	Evansville, IN-KY	0.12	0.11	0.12
Atlanta, GA	0.12	0.15	0.13	Fayetteville, NC	0.11	0.10	0.10
Atlantic City, NJ	0.12	0.16	0.14	Flint, MI	0.10	0.10	0.10
Augusta, GA-SC	0.10	0.11	0.10	Fort Collins, CO	0.09	0.10	0.09
Aurora-Elgin, IL	0.11	0.09	0.13	Ft. Lauderdale-Hollywood-Pompano, FL	0.12	0.10	0.10
Austin, TX	0.11	0.11	0.10	Fort Myers-Cape Coral, FL	0.10	0.08	0.08
Bakersfield, CA	0.16	0.16	0.16	Fort Wayne, IN	0.12	0.09	0.10
Baltimore, MD	0.13	0.14	0.16	Fort Worth-Arlington, TX	0.13	0.14	0.15
Baton Rouge, LA	0.16	0.18	0.14	Fresno, CA	0.15	0.15	0.16
Beaumont-Port Arthur, TX	0.15	0.15	0.13	Galveston-Texas City, TX	0.14	0.15	0.15
Beaver County, PA	0.10	0.10	0.11	Gary-Hammond, IN	0.11	0.12	0.12
Bellingham, WA	0.05	0.08	0.07	Grand Rapids, MI	0.13	0.14	0.15
Benton Harbor, MI			0.12	Greeley, CO	0.10	0.11	0.10
Bergen-Passaic, NJ	0.12	0.13	0.14	Green Bay, WI	0.09	0.09	0.10
Billings, MT	0.08			Greensboro-Winston Salem-High Point, NC	0.10	0.12	0.11
Birmingham, AL	0.12	0.13	0.11	Greenville-Spartanburg, SC	0.10	0.11	0.11
Boston, MA	0.12	0.11	0.13	Hamilton-Middletown, OH	0.11	0.13	0.12
Boulder-Longmont, CO	0.11	0.10	0.10	Harrisburg-Lebanon-Carlisle, PA	0.11	0.12	0.11
Bradenton, FL	0.10	0.10	0.10	Hartford, CT	0.14	0.15	0.15
Brazoria, TX		0.15	0.13	Hickory, NC		0.09	
Bridgeport-Milford, CT	0.18	0.16	0.15	Honolulu, HI	0.05	0.05	0.05
Brockton, MA	0.13	0.12	0.15	Houma-Thibodaux, LA	0.11	0.12	0.10
Buffalo, NY	0.11	0.11	0.11	Houston, TX	0.23	0.22	0.20
Canton, OH	0.12	0.11	0.12	Huntington-Ashland, WV-KY-OH	0.12	0.14	0.14
Cedar Rapids, IA	0.08	0.07	0.08	Huntsville, AL	0.09	0.09	0.11
Champaign-Urbana-Rantoul, IL	0.09	0.09	0.08	Indianapolis, IN	0.12	0.11	0.11
Charleston, SC	0.09	0.10	0.09	Iowa City, IA	0.09	0.09	0.06
Charleston, WV	0.10	0.12	0.12	Jackson, MS	0.09	0.10	0.09
Charlotte-Gastonia-Rock Hill, NC-SC	0.13	0.12	0.12	Jacksonville, FL	0.11	0.11	0.10
Chattanooga, TN-GA	0.11	0.12	0.10	Jamestown-Dunkirk, NY		0.08	0.10
Chicago, IL	0.12	0.11	0.13	Janesville-Beloit, WI	0.12	0.09	0.11
Chico, CA	0.10	0.12	0.09	Jersey City, NJ	0.12	0.18	0.14
Cincinnati, OH-KY-IN	0.12	0.15	0.14	Johnson City-Kingsport-Bristol, TN-WV	0.11	0.12	0.12
Cleveland, OH	0.12	0.12	0.13	Johnstown, PA	0.10	0.10	0.11
Colorado Springs, CO	0.09	0.09	0.09	Joliet, IL	0.10	0.09	0.12
Columbia, SC	0.10	0.11	0.11	Kalamazoo, MI			0.08
Columbus, GA-AL	0.09	0.11	0.10	Kansas City, MO-KS	0.11	0.11	0.12
Columbus, OH	0.11	0.11	0.12	Kenosha, WI	0.13	0.11	0.15
Corpus Christi, TX	0.10	0.10	0.11	Knoxville, TN	0.10	0.12	0.11
Cumberland, MD-WV		0.09	0.10	Lafayette, LA	0.10	0.11	0.08
Dallas, TX	0.13	0.14	0.12	Lafayette, IN	0.09	0.10	
Danbury, CT	0.13	0.15	0.14	Lake Charles, LA	0.13	0.13	0.12
Davenport-Rock Island-Moline, IA-IL	0.11	0.10	0.10	Lake County, IL	0.13	0.10	0.12
Dayton-Springfield, OH	0.15	0.12	0.12	Lancaster, PA	0.10	0.10	0.12

Table 4-3 (cont'd). The Highest Second Daily Maximum One-Hour Ozone Concentration (ppm) by Metropolitan Statistical Area (MSA) for the Years 1989 to 1991

MSA	1989	1990	1991	MSA	1989	1990	1991
Lansing-East Lansing, MI	0.10	0.10	0.11	Portland, OR-WA	0.09	0.15	0.11
Las Cruces, NM	0.11	0.10	0.10	Portsmouth-Dover-Rochester, NH-ME	0.11	0.10	0.13
Las Vegas, NV	0.11	0.11	0.09	Poughkeepsie, NY	0.08	0.12	0.13
Lawrence-Haverhill, MA-NH	0.12	0.10	0.13	Providence, RI	0.13	0.14	0.16
Lexington-Fayette, KY	0.11	0.11	0.10	Provo-Orem, UT	0.11	0.09	0.08
Lima, OH	0.10	0.10	0.10	Racine, WI	0.14	0.11	0.14
Lincoln, NE	0.06	0.07	0.07	Raleigh-Durham, NC	0.11	0.12	0.11
Little Rock-North Little Rock, AR	0.09	0.10	0.10	Reading, PA	0.11	0.11	0.12
Longview-Marshall, TX	0.10	0.13	0.11	Redding, CA	0.09	0.09	0.08
Lorain-Elyria, OH	0.12	0.09	0.10	Reno, NV	0.10	0.14	0.09
Los Angeles-Long Beach, CA	0.33	0.27	0.31	Richmond-Petersburg, VA	0.11	0.12	0.12
Louisville, KY-IN	0.11	0.13	0.13	Riverside-San Bernardino, CA	0.28	0.30	0.25
Lynchburg, VA		0.10	0.09	Roanoke, VA	0.10	0.09	0.10
Madison, WI	0.10	0.08	0.11	Rochester, NY	0.11	0.11	0.11
Manchester, NH	0.10	0.10	0.10	Rockford, IL	0.10	0.09	0.09
Medford, OR	0.09	0.10	0.07	Sacramento, CA	0.14	0.16	0.16
Melbourne-Titusville-Palm Bay, FL	0.10	0.09	0.09	St. Louis, MO-IL	0.13	0.13	0.12
Memphis, TN-AR-MS	0.12	0.12	0.11	Salinas-Seaside-Monterey, CA	0.11	0.09	0.09
Miami-Hialeah, FL	0.12	0.11	0.12	Salt Lake City-Ogden, UT	0.15	0.12	0.11
Middlesex-Somerset-Hunterdon, NJ	0.13	0.15	0.13	San Antonio, TX	0.11	0.10	0.11
Middletown, CT	0.17	0.16	0.17	San Diego, CA	0.19	0.17	0.18
Milwaukee, WI	0.15	0.13	0.18	San Francisco, CA	0.09	0.06	0.07
Minneapolis-St. Paul, MN-WI	0.10	0.10	0.09	San Jose, CA	0.13	0.12	0.12
Mobile, AL	0.10	0.11	0.09	San Juan, PR	0.06	0.07	0.08
Modesto, CA	0.13	0.12	0.11	Santa Barbara-Santa Maria-Lompoc, CA	0.16	0.13	0.10
Monmouth-Ocean, NJ	0.14	0.14	0.15	Santa Cruz, CA	0.08	0.08	0.10
Montgomery, AL	0.08	0.10	0.09	Santa Fe, NM	0.05	0.08	0.08
Muskegon, MI	0.14	0.13	0.15	Santa Rosa-Petaluma, CA	0.10	0.08	0.10
Nashua, NH	0.09	0.10	0.11	Sarasota, FL	0.10	0.10	0.10
Nashville, TN	0.14	0.13	0.12	Scranton-Wilkes-Barre, PA	0.11	0.11	0.13
Nassau-Suffolk, NY	0.15	0.14	0.18	Seattle, WA	0.09	0.13	0.11
New Bedford, MA	0.12	0.13	0.13	Sharon, PA	0.11	0.10	0.11
New Haven-Meriden, CT	0.15	0.16	0.18	Sheboygan, WI	0.11	0.11	0.16
New London-Norwich, CT-RI	0.14	0.16	0.14	Shreveport, LA	0.12	0.12	0.11
New Orleans, LA	0.11	0.11	0.11	South Bend-Mishawaka, IN	0.10	0.10	0.11
New York, NY	0.13	0.16	0.18	Spokane, WA		0.07	0.08
Newark, NJ	0.13	0.13	0.14	Springfield, IL	0.11	0.10	0.10
Niagara Falls, NY	0.10	0.10	0.10	Springfield, MO	0.09	0.08	0.08
Norfolk-Virginia Beach-Newport News, VA	0.10	0.11	0.11	Springfield, MA	0.13	0.12	0.13
Oakland, CA	0.13	0.12	0.12	Stamford, CT	0.16	0.14	0.15
Oklahoma City, OK	0.11	0.11	0.11	Steubenville-Weirton, OH-WV	0.11	0.09	0.12
Omaha, NE-IA	0.10	0.08	0.08	Stockton, CA	0.11	0.12	0.11
Orlando, FL	0.11	0.12	0.10	Syracuse, NY	0.10	0.11	0.11
Owensboro, KY	0.10	0.11	0.09	Tacoma, WA	0.09	0.13	0.09
Oxnard-Ventura, CA	0.17	0.15	0.16	Tallahassee, FL	0.07		0.05
Parkerburg-Marietta, WV-OH	0.12	0.11	0.12	Tampa-St. Petersburg-Clearwater, FL	0.10	0.11	0.11
Pascogoula, MS	0.10	0.11	0.10	Terre Haute, IN	0.11	0.11	0.10
Pensacola, FL	0.09	0.12	0.11	Toledo, OH	0.11	0.10	0.12
Peoria, IL	0.11	0.09	0.10	Trenton, NJ	0.14	0.14	0.15
Philadelphia, PA-NJ	0.16	0.14	0.16	Tucson, AZ	0.10	0.10	0.09
Phoenix, AZ	0.11	0.14	0.12	Tulsa, OK	0.12	0.12	0.12
Pittsburgh, PA	0.13	0.11	0.12	Utica-Rome, NY	0.09	0.10	0.10
Pittsfield, MA	0.09	0.11	0.10	Vallejo-Fairfield-Napa, CA	0.11	0.10	0.11
Portland, ME	0.13	0.13	0.14	Vancouver, WA	0.09	0.11	0.10

Table 4-3 (cont'd). The Highest Second Daily Maximum One-Hour Ozone Concentration (ppm) by Metropolitan Statistical Area (MSA) for the Years 1989 to 1991

MSA	1989	1990	1991	MSA	1989	1990	1991
Victoria, TX	0.10	0.07	0.10	Wilmington, DE-NJ-MD	0.13	0.14	0.15
Vineland-Millville-Bridgeton, NJ	0.13	0.13	0.12	Wilmington, NC		0.09	
Visalia-Tulare-Porterville, CA	0.15	0.14	0.12	Worcester, MA	0.10	0.12	0.14
Washington, DC-MD-VA	0.13	0.13	0.14	York, PA	0.10	0.12	0.11
W. Palm Beach-Boca Raton-Delray, FL	0.11	0.09	0.09	Youngstown-Warren, OH	0.11	0.10	0.12
Wheeling, WV-OH	0.11	0.11	0.11	Yuba City, CA	0.01	0.09	0.10
Wichita, KS	0.09	0.10	0.10	Yuma, AZ		0.09	0.09
Williamsport, PA	0.08	0.09	0.10				

high-oxidant urban areas where O_3 regimes contain hourly average concentrations with sharper peaks. The result would be an increase in the number of exceedances of daily maximum 8-h average concentrations ≥ 0.08 ppm, when compared to those sites experiencing sharper peaks. Lefohn et al. (1993b), using aerometric data at specific sites, observed how O_3 concentrations change when the sites change compliance status. One of the parameters examined was 4-h daily maxima. The number of exceedances for a specific daily maximum average concentration tended to decrease as fewer exceedances of the current 1-h standard were observed at a given site. The number of occurrences of the daily maximum 4-h average concentration ≥ 0.08 ppm and the number of exceedances of the current form of the standard had a positive, weak correlation ($r = 0.51$). Lefohn et al. (1993a,b) reported few changes in the shape of the average diurnal patterns as sites changed attainment status; this may have explained why Lefohn et al. (1993b) could not find evidence that the number of occurrences of the daily maximum 4-h average concentration ≥ 0.08 ppm increased when the sites experienced few high hourly average concentrations.

There has been considerable interest in possibly substituting one index for another when attempting to relate O_3 exposure with an effect. For example, using O_3 ambient air quality data, McCurdy (1988) compared the number of exceedances of 0.12 ppm and the number of occurrences of the daily maximum 8-h average concentrations ≥ 0.08 ppm and reported that a positive correlation ($r = 0.79$) existed between the second-highest 1-h daily maximum in a year and the expected number of days with an 8-h daily maximum average concentration > 0.08 ppm O_3. In this case, the predictive strength of using one O_3 exposure index to predict another is not strong.

Similar to analysis performed by McCurdy (1988), all of the hourly averaged data from rural agricultural and forested sites in the AIRS database were summarized into maximum 3-mo SUM06, second highest daily maximum hourly average concentration, and second highest daily maximum 8-h average concentration exposure indices per year for the period 1980 to 1991. For the rural agricultural sites, the correlation coefficients between the 3-mo SUM06 and the second highest daily maximum hourly average concentration and the second highest daily maximum 8-h average concentration were 0.650 and 0.739, respectively (Figure 4-5). For the rural forested sites, the correlation coefficients between the 3-mo SUM06 and the second highest daily maximum hourly average concentration and the second highest daily maximum 8-h average concentration were 0.585 and 0.683, respectively (Figure 4-6).

Table 4-4. Summary of Percentiles of Hourly Average Concentrations (ppm) for the April-to-October Period[a]

AIRS Site	Name	Year	Min.	10	30	50	70	90	95	99	Max	Number of Observations
060370016	Glendora, CA	1989	0.000	0.000	0.020	0.030	0.060	0.120	0.150	0.220	0.340	4,874
		1990	0.000	0.000	0.010	0.030	0.050	0.110	0.140	0.200	0.290	4,888
		1991	0.000	0.000	0.010	0.020	0.050	0.100	0.140	0.200	0.320	4,907
060595001	La Habra, CA	1989	0.000	0.000	0.010	0.030	0.040	0.070	0.090	0.140	0.260	4,875
		1990	0.000	0.000	0.010	0.020	0.040	0.070	0.090	0.140	0.210	4,887
		1991	0.000	0.000	0.010	0.020	0.040	0.070	0.090	0.130	0.210	4,899
060710005	San Bernardino County, CA	1989	0.000	0.020	0.050	0.060	0.090	0.140	0.160	0.200	0.270	4,871
		1990	0.000	0.020	0.040	0.060	0.080	0.120	0.150	0.180	0.330	4,899
		1991	0.000	0.020	0.040	0.060	0.080	0.120	0.140	0.190	0.270	4,905
060731001	Del Mar, CA	1989	0.000	0.020	0.040	0.040	0.050	0.070	0.080	0.120	0.250	4,814
		1990	0.000	0.020	0.030	0.040	0.050	0.060	0.070	0.100	0.170	5,060
		1991	0.000	0.020	0.040	0.050	0.050	0.060	0.070	0.100	0.150	5,017
090013007	Stratford, CT	1989	0.001	0.008	0.024	0.036	0.046	0.064	0.077	0.115	0.202	4,673
		1990	0.001	0.010	0.023	0.033	0.044	0.059	0.068	0.100	0.176	3,853
		1991	0.000	0.007	0.019	0.030	0.042	0.060	0.074	0.110	0.157	4,794
090093002	Madison, CT	1989	0.001	0.008	0.022	0.033	0.043	0.059	0.070	0.103	0.149	4,272
		1990	0.000	0.008	0.023	0.033	0.043	0.063	0.075	0.107	0.197	4,477
		1991	0.000	0.007	0.023	0.034	0.045	0.065	0.082	0.123	0.193	4,814
220330003	Baton Rouge, LA	1989	0.000	0.001	0.009	0.021	0.034	0.059	0.069	0.094	0.168	4,964
		1990	0.000	0.000	0.011	0.023	0.038	0.063	0.079	0.109	0.187	5,000
		1991	0.000	0.002	0.010	0.020	0.031	0.054	0.067	0.092	0.134	4,905
340170006	Bayonne, NJ	1989	0.001	0.001	0.008	0.021	0.036	0.059	0.074	0.099	0.147	4,815
		1990	0.001	0.001	0.009	0.022	0.036	0.058	0.073	0.106	0.185	4,939
		1991	0.001	0.002	0.011	0.024	0.038	0.065	0.082	0.110	0.167	4,943
360610063	New York, NY	1989	0.000	0.015	0.028	0.040	0.051	0.073	0.086	0.110	0.134	4,825
		1990	0.000	0.014	0.029	0.039	0.051	0.074	0.090	0.116	0.175	4,707
		1991	0.002	0.015	0.029	0.041	0.056	0.082	0.096	0.123	0.177	4,910
361030002	Babylon, NY	1989	0.001	0.004	0.015	0.027	0.039	0.060	0.073	0.101	0.156	4,407
		1990	0.000	0.006	0.017	0.027	0.040	0.060	0.075	0.105	0.146	4,876
		1991	0.001	0.005	0.018	0.030	0.044	0.067	0.081	0.111	0.217	4,873

Table 4-4 (cont'd). Summary of Percentiles of Hourly Average Concentrations (ppm) for the April-to-October Period[a]

AIRS Site	Name	Year	Min.	10	30	50	70	90	95	99	Max	Number of Observations
482010024	Harris County, TX	1989	0.000	0.000	0.010	0.020	0.030	0.060	0.070	0.110	0.230	4,728
		1990	0.000	0.000	0.010	0.020	0.040	0.070	0.090	0.130	0.220	4,274
		1991	0.000	0.000	0.000	0.020	0.030	0.060	0.080	0.110	0.170	4,322
550790085	Bayside, WI	1989	0.002	0.006	0.024	0.035	0.046	0.066	0.077	0.101	0.151	4,376
		1990	0.002	0.009	0.025	0.034	0.044	0.061	0.071	0.094	0.130	4,395
		1991	0.002	0.008	0.025	0.035	0.047	0.070	0.081	0.113	0.189	4,303

[a]See Appendix A for abbreviations and acronyms.

Table 4-5. The Highest Second Daily Maximum Eight-Hour Average Ozone Concentration (ppm) by Metropolitan Statistical Area (MSA) for the Years 1989 to 1991

MSA	1989	1990	1991	MSA	1989	1990	1991
Akron, OH	0.109	0.097	0.102	Davenport-Rock Island-Moline, IA-IL	0.102	0.071	0.086
Albany-Schenectady-Troy, NY	0.087	0.091	0.089	Dayton-Springfield, OH	0.122	0.096	0.107
Albuquerque, NM	0.078	0.089	0.077	Decatur, IL	0.084	0.077	0.087
Alexandria, LA	0.077	0.076	0.074	Denver, CO	0.089	0.086	0.080
Allentown-Bethlehem, PA-NJ	0.091	0.098	0.112	Des Moines, IA	0.073	0.051	0.056
Altoona, PA	0.077	0.090	0.094	Detroit, MI	0.103	0.091	0.111
Anaheim-Santa Ana, CA	0.146	0.135	0.110	Duluth, MN-WI	0.073	0.051	
Anderson, IN	0.084			Eau Claire, WI		0.049	
Anderson, SC			0.081	El Paso, TX	0.083	0.087	0.080
Ann Arbor, MI	0.093	0.087	0.096	Elmira, NY	0.075	0.080	0.094
Appleton-Oshkosh-Neenah, WI	0.091	0.078	0.082	Erie, PA	0.092	0.088	0.093
Asheville, NC	0.083	0.074	0.064	Eugene-Springfield, OR	0.061	0.077	0.070
Atlanta, GA	0.096	0.125	0.102	Evansville, IN-KY	0.097	0.094	0.107
Atlantic City, NJ	0.104	0.135	0.112	Fayetteville, NC	0.089	0.088	0.085
Augusta, GA-SC	0.078	0.092	0.081	Flint, MI	0.093	0.086	0.090
Aurora-Elgin, IL	0.088	0.077	0.100	Fort Collins, CO	0.076	0.076	0.077
Austin, TX	0.099	0.103	0.084	Ft. Lauderdale-Hollywood-Pompano, FL	0.089	0.078	0.064
Bakersfield, CA	0.124	0.120	0.118	Fort Myers-Cape Coral, FL	0.084	0.070	0.062
Baltimore, MD	0.103	0.111	0.127	Fort Wayne, IN	0.105	0.091	0.096
Baton Rouge, LA	0.095	0.134	0.100	Fort Worth-Arlington, TX	0.098	0.116	0.116
Beaumont-Port Arthur, TX	0.110	0.100	0.101	Fresno, CA	0.116	0.105	0.119
Beaver County, PA	0.095	0.085	0.095	Galveston-Texas City, TX	0.102	0.096	0.094
Bellingham, WA	0.038	0.068	0.059	Gary-Hammond, IN	0.102	0.122	0.101
Benton Harbor, MI			0.098	Grand Rapids, MI	0.119	0.107	0.124
Bergen-Passaic, NJ	0.093	0.097	0.106	Greeley, CO	0.080	0.080	0.081
Billings, MT	0.56			Green Bay, WI	0.095	0.074	0.079
Biloxi-Gulfport, TX			0.089	Greensboro-Winston Salem-High Point, NC	0.083	0.100	0.087
Birmingham, AL	0.088	0.105	0.088	Greenville-Spartanburg, SC	0.088	0.091	0.085
Bismark, ND	0.086	0.062	0.061	Hamilton-Middletown, OH	0.095	0.111	0.105
Bloomington-Normal, IL	0.081	0.071	0.095	Harrisburg-Lebanon-Carlisle, PA	0.091	0.108	0.100
Boston, MA	0.109	0.105	0.118	Hartford, CT	0.114	0.109	0.112
Boulder-Longmont, CO	0.082	0.084	0.083	Hickory, NC		0.080	
Bradenton, FL	0.086	0.075	0.074	Honolulu, HI	0.020	0.037	0.042
Brazoria, TX		0.101	0.107	Houma-Thibodaux, LA	0.082	0.084	0.077
Bridgeport-Milford, CT	0.139	0.114	0.121	Houston, TX	0.121	0.141	0.115
Brockton, MA	0.110	0.106	0.107	Huntington-Ashland, WV-KY-OH	0.102	0.109	0.124
Buffalo, NY	0.085	0.096	0.097	Huntsville, AL	0.072	0.080	0.082
Canton, OH	0.098	0.098	0.099	Indianapolis, IN	0.097	0.099	0.100
Carson City, NV	0.070			Iowa City, IA	0.078	0.084	0.060
Cedar Rapids, IA	0.078	0.057	0.066	Jackson, MS	0.086	0.083	0.075
Champaign-Urbana-Rantoul, IL	0.084	0.080	0.077	Jacksonville, FL	0.090	0.084	0.077
Charleston, SC	0.094	0.084	0.074	Jamestown-Dunkirk, NY		0.068	0.082
Charleston, WV	0.087	0.083	0.099	Janesville-Beloit, WI	0.097	0.081	0.090
Charlotte-Gastonia-Rock Hill, NC-SC	0.089	0.100	0.094	Jersey City, NJ	0.105	0.128	0.117
Charlottesville, VA	0.076	0.089	0.091	Johnson City-Kingsport-Bristol, TN-WV	0.083	0.100	0.080
Chattanooga, TN-GA	0.091	0.094	0.083	Johnstown, PA	0.082	0.090	0.099
Chicago, IL	0.101	0.084	0.106	Joliet, IL	0.082	0.070	0.091
Chico, CA	0.081	0.083	0.074	Kalamazoo, MI			0.071
Cincinnati, OH-KY-IN	0.106	0.119	0.115	Kansas City, MO-KS	0.090	0.089	0.089
Cleveland, OH	0.099	0.096	0.101	Kenosha, WI	0.113	0.093	0.118
Colorado Springs, CO	0.072	0.065	0.068	Knoxville, TN	0.088	0.105	0.091
Columbia, SC	0.079	0.094	0.083	Lafayette, LA	0.080	0.086	0.075
Columbus, GA-AL	0.068	0.075	0.083	Lafayette, IN	0.077	0.092	0.090
Columbus, OH	0.097	0.098	0.112	Lake Charles, LA	0.088	0.084	0.096
Corpus Christi, TX	0.083	0.085	0.075	Lake County, IL	0.092	0.082	0.102
Cumberland, MD-WV		0.070	0.076	Lancaster, PA	0.085	0.089	0.096
Dallas, TX	0.101	0.115	0.095	Lansing-East Lansing, MI	0.093	0.083	0.087
Danbury, CT	0.098	0.105	0.116	Las Cruces, NM	0.074	0.082	0.074

Table 4-5 (cont'd). The Highest Second Daily Maximum Eight-Hour Average Ozone Concentration (ppm) by Metropolitan Statistical Area (MSA) for the Years 1989 to 1991

MSA	1989	1990	1991	MSA	1989	1990	1991
Las Vegas, NV	0.084	0.082	0.075	Provo-Orem, UT	0.094	0.070	0.071
Lawrence-Haverhill, MA-NH	0.104	0.077	0.106	Racine, WI	0.110	0.090	0.118
Lewiston-Auburn, ME	0.089	0.090	0.101	Raleigh-Durham, NC	0.099	0.094	0.091
Lexington-Fayette, KY	0.097	0.097	0.088	Reading, PA	0.095	0.101	0.109
Lima, OH	0.088	0.086	0.091	Redding, CA	0.080	0.100	0.093
Lincoln, NE	0.057	0.060	0.060	Reno, NV	0.081	0.109	0.075
Little Rock-North Little Rock, AR	0.077	0.083	0.089	Richmond-Petersburg, VA	0.094	0.101	0.097
Longview-Marshall, TX	0.076	0.089	0.086	Riverside-San Bernardino, CA	0.196	0.193	0.189
Lorain-Elyria, OH	0.096	0.082	0.091	Roanoke, VA	0.077	0.075	0.078
Los Angeles-Long Beach, CA	0.188	0.170	0.178	Rochester, NY	0.094	0.097	0.103
Louisville, KY-IN	0.096	0.093	0.119	Rockford, IL	0.085	0.073	0.081
Lynchburg, VA		0.083	0.079	Sacramento, CA	0.105	0.125	0.124
Madison, WI	0.089	0.079	0.089	St. Louis, MO-IL	0.105	0.098	0.107
Manchester, NH	0.084	0.098	0.087	Salinas-Seaside-Monterey, CA	0.082	0.074	0.071
Medford, OR	0.063	0.076	0.055	Salt Lake City-Ogden, UT	0.114	0.086	0.086
Melbourne-Titusville-Palm Bay, FL	0.082	0.082	0.069	San Antonio, TX	0.100	0.080	0.085
Memphis, TN-AR-MS	0.099	0.100	0.093	San Diego, CA	0.139	0.135	0.128
Miami-Hialeah, FL	0.087	0.076	0.072	San Francisco, CA	0.064	0.056	0.054
Middlesex-Somerset-Hunterdon, NJ	0.108	0.111	0.111	San Jose, CA	0.094	0.075	0.086
Middletown, CT	0.119	0.117	0.125	San Juan, PR	0.043	0.042	0.044
Milwaukee, WI	0.115	0.100	0.118	Santa Barbara-Santa Maria-Lompoc, CA	0.129	0.129	0.075
Minneapolis-St. Paul, MN-WI	0.090	0.077	0.079	Santa Cruz, CA	0.066	0.058	0.067
Mobile, AL	0.079	0.098	0.062	Santa Fe, NM	0.049	0.069	0.076
Modesto, CA	0.101	0.106	0.091	Santa Rosa-Petaluma, CA	0.083	0.061	0.076
Monmouth-Ocean, NJ	0.118	0.107	0.122	Sarasota, FL	0.085	0.083	0.080
Montgomery, AL	0.066	0.081	0.071	Scranton-Wilkes-Barre, PA	0.088	0.096	0.111
Muskegon, MI	0.139	0.100	0.119	Seattle, WA	0.078	0.099	0.087
Nashua, NH	0.072	0.095	0.110	Sharon, PA	0.098	0.095	0.094
Nashville, TN	0.093	0.102	0.107	Sheboygan, WI	0.103	0.088	0.103
Nassau-Suffolk, NY	0.099	0.115	0.121	Shreveport, LA	0.098	0.102	0.087
New Bedford, MA	0.104	0.101	0.106	South Bend-Mishawaka, IN	0.089	0.089	0.093
New Haven-Meriden, CT	0.108	0.122	0.128	Spokane, WA		0.060	0.060
New London-Norwich, CT-RI	0.128	0.127	0.115	Springfield, IL	0.085	0.082	0.087
New Orleans, LA	0.075	0.086	0.079	Springfield, MO	0.075	0.061	0.069
New York, NY	0.111	0.119	0.133	Springfield, MA	0.123	0.113	0.117
Newark, NJ	0.108	0.107	0.119	Stamford, CT	0.113	0.112	0.115
Niagara Falls, NY	0.082	0.092	0.095	Steubenville-Weirton, OH-WV	0.094	0.075	0.098
Norfolk-Virginia Beach-Newport News, VA	0.089	0.095	0.089	Stockton, CA	0.086	0.093	0.090
Oakland, CA	0.091	0.091	0.083	Syracuse, NY	0.090	0.093	0.098
Oklahoma City, OK	0.089	0.090	0.089	Tacoma, WA	0.077	0.094	0.077
Omaha, NE-IA	0.075	0.075	0.073	Tallahassee, FL	0.072		
Orlando, FL	0.096	0.082	0.075	Tampa-St. Petersburg-Clearwater, FL	0.088	0.085	0.083
Owensboro, KY	0.096	0.104	0.077	Terre Haute, IN	0.087	0.095	0.089
Oxnard-Ventura, CA	0.147	0.119	0.129	Toledo, OH	0.093	0.084	0.107
Parkersburg-Marietta, WV-OH	0.094	0.088	0.104	Trenton, NJ	0.119	0.112	0.131
Pascagoula, MS	0.082	0.092	0.077	Tucson, AZ	0.074	0.084	0.080
Pensacola, FL	0.080	0.098	0.082	Tulsa, OK	0.093	0.094	0.097
Peoria, IL	0.087	0.075	0.088	Utica-Rome, NY	0.082	0.094	0.091
Philadelphia, PA-NJ	0.118	0.110	0.123	Vallejo-Fairfield-Napa, CA	0.076	0.074	0.078
Phoenix, AZ	0.086	0.096	0.094	Vancouver, WA	0.058	0.080	0.042
Pittsburgh, PA	0.107	0.100	0.106	Victoria, TX	0.093	0.056	0.086
Pittsfield, MA	0.075	0.094	0.095	Vineland-Millvile-Bridgeton, NJ	0.122	0.106	0.108
Portland, ME	0.124	0.109	0.134	Visalia-Tulare-Porterville, CA	0.114	0.103	0.104
Portland, OR-WA	0.071	0.111	0.092	Washington, DC-MD-VA	0.106	0.110	0.114
Portsmouth-Dover-Rochester, NH-ME	0.107	0.086	0.123	W. Palm Beach-Boca Raton-Delray, FL	0.081	0.067	0.059
Poughkeepsie, NY	0.079	0.085	0.101	Wheeling, WV-OH	0.086	0.089	0.093
Providence, RI	0.107	0.112	0.127	Wichita, KS	0.079	0.089	0.081

Table 4-5 (cont'd). The Highest Second Daily Maximum Eight-Hour Average Ozone Concentration (ppm) by Metropolitan Statistical Area (MSA) for the Years 1989 to 1991

MSA	1989	1990	1991	MSA	1989	1990	1991
Williamsport, PA	0.065	0.072	0.087	York, PA	0.091	0.108	0.103
Wilmington, DE-NJ-MD	0.105	0.110	0.118	Youngstown-Warren, OH	0.088	0.085	0.101
Wilmington, NC		0.086		Yuba City, CA	0.084	0.076	0.084
Worcester, MA	0.097	0.089	0.107	Yuma, AZ	0.080	0.075	0.070

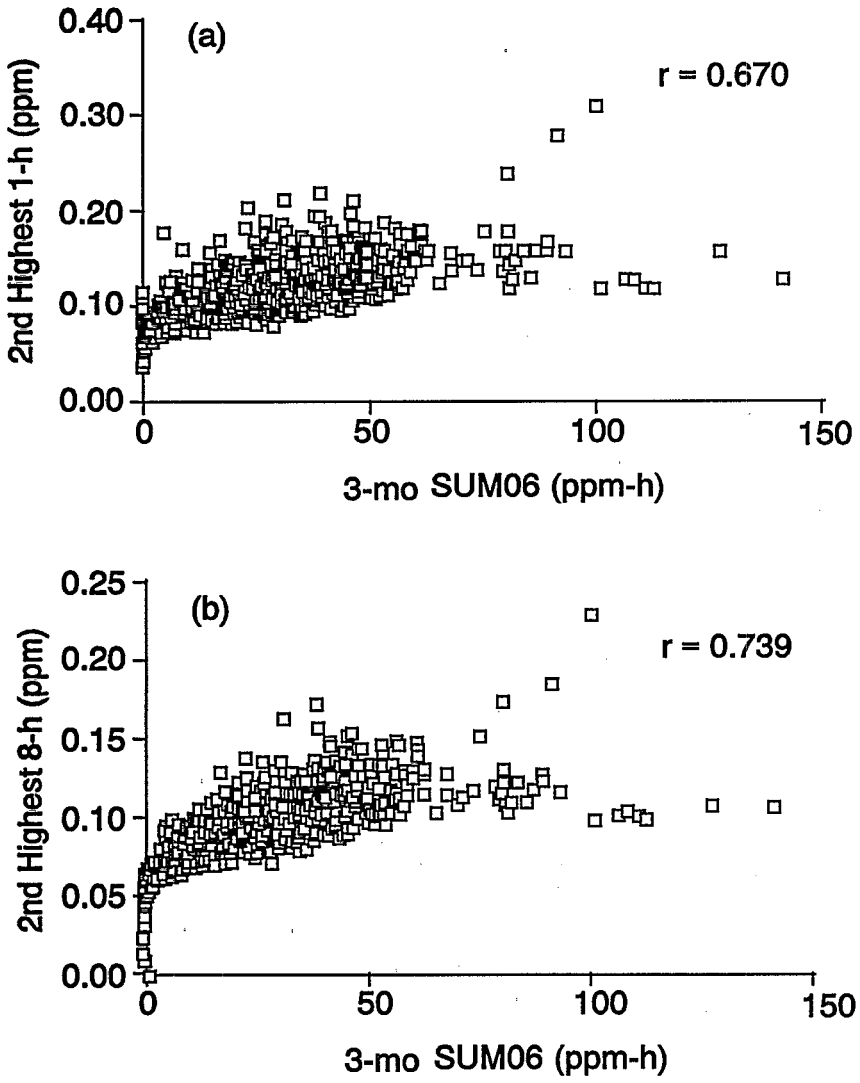


Figure 4-5. ***The relationship between (a) the second highest daily maximum hourly average ozone (O_3) concentration and the maximum 3-mo SUM06 value and (b) the second highest daily maximum 8-h average O_3 concentration and the maximum 3-mo SUM06 value for specific site years at rural agricultural sites for the 1980-to-1991 period.***

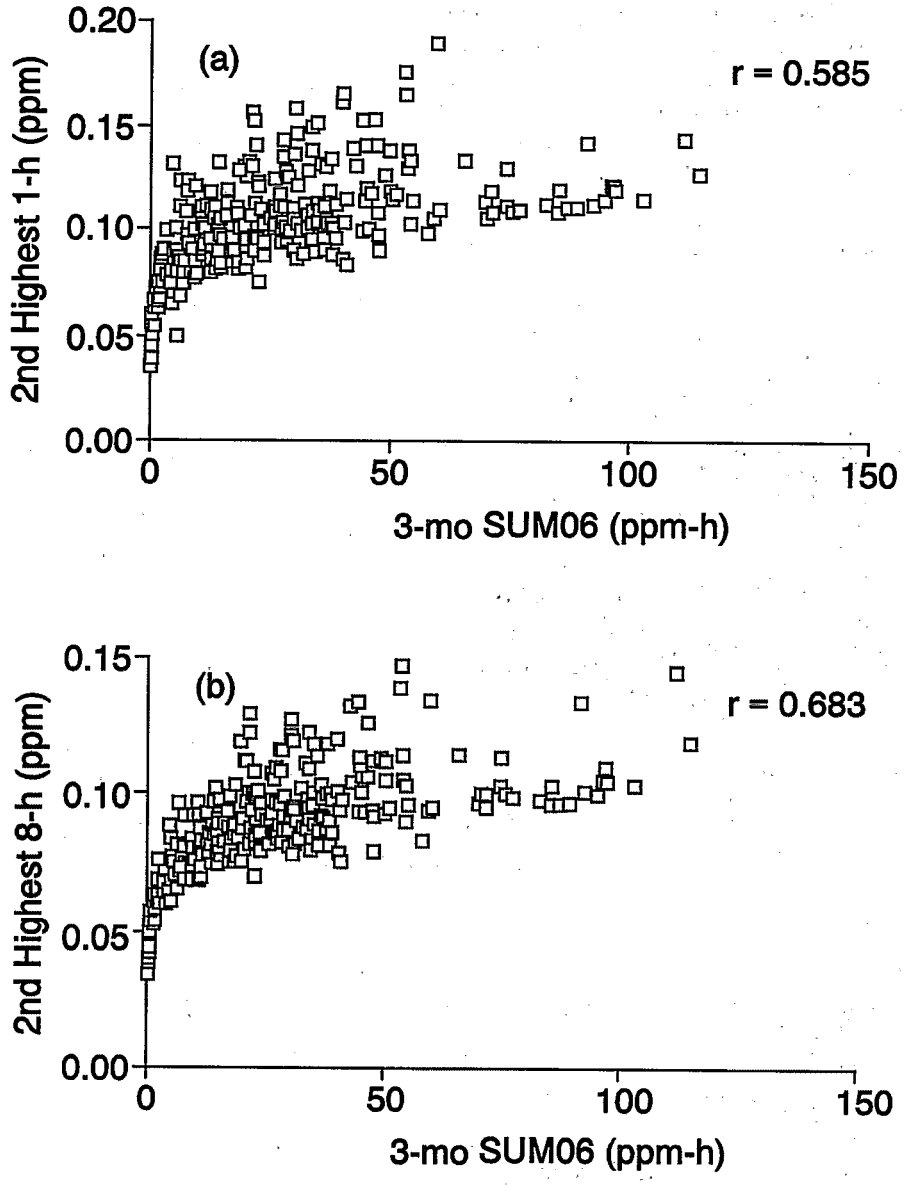


Figure 4-6. The relationship between (a) the second highest daily maximum hourly average ozone (O_3) concentration and the maximum 3-mo SUM06 value and (b) the second highest daily maximum 8-h average O_3 concentration and the maximum 3-mo SUM06 value for specific site years at rural forested sites for the 1980-to-1991 period.

One of the difficulties in attempting to use correlation analysis between indices for rationalizing the substitution of one exposure index for another to predicting an effect (e.g., SUM06 versus the second highest daily maximum hourly average concentration) is the introduction of the error associated with estimating levels of one index from another. Lefohn et al. (1989) have recommended that if a different exposure index (e.g., second highest daily maximum hourly average concentration) is to be compared to, for example, the SUM06 for

adequacy in predicting crop loss, then the focus should be on how well the two exposure indices predict crop loss using the effects model that is a function of the most relevant index, and not on how well the indices predict one another. Using data from both urban and rural O_3 monitoring sites in the midwestern United States that were located near agricultural or forested areas, Lefohn et al. (1989) reported a large amount of scatter between the second highest daily maximum hourly average concentration and the SUM06 indices. This large scatter indicated considerable uncertainty when attempting to predict a value for SUM06, given a specific second highest daily maximum hourly average concentration value. The authors reported that for a given second highest daily maximum hourly average concentration, the SUM06 values varied over a large range. Lefohn et al. (1989) concluded that such large uncertainty would introduce additional uncertainty when attempting to use the predicted exposure index to estimate an effect. The authors concluded that less error would be introduced if either of the two indices were used directly in the development of an exposure-response model.

As pointed out by the U.S. Environmental Protection Agency (1986), a familiar measure of O_3 air quality is the number or percentage of days on which some specific concentration is equalled or exceeded. This measure, however, does not shed light on one of the more important questions regarding the effects of O_3 on both people and plants: what is the possible significance of high concentrations lasting 1 h or longer and then recurring on 2 or more successive days?

The recurrence of high O_3 concentrations on consecutive days was examined in four cities (one site in each city) by the U.S. Environmental Protection Agency (1986). The numbers of multiday events were tallied by length of event (i.e., how many consecutive days) using data for the daylight hours (0600 to 2000 hours) in the second and third quarters of 1979 through 1981. These sites were selected because they included areas known to experience high O_3 concentrations (e.g., California), and because they represent different geographic regions of the country (west, southwest, and east).

Because of the importance of episodes and respites, the U.S. Environmental Protection Agency (1986) commented on the occurrences of the length of episodes and the time between episodes. The agency concluded that its analysis showed variations among sites in the lengths of episodes as well as the respite periods. In its discussion, the U.S. Environmental Protection Agency (1986) defined a day or series of days on which the daily 1-h maximum reached or exceeded the specified level as an "exposure"; the intervening day or days when that level was not reached was called a "respite". Four O_3 concentrations were selected: 0.06, 0.12, 0.18, and 0.24 ppm. At the Dallas site, for example, the value equalled or exceeded 0.06 ppm for more than 7 days in a row. The Pasadena site experienced 10 such exposures, but these 10 exposure events spanned 443 days; in Dallas, the 11 exposures involved only 168 days. At the lowest concentration ($\geq$0.06 ppm), the Dallas station recorded more short-term ($\leq$7 days) exposures (45) involving more days (159) than the Pasadena station (14 exposures over 45 days) because the daily 1-h maximum statistic in Pasadena remained above 0.06 ppm for such protracted periods.
At concentrations $\geq$0.12 ppm, the lengthy exposures at the Pasadena site resolved into numerous shorter exposures, whereas in Dallas the exposures markedly dwindled in number and duration.

4.3.3 Nonurban Area Concentrations

4.3.3.1 Sites That Experience Low Maximum Hourly Average Concentrations

It is important to establish reference points that can be used to describe the distribution of hourly average concentrations at monitoring sites experiencing low maximum concentrations. Doing so will make it possible to confirm that the hourly average concentrations in control chambers utilized for research experiments associated with human health and vegetation effects are similar to those experienced under ambient conditions. For example, there has been concern expressed that O_3 concentrations in charcoal-filtration chambers used by NCLAN did not simulate the levels in those areas of the United States that experience low maximum O_3 concentrations (see Chapter 5, Section 5.5). Heuss (1982) expressed concern that the O_3 levels in the charcoal-filtration chambers were lower than those at sites experiencing low maximum hourly average concentrations, and that the resulting agricultural loss estimates derived from the NCLAN models may have been too high.

Two possible approaches for establishing reference points have been discussed (Lefohn et al., 1990a). One method is to estimate, using mathematical models and historical data, unpolluted background levels prior to disturbance by human influence. However, there are difficulties with this approach. Background can be defined as the unpolluted conditions in preindustrial times (i.e., absolutely unpolluted air in which there was no human interference). Alternatively, a background also can be defined as the condition *currently* existing at any location that is presently free from human influence. However, almost all geographic locations on the earth have been impacted by human influences (e.g., Finlayson-Pitts and Pitts, 1986; Hong et al., 1994) (see Section 4.3.1).

It is unlikely that a single value or even a fixed range of O_3 background values can apply uniformly across North America or elsewhere in the northern or southern hemispheres. Any attempt to quantify the historical background is subject to much uncertainty for the following reasons:

1. Little is known with certainty about the nature of past unpolluted conditions.
2. Even if all anthropogenic emissions of O_3 precursors were eliminated, it is unlikely that O_3 in, for example, eastern North America, would return to preindustrial levels. Since preindustrial times, major land use changes have occurred. Because substantial amounts of natural emissions of O_3 precursors are derived from soils and vegetation, especially during the warmer months, it is probable that these land use changes have modified the emissions of O_3 precursors and, thus, changed the concentrations of O_3.

Although not representing natural O_3 background, attempts have been made, using historical data, to estimate O_3 concentrations in the late 1800s and early 1900s. Model simulations and limited observations suggest that tropospheric O_3 has increased in the northern hemisphere since the preindustrial times and future increases may be possible (Bojkov, 1986; Volz and Kley, 1988; Thompson, 1992). However, several investigators have discussed the possible confounding influences that led to a great deal of uncertainty associated with characterizing the O_3 concentrations measured in the late 1800s and early 1900s (Lisac and Grubisic, 1991; Lefohn et al., 1992c; Cartalis and Varotsos, 1994). Using

data collected over the past 30 years, consistent trends in tropospheric O_3 have not been observed across the northern hemisphere (World Meteorological Organization/United Nations Environment Program, 1994). Using ozonesonde data, observations show that tropospheric O_3 has increased above some locations in the northern hemisphere. However, in the 1980s, the trends were variable and either small or nonexistent (Logan, 1994). European measurements at some surface sites indicate an increase in O_3 concentration since earlier this century. Because of the uncertainty associated with measurements taken in the late 1880s and early 1990s, it is difficult to compare O_3 concentration levels experienced in the United States with today's levels. However, it can be concluded that O_3 levels measured around 100 years ago were lower than the values observed today at most sites in the United States, and that consistently increasing trends in O_3 concentration measured at surface levels have not been observed annually at sites monitoring O_3 in the United States.

An alternative approach (adopted by Lefohn et al., 1990a, to establish reference points) is to examine O_3 hourly average concentration data from those monitoring sites in the world that experienced low maximum hourly average values. When data from these sites, including several in North America, were characterized, and the range of hourly average concentrations compared, it was found that the distributions of hourly average concentrations for sites with the lowest hourly average concentrations were similar. The authors believed the data from these sites could be used for establishing reference points that could be compared with more polluted areas.

Is it appropriate to use sites that experience the lowest hourly average concentrations in the United States today as reference levels, or should O_3 background levels that may have existed 100 years ago be used? Although it might be argued that all sites in the United States have been affected to such a level that today's values are not relevant because an increase in O_3 concentrations has occurred since the late 1800s, two key points argue against this line of reasoning. First, as indicated above, although O_3 levels have increased since the last century, consistent trends in tropospheric O_3 have not been observed across the northern hemisphere. Thus, at some monitoring sites, O_3 levels may not show increasing trends. Second, all the increases in background levels of O_3 may not necessarily be associated with changes in anthropogenic emissions. Since preindustrial times, major land use changes, resulting from human-induced activities, have occurred. Changes in natural emissions of O_3 precursors associated with soils and vegetation in the last 100 years may be associated with these land use changes, with the result that some of the increased levels in O_3 concentrations may be attributed to sources other than anthropogenic emissions. Thus, using historical data that contain large uncertainties in the estimation of O_3 concentrations as a reference point, may yield unrealistically low hourly average concentrations.

Based on a review of available data, the U.S. Environmental Protection Agency (1989) has indicated that a reasonable estimate of O_3 background concentration near sea level in the United States today, for an annual average, is from 0.020 to 0.035 ppm. This estimate included a 0.005- to 0.015-ppm contribution from stratospheric intrusions and a 0.01-ppm contribution from photochemically affected biogenic nonmethane hydrocarbons. In addition, the U.S. Environmental Protection Agency (1989) estimated that an additional 0.010 ppm is possible from the photochemical reaction of biogenic methane. A more conservative approach would be to associate the sum of O_3 concentrations from these two processes with the differences between 0.020 to 0.035 ppb and the stratospheric intrusion contribution.

For calculating annual average concentrations, the estimate made by the U.S. Environmental Protection Agency (1989) may be valid. Pratt et al. (1983), using data from low-elevation rural sites in Minnesota and North Dakota, reported that annual average concentrations for an O_3 monitoring site in LaMoure County, ND (400 m), for 1978 through 1981, ranged from 0.030 to 0.035 ppm, whereas an O_3 monitoring site in Traverse County, MN (311 m), had a range of 0.029 to 0.035 ppm. Bower et al. (1989) reported that the remote northern Scotland site, Strath Vaich (270 m), had a 1987 to 1988 annual average O_3 concentration of 0.031 ppm.

The U.S. Environmental Protection Agency (1989) has estimated that background O_3 concentrations for a 1-h daily maximum at sea level in the United States during the summer are in the range of 0.03 to 0.05 ppm. However, the actual value may vary from site to site. Using measurements at a remote site in South Dakota, Kelly et al. (1982) estimated the background O_3 in air masses entering the midwestern and eastern United States to be 0.03 to 0.05 ppm. Lefohn and Foley (1992) reported that hourly average O_3 concentration data available for sites that experience low maximum hourly average values in the western United States indicate that, in almost all cases, the maximum hourly average concentrations were in the range of 0.060 to 0.075 ppm, and, at some of the sites, there were infrequent occurrences of hourly average concentrations below 0.02 ppm (i.e., lack of scavenging). These observations were similar to those reported for several O_3 monitoring sites experiencing low maximum hourly average O_3 concentrations for other locations in the world (Lefohn et al., 1990a; Pedersen and Lefohn, 1994).

Some vegetation researchers have used the seasonal average of the daily 7-h (0900 to 1559 hours) average as the exposure parameter in exposure-response models (Heck et al., 1982). For quantifying the effects of air pollution on crops and trees, some investigators have used controlled environment and field methods with charcoal-filtration systems (Olszyk et al., 1989). In both the design of the experiments and the analysis of the data, the 7-h (0900 to 1559 hours) seasonal mean reference point for O_3 was assumed to be 0.025 ppm. The 0.025 ppm concentration was used to estimate crop loss across the United States (Adams et al., 1985, 1989). For sites experiencing low maximum hourly average concentrations in the western United States, except for several years of O_3 measurements at Olympic National Park (Table 4-6), the 7-mo (April to October) average of the 7-h daily average concentrations ranged from 0.025 to 0.045 ppm (Altshuller and Lefohn, 1996).

Ozone hourly average concentrations were characterized at several sites located in both the western and south-central United States that experienced low maximum hourly average concentrations (Table 4-6). Redwood National Park, CA; Olympic National Park, WA; Glacier National Park, MT; Denali National Park, AK; Badlands, SD; Great Sand Dunes National Monument, CO; Theodore Roosevelt National Park, ND; and Quachita National Forest, AR, experienced no hourly average concentration $\geq$0.08 ppm for the period April to October (Altshuller and Lefohn, 1996). Except for 1988, the year in which Yellowstone National Park, WY, experienced a major forest fire, the Wyoming site experienced no hourly average concentrations $\geq$0.08 ppm. Logan (1989) has noted that O_3 hourly average concentrations above 0.08 ppm rarely are exceeded at remote western sites. In almost all cases for the above sites, the maximum hourly average concentration was $\leq$0.075 ppm. There have been some questions raised to whether the distributions experienced at those sites exhibiting low maximum hourly average concentrations in the western United States were representative of sites in the eastern and midwestern United States because of differences in biogenic precursors. The O_3 monitoring site in the

Table 4-6. Seasonal (April to October) Percentile Distribution of Hourly Ozone Concentrations, Number of Hourly Mean Ozone Occurrences ≥0.08 and ≥0.10, Seasonal Seven-Hour Average Concentrations, W126, and SUM06 Values for Sites Experiencing Low Hourly Average Concentrations with Data Capture ≥75% (Concentrations in ppm)[a]

				Percentiles								No. of	Hours		Seasonal	W126	SUM06
Location	Site/AIRS ID	Year	Min.	10	30	50	70	90	95	99	Max	Obs.	≥0.08	≥0.10	7-h	(ppm-h)	(ppm-h)
Redwood, CA	Redwood NP	1988	0.002	0.011	0.018	0.023	0.029	0.038	0.041	0.046	0.060	4,825	0	0	0.026	1.8	0.1
	060150002	1989	0.000	0.010	0.017	0.022	0.027	0.034	0.038	0.042	0.047	4,624	0	0	0.024	1.0	0.0
		1990	0.000	0.011	0.018	0.023	0.028	0.035	0.038	0.043	0.053	4,742	0	0	0.025	1.2	0.0
		1991	0.001	0.012	0.019	0.025	0.031	0.038	0.041	0.045	0.054	4,666	0	0	0.027	1.7	0.0
		1992	0.000	0.010	0.017	0.021	0.026	0.035	0.039	0.045	0.055	4,679	0	0	0.023	1.1	0.0
		1993	0.000	0.010	0.017	0.022	0.027	0.035	0.038	0.042	0.054	4,666	0	0	0.025	1.1	0.0
Olympic, WA	Olympic NP	1982	0.000	0.000	0.010	0.010	0.020	0.030	0.030	0.040	0.060	4,704	0	0	0.020	7.4	0.1
	530090012	1984	0.000	0.000	0.010	0.010	0.020	0.020	0.020	0.030	0.050	4,872	0	0	0.015	1.6	0.0
		1986	0.000	0.000	0.010	0.020	0.020	0.040	0.040	0.040	0.060	4,776	0	0	0.025	13.7	0.1
		1989	0.000	0.003	0.010	0.015	0.022	0.030	0.035	0.046	0.065	4,220	0	0	0.021	0.7	0.1
		1990	0.000	0.005	0.012	0.018	0.023	0.030	0.034	0.043	0.064	4,584	0	0	0.022	0.8	0.3
		1991	0.000	0.006	0.014	0.019	0.024	0.033	0.036	0.044	0.056	4,677	0	0	0.025	0.9	0.0
		1993	0.000	0.004	0.010	0.016	0.021	0.029	0.034	0.041	0.064	4,595	0	0	0.022	0.7	0.3
Glacier, MT	Glacier NP	1989	0.000	0.003	0.015	0.026	0.036	0.046	0.050	0.058	0.067	4,770	0	0	0.036	5.9	1.8
	300298001	1990	0.000	0.003	0.014	0.026	0.035	0.044	0.047	0.052	0.066	5,092	0	0	0.036	4.1	1.3
		1991	0.000	0.001	0.014	0.027	0.036	0.046	0.049	0.056	0.062	5,060	0	0	0.036	5.3	0.7
		1992	0.000	0.001	0.013	0.025	0.033	0.043	0.048	0.055	0.077	4,909	0	0	0.033	4.1	1.0
Yellowstone, WY	Yellowstone NP	1988	0.002	0.020	0.029	0.037	0.044	0.054	0.058	0.070	0.098	4,257	17	0	0.043	14.0	8.9
	560391010	1989	0.002	0.018	0.027	0.036	0.044	0.052	0.057	0.063	0.071	4,079	0	0	0.042	11.0	6.7
		1990	0.000	0.015	0.023	0.029	0.036	0.043	0.046	0.053	0.061	4,663	0	0	0.034	3.8	0.5
		1991	0.004	0.020	0.030	0.037	0.042	0.048	0.051	0.057	0.064	4,453	0	0	0.042	7.7	1.2
		1992	0.001	0.018	0.029	0.036	0.042	0.051	0.056	0.064	0.075	4,384	0	0	0.042	10.7	6.3
		1993	0.000	0.018	0.028	0.036	0.042	0.047	0.050	0.054	0.060	4,399	0	0	0.041	6.5	0.2
	022900003	1990	0.003	0.017	0.024	0.029	0.034	0.040	0.043	0.048	0.050	3,978	0	0	0.030	2.1	0.0
		1991	0.005	0.018	0.024	0.028	0.034	0.041	0.043	0.047	0.057	4,809	0	0	0.030	2.7	0.0
		1992	0.003	0.016	0.023	0.028	0.034	0.044	0.047	0.050	0.054	4,800	0	0	0.031	3.7	0.0
		1993	0.002	0.017	0.023	0.028	0.033	0.041	0.043	0.048	0.055	4,773	0	0	0.030	2.6	0.0

Table 4-6 (con't). Seasonal (April to October) Percentile Distribution of Hourly Ozone Concentrations, Number of Hourly Mean Ozone Occurrences ≥00.08 and ≥0.10, Seasonal Seven-Hour Average Concentrations, W126, and SUM06 Values for Sites Experiencing Low Hourly Average Concentrations with Data Capture ≥75% (Concentrations in ppm)[a]

Location	Site/AIRS ID	Year	Min.	Percentiles 10	30	50	70	90	95	99	Max	No. of Obs.	Hours ≥00.08	≥0.10	Seasonal 7-h	W126 (ppm-h)	SUM06 (ppm-h)
Badlands, SD	Badlands NP 460711010	1988	0.005	0.022	0.032	0.038	0.045	0.053	0.056	0.061	0.072	4,791	0	0	0.043	13.3	5.2
		1989	0.007	0.020	0.028	0.034	0.041	0.049	0.053	0.060	0.071	4,840	0	0	0.040	9.2	3.1
		1990	0.006	0.019	0.027	0.032	0.037	0.044	0.048	0.054	0.063	4,783	0	0	0.037	4.8	0.8
		1991	0.005	0.020	0.028	0.034	0.040	0.047	0.050	0.056	0.066	4,584	0	0	0.038	6.2	0.7
Great Sand Dunes, CO	Sand Dunes NM 080030002	1988	0.010	0.028	0.035	0.039	0.044	0.050	0.053	0.058	0.076	4,827	0	0	0.043	11.1	1.9
		1989	0.011	0.031	0.037	0.041	0.045	0.050	0.052	0.057	0.063	4,436	0	0	0.044	10.8	1.2
		1990	0.010	0.030	0.037	0.041	0.045	0.052	0.055	0.062	0.070	4,624	0	0	0.044	13.6	5.1
		1991	0.008	0.029	0.037	0.043	0.048	0.055	0.058	0.065	0.077	4,130	0	0	0.046	17.0	9.0
Theodore Roosevelt, ND, North Unit	Theodore Roosevelt NP 380530002	1984	0.000	0.017	0.025	0.032	0.039	0.047	0.050	0.059	0.068	4,923	0	0	0.038	7.0	2.8
		1985	0.000	0.019	0.026	0.032	0.038	0.046	0.049	0.054	0.061	4,211	0	0	0.038	5.0	0.1
		1986	0.004	0.017	0.027	0.033	0.039	0.047	0.050	0.056	0.062	4,332	0	0	0.039	5.5	0.4
		1989	0.004	0.023	0.032	0.039	0.045	0.054	0.058	0.065	0.073	4,206	0	0	0.046	14.2	11.0
		1992	0.005	0.019	0.027	0.033	0.039	0.047	0.050	0.056	0.063	4,332	0	0	0.040	6.1	0.8
		1993	0.004	0.018	0.025	0.031	0.037	0.045	0.048	0.055	0.064	4,281	0	0	0.038	4.6	0.7
Point Reyes, CA	Point Reyes NP 060410002	1989	0.007	0.021	0.026	0.031	0.036	0.042	0.045	0.058	0.080	4,577	1	0	0.033	4.8	2.5
		1990	0.006	0.017	0.022	0.025	0.029	0.036	0.040	0.046	0.063	4,856	0	0	0.028	1.8	0.4
		1991	0.006	0.019	0.025	0.030	0.034	0.040	0.043	0.048	0.072	4,588	0	0	0.031	3.0	0.6
		1992	0.007	0.018	0.024	0.028	0.033	0.041	0.045	0.050	0.066	4,794	0	0	0.031	3.0	0.3
Arches, UT	Arches NP 490190101	1989	0.000	0.031	0.040	0.045	0.050	0.057	0.059	0.065	0.084	4,260	2	0	0.048	21.2	12.6
		1990	0.000	0.020	0.025	0.028	0.031	0.036	0.039	0.045	0.056	4,639	0	0	0.030	1.7	0.0
Montgomery Co., AR	Quachita NF 050970001	1991	0.000	0.002	0.010	0.016	0.023	0.035	0.041	0.051	0.064	4,835	0	0	0.027	1.6	0.1
		1992	0.000	0.005	0.015	0.024	0.030	0.041	0.045	0.053	0.067	4,902	0	0	0.033	3.0	0.9
		1993	0.000	0.008	0.019	0.027	0.035	0.047	0.052	0.061	0.070	4,844	0	0	0.036	6.7	3.7

[a]See Appendix A for abbreviations and acronyms.

Quachita National Forest in Arkansas experienced distributions of hourly average concentrations similar to some of the western sites.

Evans et al. (1983) summarized O_3 hourly averaged data collected at eight stations located in eight national forests across the United States. The first three stations began operations in 1976 (Green Mountain National Forest, VT; Kisatchie National Forest, LA; and Custer National Forest, MT); the second three in 1978 (Chequamegon National Forest, WI; Mark Twain National Forest, MO; and Croatan National Forest, NC); and the last two in 1979 (Apache National Forest, AZ; and Ochoco National Forest, OR). For the period 1979 to 1983, hourly maximum average concentrations at the sites (Custer, Ochoco, and Apache national forests) were similar to the hourly average concentrations determined for six of the seven sites characterized by Lefohn and Foley (1992). In almost all cases, none of the sites experienced hourly average concentrations $\geq$0.08 ppm, and the maximum hourly average concentrations were in the range of 0.060 to 0.075 ppm. Table 4-7 summarizes the percentile distributions for the three national forest sites.

Several sites experiencing low maximum hourly average concentrations were characterized by Lefohn et al. (1990a), using various exposure indices. One of the indices used was W126 (see Section 4.1); the W126 values, calculated over an annual period, are provided in Table 4-8. The W126 values for Theodore Roosevelt National Park were in the range of 6.48 to 8.03 ppm-h. The maximum hourly average concentration reported at the site was 0.068 ppm. The W126 values at the Custer and Ochoco national forests sites ranged from 5.79 to 22.67 ppm-h. The maximum hourly average concentrations measured at each site were 0.075 and 0.080 ppm, respectively. The W126 values calculated for the Custer and Ochoco national forest sites showed greater variability from year to year than the values calculated for the South Pole, Barrow, and Theodore Roosevelt National Park sites.

As the W126 values increased, the magnitude of the year-to-year variability also increased. For 2 years of data, the W126 values calculated for the White River U-4 Oil Shale, UT, site were 19.98 and 32.10 ppm-h. The maximum hourly concentration recorded was 0.079 ppm. The W126 values calculated for the Apache National Forest site ranged from 10.24 to 81.39 ppm-h. The highest hourly average concentration was 0.090 ppm.

The 7-h (0900 to 1559 hours) average concentration has been used by vegetation researchers to characterize O_3 exposures experienced in plant chamber experiments (see Chapter 5). Because O_3 concentrations are highest during the warm-season months and, at many low-elevation sites, during daylight hours, the 7-mo seasonal, 7-h (0900 to 1559 hours) average concentration is higher than annual average values. Most remote sites outside North America experience seasonal 7-h averages of 0.025 ppm (Table 4-9) (Lefohn et al., 1990a). The seasonal average of the daily 7-h average values for the South Pole, Antarctica, range from 0.024 to 0.027 ppm. The values range from 0.022 to 0.026 ppm at Barrow, AK. In the continental United States and southern Canada, values range from approximately 0.028 to 0.050 ppm (Lefohn et al., 1990a). At an O_3 monitoring site at the Theodore Roosevelt National Park, the range of the 7-mo (April to October) average of the 7-h daily average concentrations was from 0.038 to 0.046 ppm (Table 4-6). These 7-mo seasonal averages appear to be representative of values that may occur at other sites located in the United States and other locations in the northern hemisphere. In earlier investigations, Lefohn (1984) reported 3-mo (June to August), 7-h averages of 0.048, 0.044, and 0.059 ppm at remote sites at Custer, Ochoco, and Apache national forests, respectively.

Table 4-7. Seasonal (April to October) Percentile Distribution of Hourly Ozone Concentrations, Number of Hourly Mean Ozone Occurrences ≥0.08 and ≥0.10, Seasonal Seven-Hour Average Concentrations, and W126 Values for Three "Clean" National Forest Sites with Data Capture ≥75% (Concentrations in ppm)[a]

Site	AIRS ID	Year	Min.	10	30	50	70	90	95	99	Max	No. of Obs.	Hours ≥0.08	Hours ≥0.10	Seasonal 7-h (ppm)	W126 (ppm-h)
				Percentiles												
Custer NF, MT	300870101	1978	0.000	0.010	0.020	0.035	0.040	0.050	0.055	0.060	0.070	4,759	0	0	0.033	8.3
		1979	0.010	0.025	0.035	0.040	0.045	0.050	0.055	0.060	0.075	5,014	0	0	0.043	13.2
		1980	0.010	0.025	0.035	0.040	0.050	0.055	0.060	0.065	0.070	4,574	0	0	0.043	19.7
		1983	0.010	0.025	0.035	0.040	0.045	0.050	0.055	0.060	0.065	4,835	0	0	0.042	10.7
Ochoco NF, OR	410130111	1980	0.010	0.030	0.035	0.040	0.045	0.055	0.055	0.065	0.080	4,759	5	0	0.044	16.5
		1981	0.010	0.025	0.030	0.035	0.040	0.045	0.045	0.055	0.075	4,459	0	0	0.035	4.7
		1982	0.010	0.025	0.030	0.035	0.040	0.045	0.050	0.055	0.065	4,697	0	0	0.038	7.6
		1983	0.010	0.025	0.035	0.035	0.040	0.045	0.050	0.055	0.060	4,423	0	0	0.039	6.8
Apache NF, AZ	040110110	1981	0.010	0.025	0.030	0.035	0.040	0.045	0.050	0.055	0.065	4,806	0	0	0.039	7.6
		1982	0.015	0.030	0.040	0.045	0.050	0.055	0.060	0.065	0.075	4,714	0	0	0.047	21.9
		1983	0.004	0.025	0.035	0.040	0.045	0.055	0.055	0.065	0.070	4,788	0	0	0.042	14.6

[a]See Appendix A for abbreviations and acronyms.

Table 4-8. The Value of the W126 Sigmoidal Exposure Parameter Calculated Over the Annual Period (Units in ppm-h)[a]

Site	Elevation (m)	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987
South Pole, Antarctica	2,835					2.65	3.72	3.01	2.41	3.54	2.76	4.09	
Bitumount, Alberta, Canada	350				2.99								
Barrow, AK	11					2.60	2.60	3.15	2.36	2.79	2.03	2.46	3.69
Theodore Roosevelt NP, ND	727									8.03	6.69	6.48[b]	
Custer NF, MT	1,006				14.08	22.67			12.18				
Ochoco NF, OR	1,364					19.54	5.79	9.10	8.02				
Birch Mountain, Alberta, Canada	850				19.73								
White River Oil Shale Project, UT	1,600							19.98	32.10				
Fortress Mountain, Alberta, Canada	2,103											25.04	83.89
Apache NF, AZ	2,424					81.39	10.24	27.18	17.48				
Mauna Loa, HI	3,397					27.48	45.68	33.68	48.90	19.18	32.66	24.48	
Whiteface Mountain, NY	1,483				86.50	68.30	33.75	32.03	37.82	42.94	41.36	32.07	58.33
Hohenpeissenberg, FRG	975	61.28	25.04	35.64	21.76	18.53	29.53	49.00	19.85	40.43			
American Samoa	82					0.28	0.24	0.25	0.28	0.30	0.26	0.30	0.32

[a]See Appendix A for abbreviations and acronyms.
[b]Collection did not occur during the months of October, November, and December.

Source: Lefohn et al. (1990a).

Table 4-9. The Value of the Ozone Season (Seven-Month) Average of the Daily Seven-Hour (0900 to 1559 Hours) Concentration (ppm)[a]

Site	Elevation (m)	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987
South Pole, Antarctica	2,835					0.025	0.027		0.026	0.027	0.024	0.025	
Bitumount, Alberta, Canada	350				0.028								
Barrow, AK	11					0.022	0.025	0.024	0.024	0.022	0.026	0.022	0.026
Theodore Roosevelt NP, ND	727									0.038	0.039	0.039[b]	
Custer NF, MT	1,006				0.043	0.044			0.042				
Ochoco NF, OR	1,364					0.043	0.035	0.038	0.038				
Birch Mountain, Alberta, Canada	850				0.036								
White River Oil Shale Project, UT	1,600							0.045	0.045				
Fortress Mountain, Alberta, Canada	2,103											0.041	0.050
Apache NF, AZ	2,424					0.054	0.039	0.047	0.040				
Mauna Loa, HI	3,397					0.035	0.039	0.034	0.038	0.035	0.035	0.034	
Whiteface Mountain, NY	1,483				0.049	0.046	0.040	0.034	0.041	0.044	0.043	0.043	0.045
Hohenpeissenberg, FRG	975	0.047	0.040	0.044	0.040	0.037	0.043	0.047	0.040	0.043			
American Samoa	82					0.010	0.010	0.011	0.009	0.012	0.010	0.011	

[a]See Appendix A for abbreviations and acronyms.
[b]Collection did not occur during the months of October, November, and December.

Source: Lefohn et al. (1990a).

4.3.3.2 Urban-Influenced Nonurban Areas

It is difficult to identify a set of unique O_3 distribution patterns that adequately describes the hourly average concentrations experienced at monitoring sites in nonurban locations because, as indicated earlier, many nonurban sites in the United States are influenced by local sources of pollution or long-range transport of O_3 or its precursors. Unlike the clean sites characterized by Lefohn and Jones (1986), Lefohn et al. (1990a), and Lefohn and Foley (1992), urban-influenced nonurban sites sometimes show frequent hourly average concentrations near the minimum detectable level, but almost always show occurrences of hourly average concentrations above 0.1 ppm. The frequent occurrence of hourly average concentrations near the minimum detectable level is indicative of scavenging processes (i.e., NO_x); the presence of high hourly average concentrations can be attributable to the influence of either local generation or the long-range transport of O_3. For example, Evans et al. (1983) reported that the Green Mountain (VT) and Mark Twain national forest (MO) sites were influenced by long-range transport of O_3. The U.S. Environmental Protection Agency (1986) reported that the maximum hourly average concentrations at Green Mountain (for the period 1977 to 1981) and Mark Twain (for the period 1979 to 1983) were 0.145 and 0.155 ppm, respectively. Using hourly averaged data from the AIRS database for a select number of rural monitoring sites, Table 4-10 summarizes the percentiles of the hourly average O_3 concentrations, the number of occurrences of the hourly average concentration $\geq$0.10 ppm, and the 3-mo sum of all hourly average concentrations $\geq$0.06 ppm.

As part of a comprehensive air monitoring project sponsored by the Electric Power Research Institute (EPRI), O_3 measurements were made by the chemiluminescence method from 1977 through 1979 at nine "nonurban" Sulfate Regional Experiment (SURE) Program sites and Eastern Regional Air Quality Study sites in the eastern United States. On the basis of diurnal NO_x patterns that indicated the influence of traffic emissions, five of the sites were classed as "suburban", and the other four were classed as "rural". The O_3 data from these nine stations are summarized in Table 4-11. The sites are influenced either by local sources or by transport of O_3 or its precursors. The maximum hourly average concentrations generally are higher than 0.125 ppm, and the occurrence of hourly average concentrations near minimum detectable levels indicates NO_x scavenging processes.

As part of its effort to provide long-term estimates of dry acidic deposition across the United States, the National Dry Deposition Network (NDDN) operated more than 50 sites, which included 41 in the eastern United States and 9 in the western United States, that routinely recorded hourly average O_3 concentrations. Figure 4-7 shows the locations of the NDDN sites. Edgerton and Lavery (1992) have summarized the O_3 concentrations at some of the sites for the period 1988 to 1990. Table 4-12 summarizes the 7-h (0900 to 1559 hours) growing season average concentration (May to September) for selected sites in the Midwest and the East. Fifty-nine percent of the monitoring sites listed in the table have been classified as agricultural and 36% as forested; one site was classified as commercial. As noted by the U.S. Environmental Protection Agency (1992a), 1988 was an exceptionally high O_3 concentration year, when compared with 1989 and 1990. The number of hourly O_3 concentrations $\geq$0.08 ppm is presented in Table 4-12. Edgerton and Lavery (1992) have summarized O_3 hourly average concentration data for several sites using the cumulative

Table 4-10. Summary of Percentiles, Number of Hourly Occurrences ≥0.10 ppm, and Three-Month SUM06 Values for Selected Rural Ozone Monitoring Sites in 1989 (April to October) (Concentrations in ppm)[a]

AIRS Site	Name	Min.	10	30	50	70	90	95	99	Max	Number of Hourly Values	Number of Occurrences ≥0.10	Max Uncorrected 3-mo SUM06 Value (ppm-h)
						Percentiles (ppm)							
RURAL AGRICULTURAL													
170491001	Effingham County, IL	0.000	0.009	0.023	0.036	0.046	0.063	0.070	0.081	0.104	4,600	1	25.3
180970042	Indianapolis, IN	0.001	0.006	0.021	0.034	0.046	0.063	0.072	0.085	0.103	4,592	3	25.4
240030014	Anne Arundel, MD	0.000	0.006	0.021	0.032	0.045	0.064	0.073	0.090	0.120	4,360	10	25.5
310550032	Omaha, NE	0.002	0.021	0.030	0.037	0.047	0.062	0.067	0.075	0.098	4,160	0	24.9
420070003	New Brighton, PA	0.000	0.008	0.021	0.032	0.043	0.062	0.070	0.087	0.102	5,055	4	29.4
510610002	Fauquier County, VA	0.000	0.009	0.021	0.033	0.045	0.061	0.069	0.084	0.122	5,050	5	24.6
RURAL FOREST													
060430004	Yosemite NP, CA	0.000	0.008	0.022	0.035	0.049	0.065	0.072	0.083	0.111	4,853	3	37.6
360310002	Essex County, NY	0.016	0.031	0.040	0.049	0.056	0.066	0.072	0.086	0.106	4,792	4	45.6
470090101	Smoky Mountain NP, TN	0.000	0.025	0.036	0.044	0.053	0.065	0.070	0.081	0.098	4,764	0	35.9
511870002	Shenandoah NP (Dickey Ridge), VA	0.004	0.027	0.037	0.045	0.054	0.065	0.071	0.082	0.100	4,454	1	33.5
RURAL OTHER													
040132004	Scottsdale, AZ	0.000	0.006	0.018	0.031	0.045	0.062	0.071	0.084	0.107	5,070	4	31.7
350431001	Sandoval County, NM	0.000	0.010	0.020	0.030	0.040	0.060	0.060	0.070	0.090	5,059	0	25.1
370810011	Guilford County, NC	0.004	0.010	0.023	0.034	0.046	0.063	0.070	0.083	0.113	4,853	2	27.7
371470099	Farmville, NC	0.000	0.010	0.023	0.034	0.044	0.062	0.070	0.083	0.100	4,833	2	26.4
550270001	Horicon, WI	0.002	0.019	0.029	0.037	0.047	0.062	0.070	0.088	0.111	4,142	11	24.6
551390007	Oshkosh, WI	0.002	0.016	0.028	0.038	0.048	0.063	0.070	0.084	0.121	4,206	3	27.9

[a]See Appendix A for abbreviations and acronyms.

Table 4-11. Summary of Percentiles of Hourly Average Concentrations for Electric Power Research Institute Sulfate Regional Experiment (SURE) Program Sites/Eastern Regional Air Quality Study (ERAQS) Ozone Monitoring Sites (Units in ppm)

SURE/ERAQS Name	Year	Min.	10	30	50	70	90	95	99	Max	Number of Observations
					Percentiles						
Montague, MA	1978	0.000	0.002	0.018	0.032	0.043	0.061	0.075	0.119	0.202	7,138
	1979	0.000	0.000	0.013	0.025	0.035	0.056	0.070	0.103	0.149	8,485
Scranton, PA	1978	0.000	0.015	0.031	0.040	0.048	0.062	0.073	0.094	0.126	5,461
	1979	0.000	0.011	0.022	0.030	0.040	0.061	0.074	0.097	0.132	8,313
Indian River, DE	1978	0.000	0.010	0.024	0.035	0.049	0.072	0.085	0.103	0.134	6,874
	1979	0.000	0.008	0.020	0.031	0.042	0.063	0.073	0.092	0.138	8,527
Duncan Falls, OH	1978	0.000	0.005	0.022	0.034	0.049	0.071	0.081	0.110	0.144	5,125
	1979	0.000	0.010	0.021	0.029	0.042	0.060	0.069	0.086	0.110	7,595
Rockport, IN	1978	0.000	0.008	0.021	0.032	0.044	0.066	0.078	0.101	0.145	6,849
	1979	0.000	0.008	0.019	0.028	0.038	0.055	0.064	0.083	0.104	8,391
Giles County, TN	1978	0.000	0.000	0.018	0.032	0.046	0.066	0.075	0.087	0.110	6,034
	1979	0.000	0.000	0.014	0.024	0.036	0.055	0.065	0.081	0.130	8,439
Roanoke, IN	1978	0.000	0.004	0.019	0.032	0.044	0.067	0.079	0.106	0.160	5,874
	1979	0.000	0.004	0.017	0.026	0.038	0.061	0.074	0.098	0.133	8,001
Research Triangle Park, NC	1978	0.000	0.001	0.017	0.032	0.049	0.076	0.087	0.108	0.142	7,081
	1979	0.000	0.001	0.012	0.024	0.037	0.058	0.068	0.084	0.131	8,652
Lewisburg, WV	1978	0.002	0.020	0.034	0.045	0.056	0.072	0.079	0.091	0.115	7,019
	1979	0.000	0.013	0.022	0.029	0.039	0.056	0.065	0.080	0.099	7,849

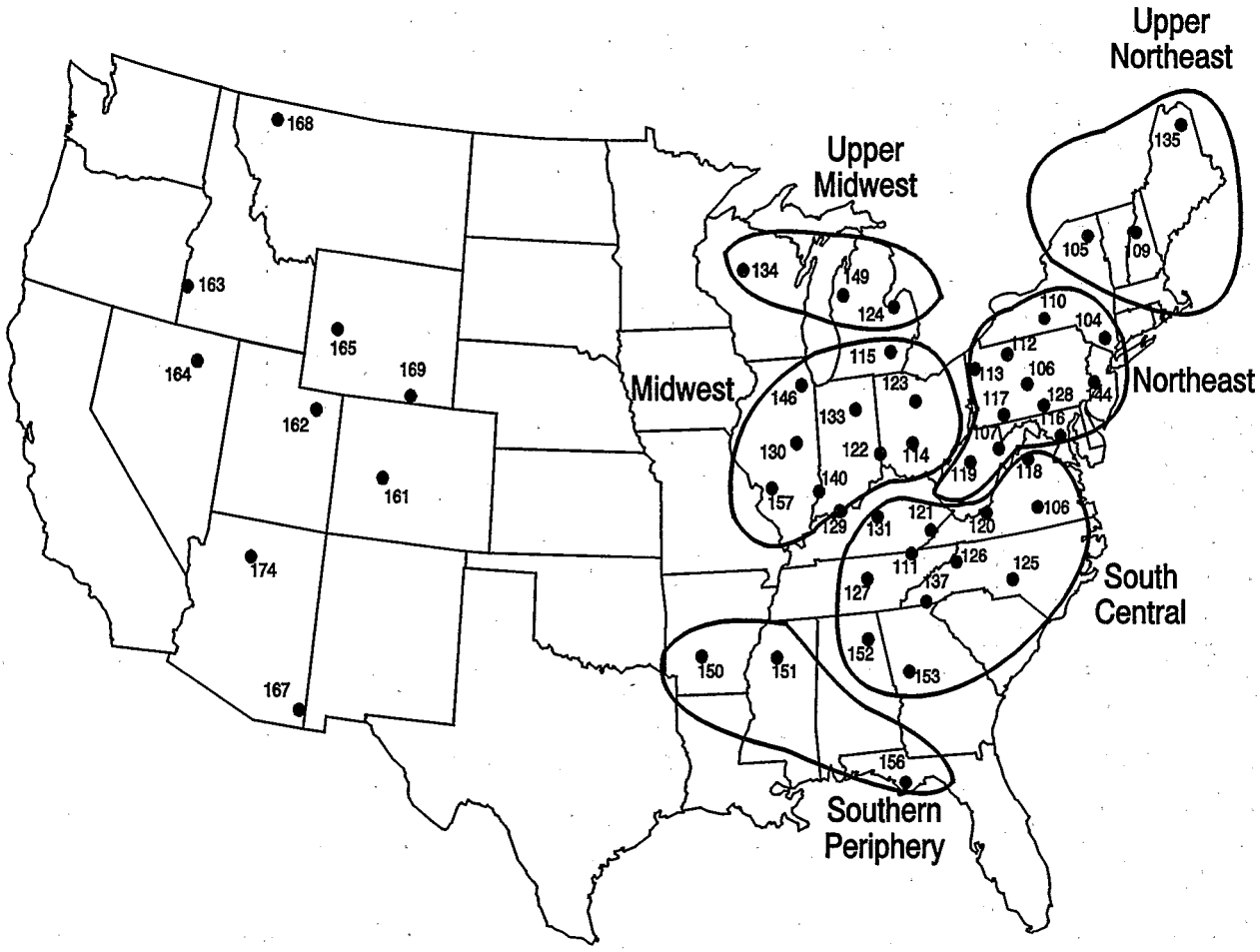


Figure 4-7. The location of National Dry Deposition Network monitoring sites as of December 1990.

Source: Edgerton and Lavery (1992).

integrated exposure index, W126, as proposed by Lefohn and Runeckles (1987). Based on evidence presented in the literature relating O_3 exposure with agricultural yield reduction, the index was proposed as a way to weight the higher hourly average concentrations greater than the lower values. The data in the table illustrate the large differences in cumulative exposure between those that occurred in 1988 and those that were experienced in 1989 and 1990. The percentile of the hourly average concentrations is summarized in Table 4-13. Although several of the monitoring sites are located in fairly remote locations in the eastern United States (based on land use characterization) the maximum hourly average concentrations reflect the transport of O_3 or its precursors into the area.

Taylor et al. (1992) have summarized the O_3 concentrations that were experienced at 10 EPRI Integrated Forest Study sites in North America. The authors reported that in 1988 all sites experienced maximum hourly average concentrations ≥ 0.08 ppm. In almost all cases, the sites experienced multiple occurrences above 0.08 ppm. This implies that,

Table 4-12. Seven-Hour Growing Season Mean, W126 Values, and Number of Hourly Ozone Concentrations ≥80 ppb for Selected Eastern National Dry Deposition Network Sites[a]

Subregion	State	Site	Land Class[b]	7-h Mean (ppb)			W126 (ppm-h)			SUM06 (ppm-h)			SUM08 (ppm-h)		
				1988	1989	1990	1988	1989	1990	1988	1989	1990	1988	1989	1990
NORTHEAST															
Connecticut Hill	NY	110	RF	55.0	48.3	45.3	75.5	40.3	36.8	86.8	47.2	35.8	44.3	5.5	3.3
Washington's Crossing	NJ	144	RA	—	52.8	52.4	—	46.0	43.7	—	52.1	48.4	—	21.2	21.3
Pennsylvania State University	PA	106	RA	59.0	46.0	51.0	63.5	25.4	42.7	65.6	28.1	45.0	32.3	5.0	11.6
Laurel Hill State Park	PA	117	RF	62.7	48.4	48.6	68.8	29.1	31.0	75.5	30.8	32.1	41.6	7.0	8.0
Beltsville	MD	116	RA	—	54.6	55.5	—	45.4	45.7	—	48.6	49.4	—	22.9	21.9
Cedar Creek State Park	WV	119	RA	59.8	44.9	48.2	50.4	19.6	24.3	56.3	19.0	23.2	27.0	4.1	6.5
UPPER NORTHEAST															
Whiteface Mountain	NY	105	RF	43.5	45.7	42.3	37.8	25.3	31.2	40.9	25.2	29.0	17.0	2.6	8.4
Ashland	ME	135	RA	—	37.9	35.3	—	9.1	8.7	—	5.4	5.8	—	0.6	0.8
MIDWEST															
Argonne National Lab	IL	146	RA	61.1	51.4	46.3	59.1	29.6	21.6	69.4	35.0	25.7	32.3	10.4	3.6
Vincennes	IN	140	RA	62.0	51.1	50.9	68.1	36.4	35.8	78.5	40.3	41.2	36.7	8.7	11.8
Oxford	OH	122	RA	65.3	53.5	51.7	91.8	48.4	46.4	103.2	55.3	51.7	56.8	15.8	17.2
UPPER MIDWEST															
Unionville	MI	124	RA	—	51.5	47.4	—	35.4	30.7	—	41.6	31.6	—	9.0	7.1
Perkinstown	WI	134	RA	—	44.2	38.8	—	19.0	11.6	—	18.3	7.6	—	0.2	0.0
SOUTH CENTRAL															
Sand Mountain	AL	152	RA	—	52.6	63.6	—	40.6	68.7	—	33.2	83.4	—	3.4	24.0
Georgia Station	GA	153	RA	—	48.1	62.6	—	28.1	69.7	—	21.8	77.7	—	4.6	28.4
Perryville	KY	129	RA	65.2	50.8	—	103.6	39.7	—	99.6	38.9	—	39.7	5.2	—
Research Triangle Park	NC	101	RC	62.3	50.8	—	62.3	31.7	—	71.0	35.5	—	20.5	6.6	—
Coweeta	NC	137	RF	55.6	41.0	47.9	44.3	16.1	21.3						
Edgar Evins State Park	TN	127	RF	—	47.2	56.1	—	26.9	44.5	—	24.4	49.2	—	1.5	8.2
Horton Station	VA	120	RF	62.3	51.4	54.4	127.6	61.2	70.6	150.7	64.0	82.8	60.2	8.5	9.2
SOUTHERN PERIPHERY															
Caddo Valley	AR	150	RF	—	46.2	49.5	—	18.5	21.0	—	15.6	25.2	—	0.2	2.3
Sumatra	FL	156	RF	—	39.8	46.3	—	17.8	20.0	—	16.5	17.4	—	1.0	0.9

[a]See Appendix A abbreviations for and acronyms.
[b]RA = Rural agricultural; RF = Rural forest; RC = Rural commercial; — = No data or insufficient data.

Source: Edgerton and Lavery (1992).

Table 4-13. Summary of Percentiles for National Dry Deposition Network Monitoring Sites (Units in ppm)

Site No.	Name	Year	Min.	10	30	50	70	90	95	99	Max	Number of Observations
						Percentiles						
RURAL AGRICULTURAL SITES												
106	Pennsylvania State University, PA	1988	0.000	0.013	0.026	0.036	0.049	0.073	0.086	0.114	0.143	4,716
		1989	0.000	0.010	0.022	0.033	0.043	0.059	0.066	0.082	0.104	5,089
		1990	0.000	0.015	0.027	0.038	0.048	0.065	0.074	0.090	0.120	5,056
116	Beltsville, MD	1989	0.002	0.003	0.014	0.029	0.044	0.068	0.081	0.096	0.131	5,062
		1990	0.000	0.001	0.015	0.027	0.041	0.067	0.080	0.103	0.137	4,597
119	Cedar Creek, WV	1988	0.000	0.008	0.017	0.029	0.044	0.069	0.082	0.108	0.134	4,938
		1989	0.001	0.006	0.013	0.024	0.037	0.056	0.065	0.082	0.172	5,044
		1990	0.001	0.007	0.014	0.024	0.038	0.057	0.067	0.085	0.116	5,025
122	Oxford, OH	1988	0.001	0.019	0.032	0.044	0.058	0.083	0.096	0.117	0.221	4,746
		1989	0.001	0.017	0.029	0.039	0.050	0.069	0.077	0.092	0.109	5,073
		1990	0.000	0.015	0.028	0.037	0.048	0.067	0.077	0.092	0.116	5,077
124	Unionville, MI	1989	0.003	0.021	0.031	0.038	0.047	0.063	0.071	0.086	0.113	5,041
		1990	0.004	0.020	0.029	0.036	0.044	0.061	0.069	0.084	0.105	5,065
129	Perryville, KY	1988	0.002	0.024	0.038	0.049	0.062	0.080	0.094	0.110	0.143	4,061
		1989	0.001	0.020	0.033	0.043	0.052	0.066	0.072	0.086	0.102	4,787
134	Perkinstown, WI	1989	0.007	0.023	0.032	0.038	0.046	0.057	0.062	0.071	0.085	5,029
		1990	0.006	0.020	0.028	0.035	0.041	0.050	0.056	0.065	0.074	5,063
135	Loring AFB/Ashland, ME	1989	0.002	0.017	0.026	0.032	0.039	0.049	0.055	0.063	0.103	5,067
		1990	0.002	0.014	0.023	0.029	0.036	0.046	0.051	0.068	0.088	5,080
140	Vincennes, IN	1988	0.000	0.007	0.024	0.036	0.052	0.076	0.089	0.104	0.120	4,908
		1989	0.000	0.009	0.025	0.036	0.047	0.064	0.072	0.085	0.112	5,065
		1990	0.000	0.009	0.025	0.035	0.045	0.062	0.073	0.089	0.110	5,084
144	Washington Crossing, NJ	1989	0.000	0.006	0.021	0.033	0.046	0.067	0.078	0.100	0.159	5,053
		1990	0.001	0.008	0.021	0.032	0.043	0.065	0.079	0.104	0.148	5,058
146	Argonne National Laboratory, IL	1988	0.000	0.004	0.019	0.032	0.046	0.073	0.085	0.103	0.146	5,037
		1989	0.000	0.005	0.019	0.029	0.041	0.061	0.070	0.088	0.126	5,055
		1990	0.000	0.004	0.017	0.028	0.039	0.057	0.065	0.077	0.097	5,033

Table 4-13 (cont'd). Summary of Percentiles for National Dry Deposition Network Monitoring Sites (Units in ppm)

Site No.	Name	Year	Min.	10	30	50	70	90	95	99	Max	Number of Observations
				Percentiles								
RURAL AGRICULTURAL SITES (cont'd)												
152	Sand Mountain, AL	1989	0.000	0.020	0.031	0.041	0.051	0.065	0.072	0.082	0.097	4,509
		1990	0.000	0.021	0.035	0.045	0.057	0.074	0.080	0.093	0.117	5,068
153	Georgia Station, GA	1989	0.002	0.014	0.025	0.034	0.045	0.062	0.069	0.082	0.118	3,540
		1990	0.002	0.021	0.034	0.044	0.056	0.073	0.084	0.102	0.144	4,814
RURAL FOREST SITES												
105	Whiteface Mountain, NY	1988	0.000	0.016	0.026	0.034	0.044	0.062	0.074	0.098	0.129	5,051
		1989	0.003	0.022	0.030	0.038	0.047	0.059	0.066	0.078	0.093	4,698
		1990	0.005	0.018	0.028	0.036	0.046	0.060	0.069	0.086	0.115	5,016
110	Ithaca, NY	1988	0.005	0.025	0.034	0.043	0.055	0.080	0.090	0.103	0.126	4,827
		1989	0.002	0.025	0.036	0.044	0.052	0.065	0.071	0.081	0.101	5,064
		1990	0.001	0.022	0.033	0.041	0.049	0.063	0.069	0.081	0.093	5,075
117	Laurel Hill, PA	1988	0.001	0.012	0.025	0.036	0.050	0.076	0.092	0.119	0.156	5,007
		1989	0.000	0.009	0.020	0.031	0.043	0.061	0.069	0.087	0.110	4,697
		1990	0.001	0.009	0.020	0.030	0.042	0.060	0.071	0.086	0.109	5,032
120	Horton Station, VA	1988	0.010	0.031	0.045	0.057	0.067	0.084	0.096	0.114	0.145	5,012
		1989	0.002	0.032	0.043	0.050	0.059	0.070	0.076	0.085	0.103	4,976
		1990	0.004	0.032	0.044	0.052	0.059	0.071	0.075	0.084	0.097	5,066
127	Edgar Evins State Park, TN	1989	0.000	0.017	0.028	0.037	0.047	0.062	0.067	0.077	0.090	5,060
		1990	0.001	0.019	0.032	0.041	0.052	0.067	0.073	0.085	0.109	5,027
137	Coweeta, NC	1988	0.001	0.010	0.022	0.034	0.047	0.065	0.072	0.094	0.145	4,182
		1989	0.001	0.007	0.016	0.025	0.037	0.055	0.061	0.071	0.094	4,275
		1990	0.000	0.008	0.018	0.029	0.043	0.059	0.064	0.072	0.085	5,046
150	Caddo Valley, AR	1989	0.002	0.005	0.016	0.028	0.041	0.057	0.063	0.075	0.102	5,046
		1990	0.002	0.004	0.015	0.029	0.041	0.057	0.065	0.077	0.094	5,078
156	Sumatra, FL	1989	0.001	0.012	0.022	0.030	0.040	0.057	0.065	0.075	0.098	4,700
		1990	0.000	0.011	0.023	0.033	0.043	0.057	0.063	0.072	0.118	4,444
RURAL COMMERCIAL SITE												
101	Research Triangle Park, NC	1988	0.000	0.004	0.020	0.035	0.050	0.072	0.084	0.111	0.137	5,030
		1989	0.000	0.004	0.019	0.030	0.042	0.063	0.071	0.083	0.121	4,893

although the sites were located in remote forested areas, the sites experienced elevated O_3 concentrations that were more than likely due to long-range transport of O_3 or its precursors.

Ozone concentrations on a seasonal basis in the Shenandoah National Park exhibit some features in common with both urban and rural areas. During some years, maximum hourly average concentrations exceed 0.12 ppm, although some sites in the park exhibit a lack of hourly average concentrations near minimum detectable level. Taylor and Norby (1985) have characterized O_3 episodes, which they defined as any day in which a 1-h mean O_3 concentration was >0.08 ppm. Based on a 4-year monitoring period in the park, the probability was 80% that any given episode during the growing season would last 2 or more days, whereas the probabilities of episodes lasting for periods greater than 3, 4, and 5 days were 30, 10, and 2%, respectively. Single-day O_3 episodes were infrequent. Taylor and Norby (1985) noted that, given the frequency of respites, there was a 50% probability that a second episode would occur within 2 weeks.

Because of a lack of air quality data collected at rural and remote locations, it has been necessary to use interpolation techniques to estimate O_3 exposures in nonurban areas. In the absence of actual O_3 data, interpolation techniques have been applied to the estimation of O_3 exposures across the United States (Reagan, 1984; Lefohn et al., 1987a; Knudsen and Lefohn, 1988). "Kriging", a mathematical interpolation technique, has been used to provide estimates of seasonal O_3 values for the NCLAN for 1978 through 1982 (May to September of each year) (Reagan, 1984). These values, along with updated values, coupled with exposure-response models were used to predict agriculturally related economic benefits anticipated by lower O_3 levels in the United States (Adams et al., 1985; Adams et al., 1989).

Kriging is a statistical tool developed by Matheron (1963) and named in honor of D.G. Krige. Although originally developed specifically for ore reserve estimation, kriging has been used for other spatial estimation applications, such as analyzing and modeling air quality data (Grivet, 1980; Faith and Sheshinski, 1979). At its simplest, kriging can be thought of as a way to interpolate spatial data much as an automatic contouring program would. In a more precise manner, kriging can be defined as a best, linear unbiased estimator of a spatial variable at a particular site or geographic area. Kriging assigns low weights to distant samples and vice versa, but also takes into account the relative position of the samples to each other and the site or area being estimated.

Figure 4-8 shows the average for the 1985 through 1987 period for the seasonal (April to October) average of the daily maximum 7- and 12-h values across the United States. The estimates made for the Rocky Mountain region had large uncertainties associated with them because of a lack of monitoring sites.

Because of the importance of the higher hourly average concentrations in eliciting injury and yield reduction for agricultural crops (U.S. Environmental Protection Agency, 1992b), kriging was used to predict O_3 exposures in the eastern United States. The sigmoidally weighted W126 exposure index was used as described earlier in this section. Lefohn et al. (1992b) used the W126 index in its kriging to characterize the O_3 exposures that occurred during the period 1985 to 1989. Figure 4-9 illustrates the integrated O_3 exposure for the 1988 and 1989 periods (data derived from work described in Lefohn et al., 1992b). Using the kriged data in the East, the 1988 exposures were the highest for the 5-year period, whereas 1989 exhibited the lowest exposures. The O_3 gradient pattern analyses described by Lefohn et al. (1992b) identified contiguous areas of persistent,

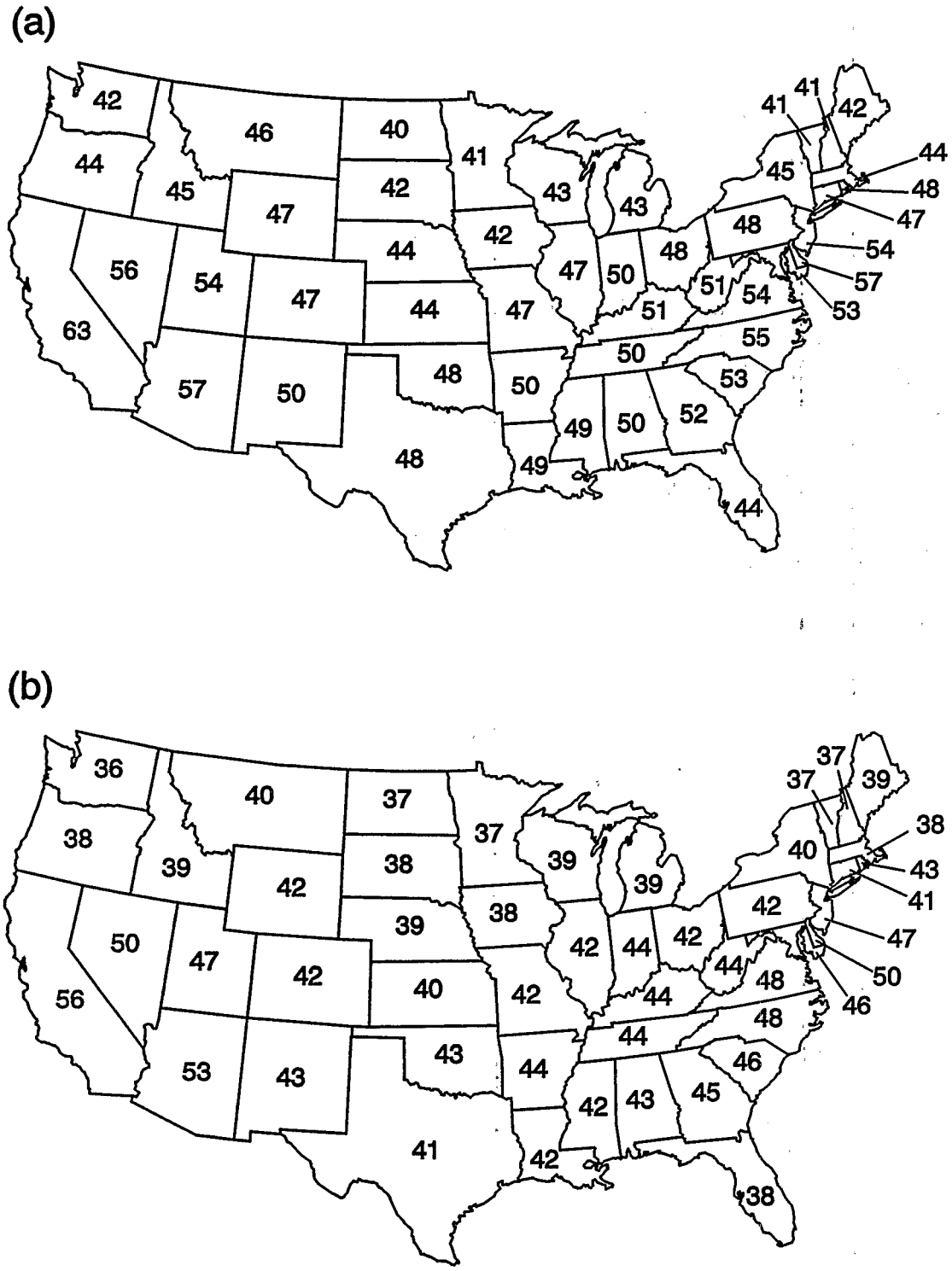


Figure 4-8. The kriged 1985 to 1986 maximum (a) 7-h and (b) 12-h average concentrations of ozone across the United States.

Source: Lefohn et al. (1991).

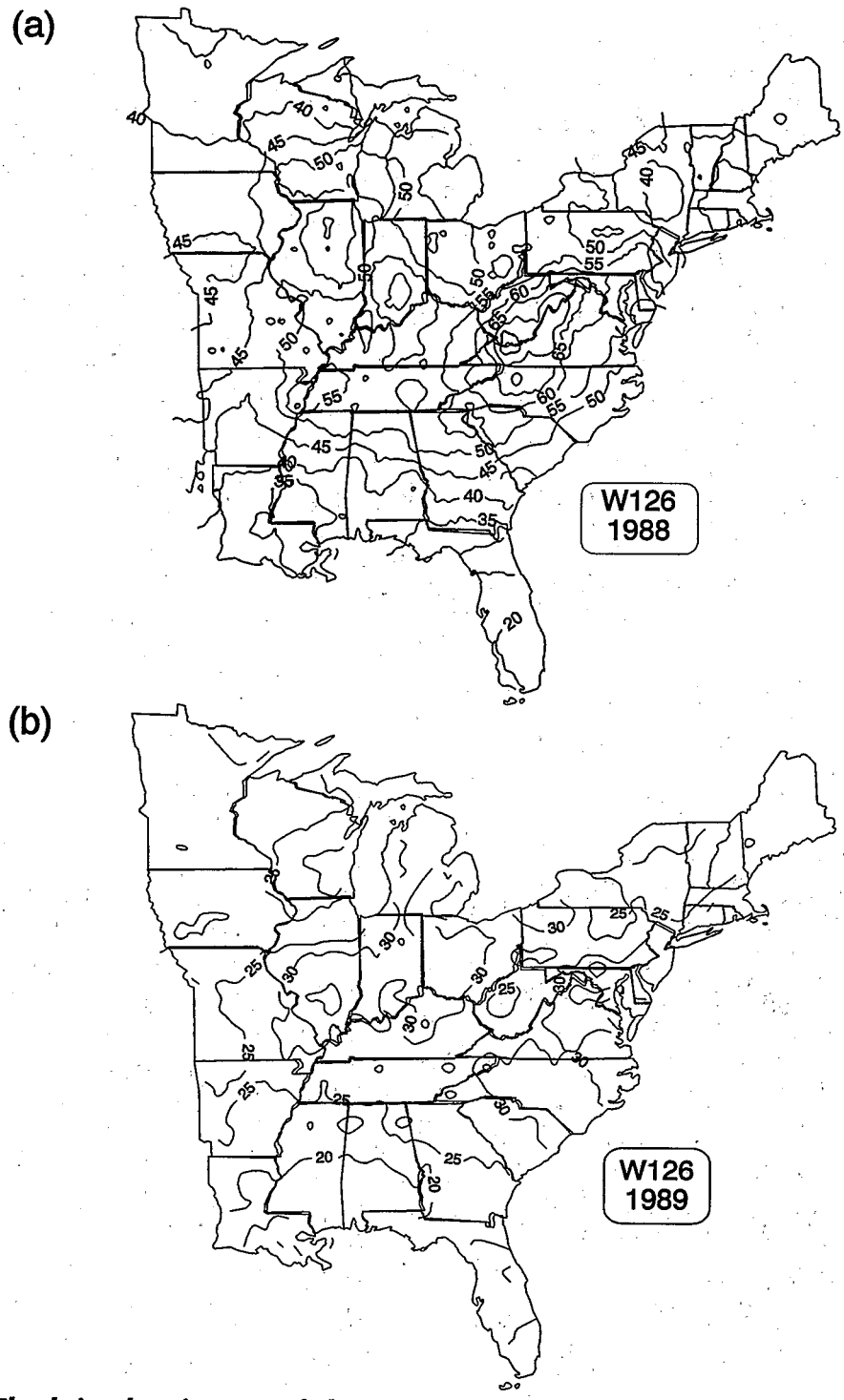


Figure 4-9. The kriged estimates of the W126 integrated ozone exposure index for the eastern United States for (a) 1988 and (b) 1989.

Source: Lefohn et al. (1992b).

relatively high seasonal O_3 values. The largest area extended from New Jersey south to northern Georgia and South Carolina. This area was roughly bounded on the west by the Appalachian Mountains. A second area, which exhibited persistent relatively high seasonal O_3 exposures, was centered over the Ohio River Valley in the region near the Kentucky-Indiana-Ohio borders. Relatively low O_3 exposures were found in Minnesota, Iowa, Wisconsin, Maine, Vermont, New Hampshire, and Florida. On a year-to-year basis, the analysis by Lefohn et al. (1992b) showed that regions that tended to be high for a specific year continued to experience O_3 exposures that were higher when compared to other regions.

4.4 Diurnal Variations in Ozone Concentrations

4.4.1 Introduction

By definition, diurnal variations are those that occur during a 24-h period. Diurnal patterns of O_3 may be expected to vary with location, depending on the balance among the many factors affecting O_3 formation, transport, and destruction. Although they vary with locality, diurnal patterns for O_3 typically show a rise in concentration from low (or levels near minimum detectable amounts) to an early afternoon peak. The 1978 criteria document (U.S. Environmental Protection Agency, 1978) ascribed the diurnal pattern of concentrations to three simultaneous processes: (1) downward transport of O_3 from layers aloft; (2) destruction of O_3 through contact with surfaces and through reaction with nitric oxide (NO) at ground level; and (3) in situ photochemical production of O_3 (U.S. Environmental Protection Agency, 1978; Coffey et al., 1977; Mohnen et al., 1977; Reiter, 1977a).

The form of an average diurnal pattern may provide information on sources, transport, and chemical formation and destruction effects at various sites (Lefohn, 1992b). Nontransport conditions will produce early afternoon peaks. However, long-range transport processes will influence the actual timing of a peak from afternoon to evening or early morning hours. Investigators have utilized diagrams that illustrate composite diurnal patterns as a means to describe qualitatively the differences in O_3 exposures between sites (Lefohn and Jones, 1986; Böhm et al., 1991). Although it might appear that composite diurnal pattern diagrams could be used to quantify the differences in O_3 exposures between sites, Lefohn et al. (1991) cautioned their use for this purpose. The average diurnal patterns are derived from long-term calculations of the hourly average concentrations, and the resulting diagram cannot identify adequately, at most sites, the presence of high hourly average concentrations and, thus, may not adequately distinguish O_3 exposure differences among sites. Logan (1989) noted that diurnal variation of O_3 did not reflect the presence of high hourly average concentrations.

Unique families of diurnal average profiles exist, and it is possible to distinguish between two types of O_3 monitoring sites. A seasonal diurnal diagram provides the investigator with the opportunity to identify whether a specific O_3 monitoring site has more scavenging than any other site. Ozone is rapidly depleted near the surface below the nocturnal inversion layer (Berry, 1964). Mountainous sites, which are above the nocturnal inversion layer, do not necessarily experience this depletion (Stasiuk and Coffey, 1974). Taylor and Hanson (1992) reported similar findings using data from the Integrated Forest Study. For the low-elevation sites, the authors reported that intraday variability was most significant due to the pronounced daily amplitude in O_3 concentration between the predawn

minimum and midafternoon-to-early evening maximum. The authors reported that the interday variation was more significant in the high-elevation sites. Ozone trapped below the inversion layer is depleted by dry deposition and chemical reactions if other reactants are present in sufficient quantities (Kelly et al., 1984). Above the nocturnal inversion layer, dry deposition generally does not occur, and the concentration of O_3 scavengers generally is lower, so O_3 concentration remains fairly constant (Wolff et al., 1987). A flat diurnal pattern is usually interpreted as indicating a lack of efficient scavenging of O_3 or a lack of photochemical precursors, whereas a varying diurnal pattern is taken to indicate the opposite. With the composite diagrams alone, it is difficult to quantify the daily or long-term exposures of O_3. For example, the diurnal patterns for two such sites are illustrated in Figure 4-10. The Jefferson County, KY, site is urban-influenced and experiences elevated levels of O_3 and NO_x. The Oliver County, ND, site is fairly isolated from urban-influenced sources and hourly average O_3 concentrations are mostly below 0.09 ppm. The flat diurnal pattern observed for the Oliver County site is usually interpreted as indicating a lack of efficient scavenging of O_3 or a lack of photochemical precursors, whereas the varying diurnal pattern observed at the Jefferson County site may be interpreted to indicate the opposite. Logan (1989) has described the diurnal pattern for several rural sites in the United States (Figure 4-11) and noted that average daily profiles showed a broad maximum from about 1200 hours until about 1800 hours at all the eastern sites, except for the peak of Whiteface Mountain, NY. Logan (1989) noted that the maximum concentrations were higher at the SURE sites than at the Western National Air Pollution Background Network sites in the East, because the latter were situated in more remote or coastal locations.

There is concern that the highest hourly average concentrations observed at rural agricultural and forested sites occur outside the most biologically active period of the day. To address this concern, a review of the hourly average data collected at all rural agricultural and forested sites in EPA's AIRS database for 1990 to 1992 was undertaken to evaluate the percentage of time hourly average concentrations ≥ 0.1 ppm occurred during the period 0900 to 1559 hours in comparison with the 24-h period. Each rural site for each of the 3 years that experienced a 3-mo SUMO6 ≥ 26.4 ppm-h (see Chapter 5, Section 5.6 for more details concerning the use of a 3-mo SUMO6 exposure index) was characterized. It was found that 70% of the rural agricultural and forested sites used in the analysis experienced at least 50% of the occurrences ≥ 0.1 ppm during the period 0900 to 1559 hours when compared to the 24-h period (Figure 4-12). When O_3 monitoring sites in California were eliminated, approximately 73% of the remaining sites experienced at least 50% of the occurrences ≥ 0.10 ppm during the daylight 7-h period when compared with the 24-h period (Figure 4-13). Reviewing Figures 4-12 and 4-13, for most rural agricultural and forested sites that experience 3-mo SUMO6 ≥ 26.4 ppm in the United States, most of the hourly average concentrations ≥ 0.1 ppm occur during the 0900 to 1559 hours period.

4.4.2 Urban Area Diurnal Patterns

The U.S. Environmental Protection Agency (1986) has discussed diurnal patterns for urban sites. Figure 4-14, reproduced from the previous document, shows the diurnal pattern of O_3 concentrations on July 13, 1979, in Philadelphia, PA. On this day a peak 1-h average concentration of 0.20 ppm, the highest for the month, was reached at 1400 hours, presumably as the result of meteorological factors, such as atmospheric mixing and local

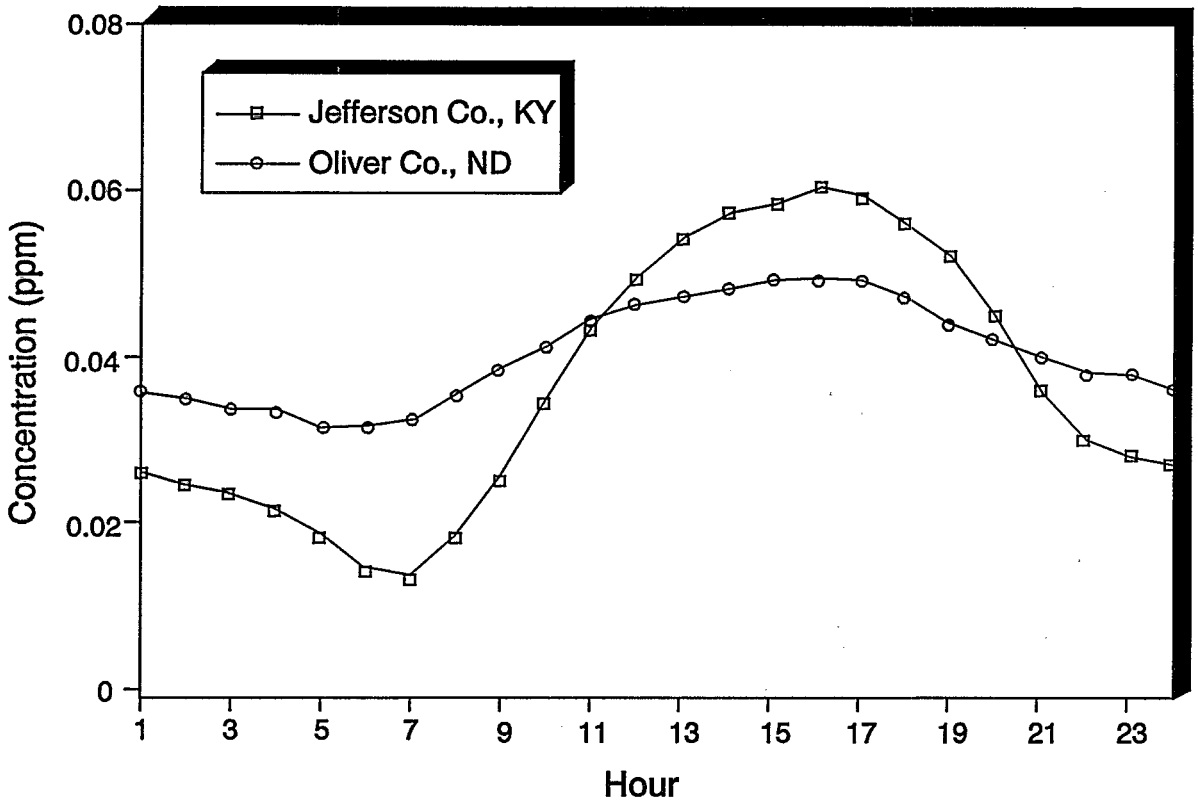


Figure 4-10. The comparison of the seasonal diurnal patterns using 1988 data for Jefferson County, KY, and Oliver County, ND.

photochemical processes. The severe depression of concentrations to below detection limits (less than 0.005 ppm) between 0300 and 0600 hours usually is explained as resulting from the scavenging of O_3 by local NO emissions. In this regard, this station is typical of most urban locations.

Diurnal profiles of O_3 concentrations can vary from day to day at a specific site, however, because of changes in the various factors that influence concentrations. Composite diurnal data (that is, concentrations for each hour of the day averaged over multiple days or months) often differ markedly from the diurnal cycle shown by concentrations for a specific day. In Figures 4-15 through 4-17 (reproduced from the previous document), diurnal data for 2 consecutive days are compared with composite diurnal data (1-mo averages of hour-by-hour measurements) at three different kinds of sites: (1) center city-commercial (Washington, DC), (2) rural-near urban (St. Louis, MO), and (3) suburban-residential (Alton, IL). Several obvious points of interest present themselves in these figures: at some sites, at least, peaks can occur at virtually any hour of the day or night, but these peaks may not show up strongly in the longer term average data; some sites may be exposed to multiple peaks during a 24-h period; and disparities, some of them large, can exist between peaks (the diurnal data) and the 1-mo average (the composite diurnal data) of hourly O_3 concentrations.

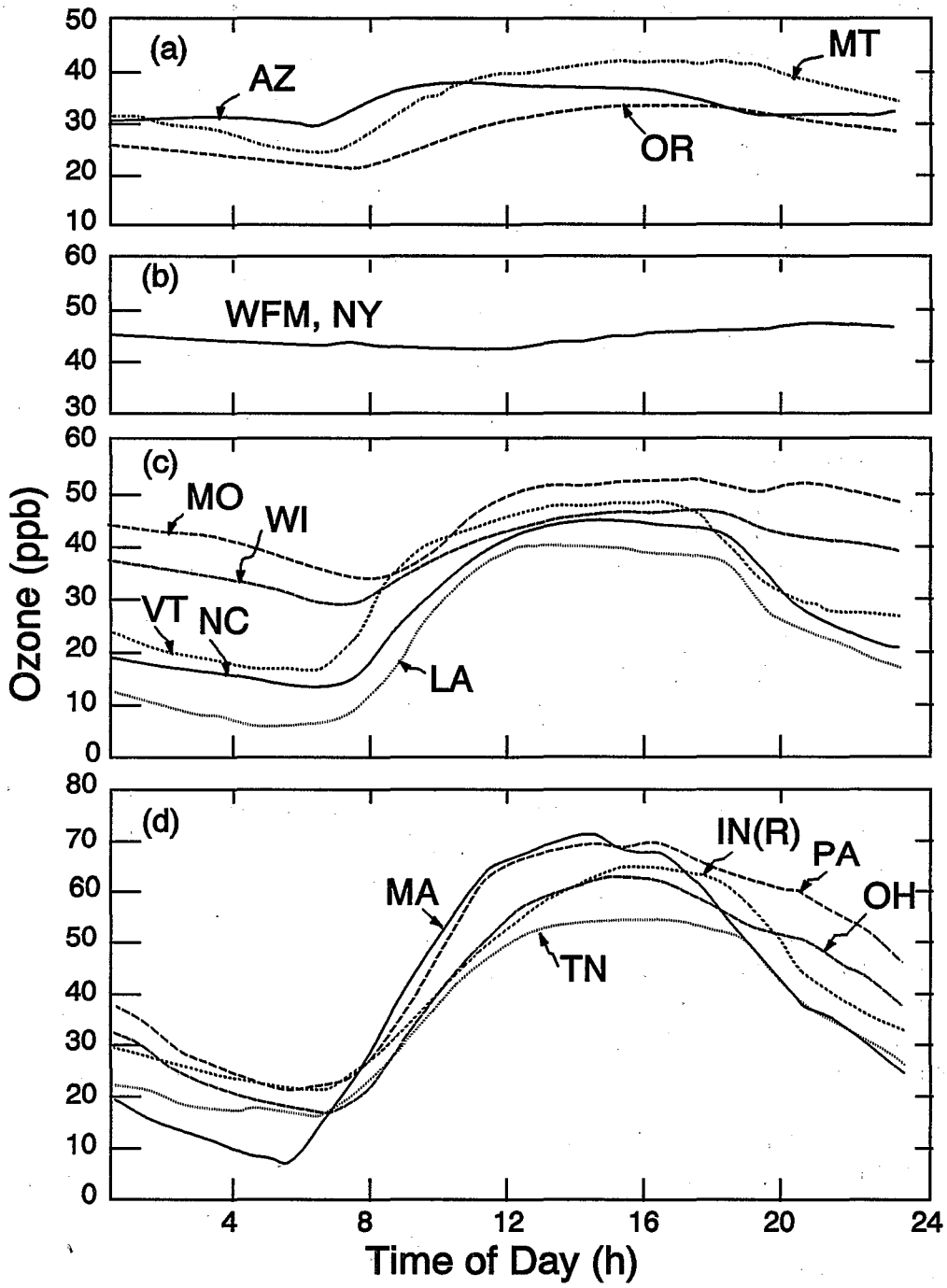


Figure 4-11. Diurnal behavior of ozone at rural sites in the United States in July. Sites are identified by the state in which they are located. (a) Western National Air Pollution Background Network (NAPBN); (b) Whiteface Mountain (WFM) located at 1.5 km above sea level; (c) eastern NAPBN sites; and (d) sites selected from the Electric Power Research Institute's Sulfate Regional Air Quality Study. IN(R) refers to Rockport.

Source: Logan (1989).

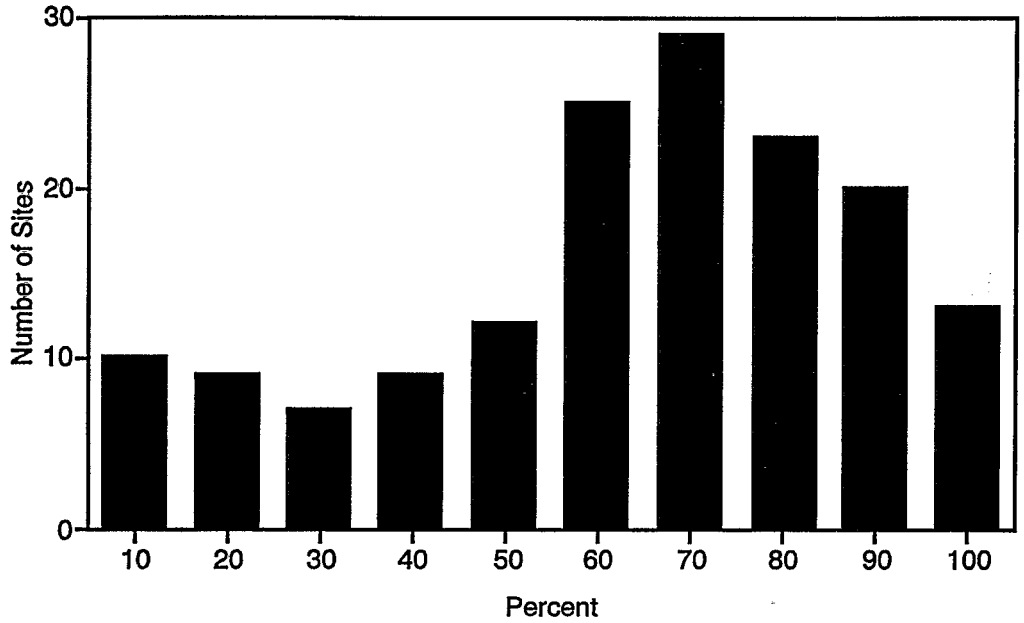


Figure 4-12. Percent of time hourly average concentrations ≥0.1 ppm occurred between 0900 and 1559 hours in comparison to 24-h period for all rural agricultural and forested sites with 3-mo SUM06 ≥26.4 ppm-h.

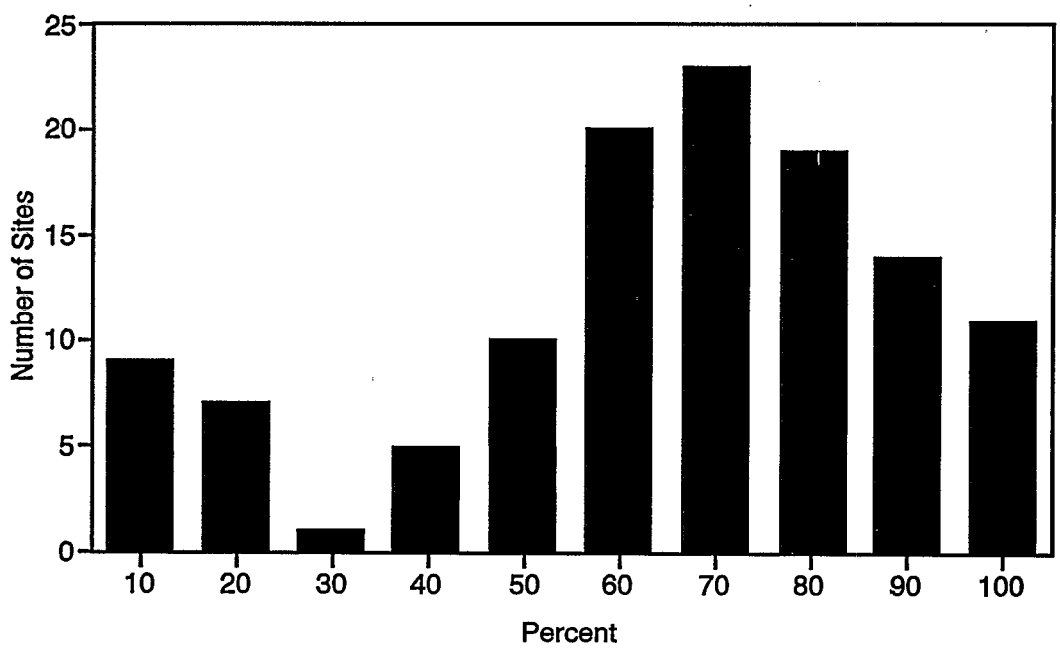


Figure 4-13. Percent of time hourly average concentrations ≥0.1 ppm occurred between 0900 and 1559 hours in comparison to 24-h period for all non-California rural agricultural and forested sites with 3-mo SUM06 ≥26.4 ppm-h.

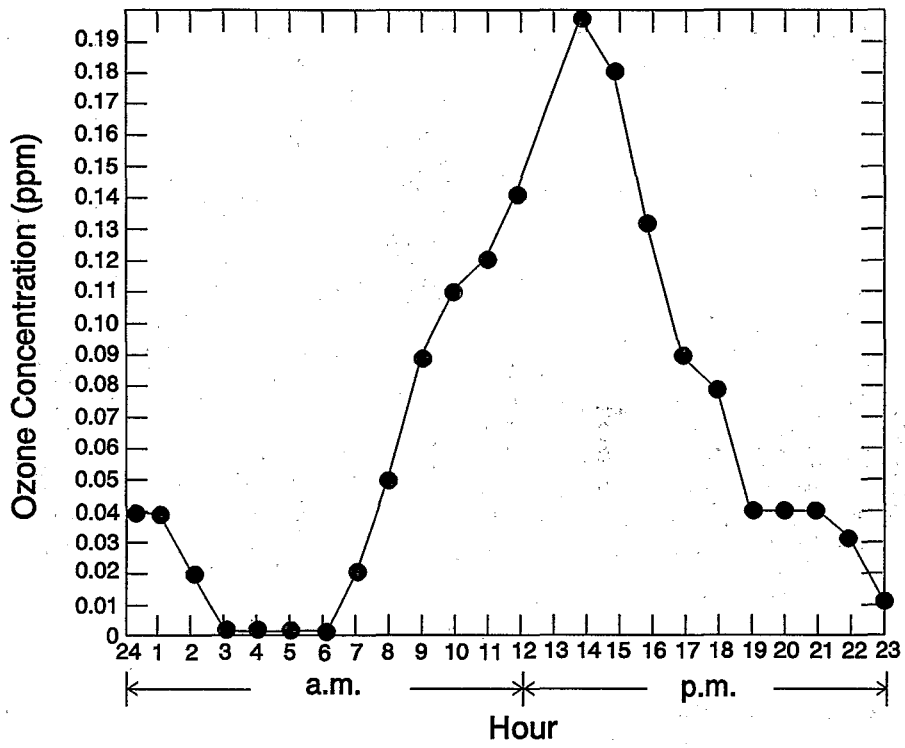


Figure 4-14. Diurnal pattern of 1-h ozone concentrations on July 13, 1979, Philadelphia, PA.

When diurnal or short-term composite diurnal O_3 concentrations are compared with longer term composite diurnal O_3 concentrations, the peaks are smoothed as the averaging period is lengthened. Figure 4-1 demonstrates the effects of lengthening the period of time over which values are averaged. This figure shows a composite diurnal pattern calculated on the basis of 3 mo. Although seasonal differences are observed, the comparison of 3-mo (Figure 4-18) with 1-mo composite diurnal concentrations (Figure 4-17) at the Alton, IL, site readily demonstrates the smoothing out of peak concentrations as the averaging period is lengthened. As indicated in the previous version of the document (U.S. Environmental Protection Agency, 1986), although this is an obvious and familiar result in the statistical treatment of monitoring data, it is highly pertinent to the protection of human health and welfare from the effects of O_3.

4.4.3 Nonurban Area Diurnal Patterns

Nonurban areas only marginally affected by transported O_3 usually have a flatter diurnal profile than sites located in urban areas. Nonurban O_3 monitoring sites experience differing types of diurnal patterns (Böhm et al., 1991; Lefohn, 1992b). As indicated earlier, O_3 concentrations at a specific location are influenced by local emissions and by long-range transport from both natural and anthropogenic sources. Thus, considerable variation of O_3 exposures among sites characterized as agricultural or forested is found and there is no preference for maximum diurnal patterns to occur in either the second or third quarter.

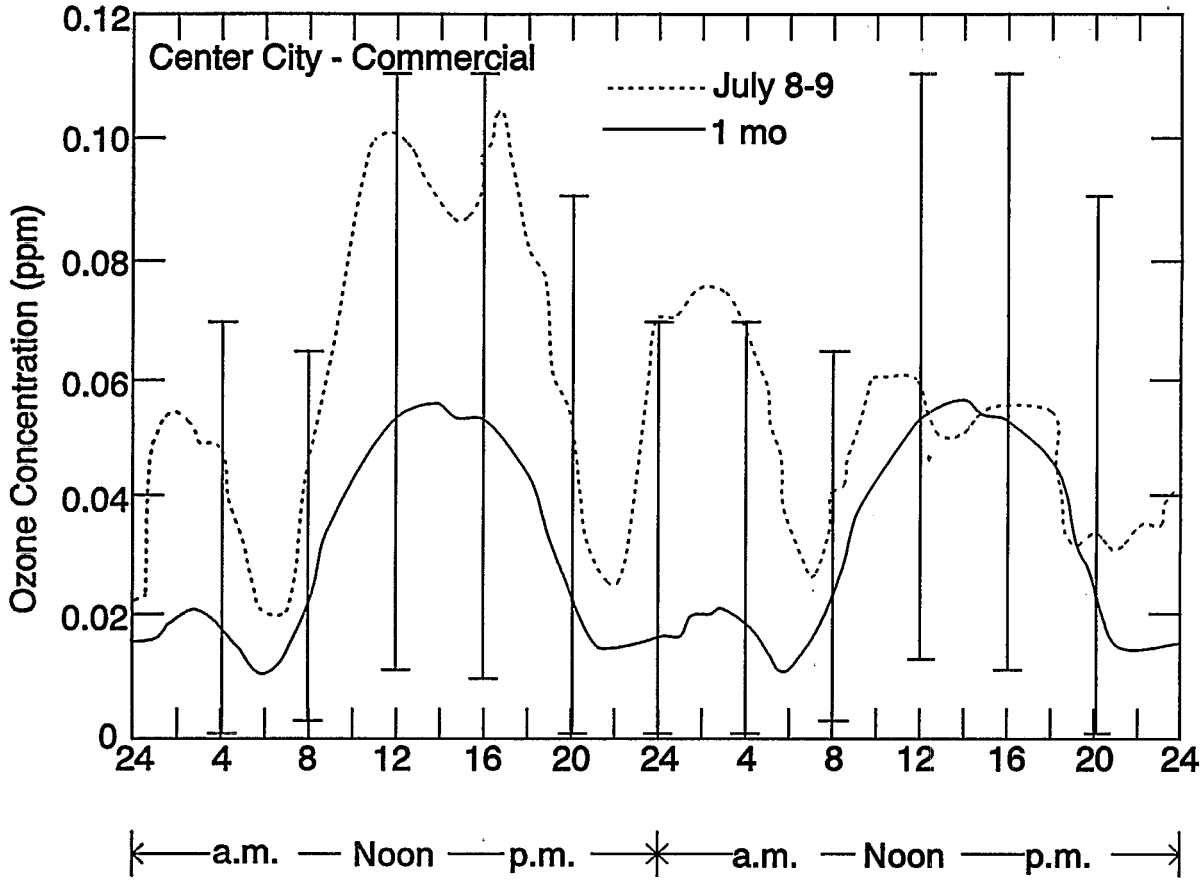


Figure 4-15. Diurnal and 1-mo composite diurnal variations in ozone concentrations, Washington, DC, July 1981.

Source: U.S. Environmental Protection Agency (1986).

The diurnal patterns for several agricultural sites have been characterized (U.S. Environmental Protection Agency, 1986). Figures 4-19 and 4-20 show some typical patterns of exposure. As discussed by U.S. Environmental Protection Agency (1986), the six sites, whose diurnal patterns are illustrated in Figure 4-18, represent counties with high soybean, wheat, or hay production. The figures show a distinct afternoon maximum with the lowest concentrations occurring in the early morning and evening hours. Quarterly composite diurnal patterns clearly show the division of the afternoon O_3 concentrations into two seasonal patterns, the low, "winter" levels in the first and fourth quarters and the high, "summer" levels in the second and third quarters of the year.

Remote forested sites experience unique patterns of O_3 concentrations (Evans et al., 1983; Lefohn, 1984). These sites tend to experience a weak diurnal pattern, with

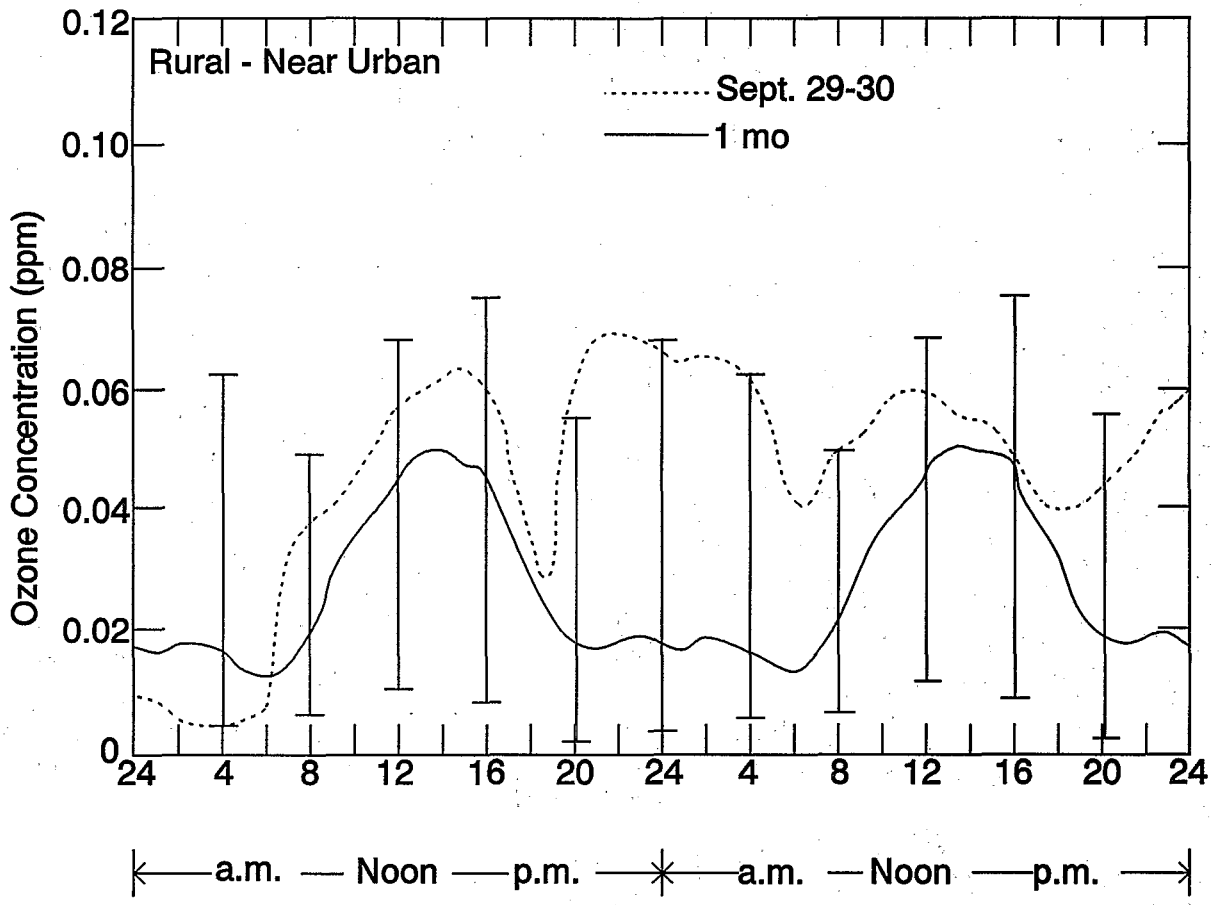


Figure 4-16. Diurnal and 1-mo composite diurnal variations in ozone concentrations, St. Louis County, MO, September 1981.

Source: U.S. Environmental Protection Agency (1986).

hourly average O_3 concentrations that occur frequently in the range of 0.04 to 0.05 ppm. Figure 4-21 shows diurnal patterns for several sites in the NDDN network that are located in forested areas. Several of the NDDN sites analyzed by Edgerton and Lavery (1992) exhibit fairly flat average diurnal patterns. Such a pattern is based on average concentrations calculated over an extended period. On a daily basis, some variation in O_3 concentration does occur from hour to hour, and, in some cases, high hourly average concentrations are experienced either during daytime or nighttime periods (Lefohn and Mohnen, 1986; Lefohn and Jones, 1986; Logan, 1989; Lefohn et al., 1990b; Taylor et al., 1992).

Lefohn et al. (1990b) characterized O_3 concentrations at high-elevation monitoring sites. The authors reported that a fairly flat diurnal pattern for the Whiteface Mountain summit site (WF1) was observed (Figure 4-22a), with the maximum hourly average concentrations occurring in the late evening or early morning hours. A similar pattern was observed for the mid-elevation site at Whiteface Mountain (WF3). The site at the base of Whiteface Mountain (WF4) showed the typical diurnal pattern expected from sites that

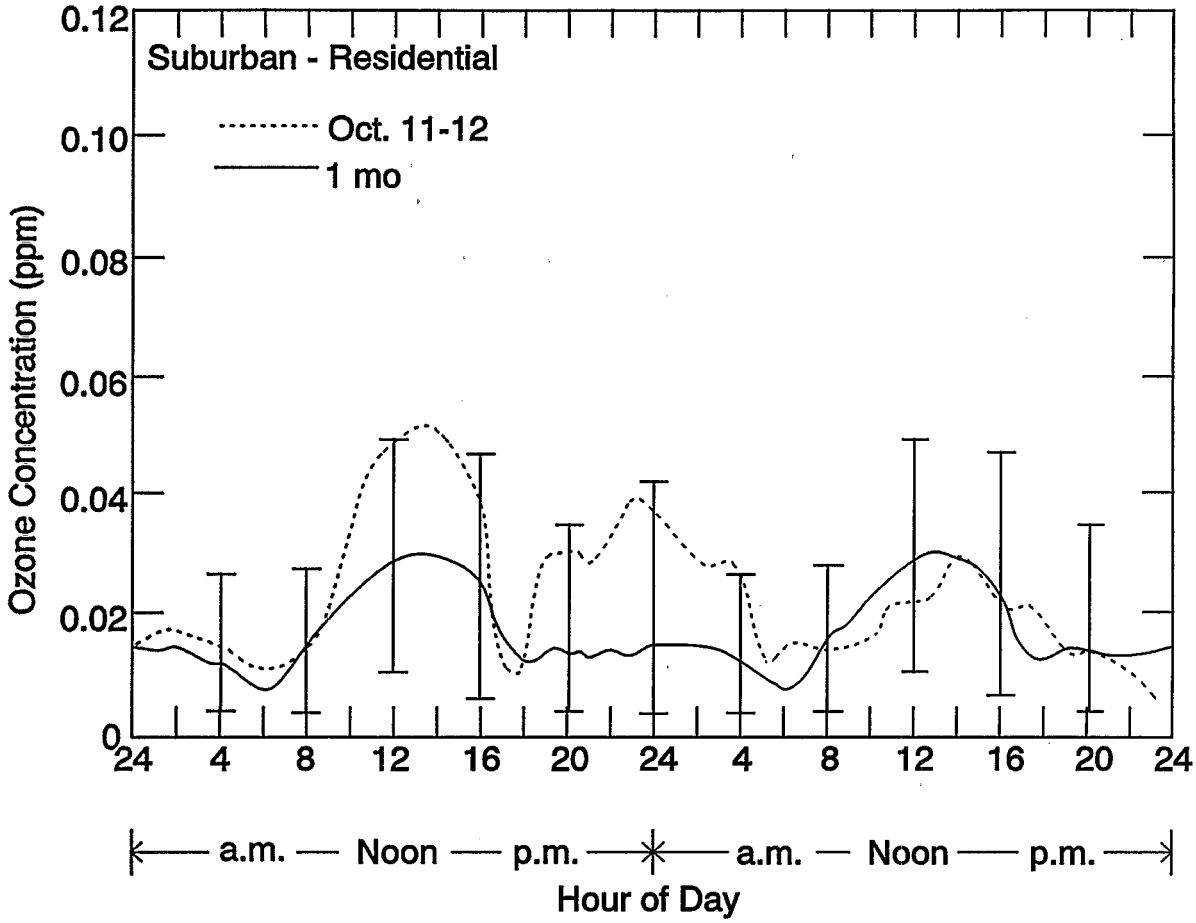


Figure 4-17. Diurnal and 1-mo composite diurnal variations in ozone concentrations, Alton, IL, October 1981 (fourth quarter).

Source: U.S. Environmental Protection Agency (1986).

experience some degree of O_3 scavenging. More variation in the diurnal pattern for the highest Shenandoah National Park sites occurred than for the higher elevation Whiteface Mountain sites, with the typical variation for urban-influenced sites in diurnal pattern at the lower elevation Shenandoah National Park site (Figure 4-22b). Aneja and Li (1992), in their analysis of the five high-elevation Mountain Cloud Chemistry Program (MCCP) sites (see Section 4.6.2 for site descriptions), noted the flat diurnal pattern typical of high-elevation sites that has been described previously in the literature. Aneja and Li (1992) noted that the peak of the diurnal patterns over the period May to October (1986 to 1988) for the five sites occurred between 1800 and 2400 hours, whereas the minimum was observed between 0900 and 1200 hours. However, it is important to note that, as indicated by Lefohn et al. (1990b), the flat diurnal pattern is not observed for all high-elevation sites.

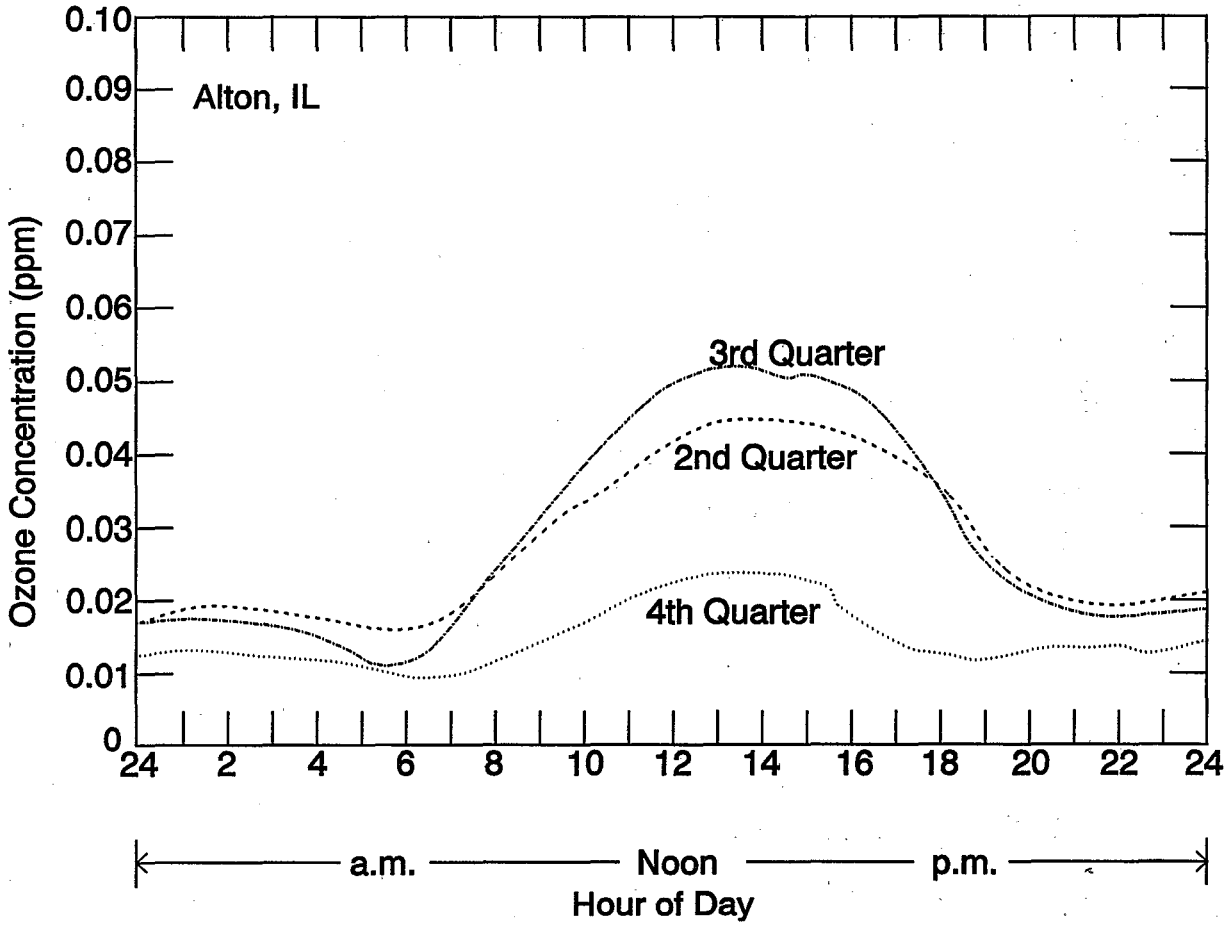


Figure 4-18. Composite diurnal patterns of ozone concentrations by quarter, Alton, IL, 1981.

4.5 Seasonal Patterns in Ozone Concentrations

4.5.1 Urban Area Seasonal Patterns

Seasonal variations in O_3 concentrations in 1981 were described by the U.S. Environmental Protection Agency (1986). The current form of the standard focuses on the highest hourly average concentrations. The description that follows uses the highest hourly average concentration as an indication of exposure. Figure 4-23 shows the 1-mo averages and the single 1-h maximum concentrations within the month for eight sites across the nation. The data from most of these sites exhibit the expected pattern of high O_3 in late spring or in summer and low levels in the winter. Data from Pomona, CA (Figure 4-23c), and Denver, CO (Figure 4-23d), show summer maxima. Tampa, FL, shows a late spring maximum but with concentrations in the fall (i.e., October) approaching those of spring (June) (Figure 4-23f). Dallas data also tend to be skewed toward higher spring concentrations; but note that November concentrations are also relatively high (Figure 4-23h). Because of seasonal humidity and storm tracks from year to year, the general weather conditions in a

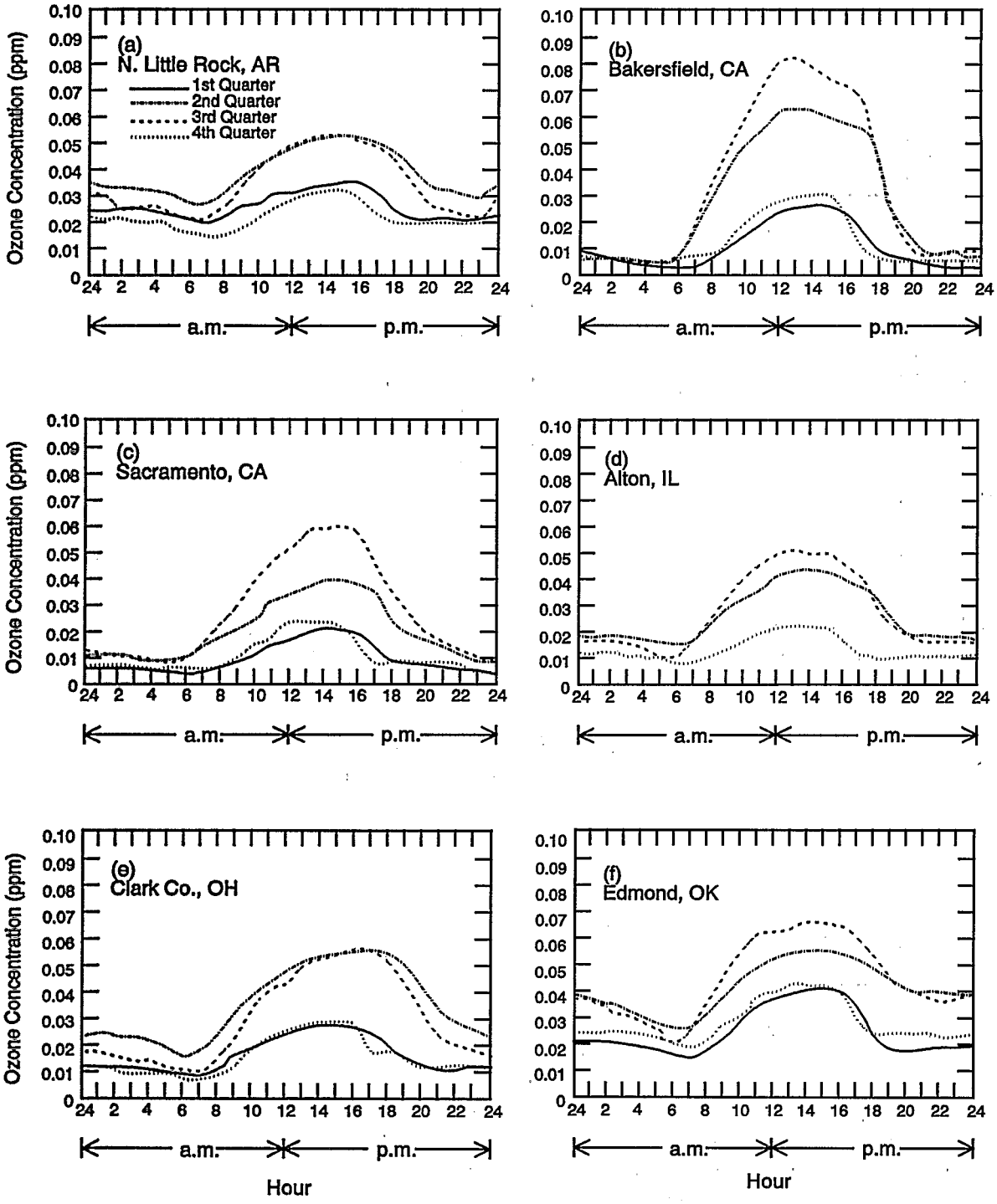


Figure 4-19. Quarterly composite diurnal patterns of ozone concentrations at selected sites representing potential for exposure of major crops, 1981.

Source: U.S. Environmental Protection Agency (1986).

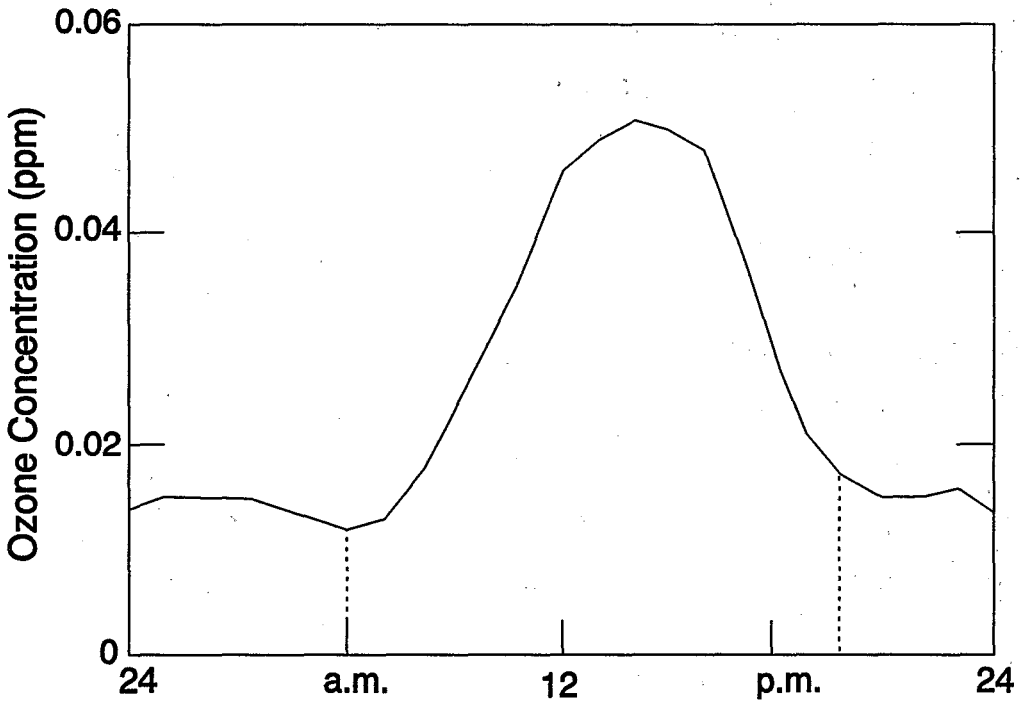


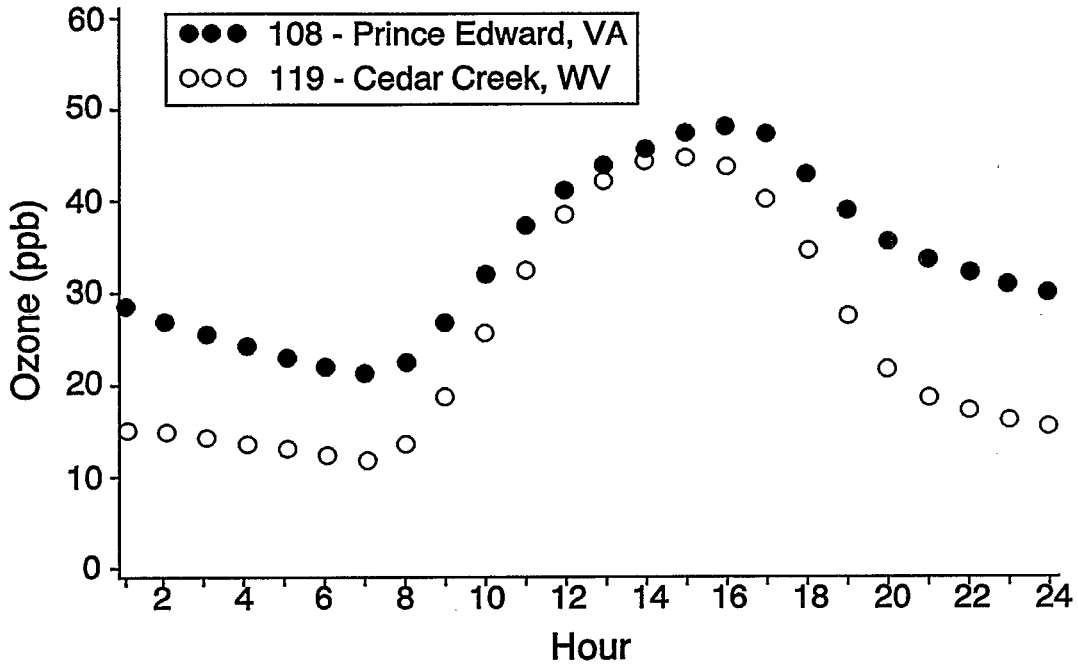
Figure 4-20. Composite diurnal ozone pattern at a rural National Crop Loss Assessment Network site in Argonne, IL, August 6 through September 30, 1980.

Source: U.S. Environmental Protection Agency (1986).

given year may be more favorable for the formation of O_3 and other oxidants than during the prior or following year. For example, 1988 was a hot and dry year during which some of the highest O_3 concentrations of the last decade occurred, whereas 1989 was a cold and wet year in which some of the lowest concentrations occurred (U.S. Environmental Protection Agency, 1992a).

4.5.2 Nonurban Area Seasonal Patterns

In the literature, several investigators have reported on the tendency for average O_3 concentrations to be higher in the second versus the third quarter of the year for many isolated rural sites (Evans et al., 1983; Singh et al., 1978). This observation has been attributed either to stratospheric intrusions or to an increasing frequency of slow-moving, high-pressure systems that promote the formation of O_3. Lefohn et al. (1990a) reported that for several clean sites, the highest values of exposure indices occurred in the third quarter rather than in the second. The results of this analysis will be discussed in the Section 4.5.3. Taylor et al. (1992) reported that for 10 forest sites in North America, the temporal patterns of O_3 on quarterly or annual periods exhibited less definitive patterns. Based on the exposure index selected, different patterns were reported. The different patterns may be



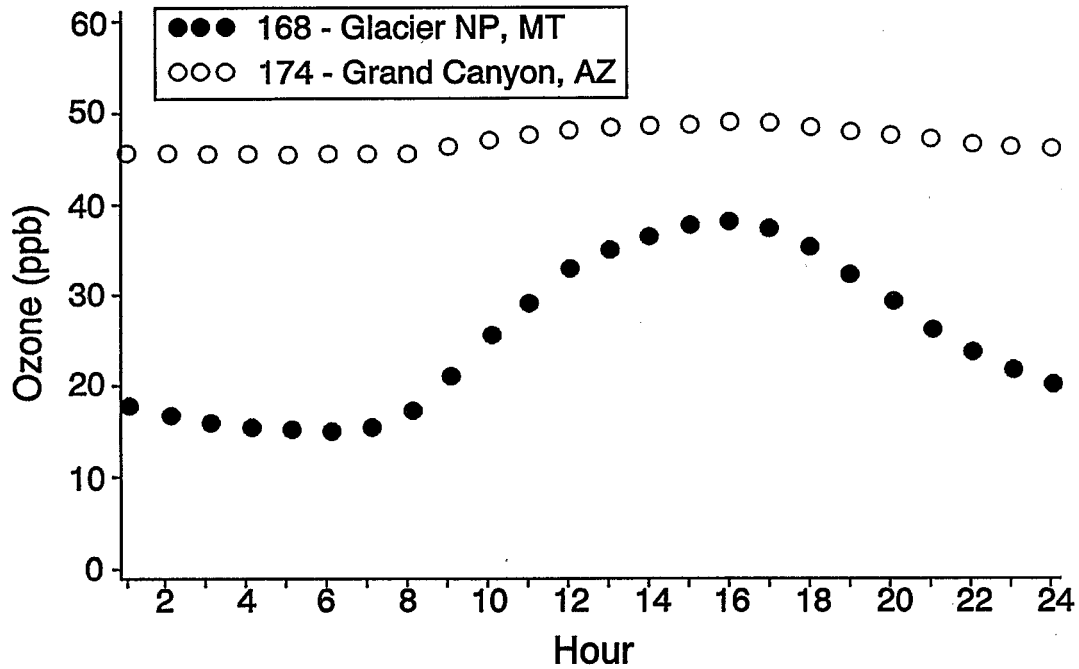


Figure 4-21. Composite diurnal ozone pattern at selected National Dry Deposition Network sites.

Source: Edgerton and Lavery (1992).

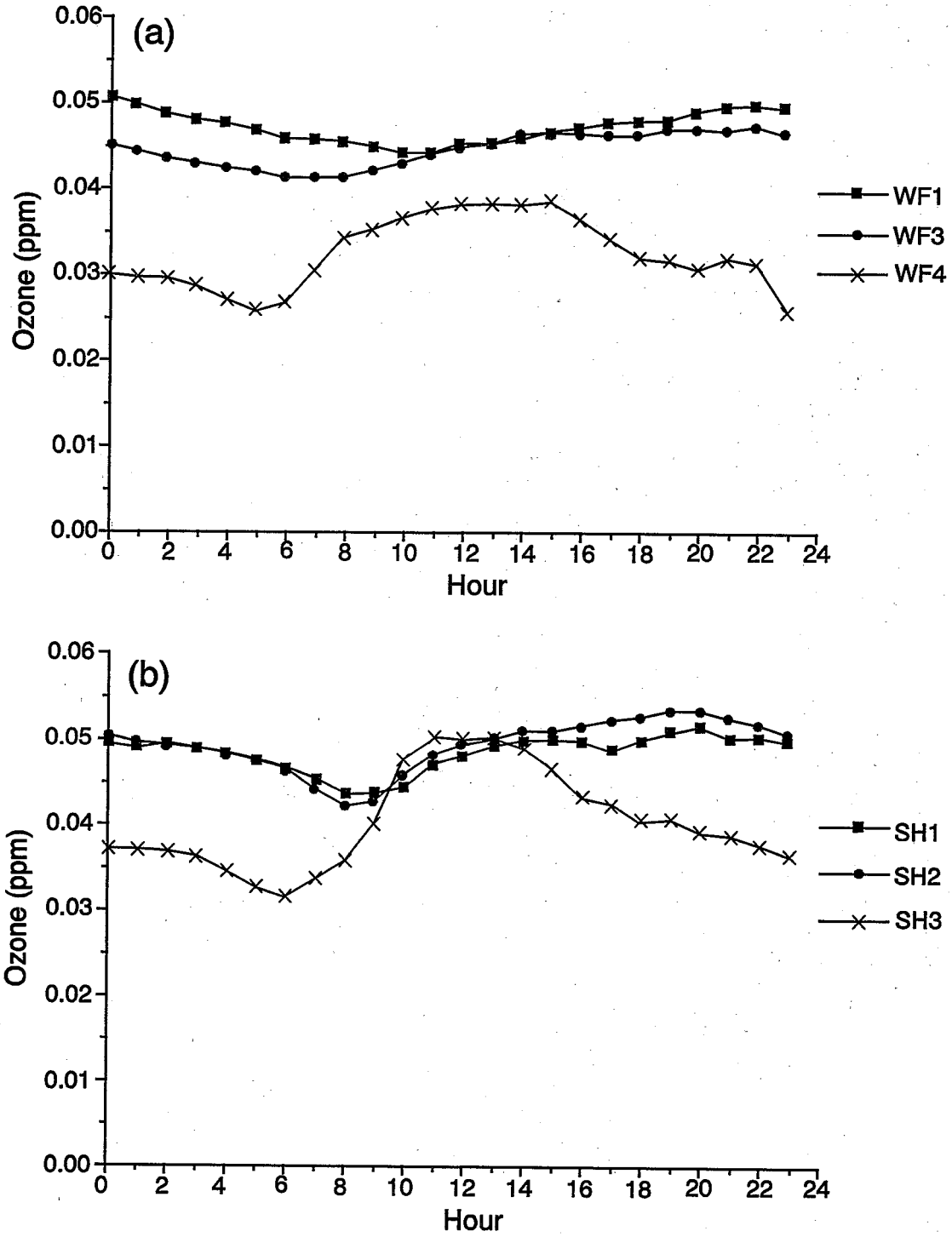


Figure 4-22. Composite diurnal pattern at (a) Whiteface Mountain, NY, and the (b) Mountain Cloud Chemistry Program Shenandoah National Park site for May to September 1987.

Source: Lefohn et al. (1990b).

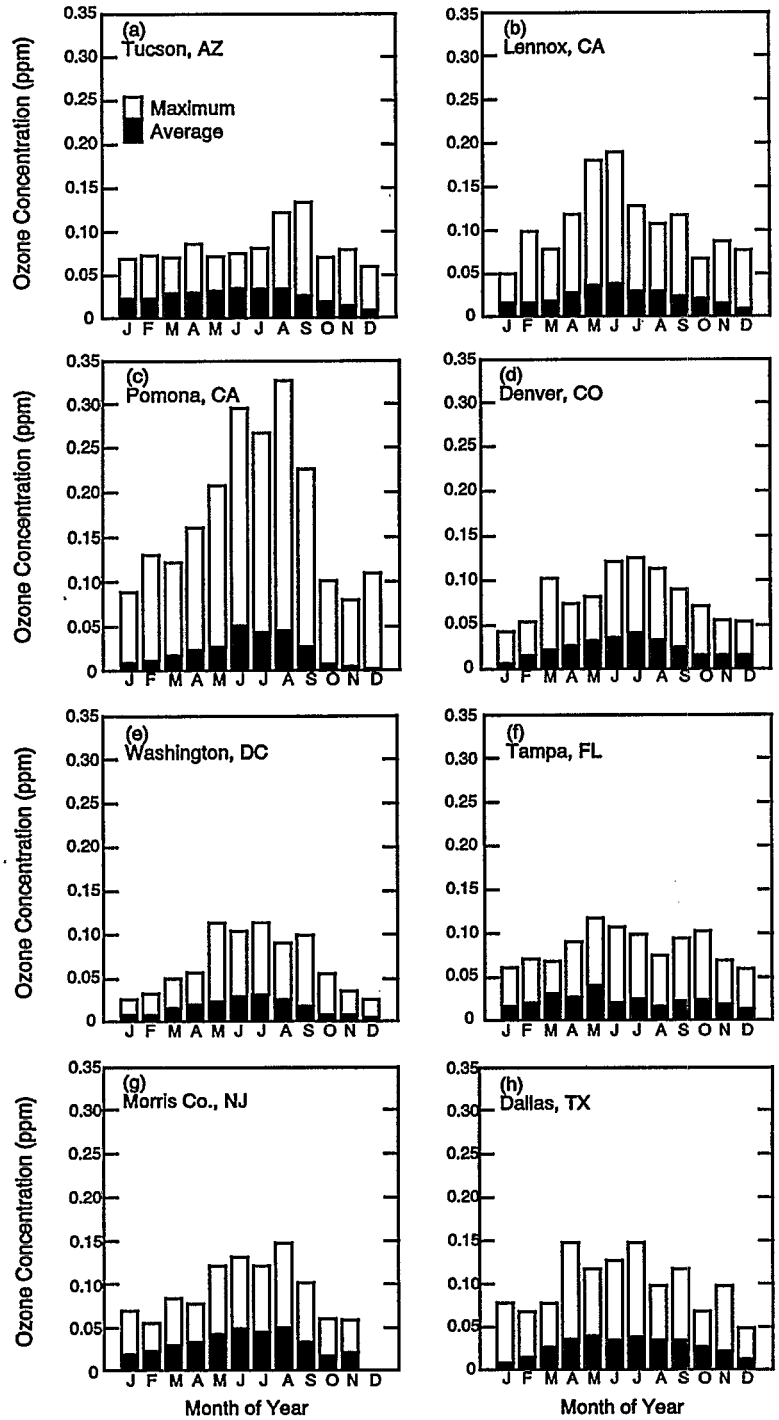


Figure 4-23. Seasonal variations in ozone concentrations as indicated by monthly averages and the 1-h maximum in each month at selected sites, 1981.

Source: U.S. Environmental Protection Agency (1986).

associated with the observations by Logan (1989) that rural O_3 in the eastern United States in the spring and summer is severely impacted by anthropogenic and possibly natural emissions of NO_x and hydrocarbons, and that O_3 episodes occur when the weather is particularly conducive to photochemical formation of O_3. Meagher et al. (1987) reported for rural O_3 sites in the southeastern United States that the daily maximum 1-h average concentration was found to peak during the summer months. Taylor and Norby (1985) reported that, in the Shenandoah National Park, the probability of a day occurring in which a 1-h mean O_3 concentration was >0.08 ppm was the same during the months of May, June, and July, whereas the probability was nearly 40% less in August. The probability of an episode during each of the remaining months of the growing season was $<5\%$. The month of July experienced both the highest frequency of episodes and the highest mean duration of exposure events.

Aneja and Li (1992) reported that the maximum monthly ozone levels occurred in either the spring or the summer (May to August), and the minimum occurred in the fall (September and October). The timing of the maximum monthly values differed across sites and years. However, in 1988, an exceptionally high O_3 concentration year, for almost all of the five sites, June was the month in which the highest monthly average concentration occurred. This was the month in which the greatest number of O_3 episodes occurred in the eastern United States.

4.5.3 Seasonal Pattern Comparisons with Sites Experiencing Low Exposures

Lefohn et al. (1990a) have characterized the O_3 concentrations that occurred at several sites in the United States that experience low maximum hourly average concentrations. The Theodore Roosevelt National Park site experienced its maximum in July for 1984 and 1985 and in May for 1986. Of the three western national forest sites evaluated by Lefohn et al. (1990a), only Apache National Forest experienced its maximum monthly mean concentration in the spring. The Apache National Forest site was above mean nocturnal inversion height, and no decrease of concentrations occurred during the evening hours. This site also experienced the highest hourly maximum concentration, as well as the highest W126 O_3 exposures. The Custer and Ochoco national forest sites experienced most of their maximum monthly mean concentrations in the summer. The White River Oil Shale site in Colorado experienced its maximum monthly mean during the spring and summer months.

The W126 sigmoidal weighting function index was also used to identify the month of highest O_3 exposure. A somewhat more variable pattern was observed than when the maximum monthly average concentration was used. For some sites, the winter/spring pattern was represented; for others, it was not. In some cases, the highest W126 exposures occurred earlier in the year than was indicated by the maximum monthly concentration. For example, in 1979, the Custer National Forest site experienced its highest W126 exposure in April, although the maximum monthly mean occurred in August. In 1980, the reverse occurred.

There was no consistent pattern for those sites located in the continental United States. The Theodore Roosevelt and Ochoco national park sites, the Custer National Forest site, and the White River Oil Shale site experienced their maximum O_3 exposures during the

spring and summer months. The sites experiencing their highest O_3 exposures in the fall-to-spring period did not necessarily experience the lowest O_3 exposures.

4.6 Spatial Variations in Ozone Concentrations

4.6.1 Urban-Nonurban Area Concentration Differences

Diurnal concentration data presented earlier indicate that peak O_3 concentrations can occur later in the day in rural areas than in urban, with the distances downwind from urban centers generally determining how much later the peaks occur. Meagher et al. (1987) reported that for five rural sites in the Tennessee Valley region of the southeastern United States, O_3 levels were found to equal or exceed urban values for the same region. Data presented in the 1978 criteria document demonstrated that peak concentrations of O_3 in rural areas generally are lower than those in urban areas, but that average concentrations in rural areas are comparable to or even higher than those in urban areas (U.S. Environmental Protection Agency, 1978). Reagan (1984) noted that O_3 concentrations measured near population-oriented areas were depressed in comparison with data collected in more isolated areas. As noted earlier, urban O_3 values are often depressed because of titration by NO (Stasiuk and Coffey, 1974). In reviewing the NCLAN's use of kriging to estimate the 7-h seasonal average O_3 levels, Lefohn et al. (1987a) found that the 7-h values derived from kriging for sites located in rural areas tended to be lower than the actual values because of the effect of using data from urban areas to estimate rural values. In addition to the occurrence of higher average concentrations and occasionally higher peak concentrations of O_3 in nonurban than in urban areas, it is well documented that O_3 persists longer in nonurban than in urban areas (Coffey et al., 1977; Wolff et al., 1977; Isaksen et al., 1978). The absence of chemical scavengers appears to be the main reason.

4.6.2 Concentrations Experienced at High-Elevation Sites

The distributions of hourly average concentrations experienced at high-elevation cities are similar to those experienced in low-elevation cities. For example, the distribution of hourly average concentrations for several O_3 sites located in Denver were similar to distributions observed at many low-elevation sites in the United States. However, as will be discussed in Section 4.6.3, for assessing the possible impacts of O_3 at high-elevation sites, the use of absolute concentrations (e.g., in units of micrograms per cubic meter) instead of mixing ratios (e.g., parts per million) may be an important consideration.

Lefohn et al. (1990b) summarized the characterization of gaseous exposures at rural sites in 1986 and 1987 at several MCCP high-elevation sites. Aneja and Li (1992) have summarized the ozone concentrations for 1986 to 1988. Table 4-14 summarizes the sites characterized by Lefohn et al. (1990b). Table 4-15 summarizes the concentrations and exposures that occurred at several of the sites for the period 1987 to 1988. In 1987, the 7- and 12-h seasonal means were similar at the Whiteface Mountain WF1 and WF3 sites (Figure 4-24a). The 7-h mean values were 0.0449 and 0.0444 ppm, respectively, and the 12-h mean values were 0.0454 and 0.0444 ppm, respectively. Note that, in some cases, the 12-h mean was slightly higher than the 7-h mean value. This resulted when the 7-h mean period (0900 to 1559 hours) did not capture the period of the day when the highest hourly mean O_3 concentrations were experienced. A similar observation was made, using the 1987

Table 4-14. Description of Mountain Cloud Chemistry Program Sites[a]

Site	Elevation (m)	Latitude			Longitude		
Howland Forest (HF1), ME	65	45°	11′		68°	46′	
Mt. Moosilauke (MS1), NH	1,000	43°	59′	18″	71°	48′	28″
Whiteface Mountain (WF1), NY	1,483	44°	23′	26″	73°	51′	34″
Shenandoah NP (SH1), VA	1,015	38°	37′	12″	78°	20′	48″
Shenandoah NP (SH2), VA	716	38°	37′	30″	78°	21′	13″
Shenandoah NP (SH3), VA	524	38°	37′	45″	78°	21′	28″
Whitetop Mountain (WT1), VA	1,689	36°	38′	20″	81°	36′	21″
Mt. Mitchell (MM1), NC	2,006	35°	44′	15″	82°	17′	15″
Mt. Mitchell (MM2), NC	1,760	35°	45′		82°	15′	

[a]See Appendix A for abbreviations and acronyms.

data, for the MCCP Shenandoah National Park sites. The 7- and 12-h seasonal means were similar for the SH1 and SH2 sites (Figure 4-24b). Based on cumulative indices, the Whiteface Mountain summit (1,483-m) site (WF1) experienced a higher exposure than the WF3 (1,026-m) site (Figure 4-24c). Both the sum of the concentrations ≥ 0.07 ppm (SUM07) and the number of hourly concentrations ≥ 0.07 ppm were higher at the WF1 site than at the WF3 site. The site at the base of the mountain (WF4) experienced the lowest exposure of the three O_3 sites. Among the MCCP Shenandoah National Park sites, the SH2 site experienced marginally higher O_3 exposures, based on the index that sums all of the hourly average concentrations (i.e., referred to as "total dose" in the figure) and sigmoidal values, than the high-elevation site (SH1; Figure 4-24d). The reverse was true for the sums of the concentrations ≥ 0.07 ppm and the number of hourly concentrations ≥ 0.07 ppm.

When the Big Meadows, Dickey Ridge, and Sawmill Run, Shenandoah National Park, data for 1983 to 1987 were compared, it again was found that the 7- and 12-h seasonal means were insensitive to the different O_3 exposure patterns. A better resolution of the differences was observed when the cumulative indices were used (Figure 4-25). There was no evidence that the highest elevation, Big Meadows, site consistently had experienced higher O_3 exposures than the other sites. In 2 of the 5 years, the highest elevation site experienced lower exposures than the Dickey Ridge and Sawmill Run sites, based on the sum of all concentration or sigmoidal indices. For 4 of the 5 years, the SUM07 index yielded the same result.

Taylor et al. (1992) indicate that the forests they monitored experienced differences in O_3 exposure. The principal spatial factors underlying this variation were elevation, proximity to anthropogenic sources of oxidant precursors, regional-scale meteorological conditions, and airshed dynamics between the lower free troposphere and the surface boundary layer. Table 4-16 summarizes the exposure values for the 10 EPRI Integrated Forest Study sites located in North America.

Table 4-15. Seasonal (April to October) Percentiles, SUM06, SUM08, and W126 Values for the Mountain Cloud Chemistry Program Sites[a]

Site	Elev. (m)	Year	Min.	10	30	50	70	90	95	99	Max	No. Obs.	SUM06	SUM08	W126
Howland Forest, ME	65	1987	0.000	0.013	0.021	0.028	0.035	0.046	0.052	0.065	0.076	4,766	5.9	0.0	7.7
(HF1)		1988	0.000	0.012	0.021	0.028	0.036	0.047	0.054	0.076	0.106	4,786	10.9	2.9	11.6
Mt. Moosilauke, NH	1,000	1987	0.006	0.027	0.036	0.045	0.053	0.065	0.074	0.086	0.102	4,077	45.0	9.5	40.1
(MS1)		1988	0.010	0.026	0.033	0.043	0.055	0.076	0.087	0.113	0.127	2,835	51.9	21.2	43.4
Whiteface Mountain, NY	1,483	1987	0.011	0.029	0.037	0.046	0.053	0.067	0.074	0.087	0.104	4,704	62.0	12.2	49.5
(WF1) (36-031-0002)		1988	0.014	0.025	0.033	0.043	0.056	0.078	0.089	0.110	0.135	4,673	65.8	40.8	56.5
Whiteface Mountain, NY (WF3)	1,026	1987	0.010	0.025	0.033	0.039	0.047	0.064	0.075	0.091	0.117	4,755	45.4	14.4	40.3
Whiteface Mountain, NY (WF4)	604	1987	0.000	0.011	0.023	0.031	0.041	0.056	0.065	0.081	0.117	4,463	23.8	5.1	21.3
Mt. Mitchell, NC	2,006	1987	0.008	0.034	0.044	0.051	0.058	0.067	0.074	0.085	0.105	3,539	59.4	7.8	46.5
(MM1)		1988	0.011	0.038	0.054	0.065	0.075	0.095	0.106	0.126	0.145	2,989	145.1	69.7	116.6
		1989	0.010	0.038	0.047	0.054	0.059	0.068	0.072	0.081	0.147	2,788	54.8	3.5	40.7
		1992	0.005	0.036	0.043	0.048	0.053	0.063	0.069	0.081	0.096	3,971	37.8	4.4	36.7
Mt. Mitchell, NC	1,760	1987	0.017	0.032	0.042	0.049	0.056	0.067	0.073	0.083	0.096	3,118	47.0	5.1	37.4
(MM2)		1988	0.009	0.029	0.041	0.050	0.060	0.080	0.092	0.110	0.162	2,992	68.7	28.1	57.7
Shenandoah Park, VA	1,015	1987	0.000	0.023	0.036	0.044	0.054	0.069	0.076	0.085	0.135	3,636	54.2	8.5	42.0
(SH1)		1988	0.006	0.024	0.036	0.047	0.058	0.077	0.087	0.103	0.140	3,959	80.9	29.6	67.2
Shenandoah Park, VA	716	1987[b]	0.003	0.027	0.040	0.049	0.059	0.071	0.077	0.086	0.145	2,908	55.7	7.8	41.8
(SH2)														55.8	
Shenandoah Park, VA	524	1987	0.000	0.018	0.029	0.037	0.047	0.061	0.068	0.080	0.108	3,030	23.1	2.6	19.2
(SH3)		1988	0.006	0.020	0.031	0.040	0.051	0.067	0.076	0.097	0.135	4,278	52.3	15.6	44.2
Whitetop Mountain, VA	1,689	1987	0.011	0.038	0.051	0.059	0.066	0.078	0.085	0.096	0.111	4,326	147.7	32.4	105.7
(WT1)		1988	0.000	0.030	0.046	0.058	0.068	0.084	0.094	0.119	0.163	3,788	133.8	51.0	102.8

[a]See Appendix A for abbreviations and acronyms.
[b]Calculations based on a May to September season.

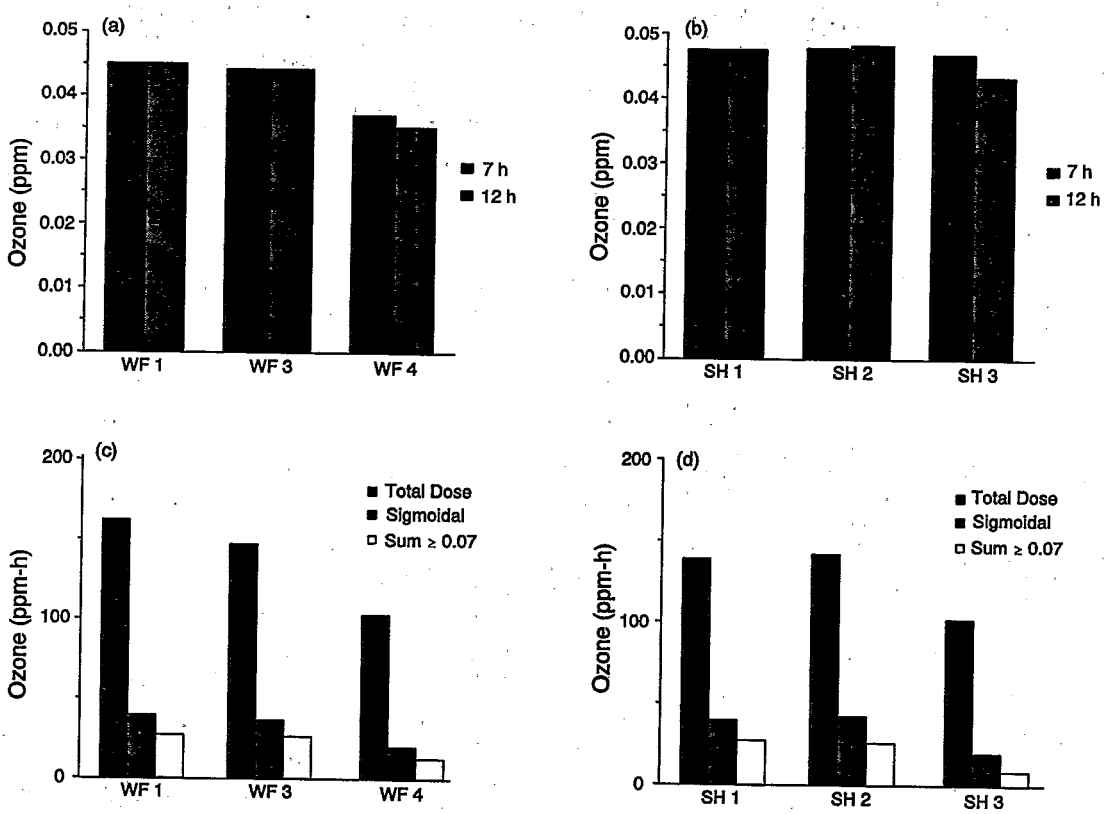


Figure 4-24. Seven- and 12-h means at (a) Whiteface Mountain and (b) Shenandoah National Park for May to September 1987 and integrated exposures at (c) Whiteface Mountain and (d) Shenandoah National Park for May to September 1987.

Source: Lefohn et al. (1990b).

4.6.3 Other Spatial Variations in Ozone Concentrations

Despite relative intraregional homogeneity, evidence exists for intracity variations in concentrations that are pertinent to potential exposures of human populations and to the assessment of actual exposures sustained in epidemiologic studies. Two illustrative pieces of data are presented in this section: (1) a case of relative homogeneity in a city with a population under 500,000 (New Haven, CT) and (2) a case of relative inhomogeneity of concentrations in a city of greater than 9 million population (New York City).

As described in the previous version of the criteria document (U.S. Environmental Protection Agency, 1986), the percentiles of the hourly average concentrations for a New Haven site and two other monitoring stations that were operating at the time in the same county, one in Derby, 9 mi west of New Haven, and one in Hamden, 6 mi north of New Haven, generally are similar. Table 4-17 shows the monitoirng data and time of the maximum hourly concentrations by quarter at these three sites.

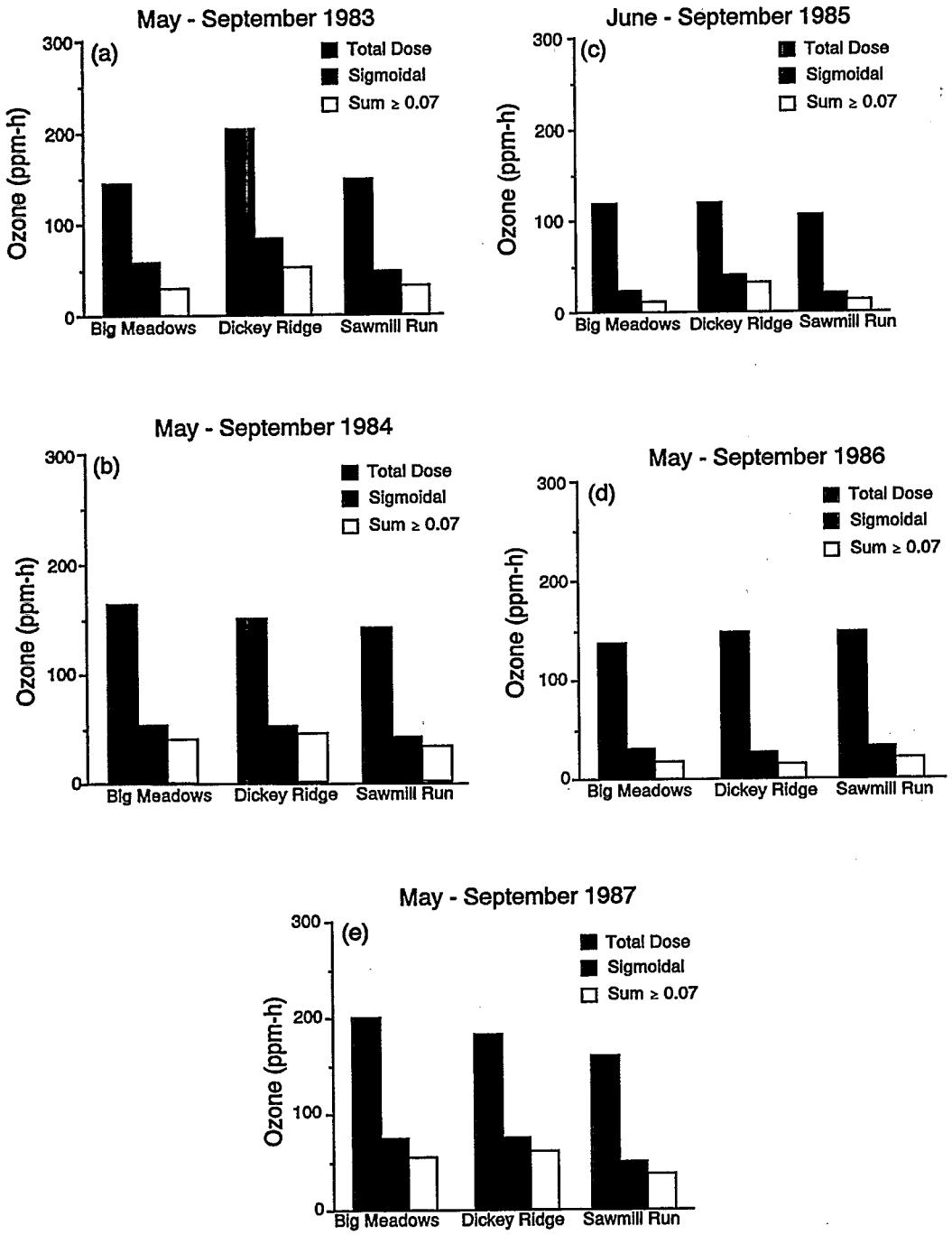


Figure 4-25. Integrated exposures for three non-Mountain Cloud Chemistry Program Shenandoah National Park sites, 1983 to 1987.

Source: Lefohn et al. (1990b).

Table 4-16. Summary Statistics for 11 Integrated Forest Study Sites[a]

Site	Year	Quarter	24-h (ppb)	12-h (ppb)	7-h (ppb)	1-h Max (ppb)	SUM06 (ppm-h)	SUM08 (ppm-h)
HIGH ELEVATION SITES								
Whiteface Mountain, NY	1987	2	42	43	42	104	13.2	2.5
	1987	3	45	44	43	114	30.1	11.8
	1988	2	49	50	49	131	33.5	13.9
	1988	3	44	43	43	119	22.6	10.4
Great Smoky Mountain NP	1987	2	54	52	49	99	57.1	10.9
	1987	3	53	51	49	95	34.3	8.8
	1988	2	71	70	68	119	126.3	61.2
	1988	3	59	57	55	120	74.7	22.2
Coweeta Hydrologic Lab, NC	1987	2	50	48	47	85	32.4	2.6
	1987	3	47	44	42	95	24.1	2.4
	1988	2	61	59	59	104	81.6	18.5
	1988	3	57	54	51	100	63.6	19.8
LOW ELEVATION SITES								
Huntington Forest, NY	1987	2	36	42	42	88	9.8	0.9
	1987	3	24	32	33	76	5.4	0.2
	1988	2	40	46	46	106	19.2	6.1
	1988	3	37	46	48	91	18.6	2.7
Howland, MA	1987	2	34	39	39	69	1.9	0.0
	1987	3	26	32	31	76	3.8	0.0
	1988	2	36	41	41	90	8.1	2.9
	1988	3	24	30	30	71	1.7	0.0
Oak Ridge, TN	1987	2	42	53	50	112	39.5	13.5
	1987	3	29	44	41	105	24.3	9.0
	1988	2	40	57	58	104	26.4	9.8
	1988	3	32	47	51	122	19.7	7.7

Table 4-16 (cont'd). Summary Statistics for 11 Integrated Forest Study Sites[a]

Site	Year	Quarter	24-h (ppb)	12-h (ppb)	7-h (ppb)	1-h Max (ppb)	SUM06 (ppm-h)	SUM08 (ppm-h)
LOW ELEVATION SITES (cont'd)								
Thompson Forest, WA	1987	2	36	43	41	103	10.7	3.6
	1987	3	30	36	34	94	10.3	2.1
	1988	2	32	39	37	103	8.1	2.3
	1988	3	32	39	36	140	13.5	6.7
B.F. Grant Forest, GA	1987	2	32	46	48	99	26.1	5.1
	1987	3	33	52	54	102	31.3	10.3
	1988	2	47	63	64	127	53.1	21.9
	1988	3	32	47	48	116	24.1	7.4
Gainesville, FL	1987	2	42	53	50	[b]	[b]	[b]
	1987	3	29	44	41	[b]	[b]	[b]
	1988	2	35	48	51	84	23.4	0.5
	1988	3	20	29	30	70	1.9	0.1
Duke Forest, NC	1987	2	38	48	52	100	29.2	7.8
	1987	3	52	59	50	124	[b]	[b]
	1988	2	54	69	75	115	[b]	[b]
	1988	3	38	51	54	141	52.9	23.4
Nordmoen, Norway	1987	2	32	40	41	75	2.4	0.0
	1987	3	14	18	20	32	0.0	0.0
	1988	2	22	28	29	53	0.0	0.0
	1988	3	11	15	16	30	0.0	0.0

[a]See Appendix A for abbreviations and acronyms.
[b]Data were insufficient to calculate statistic.

Source: Adapted from Taylor et al. (1992).

Table 4-17. Quarterly Maximum One-Hour Ozone Values at Sites in and Around New Haven, Connecticut, 1976 (Chemiluminescence Method, Hourly Values in ppm)

	Quarter of Year			
	1	2	3	4
New Haven, CT				
No. measurements	10	1,964	2,079	66
Max 1-h, ppm	0.045	0.274	0.235	0.066
Time of day	1100 hours	1400 hours	1400 hours	1000 hours
Date	March 29	June 24	August 12	October 3
Derby, CT				
No. measurements	11	2,140	2,187	1,360
Max 1-h, ppm	0.015	0.280	0.290	0.060
Time of day	2300 hours	1400 hours	1400 hours	1900 hours
Date	March 31	June 24	August 12	December 20
Hamden, CT				
No. measurements	56	2,065	1,446	286
Max 1-h, ppm	0.050	0.240	0.240	0.065
Time of day	2400 hours	1500 hours	1300 hours	1500 hours
Date	March 29	June 24	July 20	October 7

Source: U.S. Environmental Protection Agency (1986).

The source of much of the O_3 experienced in the New Haven area is the greater New York area (e.g., Wolff et al., 1975; Cleveland et al., 1976a,b). An urban plume transported over the distance from New York City to New Haven would tend to be relatively well-mixed and uniform, such that intracity variations in New Haven probably would be minimal.

As indicated in the previous version of the criteria document (U.S. Environmental Protection Agency, 1986), intracity differences in O_3 concentrations also have been reported by Kelly et al. (1986) for a 1981 study in Detroit, MI. Ozone concentrations were measured for about 3 mo at 16 sites in the metropolitan Detroit area and in nearby Ontario, Canada. Values at 15 sites were correlated with those at a site adjacent to the Detroit Science Center, about 3 km north of the central business district in Detroit. In general, the correlation decreased as distance from the Science Center site increased; and, in general, the actual concentrations increased with distance from that site toward the north-northeast. The highest O_3 concentrations were recorded at sites about 10 to 70 km north-northeast of the urban core. At greater distances or in other directions, O_3 maxima decreased.

Chicago is an example where O_3 concentrations increase as the distance from the inner city increases. Figure 4-26 shows, for a number of O_3 monitoring sites, the number of days in 1991 that the maximum hourly average concentration was greater than 0.1 ppm. The greatest number of exceedances of the daily maximum 1-h concentration of 0.1 ppm was to the north of the city.

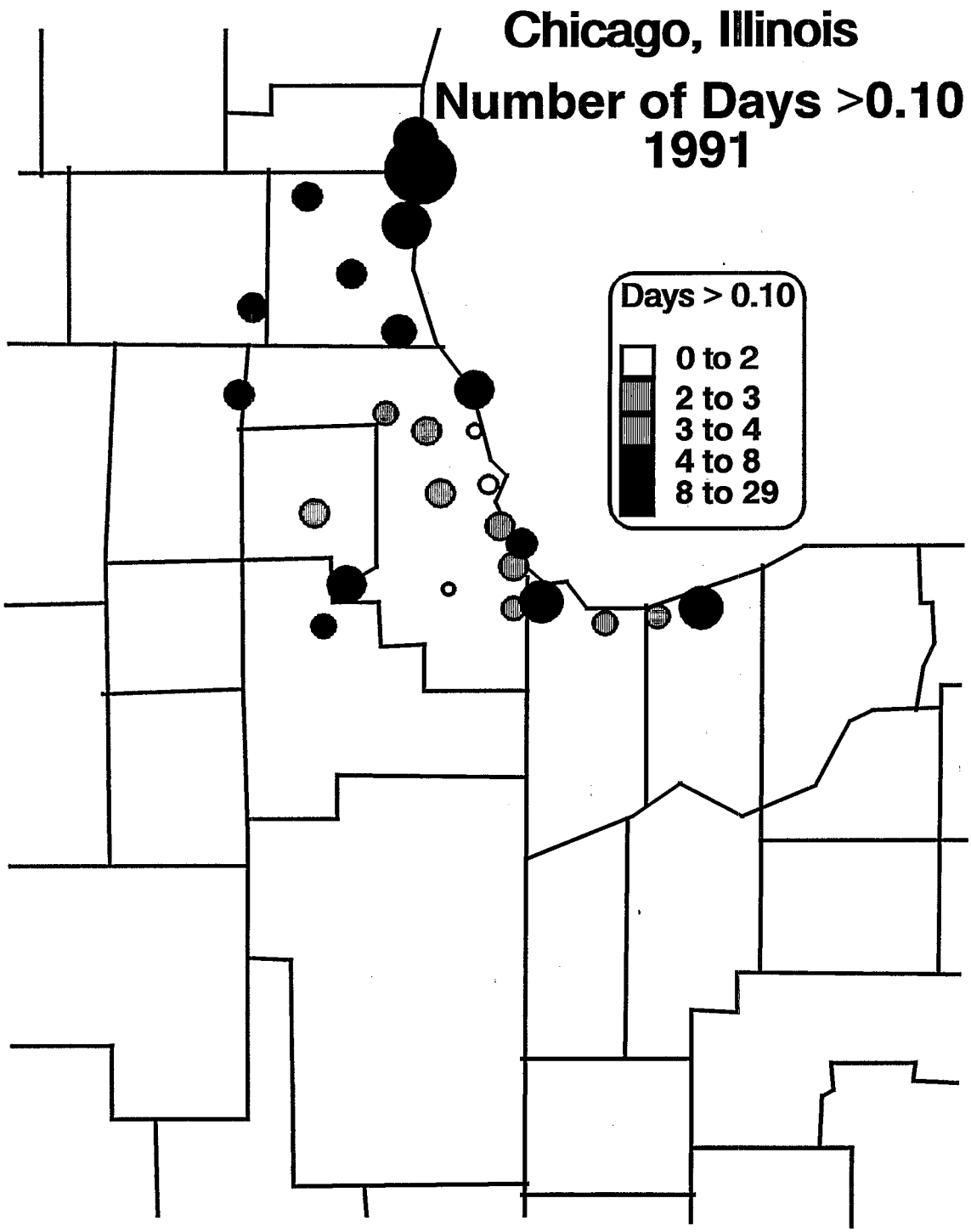


Figure 4-26. Number of days in 1991 for which the maximum hourly average ozone concentration was greater than 0.1 ppm at Chicago, IL.

Concentrations of O_3 vary with altitude and with latitude. Although a number of reports contain data on O_3 concentrations at high altitudes (e.g., Coffey et al., 1977; Reiter, 1977b; Singh et al., 1977; Evans et al., 1985; Lefohn and Jones, 1986), fewer reports are available that present data for different elevations in the same locality. There appears to be no consistent conclusion concerning the relationship between O_3 exposure and elevation.

Wolff et al. (1987) reported, for a short-term study at High Point Mountain in northwestern New Jersey, that both the daily maximum and midday O_3 concentrations were similar at different altitudes, but that the O_3 exposures increased with elevation. Wolff et al. (1987) conducted a study of the effects of altitude on O_3 concentrations at three sites located at three separate elevations on High Point Mountain in northwestern New Jersey. Data for several days indicate that in mid-July, when atmospheric mixing was good, vertical profiles were nearly constant, with concentrations increasing only slightly with elevation. Likewise, the daily O_3 maxima were similar at different elevations. At night, however, O_3 concentrations were nearly zero in the valley (i.e., the lowest-elevation site) and increased with elevation. Comparison of the O_3 exposures at the three sites (number of hours >0.08 ppm) showed that greater cumulative exposures were sustained at the higher elevations. Comparable data from an urban area (Bayonne) about 80 km southeast of High Point Mountain showed that the cumulative exposures were higher at all three of the mountain sites than in the urban area (Wolff et al., 1987). The investigators concluded from their concentration and meteorological data that elevated, mountainous sites in the eastern United States may be expected to be exposed to higher O_3 concentrations than valley sites throughout the year.

Winner et al. (1989) reported that, for three Shenandoah National Park sites (i.e., Big Meadows, Dickey Ridge, and Sawmill Run), the 24-h monthly mean O_3 concentrations tended to increase with elevation, but that the number of elevated hourly occurrences equal to or above selected thresholds did not. The authors reported that the highest elevation site (Big Meadows) experienced a smaller number of concentrations at or below the minimum detectable level than did the other two sites. The larger number of hourly average concentrations that occurred at or below the minimum detectable level at both Dickey Ridge and Sawmill Run resulted in lower 24-h averages at these sites.

Lefohn et al. (1990b), characterizing the O_3 exposures at several high-elevation sites, reported that, based on cumulative indices, the Whiteface Mountain summit site (WF1) experienced a slightly higher exposure than the lower elevation Whiteface Mountain (WF3) site. The site at the base of Whiteface Mountain (WF4) experienced the lowest exposure of the three O_3 sites. Among the MCCP Shenandoah National Park sites, the SH2 site experienced higher O_3 exposures than the high-elevation site (SH1). The "total dose" (correctly referred to as the sum of all hourly average concentrations) and sigmoidal (W126) indices were slightly higher at the SH2 than at the SH1 site. The data capture at the two sites for the 5-mo period was similar. However, the sum of the concentrations ≥ 0.07 ppm and the number of hourly concentrations ≥ 0.07 ppm were slightly higher at the SH1 than at the SH2 site. For the Whiteface Mountain sites, both the sum of the concentrations ≥ 0.07 ppm (SUM07) and the number of hourly concentrations ≥ 0.07 ppm were higher at the WF1 site than at the WF3 site.

When the Big Meadows, Dickey Ridge, and Sawmill Run, Shenandoah National Park, data for 1983 to 1987 were compared, a higher resolution of the differences among the regimes was observed when the cumulative indices were used. No specific trend could be identified that showed the highest elevation site, Big Meadows, had consistently experienced

higher O_3 exposures than the lower elevation sites. In 2 of the 5 years, the highest elevation site experienced lower exposures than the Dickey Ridge and Sawmill Run sites, based on the sum of all concentrations or sigmoidal indices. For 4 of the 5 years, the SUM07 index yielded the same result.

An important issue for assessing possible impacts of O_3 at high-elevation sites that requires further attention is the use of mixing ratios (e.g., parts per million) instead of absolute concentration (e.g., in units of micrograms per cubic meter) to describe O_3 concentration. In most cases, mixing ratios or mole fractions are used to describe O_3 concentrations. Lefohn et al. (1990b) have pointed out that the manner in which concentration is reported may be important when assessing the potential impacts of air pollution on high-elevation forests. Concentration varies as a function of altitude. Although the change in concentration is small when the elevational difference between sea level and the monitoring site is small, it becomes substantial at high-elevation sites. Given the same part-per-million value experienced at both a high- and low-elevation site, the absolute concentrations (i.e., micrograms per cubic meter) at the two elevations will be different. Because both O_3 and ambient air are gases, changes in pressure directly affect their volume. According to Boyle's law, if the temperature of a gas is held constant, the volume occupied by the gas varies inversely with the pressure (i.e., as pressure decreases, volume increases). This pressure effect must be considered when measuring absolute pollutant concentrations. At any given sampling location, normal atmospheric pressure variations have very little effect on air pollutant measurements. However, when mass/volume units of concentration are used and pollutant concentrations measured at significantly different altitudes are compared, pressure (and, hence, volume) adjustments are necessary. In practice, the summit site at Whiteface Mountain had a slightly higher O_3 exposure than the two low-elevation sites (Lefohn et al., 1991). However, at Shenandoah National Park sites, the higher elevation site experienced lower exposures than lower elevation sites in some years.

These exposure considerations are trivial at low-elevation sites. However, when one compares exposure-effects results obtained at high-elevation sites with those from low-elevation sites, the differences may become significant (Lefohn et al., 1990b). In particular, assuming that the sensitivity of the biological target is identical at both low and high elevations, some adjustment will be necessary when attempting to link experimental data obtained at low-elevation sites with air quality data monitored at the high-elevation stations.

4.7 Indoor Ozone Concentrations

Most people in the United States spend a large proportion of their time indoors. A knowledge of actual exposures of populations to indoor levels of O_3 is essential for the interpretation and use of results associated with epidemiological studies. However, essentially all routine air pollution monitoring is done on outdoor air. Until the early 1970s, very little was known about the O_3 concentrations experienced inside buildings. The ratio of the indoor/outdoor (I/O) O_3 concentrations is a parameter that has been widely used for studying the indoor and outdoor relationships, sources, and exposure patterns of O_3. However, the database on this subject is not large, and a wide range of I/O O_3 concentration relationships can be found in the literature. The only significant source of O_3 in indoor residential air is infiltration of outdoor O_3, with ventilation rates affecting the flow of air between indoor and outdoor (Zhang and Lioy, 1994).

Reported I/O values for O_3 are highly variable (U.S. Environmental Protection Agency, 1986) and range from <0.1 to 0.8 for various indoor environments and ventilation rates (Weschler et al., 1989). Unfortunately, the number of experiments and kinds of structures examined to date provide only limited data for use in modeling indoor exposures. Data were summarized by Yocom (1982) describing studies of indoor-outdoor gradients in buildings and residences for either O_3 or photochemical oxidant. The results were highly variable. A relatively large number of factors can affect the difference in O_3 concentrations between the inside of a structure and the outside air. In general, outside air infiltration or exchange rates, interior air circulation rates, and interior surface composition (e.g., rugs, draperies, furniture, walls) affect the balance between replenishment and decomposition of O_3 within buildings (U.S. Environmental Protection Agency, 1986). Although indoor concentrations of O_3 will almost invariably be less than outdoors, the fact that people spend more time indoors than outdoors may result in greater overall indoor exposures.

Cass et al. (1991) have discussed the importance of protecting works of art from damage due to O_3. Experiments show that the fading of artists' pigments in the presence of O_3 is directly related to the product of concentration times duration of exposure. Druzik et al. (1990) reported that, in a survey of 11 museums, galleries, historical houses, and libraries in Southern California, facilities with a high air exchange with the outdoors and no pollutant removal system have indoor O_3 concentrations more than two-thirds those of outdoor concentrations. The author reported that museums with conventional air-conditioning systems showed indoor O_3 concentrations about 30 to 40% of those outside, whereas museums with no forced ventilation system, where slow air infiltration provides the only means of air exchange, have indoor O_3 levels typically 10 to 20% of those outdoors. Several other studies have been reported in the literature and Table 4-18 lists the I/O ratios reported from these efforts as well as those from earlier years.

Automobiles and other vehicles constitute another indoor environment in which people may spend appreciable amounts of time. As with buildings, the mode of ventilation and cooling helps determine the inside concentrations. The U.S. Environmental Protection Agency (1986) describes studies for the I/O ratios. In one study reported by Contant et al. (1985), the I/O ratios from 49 measurements inside vehicles were 0.44 for the mean, 0.33 for the median, and 0.56 for maximum concentrations measured. Chan et al. (1991) reported an I/O ratio of 0.20 for median in-vehicle concentrations (0.011 ppm) and time-matched fixed-site measurements (0.051 ppm).

At present, there are no long-term monitoring data on indoor air pollutant concentrations comparable to the concentration data available for outdoor locations. Thus, for estimates of the exposure of building or vehicle occupants to O_3 and other photochemical oxidants, it is necessary to rely on extrapolations of very limited I/O data.

4.8 Estimating Exposure to Ozone

4.8.1 Introduction

Human exposure represents the joint occurrence of an individual being located at point (x,y,z) during time t, with the simultaneous presence of an air pollutant at concentration $C_{x,y,z}(t)$ (U.S. Environmental Protection Agency, 1991). Consequently, an individual's exposure to an air pollutant is a function of location as well as time. If a volume at a location can be defined such that air pollutant concentrations within it are homogeneous yet

Table 4-18. Summary of Reported Indoor-Outdoor Ozone Ratios

Structure	Indoor-Outdoor Ratio	Reference
Hospital	0.67[a]	Thompson (1971)
Residence (with evaporative cooler)	0.60[a]	Thompson et al. (1973)
Office		
(air-conditioned; 100% outside air intake)	0.80 ± 0.10	Sabersky et al. (1973)
(air-conditioned; 70% outside air intake)	0.65 ± 0.10	Sabersky et al. (1973)
Office	0.66	Shair and Heitner (1974)
	0.54	Shair and Heitner (1974)
Office/Lab	0.62	Hales et al. (1974)
Residence	0.70	Sabersky et al. (1973)
Residence	0.50-0.70	Moschandreas et al. (1978)
Two offices	0.30	Moschandreas et al. (1978)
Residence		Moschandreas et al. (1981)
(gas stoves)	0.19	
(all electric)	0.20	
Office	0.29	Moschandreas et al. (1978)
School room	0.19 (max concentration)	Berk et al. (1980)
Residence	0.10-0.25	Berk et al. (1981)
Residences (1 each)		Stock et al. (1983)
(air-conditioned)	0.00-0.09	
(100% outside air; no air-conditioning)	1.00	
Residences (12) (air-conditioned)	0.21 (mean concentration) 0.12 (med. concentration) 0.59 (max concentration)	Contant et al. (1985)
Residences (41)	0.30	Lebowitz et al. (1984)
Residences (6)		Zhang and Lioy (1994)
(window open)	0.59 ± 0.16	
(window closed)	0.26 ± 0.12	
(air-conditioning)	0.28 ± 0.12	
Art gallery	0.50	Shaver et al. (1983)
Art gallery (three modes of ventilation in each 24-h period: recirculation, mixture of recirculated and outside air, and 100% outside air)	0.70 ± 0.10 (mean concentration)	Davies et al. (1984)
Museums	<0.10	Shaver et al. (1983)

Table 4-18 (cont'd). Summary of Reported Indoor-Outdoor Ozone Ratios

Structure	Indoor-Outdoor Ratio	Reference
Museum	0.45	Nazaroff and Cass (1986)
Museums		Druzik et al. (1990)
(with high air exchange, but no air-conditioning)	0.69-0.84 (1 h) 0.50-0.87 (8 h)	
(with no air-conditioning and with low air exchange rate)	0.10-0.59 (1 h) 0.10-0.58 (8 h)	
(with natural convection-induced air-exchange system)	0.33-0.49 (1 h) 0.28-0.40 (8 h)	
(with conventional air-conditioning system but with no activated carbon air filtration)	0.24-0.40 (1 h) 0.25-0.41 (8 h)	
(with activated carbon air-filtration system)	0.03-0.37 (1 h) 0.03-0.31 (8 h)	

[a]Measured as total oxidants.

potentially different from other locations, the volume may be considered a "microenvironment" (Duan, 1982). Microenvironments may be aggregated by location (i.e., indoor or outdoor) or activity performed at a location (i.e., residential, commercial) to form microenvironment types. Also, activity has two major dimensions: location and exertion. Various microenvironments can have different levels of ventilation that will significantly influence the delivered dose.

Air Quality Criteria for Carbon Monoxide (U.S. Environmental Protection Agency, 1991) discusses the difference between individual and population exposures. The document notes that Sexton and Ryan (1988) define the pollutant concentrations experienced by a specific individual during normal daily activities as "personal" or "individual" exposures. A personal exposure depends on the air pollutant concentrations that are present in the location through which the person moves, as well as on the time spent at each location. Because time-activity patterns can vary substantially from person to person, individual exposures exhibit wide variability (U.S. Environmental Protection Agency, 1991). Thus, although it is a relatively straightforward procedure to measure any one person's exposure, many such measurements may be needed to quantify exposures for a defined group. The daily activities of a person in time and space define the individual's activity pattern. Accurate estimates of air pollution exposure generally require that an exposure model account for the activity patterns of the population of interest.

From a public health perspective, it is important to determine the "population exposure", which is the aggregate exposure for a specified group of people (e.g., a community or an identified occupational cohort). Because exposures are likely to vary substantially between individuals, specification of the distribution of personal exposures

within a population, including the average value and the associated variance, is often the focus of exposure assessment studies.

In many cases, the upper tail of the distribution, which represents those individuals exposed to the highest concentrations, is frequently of special interest because the determination of the number of individuals who experience elevated pollutant levels can be critical for health risk assessments. This is especially true for pollutants for which the relationship between dose and response is highly nonlinear. Runeckles and Bates (1991) have pointed out the importance of peak concentrations in eliciting adverse human effects. As indicated in Section 4.1, results using controlled human exposures have shown the possible importance of concentration in relation to duration of exposure and inhalation rate. The implication of the importance of concentration can be translated into the conclusion that the simple definition of exposure (i.e., equal to concentration multiplied by time) may be too simplified.

Several human exposure models have been developed for most cases, because it is not possible to estimate population exposure solely from fixed-station data. Some of these models include information on human activity patterns (i.e., the microenvironments people visit and the time they spend there). These models also contain submodels depicting the sources and concentrations likely to be found in each microenvironment, including indoor, outdoor, and in-transit settings.

4.8.2 Fixed-Site Monitoring Information Used To Estimate Population and Vegetation Exposure

Based on the information provided in earlier sections in this chapter, fixed-site monitors alone cannot accurately depict population exposures for most cases, because indoor and in-transit concentrations of O_3 may be significantly different from ambient O_3 concentrations, and ambient outdoor concentrations of O_3 that people come in contact with may vary significantly from O_3 concentrations measured at fixed-site monitors. Fixed-site monitors measure concentrations of pollutants in ambient air. Ambient air as noted by the U.S. Environmental Protection Agency (1991) is defined in the *U.S. Code of Federal Regulations* (1991) as air that is "external to buildings, to which the general public has access." But the nature of modern urban lifestyles in many countries, including the United States, is that people spend an average of over 20 h per day indoors (Meyer, 1983). Reviews of studies summarized in Section 4.7 show that indoor O_3 concentration measurements vary significantly from simultaneous measurements in ambient air. The difference between indoor and outdoor air quality and the amount of time people spend indoors reinforce the conclusion that using ambient air quality measurements alone does not provide accurate estimates of population exposure in most cases.

It is assumed that exposure for vegetation is the same as the concentration information provided at fixed monitors in the field (see Sections 5.5 and 5.6). In some cases, because of foliar scavenging and height differences between the vegetation canopy and the pollutant monitor, the measured concentration is not equivalent to the vegetation exposure.

A subgroup, children attending summer camp, has been studied by several investigators to evaluate the influence of ambient air pollution on respiratory health and function. Because children are predominantly outdoors and relatively active while at camp, they provide a unique opportunity to examine the relationships between respiratory health and

function and concurrent air pollution levels. Children may be at potentially increased risk from air pollution by virtue of their lifestyle patterns, which often involve several hours of outdoor exercise, regardless of air quality, during daylight hours.

For campers, attempts have been made to estimate human exposure to O_3 using types of activity patterns (Mage et al., 1985; Paul et al., 1987). Mage et al. (1985) developed an objective approach to estimate the dose delivered to the lung of a 12-year-old camper by using pulmonary minute volume associated with a specific activity, the fractional penetration beyond the trachea, and infiltration of ozone indoors. Lioy and Dyba (1989) have applied the parameters used by Mage et al. (1985) to predict the delivered O_3 dose over a 4-day episodic period. The schedule of a hypothetical camper was matched to the actual O_3 concentrations, and the predicted doses were estimated.

Several studies involving children attending summer camp have been summarized in Chapter 7. In one study, Avol et al. (1990) reported that O_3 levels at a Southern California summer camp, located 190 km southeast of Los Angeles, CA, rose gradually throughout each day, displaying a "broad peak" between 1000 and 2000 hours each day. Daily maxima typically occurred in late afternoon (1500 to 1700 hours); subsequently, concentrations gradually declined to overnight O_3 levels of 0.025 to 0.050 ppm. Spektor et al. (1991) investigated the pulmonary function of 46 healthy children on at least 7 days for each child during a 4-week period at a northwestern New Jersey residential summer camp in 1988. The daily levels of 1-h peak O_3 and the 12-h average hygroden ion (H^+) concentrations are shown in Figure 4-27. On 5 of these days, the current NAAQS of 0.12 ppm was exceeded. The maximum hourly concentration attained during the study was 0.15 ppm. The year 1984 was a milder O_3 exposure year and Figure 4-28 summarizes the maximal 1-h O_3 concentrations at Fairview Lake during a 1984 study period (Spektor et al., 1988).

4.8.3 Personal Monitors

A personal exposure profile can be identified by using a personal exposure monitor. McCurdy (1994) has described the development of personal exposure monitors by several companies. However, few data are available describing personal exposures for individuals using these monitors. An example of a pilot study using a personal exposure monitor was described for assessing O_3 exposure in 23 children by Liu et al. (1993). The accuracy of the monitor was within 20% of the actual value. The authors collected indoor, outdoor, and personal O_3 concentration data as well as time-activity data in State College, PA. Results from the pilot study demonstrated that fixed-site ambient measurements may not adequately represent individual exposures. Outdoor O_3 concentrations showed substantial spatial variation between rural and residential regions. In addition, Liu et al. (1993) reported that models based on time-weighted indoor and outdoor concentrations explained only 40% of the variability in personal exposures. When the model used included observations for only those participants who spent the majority of their day in or near their homes, an R^2 of 0.76 resulted when estimates were regressed on measured personal exposures. The authors concluded that contributions from diverse indoor and outdoor microenvironments should be considered to estimate personal O_3 exposure accurately. From these results, it is clear that additional data are needed to better quantify the O_3 exposures to which populations are exposed.

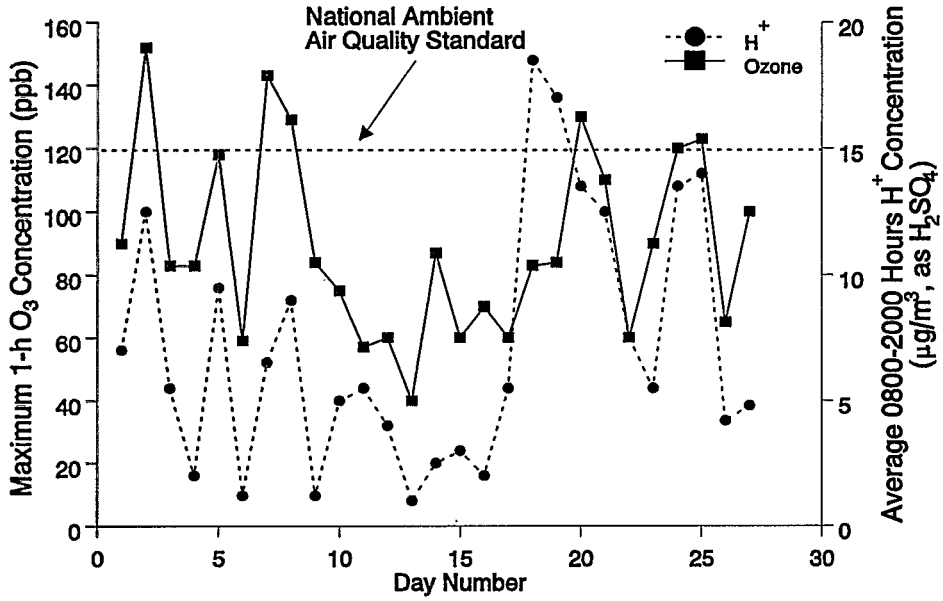


Figure 4-27. Maximum 1-h ozone (O^3) concentrations (in parts per billion) and average 0800 to 2000 hours strong acid concentrations (expressed as micrograms per cubic meter of sulfuric acid [H_2SO_4]) for each day that pulmonary function data were collected at Fairview Lake camp in 1988. The correlation coefficient between O_3 and the hydrogen ion (H^+) was 0.56.

Source: Spektor et al. (1991).

4.8.4 Population Exposure Models

McCurdy (1994) has reviewed the current status of human exposure modeling. The author describes two distinct types of O_3 exposure models: (1) those that focus narrowly on predicting indoor O_3 levels and (2) those that focus on predicting O_3 exposures on a community-wide basis. The models that predict indoor O_3 levels have been described by Sabersky et al. (1973), Shair and Heitner (1974), Nazaroff and Cass (1986), and Hayes (1989, 1991). McCurdy (1994) discusses four distinct models that predict O_3 exposure on a community-wide basis. These models and their distinguishing features are:

1. pNEM/O_3 based on the National Air Quality Standards Exposure Model (NEM) series of models (Paul et al., 1987; Johnson et al., 1990; McCurdy et al., 1991).
   - Uses mass-balance approach and seasonal considerations for I/O ratio estimation.
   - Variables affecting indoor exposure obtained by Monte Carlo sampling from empirical distributions of measured data.

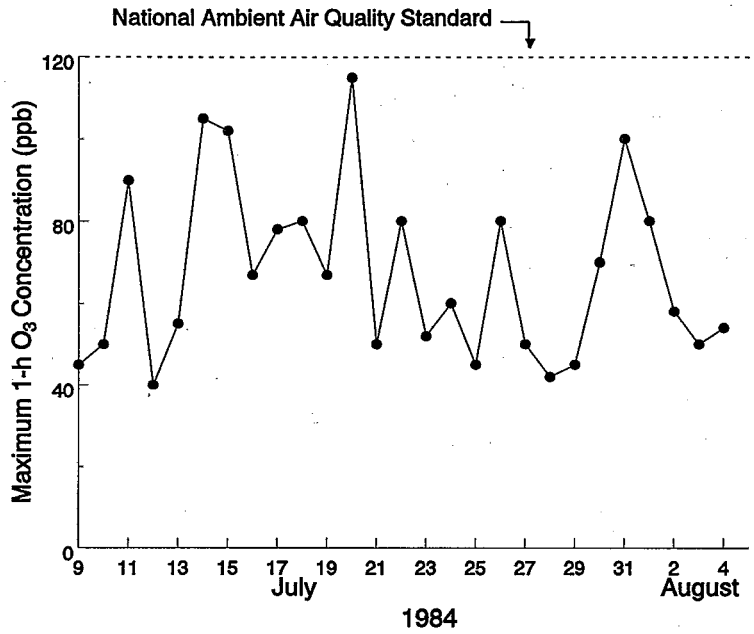


Figure 4-28. Maximal 1-h ozone concentrations at Fairview Lake during the study period.

Source: Spektor et al. (1988).

2. Systems Applications International (SAI)/NEM (Hayes et al., 1984; Hayes and Lundberg, 1985; Austin et al., 1986; Hayes et al., 1988; Hayes and Rosenbaum, 1988).
   - More districts and microenvironments and more detailed mass-balance model than pNEM/O_3.
   - Human activity data outdated and inflexible.
3. Regional Human Exposure Model (REHEX) (Lurmann and Colome, 1991; Winer et al., 1989; Lurmann et al., 1989; Lurmann et al., 1990).
   - More detailed geographic resolution than NEM.
   - Uses California-specific activity data and emphasizes in-transit and outdoor microenvironments.
4. Event probability exposure model (EPEM) (Johnson et al., 1992).
   - Estimates probability that a randomly selected person will experience a particular exposure regime.
   - Lacks multiday continuity.

McCurdy (1994) points out that all four models are related to the NEM. The NEM is an EPA exposure model developed in the 1980s (Biller et al., 1981). Outdoor air quality data are obtained from monitoring or modeling data. In most applications of NEM, fixed-site monitoring data are used. The hourly average values are transformed by a suitable relationship so that they better represent air quality outside of the various microenvironments of interest. McCurdy (1994) points out that the important point of the NEM spatial

dimension is that people can be assigned to a monitor using U.S. census data. In addition, community trips can be assigned among the districts, grid cells, or neighborhood types using census data. Thus, the NEM model simulates the movement of people through space for work-trip purposes. Interested readers are referred to McCurdy (1994) for further discussion of the pNEM model.

4.8.5 Concentration and Exposures Used in Research Experiments

It is important to adequately characterize the exposure patterns that result in vegetation and human health effects. Hourly average concentrations used in many of the high treatment experimental studies did not necessarily mimic those concentrations observed under ambient conditions. Although the ramifications of this observation on the effects observed are not clear, it was pointed out that the highest treatments used in many of the open-top chamber experiments were bimodal in the distribution of the hourly average concentrations. In other experiments designed to assess the effects of O_3 on vegetation, constant concentration (i.e., square wave) exposures were implemented. As has been discussed in earlier sections of this chapter, hourly average concentrations change by the hour and square wave exposure regimes do not normally occur under ambient conditions. In addition to the exposures used at the highest treatment levels, there is concern that the hourly average concentrations used in the control treatments may be lower than those experienced at isolated sites in the United States or in other parts of the world. Although the ramifications of using such exposure regimes are unclear, there is some concern that the use of atypically low control levels may result in an overestimation of vegetation yield losses when used as the baseline for evaluating the effects of treatments at higher concentrations (Lefohn and Foley, 1992).

For assessing the human health effects of O_3 exposure, a series of studies has explored prolonged 6.6-h O_3 exposures at low levels (i.e., 0.08 to 0.12 ppm) (Horstman et al., 1990). McDonnell et al. (1991), using similar hourly average concentration regimes, have confirmed the findings reported by Horstman et al. (1990). All the research investigations using 6.6-h durations have applied constant concentrations during the exposure period. If, as indicated in the introduction of this chapter, concentration is more important than duration and ventilation rate, different human health effects may occur as a result of different exposure regimes that have identical 6.6-h average concentrations. Because of this, it is important to explore the different types of exposure regimes that occur under ambient conditions during an 8-h episode.

Lefohn and Foley (1993) reported on an analysis of hourly average data for O_3 monitoring sites that never experienced an exceedance of an hourly average concentration $\geq$0.12 ppm and that experienced 8-h daily maximum average concentrations $>$0.08 ppm. For those monitoring sites that met the above two criteria, they identified the number of times the 8-h daily maximum average concentration exceeded 0.08 ppm during the monitoring year. For the period 1987 to 1989, there were 925 exposure regimes identified from 166 site-years of data that met the above criteria. The data were then organized into the following seven categories:

I. The occurrence of 8-h daily maximum averages $>$0.08 ppm and $<$0.09 ppm;

II. The occurrence of 8-h daily maximum averages >0.08 ppm but ≤0.082 ppm, which contained only hourly average concentrations >0.08 ppm but ≥0.082 ppm;
III. 8-h daily maximum averages >0.08 ppm, which contained hourly average concentrations <0.09 ppm;
IV. 8-h daily maximum averages >0.08 ppm and <0.09 ppm, which contained at least one hourly average concentration ≥0.09 ppm but <0.10 ppm;
V. 8-h daily maximum averages >0.08 ppm and <0.09 ppm, which contained at least one hourly average concentration ≥0.10 ppm;
VI. 8-h daily maximum averages <0.08 ppm, which contained at least one hourly average concentration ≥0.09 ppm but <0.10 ppm; and
VII. 8-h daily maximum averages <0.08 ppm, which contained at least one hourly average concentration ≥0.10 ppm.

Figure 4-29 summarizes the results of the analysis. The results indicated that there was a poor relationship between the value of the 8-h daily maximum average concentration and the frequency of occurrence of hourly average concentrations within specific ranges (e.g., between 0.09 and 0.10 ppm). In no case could the authors identify a monitoring site that experienced the square-wave type of exposure that was described in Category II (i.e., the occurrence of 8-h daily maximum averages >0.08 ppm but ≤0.082 ppm). Lefohn and Foley (1993) concluded that the square wave exposures used in the 6.6-h human health effects experiments were not found under ambient conditions. The authors identified 453 additional exposure regimes, where the 8-h daily maximum average was <0.08 ppm but experienced maximum hourly average concentrations ≥0.09 ppm. Thus, if hourly average concentrations ≥0.08 ppm are of concern for affecting human health, there will be instances where occurrences above this threshold are evident, but the 8-h average value is below 0.08 ppm.

4.9 Concentrations of Peroxyacetyl Nitrates in Ambient Atmospheres

4.9.1 Introduction

The biological effects of PAN in human exposures, toxicological studies of animals, and plant response and yield have been considered previously (U.S. Environmental Protection Agency, 1986). Controlled human exposure studies involving O_3 and O_3 + PAN are discussed elsewhere in this document (Chapter 7, Section 7.2.6.3). Some effects on respiratory parameters have been reported in one study, but not in others. However, the PAN concentrations used in these studies have been well above the maximum ambient concentrations usually experienced many years ago within the Los Angeles Basin (U.S. Environmental Protection Agency, 1986) and, more importantly, above the maximum ambient concentrations in the more recent measurements considered in this section.

The PANs are of importance as reservoirs for NO_2 as NO_x is depleted relative to VOCs in plumes moving downwind into less polluted areas (Chapter 3, Section 3.2.4). In performance evaluation of ozone air quality models, measured concentrations of PANs are useful in model evaluation (Chapter 3, Section 3.6.4.2).

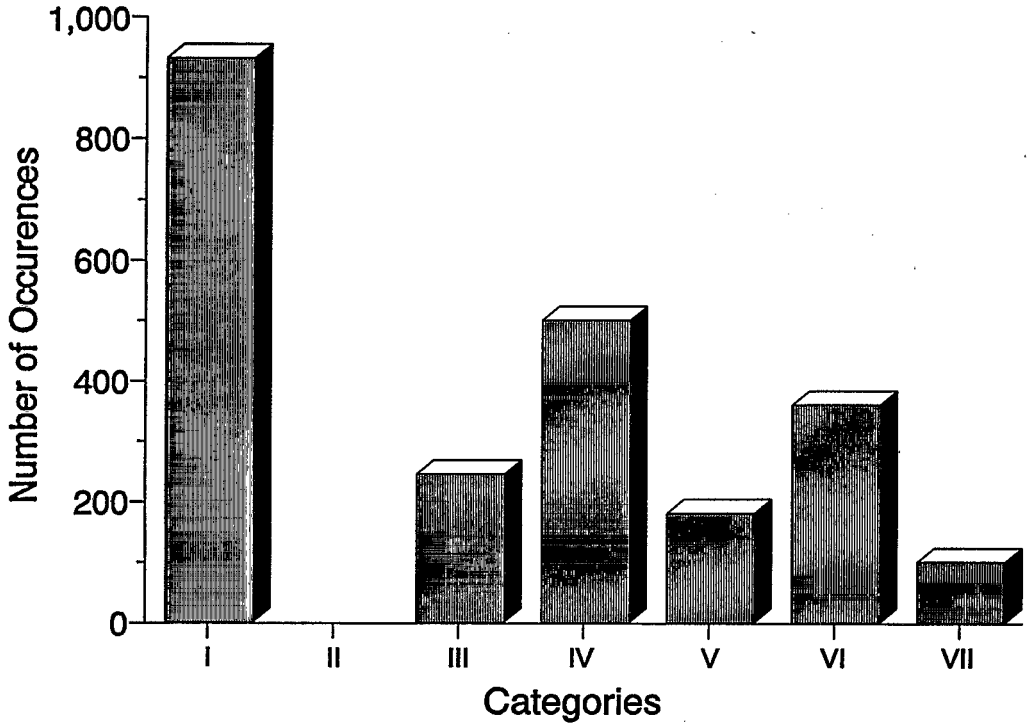


Figure 4-29. The number of occurrences for each of the seven categories described in text.

In the previous air quality criteria for O_3 and other photochemical oxidants (U.S. Environmental Protection Agency, 1986), extensive tabulations of PAN and peroxypropionyl nitrate (PPN, CH3CH2C(O)OONO2) concentrations were given based on measurements made between 1965 and 1981 from references up to 1983. In the present work, references from 1983 to the present are used for measurements of PANs in urban and rural locations. The urban area measurements are from the United States, Canada, France, Greece, and Brazil. The use of measurements from aboard serve to illustrate or support certain U.S. results as well as to demonstrate the widespread presence of PANs in the atmosphere. These PAN measurements usually were of limited duration, and the results should not be assumed to be comparable to those obtained at the O_3 monitoring sites discussed earlier in this chapter (see also Section 4.10).

4.9.2 Urban Area Peroxyacetyl Nitrate Concentrations

For urban sites, the prior criteria document for ozone and other photochemical oxidants contains a number of tables tabulating measurements of PAN, PPN, and PPN to PAN and PAN to O_3 ratios (Altshuller, 1983; U.S. Environmental Protection Agency, 1986). Based on comparisons of PAN measurements in Los Angeles in 1980 with those made in the 1960s, it was uncertain whether PAN concentrations had decreased. In the Los Angeles area, the average and maximum PAN concentrations reported ranged from 1.6 to 31 ppb and from 6 to 214 ppb, respectively. The wide variations, at least in part, were associated with

the range of years, different seasons, and differing average times among studies. On average, the PPN to PAN ratios among studies in Los Angeles ranged from 0.15 to 0.20, whereas the PAN to O_3 ratios among studies ranged from 0.04 to 0.20. In the earlier PAN measurement results, studies conducted in the South Coast Air Basin predominated.

The average PAN concentrations measured in other cities usually were lower than in the Los Angeles area, whereas the maximum PAN concentrations overlapped with the lower end of range in Los Angeles. The PPN and PAN ratios in other cities ranged from 0.1 to 0.4, whereas the PAN to O_3 ratios were in the 0.01 to 0.05 range.

Seasonally, PAN to O_3 ratios tended to be somewhat higher in the winter. The diurnal characteristics of O_3 and of PAN were similar, but not identical.

The urban area measurement results are tabulated in Table 4-19. The earlier maximum PAN concentrations reported usually were substantially higher than those given in Table 4-19. A possible exception occurs for the Claremont, CA, results. Measurements of PAN and PPN were made in 1989 and 1990 at sites downwind of Los Angeles: Perrin, 90 km to the east-southeast, and Palm Springs, 120 km to the east (Grosjean and Williams, 1992). The concentrations of PAN and PPN were high, and the concentration maxima occurred during the evening hours, which was consistent with downwind transport from the Los Angeles area rather than from local sources.

In Southern California, the maximum PAN concentrations appear to be more evenly distributed spatially during the fall than during the summer (Williams and Grosjean, 1990). At coastal and central locations, the PAN maxima during the fall were comparable to those observed at inland locations during the summer.

As observed previously, PAN concentrations in other U.S. cities and in cities in other countries tend to be substantially lower than in Los Angeles and its surrounding urban areas (Table 4-19). An exception occurs for the measurements from Paris (Tsalkani et al., 1991). Maximum PAN concentrations in the 20 to 35 ppb range were observed.

In measurements made in 1992 in Atlanta, GA, at the Georgia Institute of Technology campus site, not only were PAN and PPN measured, but very occasionally peroxymethacryloyl nitrate (MPAN, $CH_2{=}C(CH_3)\ C(O)OONO_2$) (a product of the atmospheric photooxidation of local biogenic sources of isoprene) was observed (Williams et al., 1993). Maximum diurnal concentrations of peroxyacyl nitrates and O_3 occur in late afternoon and early evening. The average MPAN concentration was 0.3 ppb, and the maximum value was 0.5 ppb and constituted about 15% of the concurrent PAN concentrations.

In a study in Rio de Janeiro performed to investigate the effects of the use of ethanol or ethanol-containing fuel on PAN concentrations, the maximum PAN concentration reached 5.4 ppb (Tanner et al., 1988). However, this maximum concentration is well below the maximum concentrations reported in and around Los Angeles, and it falls within the maximum PAN values reported for a number of other cities (Table 4-19).

4.9.3 Concentration of Peroxyacetyl Nitrate and Peroxypropionyl Nitrate in Rural Areas

Prior measurements of nonurban PAN and PPN concentrations and PAN to O_3 ratios are available (Altshuller, 1983; U.S. Environmental Protection Agency, 1986). At nonurban sites that are not impacted by urban plumes, PAN and PPN concentrations are

Table 4-19. Summary of Measurements of Peroxyacetyl Nitrate and Peroxypropionyl Nitrate in Urban Areas[a]

Site	Month/ Year	Number of Days Sampled	PAN Concentration (ppb) Average/Mean	Max	PPN Concentration (ppb) Average/Mean	Max	Reference
Long Beach, CA	6-12/1987	16	NA	16	NA	NA	Williams and Grosjean (1990)
Anaheim, CA	6-12/1987	14	NA	19	NA	NA	Williams and Grosjean (1990)
Los Angeles, CA	6-12/1987	16	NA	13	NA	NA	Williams and Grosjean (1990)
Burbank, CA	6-12/1987	16	NA	19	NA	NA	Williams and Grosjean (1990)
Azusa, CA	6-9/1987	11	NA	13	NA	NA	Williams and Grosjean (1990)
Claremont, CA	6-9/1987	10	NA	30	NA	NA	Williams and Grosjean (1990)
Perrin, CA	6/1989 to 6/1990	NA	1.6	9.1	NA	0.73	Grosjean and Williams (1992)
Palm Springs, CA	6/1989 to 6/1990	NA	1.6	7.6	NA	0.42	Grosjean and Williams (1992)
Downey, CA	2/1984	10	1.2	6.7	0.06	0.40	Singh and Salas (1989)
Boulder, CO	5, 6 and	12	0.63	2.0	0.08	0.3	Ridley et al. (1990)
	8, 9/1987	45	0.59	3.8	0.07	0.6	
Denver, CO	3/1984	9	0.64	2.0	0.02	0.09	Singh and Salas (1989)
Houston, TX	3/1984	9	0.75	7.9	0.045	0.54	Singh and Salas (1989)
Philadelphia, PA	4/1983	19	1.1	3.7	0.14	0.50	Singh and Salas (1989)
Staten Island, NY	4/1983	7	1.6	5.5	0.21	0.90	Singh and Salas (1989)
Atlanta, GA	7, 8/1992	36	0.71	2.9	0.14	0.37	Williams et al. (1993)
Edmonton, Alberta, Canada	12/1983 to 4/1984	66		7.5	NA	NA	Peake et al. (1988)
Calgary, Alberta, Canada	7/1981 to 2/1982	213	0.14	6.6	NA	NA	Peake and Sandhu (1983)
University of Calgary, Alberta, Canada	10/1980 to 8/1981	175	0.22	2.4	NA	NA	Peake and Sandhu (1983)
Simcoe, Ontario, Canada	6/1980 to 3/1981	191	1.3	5.6	NA	NA	Corkum et al. (1986)

Table 4-19 (cont'd). Summary of Measurements of Peroxyacetyl Nitrate and Peroxypropionyl Nitrate in Urban Areas[a]

Site	Month/ Year	Number of Days Sampled	PAN Concentration (ppb) Average/Mean	Max	PPN Concentration (ppb) Average/Mean	Max	Reference
Rio de Janeiro							
Vila Isabel	7/1985	8	NA	5.4	NA	1.0	Tanner et al. (1988)
PUC/RJ	7/1985	4	NA	3.3	NA	0.6	Tanner et al. (1988)
Athens, Greece	2-11/1985	113	NA	3.7	NA	NA	Tsani-Bazaca et al. (1988)
Paris, France	11/1985 to 11/1986	NA	1.1	20.5	NA	NA	Tsalkani et al. (1991)

[a]See Appendix A for abbreviations and acronyms.

much lower than those in urban areas. Average PAN concentrations ranged between 0.1 and 1.0 ppb, whereas the PAN to O_3 ratios were at or below 1.

Concentrations of PAN, PPN, and other peroxyacyl nitrates have been reported (Table 4-20) at Tanbark Flat, CA, 35 km northeast of Los Angeles, during 1989, 1990, and 1991 and at Franklin Canyon, CA, 25 km west of Los Angeles, during 1991 (Grosjean and Williams, 1992; Grosjean et al., 1993). As indicated by the results tabulated in Table 4-20, the concentrations were high at these mountain sites, the PPN to PAN ratios were relatively high, and the concentration maxima occurred during the afternoon hours. These concentration levels of PAN and PPN are attributed to downwind transport from the Los Angeles urban area. The MPAN was occasionally detected with average concentrations of 1.2 ppb at Tanbark Flat and 1.0 ppb at Franklin Canyon in 1991.

At Tanbark Flat, the O_3 and PAN diurnal concentration patterns were similar to those in upwind urban areas. The PAN to O_3 ratios at the O_3 maximum were as follows: 1989, 0.05; 1990, 0.08; 1991, 0.05; all the ratios are within the same range as at sites in urban areas in and around Los Angeles.

Additional measurements of PAN and PPN or other peroxyacyl nitrates are available over a period of years at Niwot Ridge, CO, just west of the Denver-Boulder area; at Point Arena, CA; and at a forest site, Scotia, PA (Ridley et al., 1990). The concentrations reported at all of these sites are much lower than the mountain sites in California. The Niwot Ridge site concentrations, which show the effects of easterly upslope flow of air parcels from Denver-Boulder, are still low compared to the sites downwind of the urban Los Angeles area (Table 4-20).

The PAN concentrations at the Scotia rural site in the eastern United States tend to be somewhat higher than the Niwot Ridge or Point Arena sites (Table 4-20). This difference may relate to higher regional precursor concentration levels.

4.10 Concentration and Patterns of Hydrogen Peroxide in the Ambient Atmosphere

Efforts to measure H_2O_2 began in the 1970s, but the early reports of H_2O_2 concentrations above 10 ppb and even 100 ppb appear to be in error because of the artifact H_2O_2 generated within the presence of O_3 (Chapter 3, Section 3.5.1.3). Subsequent measurements of H_2O_2 in the 1980s resulted in maximum H_2O_2 concentrations at or below 5 ppb and mean concentrations at or below 1 ppb (Sakugawa et al., 1990).

Studies comparing more recent methods for measuring H_2O_2, which were conducted in North Carolina, indicated differences among measurement methods in synthetic mixtures of H_2O_2, including possible interferences, and in the ambient atmosphere of up to about $\pm 25\%$ (Kleindienst et al., 1988). However, results from the same study from mixtures irradiated in a smog chamber produced larger differences among methods, especially for the luminol technique compared to the fluorescence technique and with tunable-diode laser absorption spectroscopy. Another comparison study conducted in California resulted in differences between methods for measuring H_2O_2 that varyied by a factor or two (Lawson et al., 1988). In the measurements of H_2O_2 discussed below, the cryogenic fluorescence method or the scrubber-coil fluorescence method generally was used.

Table 4-20. Summary of Measurements of Peroxyacetyl Nitrate and Peroxypropionyl Nitrate in Rural Areas[a]

Site	Month/ Year	Number of Days Sampled	PAN Concentration (ppb) Average/Mean	Max	PPN Concentration (ppb) Average/Mean	Max	Reference
Tanbark Flat, CA	8-10/1989	69	2.9	>16.1	0.75	5.1	Williams and Grosjean (1991)
	8,9/1990	34	4.8	22.0	0.76	4.3	Grosjean et al. (1993)
	8/1991	22	2.8	12.8	0.43	2.66	Grosjean et al. (1993)
Franklin Canyon, CA	9/1991	9	1.6	7.0	0.18	1.15	Grosjean et al. (1993)
Niwot Ridge, CO	7/1984	16	0.28	2.3	0.016	0.17	Singh and Salas (1989)
	6, 7/1984	23	≈0.25	NA	NA	NA	Fahey et al. (1986)
	8, 9/1984	21	≈0.25	NA	NA	NA	Fahey et al. (1986)
	6, 7/1987	46	0.81 ([b]) 0.21 ([c])	3.2	0.08 ([b]) 0.01 ([c])	0.45	Ridley et al. (1990)
Point Arena, CA	1/1984	14	0.12	1.1	0.005	0.07	Singh and Salas (1989)
	Spring 1985	NA	0.05	NA	NA	NA	Ridley (1991)
Scotia, PA	Summer 1986	NA	≈0.6	NA	NA	NA	Ridley (1991)
	6-8/1988	47	1.0	NA	NA	NA	Buhr et al. (1990)
Kananaskis Valley, Alberta, Canada	9/1979, 4/1982, 6-8/1982	NA	≈0.5	2.3	NA	NA	Peake et al. (1983)
Frijoles Mesa, NM	10/1987 to 1/1989	NA	0.26	1.9	NA	NA	Gaffney et al. (1993)

[a]See Appendix A for abbreviations and acronyms.
[b]Flow from Boulder-Denver area.
[c]Flow across the Rockies.

Based on interpretation of a compilation of H_2O_2 measurements made between 1984 and 1988 at a number of urban locations, at rural/remote locations, and on aircraft flights, it was concluded that the higher H_2O_2 concentrations were associated with the following measurement conditions: in afternoon hours, during summer months, at rural locations, and at lower latitudes (Sakugawa et al., 1990; Van Valin et al., 1987). The H_2O_2 concentrations increase from the surface to the top of the boundary layer (Daum et al., 1990). Available values for mean H_2O_2 concentrations at three U.S. locations were (1) at the summit of Whitetop Mountain, VA: summer, 0.80 ppb; winter, 0.15 ppb (Olszyna et al., 1988); (2) at the summit of Whiteface Mountain: 1986, 0.6 ppb; 1987; 0.8 ppb (Mohnen and Kadlecek, 1989); and (3) at Westwood, CA: summer, $\approx$1.0 ppb; winter, 0.2 ppb (Sakugawa and Kaplan, 1989). At Westwood, the highest correlation with various parameters was found for solar radiation consistent with the higher H_2O_2 concentrations being observed in the afternoon during the late spring and early summer months (Sakugawa and Kaplan, 1989). In the same study, the average H_2O_2 concentrations were observed to increase from Westwood, near the coast in the Los Angeles Basin, to Duarte, inland; at Daggett in the Mohave Desert; and at Sky Mountain and Lake Gregory in the San Bernadino Mountains. The ratios of O_3 to H_2O_2 concentrations at the these sites were $\geq$100.
In subsequent measurements, the same relationship in H_2O_2 concentrations between Westwood and the other California sites listed above was observed (Sakugawa and Kaplan, 1993). Unlike the results at several urban sites and other mountain sites, it was reported that the highest diurnal H_2O_2 concentrations at Lake Gregory in the San Bernardino Mountains were observed during the nighttime hours (Sakugawa and Kaplan, 1993).

4.11 Co-occurrence of Ozone

4.11.1 Introduction

There have been several attempts to characterize air pollutant mixtures (Lefohn and Tingey, 1984; Lefohn et al., 1987b). Pollutant combinations can occur at or above a threshold concentration either together or temporally separated from one another. For example, for characterizing the different types of co-occurrence patterns, Lefohn et al. (1987b) grouped air quality data within a 24-h period starting at 0000 hours and ending at 2359 hours. Patterns that showed air pollutant pairs appearing at the same hour of the day at concentrations equal to or greater than a minimum hourly mean value were defined as "simultaneous-only" daily co-occurrences. When pollutant pairs occurred at or above a minimum concentration during the 24-h period, without occurring during the same hour, a "sequential-only" co-occurrence was defined. During a 24-h period, if the pollutant pair occurred at or above the minimum level at the same hour of the day and at different hours during the period, the co-occurrence pattern was defined as "complex-sequential".
A co-occurrence was not indicated if one pollutant exceeded the minimum concentration just before midnight and the other pollutant exceeded the minimum concentration just after midnight. As will be discussed below, studies of the joint occurrence of gaseous NO_2/O_3 and SO_2/O_3 reached two conclusions: (1) the co-occurrence of two-pollutant mixtures lasted only a few hours per episode, where an episode was defined by the threshold concentration used, and (2) the time between episodes is generally long (i.e., weeks, sometimes months) (Lefohn and Tingey, 1984; Lefohn et al., 1987b).

For exploring the co-occurrence of O_3 and other pollutants (e.g., acid precipitation, acidic cloudwater, and acidic sulfate aerosols), there are limited data available. In most cases, routine monitoring data are not available from which to draw general conclusions. However, published results are reviewed and summarized for the purpose of assessing an estimate of the possible importance of co-occurrence patterns of exposure.

4.11.2 Nitrogen Oxides

Ozone occurs frequently at concentrations ≥ 0.03 ppm at many rural and remote monitoring sites in the United States (Evans et al., 1983; Lefohn, 1984; Lefohn and Jones, 1986). Therefore, for many rural locations in the United States, the co-occurrence patterns observed by Lefohn and Tingey (1984) for O_3 and NO_2 were defined by the presence or absence of NO_2. As anticipated, Lefohn and Tingey (1984) reported that most of the sites analyzed experienced fewer than 10 co-occurrences (when both pollutants were present at an hourly average concentration ≥ 0.05 ppm). However, the authors did note that several urban monitoring sites in the South Coast Air Basin experienced more than 450 co-occurrences. The rural sites of Riverside, Fontana, and Rubidoux, CA, had more than 100 co-occurrences. Denver and San Jose, CA, also experienced more than 100 co-occurrences of O_3/NO_2. Lefohn and Tingey (1984) reported that for Rubidoux, because NO_2 concentration maxima tended to peak in the evenings or early morning, the co-occurrences were present at these times. For more moderate areas of the country, Lefohn et al. (1987b) reported that even with a threshold of 0.03 ppm O_3, the number of co-occurrences with NO_2 was small.

4.11.3 Sulfur Dioxide

Because elevated SO_2 concentrations are mostly associated with industrial activities (U.S. Environmental Protection Agency, 1992a), co-occurrence observations are usually associated with monitors located near these types of sources. Lefohn and Tingey (1984) reported that, for the rural and nonrural monitoring sites investigated, most sites experienced fewer than 10 co-occurrences of SO_2 and O_3. Only Rockport, IN, and Paradise No. 21 (KY) had more than 40 co-occurrences during the monitoring period (48 and 45, respectively). The monitors at these two sites were influenced by the local sources. The authors noted that at Fontana there were numerous O_3 episodes above 0.05 ppm, and there was a high probability that when the SO_2 hourly average concentrations rose above 0.05 ppm, both pollutants would be present at levels equal to or greater than 0.05 ppm.

Meagher et al. (1987) reported that several documented O_3 episodes at specific rural locations appeared to be associated with elevated SO_2 levels. The investigators defined the co-occurrence of O_3 and SO_2 to be when hourly mean concentrations were equal to or greater than 0.10 and 0.01 ppm, respectively. On reviewing the hourly mean O_3 and SO_2 data used by Lefohn et al. (1987b) in 1980 (using a threshold of 0.05 ppm for both pollutants), the Paradise No. 23 (KY); Giles County, TN; Murphy Hill (reported as Marshall County by Meagher et al., 1987), AL; and Saltillo (reported as Hardin Co. by Meagher et al., 1987), TN, sites experienced fewer than 7 days over a 153-day period for a co-occurrence of any form (i.e., simultaneous only, sequential, and complex co-occurrence). Thus, as reported by Lefohn et al. (1987b), the co-occurrence pattern of O_3 and SO_2 was infrequent.

The above discussion was based on the co-occurrence patterns associated with the presence or absence of hourly average concentrations of pollutant pairs. Taylor et al. (1992) have discussed the joint occurrence of O_3, nitrogen, and sulfur in forested areas using cumulative exposures of O_3 with data on dry deposition of sulfur and nitrogen. The authors concluded in their study that the forest landscapes with the highest loadings of sulfur and nitrogen via dry deposition tended to be the same forests with the highest average O_3 concentrations and largest cumulative exposure. Although the authors concluded that the joint occurrences of multiple pollutants in forest landscapes were important, nothing was mentioned about the hourly co-occurrences of O_3 and SO_2 or O_3 and NO_2.

4.11.4 Acidic Sulfate Aerosols

Acid sulfates, which are usually composed of sulfuric acid (H_2SO_4), ammonium bisulfate, and ammonium sulfate, have been measured at a number of locations in North America. Acidic sulfate and neutralized species can accumulate and range in concentration from 0 to 50 $\mu g/m^3$ at a specific location or a number of locations simultaneously (Lioy, 1989). For many summertime studies, peaks of H_2SO_4 or H^+ appear to be associated with the presence of a slow-moving high pressure system (Lioy and Waldman, 1989). Acid sulfates are found primarily in the fine particle size range (<2.5 μm in diameter). Lioy (1989) reports that the acidic sulfate concentrations measured in the summertime can be found at 20 $\mu g/m^3$ for over an hour and can be found at high concentrations of 10 to 20 $\mu g/m^3$ for 6 to 24 h at one or more sites (Lioy, 1989). Acidic sulfate aerosol concentrations can occur at concentrations in the summertime above 10 $\mu g/m^3$ for periods longer than 5 h (Lioy, 1989). As has been discussed earlier in this chapter, the highest O_3 exposures for sites affected by anthropogenically derived photooxidant precursors are expected to occur during the late spring and summer months. Thus, the potential for O_3 and acidic sulfate aerosols to co-occur at some locations in some form (i.e., simultaneously, sequentially, or complex-sequentially) is real. Our knowledge of the potential exposure of the co-occurrence of acidic sulfate aerosols and O_3 is limited because routine monitoring data for acidic aerosols are not available. Information on the co-occurrence patterns is limited to research studies and some of the results of these studies are provided in this section.

Spektor et al. (1991) investigated the effects of single- and multiday O_3 exposures on respiratory function in active normal children aged 8 to 14 years at a northwestern New Jersey residential summer camp in 1988. During the investigation, the authors measured daily levels of 1-h peak O_3 and the 12-h average H^+ concentrations. On 7 days, the acid aerosol concentrations (reported as H_2SO_4) were higher than 10 $\mu g/m^3$, reaching a 12-h maximum of 18.6 $\mu g/m^3$. Figure 4-27 shows the relationship between daily maximum O_3 and daily 12-h average H^+ concentrations. Thurston et al. (1992) reported occurrences in 1988 of maximum 24-h average concentrations of H^+ as high as 18.7 $\mu g/m^3$ (Buffalo, NY) and a maximum daily hourly average concentration of 0.164 ppm. Although lower than Buffalo, high O_3 or H^+ values were reported by the investigators for Albany and White Plains, NY. It is unclear whether the O_3 or H^+ maximum concentrations occurred simultaneously; however, it is clear that high concentrations could occur either sequentially, complex-sequentially, or simultaneously. Evidence exists in the literature indicating that hourly co-occurrences are experienced. Raizenne and Spengler (1989) described an episodic co-occurrence pattern in 1986 of high hourly averaged concentrations of O_3 and H_2SO_4 that occurred at a residential summer camp located on the north shore of Lake Erie, Ontario,

(Figure 4-30). Thurston et al. (1994) conducted a study of ambient acidic aerosols in the Toronto, Ontario, metropolitan area in July and August of 1986, 1987, and 1988, and reported on the fine particle (aerodynamic equivalent diameter <2.5 μm) samples collected twice per day. The authors reported that their results indicated that acidic aerosol episodes (i.e., $H^+ \geq 100$ nmol/m^3) occurred routinely during the summer months and that H^+ peaks were correlated with sulfate (SO_4) episodes. Figure 4-31 illustrates the relationship among SO_4, H^+, and O_3.

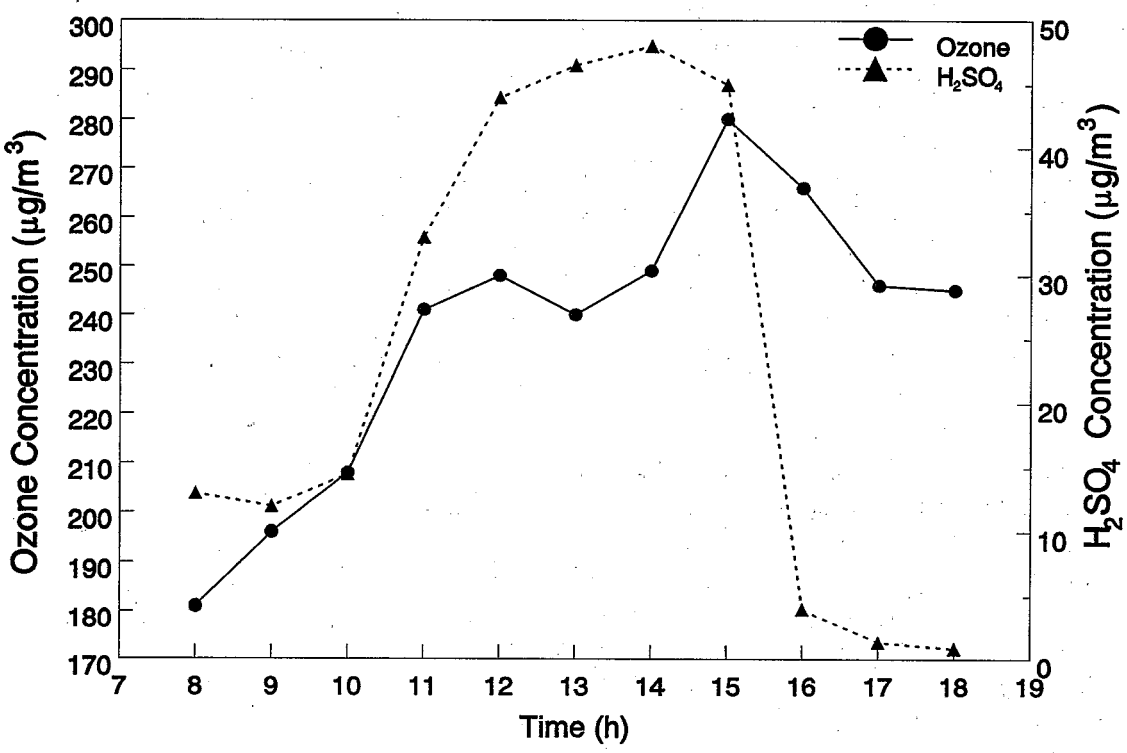


Figure 4-30. The co-occurrence pattern of ozone and sulfuric acid (H_2SO_4) for July 25, 1986, at a summer camp on the north shore of Lake Erie, Ontario, Canada.

Source: Raizenne and Spengler (1989).

4.11.5 Acid Precipitation

Concern has been expressed about the possible effects on vegetation from co-occurring exposures of O_3 and acid precipitation (Prinz et al., 1985; National Acid Precipitation Assessment Program, 1987; Prinz and Krause, 1988). Little information has been published concerning the co-occurrence patterns associated with the joint distribution of O_3 and acidic deposition (i.e., H^+). Lefohn and Benedict (1983) reviewed EPA's SAROAD monitoring data for 1977 through 1980 and, using National Atmospheric Deposition Program (NADP) and EPRI wet deposition data, evaluated the frequency distribution of pH events for 34 NADP and 8 EPRI chemistry monitoring sites located across the United States. Unfortunately, there were few sites where O_3 and acidic deposition were comonitored.

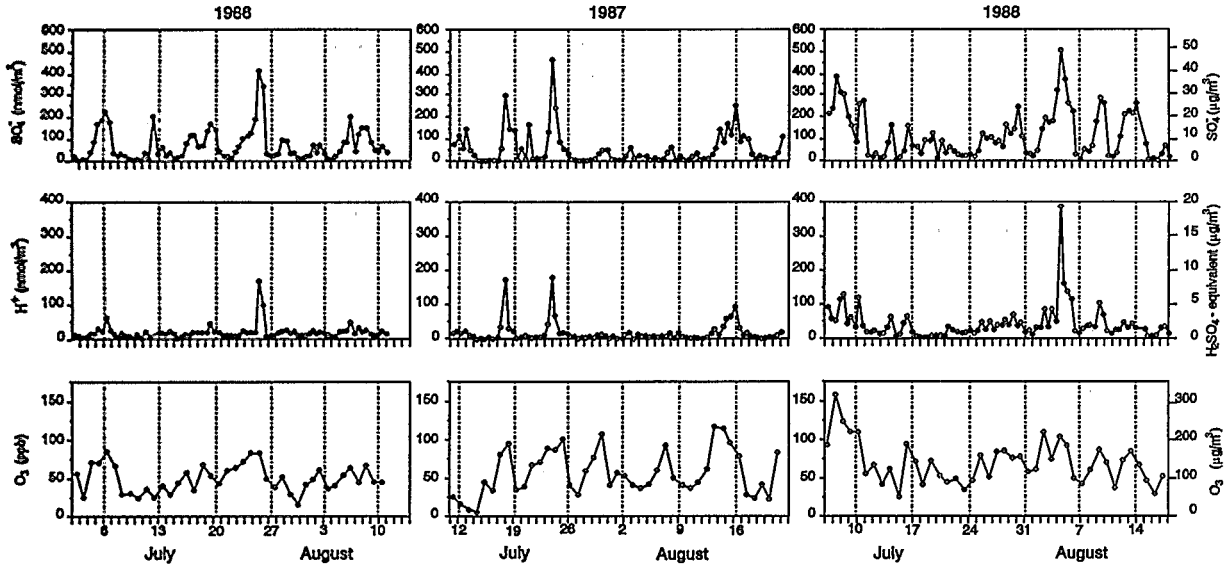


Figure 4-31. Sulfate (SO_4^-), hydrogen ion (H^+), and ozone (O_3) measured at Breadalbane Street (Site 3) in Toronto during July and August 1986, 1987, and 1988.

Source: Thurston et al. (1994).

As a result, Lefohn and Benedict (1983) focused their attention on O_3 and acidic deposition monitoring sites that were closest to one another. In some cases, the sites were as far apart as 144 km. Using hourly O_3 monitoring data and weekly and event acidic deposition data from the NADP and EPRI databases, the authors identified specific locations where the hourly mean O_3 concentrations were ≥ 0.1 ppm and 20% of the wetfall daily or weekly samples were below pH 4.0. Elevated levels of O_3 were defined as hourly mean concentrations equal to or greater than 0.1 ppm. Although for many cases, experimental research results of acidic deposition on agricultural crops show few effects at pH levels above 3.5 (National Acid Precipitation Assessment Program, 1987), it was decided to use a pH threshold of 4.0 to take into consideration the possibility of synergistic effects of O_3 and acidic deposition.

Based on their analysis, Lefohn and Benedict (1983) reported five sites where there may be the potential for agricultural crops to experience additive, less than additive, or synergistic (i.e., greater than additive) effects from elevated O_3 and H^+ concentrations. The authors stated that they believed, based on the available data, the greatest potential for interaction between acid rain and O_3 concentrations in the United States, with possible effects on crop yields, may be in the most industrial areas (e.g., Ohio and Pennsylvania). However,they cautioned that, because no documented evidence existed to show that pollutant interaction had occurred under field growth conditions and ambient exposures, their conclusions should only be used as a guide for further research.

In their analysis, Lefohn and Benedict (1983) found no colocated sites. The authors rationalized that data from non-co-monitoring sites (i.e., O_3 and acidic deposition)

could be used because O_3 exposures are regional in nature. However, work by Lefohn et al. (1988a) has shown that hourly mean O_3 concentrations vary from location to location within a region, and that cumulative indices, such as the percent of hourly mean concentrations $\geq$0.07 ppm, do not form a uniform pattern over a region. Thus, extrapolating hourly mean O_3 concentrations from known locations to other areas within a region may provide only qualitative indications of actual O_3 exposure patterns.

In the late 1970s and the 1980s, both the private sector and the government funded research efforts to better characterize gaseous air pollutant concentrations and wet deposition. The event-oriented wet deposition network, EPRI/Utility Acid Precipitation Study Program, and the weekly oriented sampling network, NADP, provided information that can be compared with hourly mean concentrations of O_3 collected at several comonitored locations. No attempt was made to include H^+ cloud deposition information. In some cases, for mountaintop locations (e.g., Clingman's Peak, Shenandoah, Whiteface Mountain, and Whitetop Mountain), the H^+ cloud water deposition is greater than the H^+ deposition in precipitation (Mohnen, 1989), and the co-occurrence patterns associated with O_3 and cloud deposition will be different than those patterns associated with O_3 and deposition in precipitation.

Smith and Lefohn (1991) explored the relationship between O_3 and H^+ in precipitation, using data from sites that monitored both O_3 and wet deposition simultaneously and within one minute latitude and longitude of each other. The authors reported that individual sites experienced years in which both H^+ deposition and total O_3 exposure were at least moderately high (i.e., annual H^+ deposition $\geq$0.5 kg ha^{-1} and an annual O_3 cumulative, sigmoidally weighted exposure (W126) value $\geq$50 ppm-h). With data compiled from all sites, it was found that relatively acidic precipitation (pH $\leq$ 4.31 on a weekly basis or pH $\leq$ 4.23 on a daily basis) occurred together with relatively high O_3 levels (i.e., W126 values $\geq$ 0.66 ppm-h for the same week or W126 values $\geq$ 0.18 ppm-h immediately before or after a rainfall event) approximately 20% of the time, and highly acidic precipitation (i.e, pH $\leq$ 4.10 on a weekly basis or pH $\leq$ 4.01 on a daily basis) occurred together with a high O_3 level (i.e., W126 values $\geq$ 1.46 ppm-h for the same week or W126 values $\geq$ 0.90 ppm-h immediately before or after a rainfall event) approximately 6% of the time. Whether during the same week or before, during, or after a precipitation event, correlations between O_3 level and pH (or H^+ deposition) were weak to nonexistent. Sites most subject to relatively high levels of both hydrogen ion and O_3 were located in the eastern portion of the United States, often in mountainous areas.

4.11.6 Acid Cloudwater

In addition to the co-occurrence of O_3 and acid precipitation, results have been reported on the co-occurrence of O_3 and acidic cloudwater in high-elevation forests. Vong and Guttorp (1991) characterized the frequent O_3-only and pH-only, single-pollutant episodes, as well as the simultaneous and sequential co-occurrences of O_3 and acidic cloudwater. The authors reported that both simultaneous and sequential co-occurrences were observed a few times each month above the cloud base. Episodes were classified by considering hourly O_3 average concentrations $\geq$0.07 ppm and cloudwater events with pH $\leq$ 3.2. The authors reported that simultaneous occurrences of O_3 and pH episodes occurred two to three times per month at two southern sites (Mitchell, NC, and Whitetop, VA) and the two northern sites (Whiteface Mountain, NY, and Moosilauke, NH) averaged

one episode per month. No co-occurrences were observed at the central Appalachian site (Shenandoah, VA), due to a much lower cloud frequency. Vong and Guttorp (1991) reported that the simultaneous occurrences were usually of short duration (mean = 1.5 h/episode) and were followed by an O_3-only episode. As would be expected, O_3-only episodes were longer than co-occurrences and pH episodes, averaging an 8-h duration.

4.12 Summary

Ozone is a pervasive compound that is detected at all monitoring locations throughout the world. To obtain a better understanding of the potential for ambient O_3 exposures affecting human health and vegetation, hourly average concentration information is summarized for urban, rural forested, and rural agricultural areas in the United States.

The distribution of O_3 or its precursors at a rural site near an urban source is affected by wind direction (i.e., whether the rural site is located up- or downwind from the source). It is difficult to apply land-use designations to the generalization of exposure regimes that may be experienced in urban versus rural areas, because the land use characterization of "rural" does not imply that a specific location is isolated from anthropogenic influences. Rather, the characterization implies only the current use of the land. Because it is possible for urban emissions, as well as O_3 produced from urban area emissions, to be transported to more rural downwind locations, elevated O_3 concentrations can occur at considerable distances from urban centers. Urban O_3 concentration values often are depressed because of titration by NO. Because of the absence of chemical scavenging, O_3 tends to persist longer in nonurban than in urban areas, and exposures may be higher in nonurban than in urban locations.

For vegetation, as indicated in Chapter 5 (Section 5.5), extensive research has focused on identifying exposure indices with a firm foundation on biological principles. Many of these exposure indices have been based on research results indicating that the magnitude of vegetation responses to air pollution is more an effect of the magnitude of the concentration than the length of the exposure. For O_3, the short-term (1- to 8-h), high concentration exposures (>0.1 ppm) have been identified by many researchers as being more important than long-term, low concentration exposures in producing visible injury to plants (see Chapter 5 for further discussion). Similarly, for human health considerations, results using controlled human exposures have shown the possible importance of concentration in relation to duration of exposure and inhalation rate.

In summarizing the hourly average concentrations in this chapter, specific attention is given to the relevance of the exposure indices used. For example, for human health considerations, concentration (or exposure) indices such as the daily maximum 1-h average concentrations, as well as the number of daily maximum 4- or 8-h average concentrations above a specified threshold, are used to characterize information in the population-oriented locations. For vegetation, several different types of exposure indices are used. Because much of the NCLAN exposure information is summarized in terms of 7-h average concentrations, this exposure index is used. However, because peak-weighted, cumulative indices (i.e., exposure parameters that sum the products of hourly average concentrations multiplied by time over an exposure period have shown considerable promise in relating exposure and vegetation response (see Chapter 5, Section 5.5), several exposure

indices that use either a threshold or a sigmoidally weighted scheme are used in this chapter to provide insight concerning the O_3 exposures that are experienced at a select number of rural monitoring sites in the United States. The peak-weighted cumulative exposure indices such as SUM06, SUM08, and W126 are used.

Ozone hourly average concentrations have been recorded for many years by the State and local air pollution agencies who report their data to EPA. The 10-year (1983 to 1992) composite average trend for the second highest daily maximum hourly average concentration during the O_3 season for 509 trend sites and a subset of 196 NAMS sites, shows that the 1992 composite average for the trend sites is 21% lower than the 1983 average and 20% lower for the subset of NAMS sites. The 1992 value is the lowest composite average of the past 10 years. The 1992 composite average is significantly less than all the previous 9 years, 1983 to 1991. The relatively high O_3 concentrations in 1983 and 1988 were attributable, in part, to hot, dry, stagnant conditions in some areas of the country that were especially conducive to O_3 formation.

From 1991 to 1992, the composite mean of the second highest daily maximum 1-h O_3 concentrations decreased 7% at the 672 sites and 6% at the subset of 222 NAMS sites. Also, from 1991 to 1992, the composite average of the number of estimated exceedances of the O_3 standard decreased by 23% at the 672 sites, and 19% at the 222 NAMS sites. Nationwide VOC emissions decreased 3% from 1991 to 1992 (U.S. Environmental Protection Agency, 1993). The composite average of the second daily maximum concentrations decreased in 8 of the 10 EPA regions from 1991 to 1992, and remained unchanged in Region VII. Except for Region VII, the 1992 regional composite means are lower than the corresponding 1990 levels. Although meteorological conditions in the east during 1993 were more conducive to O_3 formation than those in 1992, the composite mean level for 1993 was the second lowest composite average for the decade (1984 to 1993).

Information is provided in this chapter on methods used for investigating techniques for adjusting O_3 trends for meteorological influences. Historically, the long-term O_3 trends in the United States characterized by EPA have emphasized air quality statistics that are closely related to the NAAQS. Information is provided on the use of alternative indices. Besides EPA, additional investigators have assessed trends at several locations in the United States, and information is provided for both urban and rural areas.

Interest has been expressed in characterizing O_3 exposure regimes for sites experiencing daily maximum 8-h concentrations above specific thresholds (e.g., 0.08 or 0.10 ppm). Documented evidence has been published showing the occurrence, at some sites, of multihour periods within a day of O_3 at levels of potential health effects. Although most of these analyses were made using monitoring data collected from sites in or near nonattainment areas, one analysis showed that at five sites, two in New York state, two in rural California, and one in rural Oklahoma, an alternative O_3 standard of an 8-h average of 0.10 ppm would be exceeded even though the existing 1-h standard would not be. The study indicated the occurrence at these five sites, none of which was in or near a nonattainment area, of O_3 concentrations showing only moderate peaks but showing multihour levels above 0.10 ppm.

An important question is whether an improvement in O_3 levels would produce distributions of 1-h O_3 concentrations that result in a broader diurnal profile than those seen in high-oxidant urban areas where O_3 regimes contain hourly average concentrations with sharper peaks. The result would be an increase in the number of exceedances of daily maximum 8-h average concentrations ≥ 0.08 ppm, when compared to those sites

experiencing sharper peaks. One research effort observed, using aerometric data at specific sites, how O_3 concentrations change when the sites change compliance status. One of the parameters examined was 4-h daily maxima. The number of exceedances for a specific daily maximum average concentration tended to decrease as fewer exceedances of the current 1-h standard were observed at a given site. The number of occurrences of the daily maximum 4-h average concentration $\geq$0.08 ppm and the number of exceedances of the current form of the standard had a positive, weak correlation ($r = 0.51$). The investigators reported few changes in the shape of the average diurnal patterns as sites changed attainment status. The lack of a change in shape may have explained why the investigators could not find evidence that the number of occurrences of the daily maximum 4-h average concentration $\geq$0.08 ppm increased when the sites experienced few high hourly average concentrations.

There has been considerable interest in possibly substituting one index for another when attempting to relate O_3 exposure with an effect. For example, using O_3 ambient air quality data, the number of exceedances of 0.125 ppm and the number of occurrences of the daily maximum 8-h average concentrations $\geq$0.08 ppm have been compared with the result that a positive correlation ($r = 0.79$) existed between the second-highest 1-h daily maximum in a year and the expected number of days with an 8-h daily maximum average concentration $>$0.08 ppm O_3. However, there was not much predictive strength in using one O_3 exposure index to predict another. Similarly, the maximum 3-mo SUM06, second highest daily maximum hourly average concentration, and second highest daily maximum 8-h average concentration exposure indices were compared. For the rural agricultural and forest sites, the correlations among the indices were not strong.

One of the difficulties in attempting to use correlation analysis between indices for rationalizing the substitution of one exposure index for another for predicting an effect (e.g., SUM06 versus the second highest daily maximum hourly average concentration) is the introduction of the error associated with estimating levels of one index from those of another. Evidence has been presented in the literature for recommending that, if a different exposure index (e.g., second highest daily maximum hourly average concentration) is to be compared to, for example, the SUM06 for adequacy in predicting crop loss, then the focus should be on how well the two exposure indices predict crop loss using the effects model that is a function of the most relevant index and not on how well the indices predict one another. Less error would be introduced if either of the two indices were used directly in the development of an exposure-response model.

The EPA has indicated that a reasonable estimate, as an annual average, of O_3 background concentration near sea level in the United States today is from 0.020 to 0.035 ppm; this estimate included a 0.005 to 0.015 ppm contribution from the stratosphere. The EPA concluded that a reasonable estimate of natural O_3 background concentration for a 1-h daily maximum at sea level in the United States during the summer is on the order of 0.03 to 0.05 ppm. Reviewing data from sites that appear to be isolated from anthropogenic sources, it has been reported that, in almost all cases, none of the sites experienced hourly average concentrations $\geq$0.08 ppm and that the maximum hourly average concentrations were in the range of 0.060 to 0.075 ppm. Using data from these sites, in the continental United States, the 7-mo (April to October) average of the 7-h daily average concentrations range from approximately 0.025 to 0.045 ppm. At an O_3 monitoring site at the Theodore Roosevelt National Park, 7-mo (April to October) averages of the 7-h daily average concentrations of 0.038, 0.039, and 0.039 ppm, respectively, were experienced in 1984, 1985, and 1986. These 7-h seasonal averages appear to be representative of the 8-h daily

average O_3 concentrations that may occur at other fairly clean sites in the United States and other locations in the northern hemisphere.

Diurnal variations are those that occur during a 24-h period. Diurnal patterns of O_3 may be expected to vary with location, depending on the balance among the many factors affecting O_3 formation, transport, and destruction. Although they vary with locality, diurnal patterns for O_3 typically show a rise in concentration from low levels or levels near minimum detectable amounts to an early afternoon peak. The diurnal pattern of concentrations can be ascribed to four simultaneous processes: (1) downward transport of O_3 from layers aloft, (2) destruction of O_3 through contact with surfaces and through reaction with NO at ground level, (3) in situ photochemical production of O_3, and (4) horizontal transport of O_3 and its precursors.

Although it might appear that composite diurnal pattern diagrams could be used to quantify the differences in O_3 exposures among sites, caution has been expressed in their use for this purpose. The average diurnal patterns are derived from long-term calculations of the hourly average concentrations, and the resulting diagram cannot adequately identify, at most sites, the presence of high hourly average concentrations; thus, they may not be adequate to distinguish O_3 exposure differences among sites. Unique families of diurnal average profiles exist, and it is possible to distinguish between two types of O_3 monitoring sites. A seasonal diurnal diagram provides the investigator with the opportunity to identify whether a specific O_3 monitoring site has more scavenging than any other site. For low-elevation sites, intraday variability is most significant due to the pronounced daily amplitude in O_3 concentration between the predawn minimum and midafternoon to early-evening maximum, whereas interday variation is more significant in the high-elevation sites.

Seasonal variations in O_3 concentrations in urban areas usually show the pattern of high O_3 in late spring or in summer and low levels in the winter. Because of temperature, relative humidity, and seasonal changes in storm tracks from year to year, the general weather conditions in a given year may be more favorable for the formation of O_3 and other oxidants than during the prior or following year. For example, 1988 was a hot and dry year in which some of the highest O_3 concentrations of the last decade occurred, whereas 1989 was a cold and wet year in which some of the lowest concentrations occurred.

Several investigators have reported on the tendency for average O_3 concentrations to be higher in the second than in the third quarter of the year for many isolated rural sites. This observation has been attributed to either stratospheric intrusions or an increasing frequency of slow-moving, high-pressure systems that promote the formation of O_3. However, for several clean rural sites, the highest exposures have occurred in the third quarter rather than in the second. For rural O_3 sites in the southeastern United States, the daily maximum 1-h average concentration was found to peak during the summer months. For sites located in rural areas, but not isolated from anthropogenic sources of pollution, the different patterns may be associated with anthropogenic emissions of NO_x and hydrocarbons.

Concentrations of O_3 vary with altitude and with latitude. There appears to be no generalizable conclusion concerning the relationship between O_3 exposure and elevation. The differences in exposure occur when one site is above the natural inversion and the other is not. An important issue for assessing possible impacts of O_3 at high-elevation sites that requires further attention is the use of mixing ratios (e.g., parts per million) instead of absolute concentration (e.g., in units of micrograms per cubic meter) to describe O_3 concentration. In most cases, mixing ratios, or mole fractions, are used to describe O_3 concentrations. The manner in which concentration is reported may be important when

assessing the potential impacts of air pollution on high-elevation forests. Concentration varies as a function of altitude. Although the change in concentration is small when the elevational difference between sea level and the monitoring site is small, it becomes substantial at high-elevation sites. Given the same part-per-million value registering at both a high- and low-elevation site, the absolute concentrations (i.e., micrograms per cubic meter) at the two elevations will be different. Because both pollutants and ambient air are gases, changes in pressure directly affect their volume. This pressure effect must be considered when measuring absolute pollutant concentrations. Although these exposure considerations are trivial at low-elevation sites, when one compares exposure-effects results obtained at high-elevation sites with those from low-elevation sites, the differences may become significant.

Most people in the United States spend a large proportion of their time indoors. Until the early 1970s, very little was known about the O_3 concentrations experienced inside buildings. Even to date, the database on this subject is not extensive, and a wide range of I/O O_3 concentration relationships can be found in the literature. Reported I/O values for O_3 are highly variable. A relatively large number of factors can affect the difference in O_3 concentrations between the inside of a structure and the outside air. In general, outside air infiltration or exchange rates, interior air circulation rates, and interior surface composition (e.g., rugs, draperies, furniture, walls) affect the balance between replenishment and decomposition of O_3 within buildings. The I/O O_3 concentration ratios generally fall in the range of 0.1 to 0.7, and indoor concentrations of O_3 will almost invariably be less than those outdoors.

It is important that accurate estimates of both human and vegetation exposure to O_3 be available for assessing the risks posed by the pollutant. Examples are provided on how both fixed-site monitoring information and human exposure models are used to estimate risks associated with O_3 exposure.

In many cases, the upper tail of the distribution, which represents those individuals exposed to the highest concentrations, often generates special interest because the determination of the number of individuals who experience elevated pollutant levels can be critical for health risk assessments. This is especially true for pollutants for which the relationship between dose and response is highly nonlinear.

Because it is not possible to estimate population exposure, in most cases, solely from fixed-station data, several human exposure models have been developed. Some of these models include information on human activity patterns (i.e., the microenvironments people visit and the times they spend there). These models also contain submodels depicting the sources and concentrations likely to be found in each microenvironment, including indoor, outdoor, and in-transit settings.

A subgroup that has been studied by several investigators to assess the influence of ambient air pollution on their respiratory health and function is children attending summer camp. Because children are predominantly outdoors and relatively active while at camp, they provide a unique opportunity to assess the relationships between respiratory health and function and concurrent air pollution levels. Examples are provided on the type of exposure patterns that children experience.

A personal exposure profile can be identified by using a personal exposure monitor. Few data are available for individuals using personal exposure monitors. Results from a pilot study demonstrated that fixed-site ambient measurements may not adequately represent individual exposures. Outdoor O_3 concentrations showed substantial spatial

variation between rural and residential regions. The study showed that the use of fixed-site measurements could result in an error as high as 127%. In addition, the study showed that models based on time-weighted I/O concentrations explained only 40% of the variability in personal exposures. The investigators concluded that contributions from diverse indoor and outdoor microenvironments could estimate personal O_3 exposure accurately.

The field of human exposure modeling is relatively young, with the first rigorous exposure modeling analyses appearing in the mid-1970s and the theoretical constructs regarding human exposure to environmental pollution being published in the early 1980s. Two distinct types of O_3 exposure models exist: (1) those that focus narrowly on predicting indoor O_3 levels and (2) those that focus on predicting O_3 exposures on a community-wide basis. The following four distinct models address the prediction of O_3 exposures on a community-wide basis: (1) pNEM/O_3, (2) SAI/NEM, (3) REHEX, and (4) EPEM. All four O_3 exposure models are derived from the NEM (NAAQS exposure model), which was first developed in the early 1980s. Most applications of NEM use fixed-site monitoring data, U.S. census data, and human-activity data. The calculations result in an estimate of the O_3 concentration experienced by an individual in each microenvironment that the person inhabits.

The hourly average concentrations used in many of the high-treatment experimental vegetation and human health effects studies did not necessarily simulate those concentrations observed under ambient conditions. Although the ramifications of this observation on the effects observed are not clear, it has been pointed out that the highest treatments used in many of the vegetation open-top chamber experiments were bimodal in the distribution of the hourly average concentrations. In other experiments designed to assess the effects of O_3 on vegetation, constant concentration (i.e., square wave) exposures were implemented. As discussed in earlier sections of this Chapter, square wave exposure regimes do not normally occur under ambient conditions. Similar square wave exposures have been used in human health effects studies. In addition to the exposures used at the highest treatment levels for vegetation experiments, there is concern that the hourly average concentrations used in the charcoal-filtered control treatments may be lower than those experienced at isolated sites in the United States and in other parts of the world. Although the ramifications of using such exposure regimes is unclear, there is some concern that the use of atypically low control levels may result in an overestimation of vegetation yield losses when used as the baseline for evaluating the effects of treatments at higher concentrations.

Published data on the concentrations of photochemical oxidants other than O_3 in ambient air are neither comprehensive nor abundant. A review of the data shows that PAN and PPN are the most abundant of the non-O_3 oxidants in ambient air in the United States, other than the inorganic nitrogenous oxidants such as NO_2 and possibly nitric acid. At least one study has reported that a higher homologue of the series, peroxybenzoyl nitrate (PBzN), like PAN, is a lachrymator. No unambiguous identification of PBzN in the ambient air of the United States has been made.

Given the information available on PAN, the concentrations of PAN that are of most concern are those to which vegetation could potentially be exposed, especially during daylight hours in agricultural areas. These are followed in importance by concentrations both indoors and outdoors, in urban and nonurban areas, to which human populations potentially could be exposed. Most of the available data on concentrations of PAN and PPN in ambient air are from urban areas. The levels to be found in nonurban areas will be highly dependent on the transport of PAN and PPN or their precursors from urban areas, because

the concentrations of the NO_x precursors to these compounds are considerably lower in nonurban than in urban areas.

There have been several attempts to characterize air pollutant mixtures. Pollutant combinations can occur at or above a threshold concentration either together or temporally separated from one another. Studies of the joint occurrence of gaseous NO_2/O_3 and SO_2/O_3 have concluded that the co-occurrence of two-pollutant mixtures lasted only a few hours per episode, and that the time between episodes is generally long (i.e, weeks, sometimes months). Using hourly averaged data collected at rural sites for vegetation considerations, the periods of co-occurrence represent a small portion of the potential plant growing period. For human ambient exposure considerations, the simultaneous co-occurrence of NO_2/O_3 was infrequent in most cases. However, for several sites located in the South Coast Air Basin, more than 450 simultaneous co-occurrences of each pollutant, at hourly average concentrations $\geq$0.05 ppm, were present. Although the focus of co-occurrence research has been on patterns associated with the presence or absence of hourly average concentrations of pollutant pairs, some researchers have discussed the joint occurrence of O_3, nitrogen, and sulfur in forested areas, combining cumulative exposures of O_3 with data on dry deposition of sulfur and nitrogen. One study reported that several forest landscapes with the highest dry deposition loadings of sulfur and nitrogen tended to experience the highest average O_3 concentrations and the largest cumulative exposure. Although the investigators concluded that the joint occurrences of multiple pollutants in forest landscapes were important, nothing was mentioned about hourly co-occurrences of O_3 and SO_2 or O_3 and NO_2.

Knowledge of the potential exposure of the co-occurrence of acidic sulfate aerosols and O_3 is limited because routine monitoring data for acidic aerosols are not available. Information on the co-occurrence patterns is limited to research studies; some of the results are provided in this chapter. Acid sulfates, which are composed of H_2SO_4, ammonium bisulfate, and ammonium sulfate, have been measured at a number of locations in North America. Acidic sulfate and neutralized species can accumulate and range in concentration from 0 to 50 $\mu g/m^3$ at a specific location or a number of locations simultaneously. For many summertime studies, peaks of H_2SO_4 or H^+ appear to be associated with the presence of a slow-moving high pressure system. Acid sulfates are found primarily in the fine particle size range (<2.5 μm in diameter). The acidic sulfate concentrations measured in the summertime can be found at 20 $\mu g/m^3$ for over an hour and at high concentrations of 10 to 20 $\mu g/m^3$ for 6 to 24 h at one or more sites. Acidic sulfate aerosol concentrations can occur at concentrations in the summertime above 10 $\mu g/m^3$ for periods longer than 5 h. The highest O_3 exposures for sites affected by anthropogenically derived photooxidant precursors are expected to occur during the late spring and summer months. Thus, the potential for O_3 and acidic sulfate aerosols to co-occur at some locations in some form (i.e., simultaneously, sequentially, or complex-sequentially) is real and requires further characterization.

Concern has been expressed about the possible effects on vegetation from co-occurring exposures of O_3 and acid precipitation. One study explored the relationship between O_3 and H^+ in precipitation, using data from sites that monitored both O_3 and wet deposition simultaneously and within one minute latitude and longitude of each other. The investigators reported that individual sites experienced years in which both H^+ deposition and total O_3 exposure were at least moderately high (i.e., annual H^+ deposition $\geq$0.5 kg ha^{-1} and an annual O_3 cumulative sigmoidally weighted exposure (W126) value $\geq$50 ppm-h). Based on data compiled from all sites, relatively acidic precipitation (pH $\leq$ 4.31 on a

weekly basis or pH $\leq$ 4.23 on a daily basis) occurred together with relatively high O_3 levels (i.e., W126 values $\geq$ 0.66 ppm-h for the same week or W126 values $\geq$ 0.18 ppm-h immediately before or after a rainfall event) approximately 20% of the time, and highly acidic precipitation (i.e, pH $\leq$ 4.10 on a weekly basis or pH $\leq$ 4.01 on a daily basis) occurred together with a high O_3 level (i.e., W126 values $\geq$ 1.46 ppm-h for the same week or W126 values $\geq$0.90 ppm-h immediately before or after a rainfall event) approximately 6% of the time. Whether during the same week or before, during, or after a precipitation event, correlations between O_3 level and pH (or H^+ deposition) were weak to nonexistent. Sites most subject to relatively high levels of both H^+ and O_3 were located in the eastern portion of the United States, often in mountainous areas.

The co-occurrence of O_3 and acidic cloudwater in high-elevation forests has been characterized. The frequent O_3-only and pH-only, single-pollutant episodes, as well as the simultaneous and sequential co-occurrences of O_3 and acidic cloudwater, have been reported. Both simultaneous and sequential co-occurrences were observed a few times each month above the cloud base.

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Appendix A

Abbreviations and Acronyms

ADOM	Acid Deposition and Oxidant Model
AGL	Above ground level
AIRS	Aerometric Information Retrieval System
AM	Alveolar macrophage
AQCD	Air Quality Criteria Document
AQCR	Air Quality Control Region
AUSPEX	Atmospheric Utility Signatures, Predictions, and Experiments
C	Carbon
C	Concentration
CA	Chromotropic acid
CAA	Clean Air Act
CAAA	Clean Air Act Amendments of 1990
CAL-RAMS	Coast and Lake Regional Atmospheric Modeling System
CAR	Centriacinar region
CASAC	Clean Air Scientific Advisory Committee
CBM	Carbon-bond mechanism
CCM	Community Climate Model
CFC	Chlorofluorocarbon
CH_3OH	Methanol
CH_4	Methane
CI	Chemical ionization
CIT	California Institute of Technology
CL	Chemiluminescence
CMB	Chemical mass balance
CNG	Compressed natural gas
CO	Carbon monoxide
CO_2	Carbon dioxide
CTWM	Complex Terrain Wind Model
DIAL	Differential absorption lidar

DNPH	2,4-Dinitrophenylhydrazine
DOAS	Differential optical absorption spectrometry
DWM	Diagnostic Wind Model
ECD	Electron capture detection
EKMA	Empirical Kinetic Modeling Approach
EMS	Emissions Modeling System
EPA	U.S. Environmental Protection Agency
EPEM	Event Probability Exposure Model
EPRI	Electric Power Research Institute
EPS	Emissions Preprocessor System
ERAQS	Eastern Regional Air Quality Study
ETBE	Ethyl-tertiary-butyl ether
EtOH	Ethanol
FDDA	Four-dimensional data assimilation
$FeSO_4$	Ferrous sulfate
FEV_1	Forced expiratory volume in 1 s
FVC	Forced vital capacity
FID	Flame ionization detection
FTIR	Fourier transform infrared absorption spectroscopy
GC	Gas chromatography
GMEP	Geocoded Model of Emissions and Projections
GPT	Gas-phase titration
H^+	Hydrogen ion
HC	Hydrocarbon
HCFC	Hydrochlorofluorocarbon
HCHO	Formaldehyde
HNO_2	Nitrous acid
HNO_3	Nitric acid
HO_2	Hydroperoxyl
H_2O_2	Hydrogen peroxide
HPLC	High-performance liquid chromatography
H_2SO_4	Sulfuric acid
IC	Ion chromatography
ID	Identification (number)
I/O	Indoor/outdoor
IR	Infrared radiation

IR	Incremental reactivity
LMOS	Lake Michigan Oxidant Study
LPG	Liquified petroleum gas
MBTH	3-Methyl-2-benzothiazolone hydrazone
MCCP	Mountain Cloud Chemistry Program
MM4/MM5	Mesoscale Model, versions 4 and 5
MOBILE	U.S. Environmental Protection Agency emissions model for mobile sources
MODELS 3	Modeling framework that consolidates all of the U.S. Environmental Protection Agency's three-dimensional photochemical air quality models
MPAN	Peroxymethacryloyl nitrate
MSA	Metropolitan Statistical Area
MSCET	Month and state current emissions trends
MTBE	Methyl-tertiary-butyl ether
NA	Not available
NAAQS	National Ambient Air Quality Standards
NADP	National Atmospheric Deposition Program
NAMS	National Air Monitoring Station
NAPAP	National Acid Precipitation Assessment Program
NAPBN	Western National Air Pollution Background Network
NAS	National Academy of Sciences
NBKI	Neutral buffered potassium iodide
NBS	National Bureau of Standards; now National Institute of Standards and Technology
NCAR	National Center for Atmospheric Research
NCLAN	National Crop Loss Assessment Network
NDDN	National Dry Deposition Network
NEM	National Air Quality Standards Exposure Model
NF	National forest
NH_3	Ammonia
NH_4HSO_4	Ammonium bisulfate
NH_4OH	Ammonium hydroxide
$(NH_4)_2SO_4$	Ammonium sulfate
NIST	National Institute of Standards and Technology
NM	National monument

NMHC	Nonmethane hydrocarbon
NMOC	Nonmethane organic compound
NO	Nitric oxide
NO_2	Nitrogen dioxide
N_2O	Nitrous oxide
NO_3^-	Nitrate
NO_x	Nitrogen oxides
NP	National park
NPN	*n*-propyl nitrate
NTP	National Toxicology Program
O_3	Ozone
OAQPS	Office of Air Quality Planning and Standards
Obs.	Observations
OH	Hydroxyl
OHBA	Hydroxybenzoic acid
PAMS	Photochemical Aerometric Monitoring System
PAN	Peroxyacetyl nitrate
PANs	Peroxyacyl nitrates
PAR	Proximal alveolar region
PBL	Planetary boundary layer
PBzN	Peroxybenzoyl nitrate
PDFID	Cryogenic preconcentration-direct flame ionization detection
PF/TPLIF	Photofragmentation two-photon laser-induced fluorescence
pH	Hydrogen ion concentration
PL	Liquid-phase vapor pressure
PLANR	Practice for Low-cost Application in Nonattainment Regions
PMN	Polymorphonuclear leukocyte (also called neutrophil)
ppmC	Parts per million carbon
PPN	Peroxypropionyl nitrate
PSD	Passive sampling device
PVOC	Polar volatile organic compound
Q_E	Latent heat flux
Q_H	Heat flux
r	Linear regression correlation coefficient
R^2	Multiple correlation coefficient
RADM	Regional Acid Deposition Model

RAPS	Regional Air Pollution Study
REHEX	Regional Human Exposure Model
RMSD	Root-mean-square difference
ROG	Reactive organic gas
ROM	Regional Oxidant Model
ROMNET	Regional Ozone Modeling for Northeast Transport program
RT	Respiratory tract
SAB	Science Advisory Board
SAI	Systems Applications International
SAPRC	Statewide Air Pollution Research Center, University of California, Riverside
SARMAP	San Joaquin Valley Air Quality Study (SJVAQS)/Atmospheric Utility Signatures, Predictions, and Experiments (AUSPEX) Regional Model Adaptation Project
SAROAD	Storage and Retrieval of Aerometric Data (U.S. Environmental Protection Agency centralized database; superseded by Aerometric Information Retrieval System [AIRS])
SCAQS	South Coast Air Quality Study (California)
SIP	State Implementation Plan
SLAMS	State and Local Air Monitoring Station
SJVAQS	San Joaquin Valley Air Quality Study
SO_2	Sulfur dioxide
SO_4^{2-}	Sulfate
SOS	Southern Oxidant Study
SRM	Standard reference material
SRP	Standard reference photometer
STEM-II	Sulfur Transport Eulerian Model (version II)
SUM06	Seasonal sum of all hourly average concentrations $\geq$0.06 ppm
SUM07	Seasonal sum of all hourly average concentrations $\geq$0.07 ppm
SUM08	Seasonal sum of all hourly average concentrations $\geq$0.08 ppm
SURE	Sulfate Regional Experiment Program
T	Temperature
TAMS	Toxic Air Monitoring Study (U.S. Environmental Protection Agency)
TDLAS	Tunable-diode laser absorption spectroscopy
TEA	Triethanolamine
Tg	Teragram

TGTP	The Global Thinking Project
TNMHC	Total nonmethane hydrocarbons
TPLIF	Two-photon laser-induced fluorescence
TTFMS	Two-tone frequency-modulated spectroscopy
UAM	Urban Airshed Model
UV	Ultraviolet
UV-B	Ultraviolet radiation of wavelengths 280 to 320 nm
VMT	Vehicle miles traveled
VOC	Volatile organic compound
$\dot{V}_E$	Minute ventilation; expired volume per minute
WFM	White Face Mountain
WMO/UNEP	World Meteorological Organization/United Nations Environment Program
W126	Cumulative integrated exposure index with a sigmoidal weighting function

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Volume II of III

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Air Quality Criteria for Ozone and Related Photochemical Oxidants

Volume II of III

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Preface

In 1971, the U.S. Environmental Protection Agency (EPA) promulgated National Ambient Air Quality Standards (NAAQS) to protect the public health and welfare from adverse effects of photochemical oxidants. In 1979, the chemical designation of the standards was changed from photochemical oxidants to ozone (O_3). This document focuses primarily on the scientific air quality criteria for O_3 and, to a lesser extent, on those for other photochemical oxidants such as hydrogen peroxide and the peroxyacyl nitrates.

The EPA promulgates the NAAQS on the basis of scientific information contained in air quality criteria issued under Section 108 of the Clean Air Act. The previous O_3 criteria document, *Air Quality Criteria for Ozone and Other Photochemical Oxidants*, was released in August 1986 and a supplement, *Summary of Selected New Information on Effects of Ozone on Health and Vegetation*, was released in January 1992. These documents were the basis for a March 1993 decision by EPA that revision of the existing 1-h NAAQS for O_3 was not appropriate at that time. That decision, however, did not take into account some of the newer scientific data that became available after completion of the 1986 criteria document. The purpose of this revised air quality criteria document for O_3 and related photochemical oxidants is to critically evaluate and assess the latest scientific data associated with exposure to the concentrations of these pollutants found in ambient air. Emphasis is placed on the presentation of health and environmental effects data; however, other scientific data are presented and evaluated in order to provide a better understanding of the nature, sources, distribution, measurement, and concentrations of O_3 and related photochemical oxidants and their precursors in the environment. Although the document is not intended to be an exhaustive literature review, it is intended to cover all pertinent literature available through 1995.

This document was prepared and peer reviewed by experts from various state and Federal governmental offices, academia, and private industry and reviewed in several public meetings by the Clean Air Scientific Advisory Committee. The National Center for Environmental Assessment (formerly the Environmental Criteria and Assessment Office) of EPA's Office of Research and Development acknowledges with appreciation the contributions provided by these authors and reviewers as well as the diligence of its staff and contractors in the preparation of this document at the request of the Office of Air Quality Planning and Standards.

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5

Environmental Effects of Ozone and Related Photochemical Oxidants

5.1 Introduction

Analyses of photochemical oxidants in the ambient air have revealed the presence of a number of phytotoxic compounds, including ozone (O_3), peroxyacyl nitrates (PANs), and nitrogen dioxide (NO_2). Ozone, the most prevalent photochemical oxidant, has been studied the most, and its effects are understood better than those of other photochemically derived oxidants. Ozone affects vegetation throughout the United States, impairing crops, native vegetation, and ecosystems more than any other air pollutant (Heck et al., 1980). The phytotoxicity of nitrogen oxides has been assessed in Air Quality Criteria for Oxides of Nitrogen (U.S. Environmental Protection Agency, 1993) and will not be discussed here. On the basis of concentration, the PANs are more toxic than O_3, with peroxyacetyl nitrate (PAN) being about 10 times more phytotoxic than O_3 (Darley et al., 1963; Taylor and MacLean, 1970; Pell, 1976). Although more phytotoxic than O_3, PANs generally occur at significantly lower ambient concentrations and are distributed less widely than those of O_3. Ambient concentrations of O_3 and PAN, as well as their concentration ratios, are discussed in detail in Chapter 4.

The effects of photochemical oxidants were observed first as foliar injury on vegetation growing in localized areas in Los Angeles County, CA (Middleton et al., 1950). In these early reports, foliar injury was described as glazing, silvering, and bronzing of the lower leaf surface of leafy vegetables and as transverse bands of injury on monocotyledonous species. Subsequent studies showed that these symptoms of photochemical oxidant injury were caused by PAN (Taylor et al., 1960). The characteristic O_3 stipple on grape (*Vitis labruscana*) leaves reported in the late 1950s was the first observation of O_3 injury to vegetation in the field (Richards et al., 1958). Subsequent studies with tobacco (*Nicotiana tabacum*) and other crops confirmed that O_3 was injuring vegetation at sites near urban centers (Heggestad and Middleton, 1959; Daines et al., 1960). It now is recognized that vegetation at rural sites may be injured by O_3 transported long distances from urban centers (Edinger et al., 1972; Heck et al., 1969; Heck and Heagle, 1970; Wolff et al., 1977a,b,c, 1980; Wolff and Lioy, 1980; Kelleher and Feder, 1978; Miller et al., 1972; Skelly et al., 1977; Skelly, 1980; Garner et al., 1989; see also Chapters 3 and 4). Concentrations of O_3 in polluted air masses often remain high for prolonged periods in rural areas, increasing the concern over possible effects on agriculture, forests, and native ecosystems.

Exposure to tropospheric O_3 can cause injury and premature mortality of plant tissues after entering the plant because O_3 has strong oxidizing properties and reacts with

cellular components. The effects of O_3 on terrestrial ecosystems begin with the responses of individual plants (Figure 5-1). Effects are initiated within the plant by reactions between O_3 or its metabolites and cellular constituents that influence biochemical and physiological processes and alter plant growth. Plant sensitivity to O_3 varies widely among individuals and among species. Sensitivity is determined both by genetic composition of the plant and environmental conditions. Plant response also is influenced by factors such as pollutant concentration, duration of exposures, plant nutrition, developmental stage, climate, insects, and diseases (See Sections 5.3 and 5.4).

Changes in foliar pigmentation and development of injured tissues are usually the first visible sign of injurious O_3 exposures and indicate impairment of physiological processes with the leaves. To affect metabolic processes within the cell, sufficient amounts of O_3 from the atmosphere must be able to enter the plant through the leaf stomata and dissolve in the aqueous layer lining the air spaces. Ozone and its decomposition products then diffuse through the cell membrane, where they can react with cellular components (unless the plant is able to detoxify or metabolize O_3 or its metabolites) (Section 5.3; Tingey and Taylor, 1982).

Ozone can affect all aspects of plant growth (Figure 5-1). Plants accumulate, store, and use carbon compounds to build their structure and maintain physiological processes (Waring and Schlesinger, 1985). Within the leaf, carbon dioxide (CO_2) absorbed from the atmosphere is converted to carbohydrates during the process of photosynthesis. The water and minerals necessary for growth are absorbed by plants from the soil. Growth and seed formation depend not only on the rate of photosynthesis and uptake of water and nutrients, but also on the subsequent metabolic processes and the allocation of the carbohydrates produced during photosynthesis. Most plants require a balance of resources (i.e, energy, water, mineral nutrients) to maintain optimal growth, but these are seldom available in natural environments (Chapin et al., 1987). Plants compensate for injury or stress by allocating their available resources to the point of injury or stress (McLaughlin et al., 1982; Miller et al., 1982; Tingey et al., 1976b). Altering the allocation of carbohydrates has been shown to decrease plant vigor, to increase susceptibility to insect pests and fungal pathogens, to interfere with mycorrhizal formation, and to reduce plant growth and reproduction (McLaughlin et al., 1982; Miller et al., 1982; U.S.Environmental Protection Agency, 1986; Garner et al., 1989).

Most of the available information concerning the effects of O_3 on vegetation is the result of exposure-response studies of important agricultural crops and some selected forest tree species, usually as seedlings. Through the years, crop plants, because of human food demand, usually have been selected for their productivity. They are grown as monocultures, fertilized, weeded, and frequently irrigated. In other words, competition for water nutrients, space, and light is minimized greatly when compared with plants growing in natural conditions, particularly in ecosystems. Trees for timber and paper also are grown on plantations under conditions favoring the greatest production.

Some O_3 exposures (concentration and duration) result in visible foliar injury to the plant without growth reduction; other exposures result in growth reduction and decrease in productivity without visible injury, whereas some exposures result in both. Data is presented in Section 5.6 that deals with the impact of different concentrations and exposure durations from many different experimental exposure-response studies on the growth of a variety of cultivated crops, ornamental species, and natural vegetation.

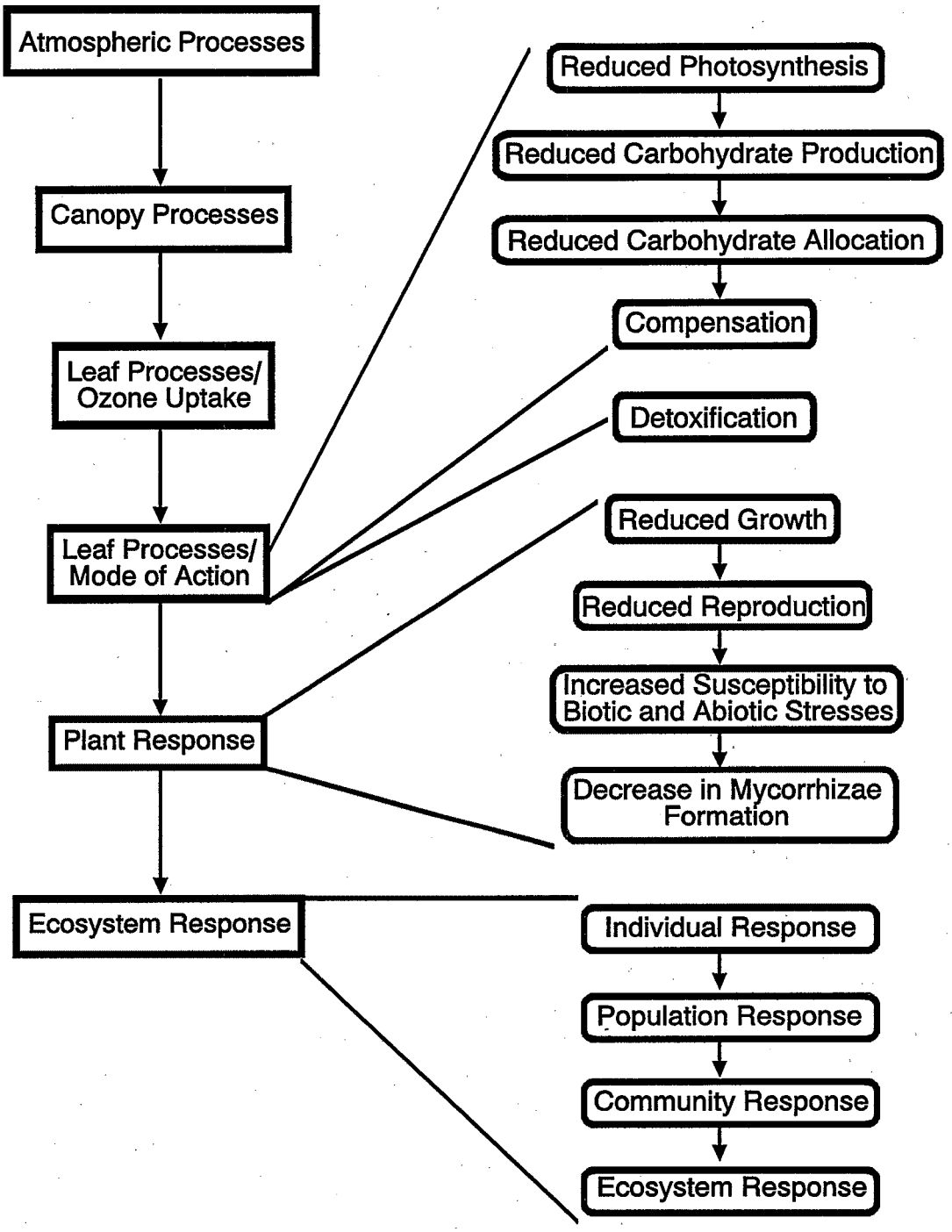

Figure 5-1. Leaf absorption and possible functional changes that may occur within the plant. Ecosystem response begins at the level of the individual and is propagated to the more complex level of organization.

The number of crop species and cultivars for which information regarding O_3 effects exists encompasses a mere fraction of the total of those cultivated as crops or found growing in natural communities. It is not possible to predict the sensitivity of the species and cultivars that have not been investigated, except in very general terms, because of the wide range of sensitivities to O_3 known to exist among crop cultivars and species that have been studied. Attempts to develop a general framework of response covering a range of species using the fragmented knowledge available have not been successful.

For many years, attempts have been made to develop mathematical equations that quantify the relationship between pollutant exposure and agricultural crop yield. The advantages and limitations of the various indices that have been developed to aid in predicting the effects of O_3 on crop yield are discussed in Section 5.5.

Organisms, not ecosystems, respond to O_3 exposure (Sigal and Suter, 1987). The only well-documented study of ecosystem change is that of the San Bernardino Mixed Forest ecosystem in Southern California where the impact of O_3 on the keystone species, ponderosa and Jeffrey pine (*Pinus jeffreyi*), resulted in the reversion of the forest to a simpler stage (Miller et al., 1982; Miller, 1984; U.S. Environmental Protection Agency, 1978, 1986). In other regions of the United States, most of the data available for assessing ecosystem responses deals with the responses of individuals to experimental O_3 exposures. Studies within the forests of the eastern United States, have dealt chiefly with the response in the field of eastern white pine (*Pinus strobus*) (McLaughlin et al., 1982; Skelly, 1980; Skelly et al., 1984). No long-term studies exist that deal with the impacts of O_3 on the various ecosystems components and how and whether these impacts alter ecosystem structure and functions. Therefore, the determination of the impact of O_3 on eastern forest ecosystems is difficult, if not impossible (see Section 5.7).

Plant populations are affected if they include many sensitive individuals. Removal of sensitive individuals within populations, or stands, if large in number, ultimately can change community and ecosystem structure (Figure 5-1). Structural changes that alter the ecosystem functions of energy flow and nutrient cycling can arrest or reverse ecosystem development (Odum, 1985).

The sequential organization of this chapter begins first with the methodologies (Section 5.2) that have been used to obtain the information presented and discussed in this chapter. Next, Section 5.3 explains the known biochemical and physiological changes that occur within the leaf cells after O_3 entry into the plants and how these chemical responses affect plant vigor, growth, and reproduction. Factors within and external to plants influence their response to O_3 and other stresses. These factors, as observed during experimental exposures and in the field, can modify functional growth responses of plants to O_3 (see Section 5.4). The development of indices or exposure statistics that may be used in quantifying and predicting crop responses to O_3 exposures are found in Section 5.5. Data obtained from many experimental exposure-response studies using methodologies presented in Section 5.2 and the basis for the development of the indices discussed in Section 5.5 are presented in Section 5.6. The information available on the ecosystem effects of O_3 and the data needed for more definitive assessments are found in Section 5.7. The costs to the nation of O_3 exposure of crops and ecosystems is discussed in Section 5.8. The scientific names of the plants cited in this chapter are presented in Appendix B. Section 5.10 discusses the effects of O_3 on nonbiological materials.

5.2 Methodologies Used in Vegetation Research

5.2.1 Fumigation Systems

The methodologies used in vegetation research have become more sophisticated over the years as new technology has developed. New exposure systems have been devised with pollutant dispensing systems that make it possible to more nearly duplicate the exposures plants receive in the field. These systems and their good points and shortcomings are discussed below.

Ozone fumigation plant-response studies require the fumigation of well-characterized vegetation to varying O_3 regimes. The variation in O_3 regimes may be achieved by controlled fumigation, chemical/mechanical exclusion or natural gradients of O_3. Controlled O_3 fumigation systems are designed to maintain a modified gaseous atmosphere around a plant for a period of exposure, for the purpose of monitoring plant responses to that modified gaseous atmosphere. All fumigation systems share some common features: general plant growth conditions (light, temperature, humidity, CO_2, and soil moisture) must be met, and differential concentrations of O_3 generated either artificially or naturally must be supplied to the vegetation and maintained during the exposure period. Exposure systems have been established in controlled environments, greenhouses, and the field. Many of these were described in the earlier criteria document, Air Quality Criteria for Ozone and Other Photochemical Oxidants (U.S. Environmental Protection Agency, 1986). More recent reviews of wet and dry deposition exposure systems have refined the knowledge of the strengths and limitations of experimental approaches for studying the effects of O_3, alone or in combination with other pollutants, on crops and trees (Hogsett et al., 1987a,b; Grünhage and Jäger, 1994a; Manning and Krupa, 1992). Controlled fumigation systems may range from cuvettes, which enclose leaves or branches (Bingham and Coyne, 1977; Legge et al., 1978), to a series of tubes with calibrated orifices spatially distributed over a field to emit gaseous pollutants to a plant canopy (Lee et al., 1978). Systems that exclude O_3 by mechanical or chemical means have been used, as have natural gradients of O_3, to evaluate vegetation response to ambient O_3.

5.2.1.1 Methodologies Discussed in the Air Quality Criteria for Ozone and Other Photochemical Oxidants (U.S. Environmental Protection Agency, 1986)

Controlled Environment Exposure Systems

Controlled environment fumigation systems are those in which light sources and control of temperature and relative humidity are artificial. Light quality and quantity are likely to be lower than in ambient environments, usually resulting in lower photosynthetically active radiation (PAR). Temperature and relative humidity likely will be more consistent in a controlled environment than in ambient air. Controlled environment exposure systems are typified by the widely used continuous stirred tank reactor (CSTR), a system originally designed for mass balance studies of O_3 flux to vegetation. The CSTR chambers have distinct advantages for gas exchange studies because fluxes can be calculated readily when controlling for environmental and pollutant conditions. The rapid air mixing minimizes horizontal and vertical gradients within chambers as well as leaf boundary layer resistance. Disadvantages of CSTR chambers include the following: the artificial pollution and growing conditions may not represent natural exposure conditions, the rapid air movement may cause wind injury to sensitive plants, the size of chambers restricts the study of large plants, and lighting systems are problematic and provide subambient levels of PAR. Although CSTR

chambers are useful for evaluating O_3 effects on physiological processes, it is not possible to extrapolate the data to field situations.

Greenhouse system designs are similar to those found in controlled environments, except that light, temperature, and relative humidity conditions fluctuate with those occurring in the greenhouse. Thus, greenhouse system designs are related more closely to field studies than are controlled environments, but plant culture and environmental conditions are still quite different from those of field exposure chambers, making direct extrapolation difficult. These studies are, however, more applicable to phytotoxicity of O_3 to greenhouse grown ornamental and floriculture crops (U.S. Environmental Protection Agency, 1986). Some greenhouse exposure systems use activated charcoal filtration to remove pollutants from the incoming air prior to the addition of experimental O_3 and either vent directly to the outside or use charcoal filtration of the outgoing air to prevent contamination of the greenhouse air supply. Other greenhouse exposure systems filter neither incoming nor outgoing air.

Field Exposure Systems

Fumigation of plants with O_3 in the field is most frequently carried out using open-top chambers (OTCs). There are many designs, each produces an environment that differs in some degree from the ambient air (Unsworth et al., 1984a,b). The most widely utilized design (U.S. Environmental Protection Agency, 1986) consists of a cylindrical aluminum frame, covered with transparent film. The bottom half of the transparent covering is double layered, with the inside panel perforated. Charcoal- and particulate-filtered air, nonfiltered air, or O_3-supplemented air is blown into the bottom layer, forced through the perforations into the plant canopy, and then escapes through the top of the chamber. The positive pressure maintained by the forced movement of air up through the chamber minimizes influx of ambient air into the chamber through the open top. The design of these chambers has been modified with frusta to reduce such incursions by ambient air, making the chambers more viable under windy conditions. Moveable canopies have been added so that rain exclusion studies can be carried out. Finally, these chambers have been modified in shape or increased in size so that species such as mature trees and grapevines can be enclosed. The OTC exposure system was employed in the National Crop Loss Assessment Network (NCLAN) from 1980 to 1988, and a description and discussion of the chambers is provided in Section 6.2.4 of the 1986 criteria document (U.S. Environmental Protection Agency, 1986).

The main advantage of OTCs is the ability to provide an enclosed environmental area for an increased range of treatments at near-ambient environmental conditions, while excluding ambient pollutants. Most current OTC designs have been used widely and successfully for studying the impact of O_3 on crops over a growing season (e.g., NCLAN program), but have diameters and heights that limit their use for larger plants. Although the OTCs provide for the least amount of environmental modification of any outdoor chamber, the OTC still may alter the microclimate sufficiently to have a significant effect on plant growth under pollutant stress. The OTC effects on the microclimate include reductions in light intensity, wind velocity, rainfall, and dew formation and persistence, and increases in air temperature and possibly relative humidity (Hogsett et al., 1987a; Heagle et al., 1988a; McLeod and Baker, 1988; Heck et al., 1994). For plants taller than 120 cm, there is more air movement near the bottom of the plant canopy than near the top during calm periods (Heagle et al., 1979c; Weinstock et al., 1982).

Exhaustive comparisons have been made among plants grown in carbon-filtered (CF) chambers, NF chambers, and similarly sized and located ambient air (AA) plots. Much attention has been paid to the potential for differences in productivity between AA and NF plants because of the modification of microclimate in OTCs (Manning and Krupa, 1992). For NCLAN studies, plants in NF chambers were frequently taller than AA plants (Albaugh et al., 1992; Olszyk et al., 1980; Heagle et al., 1979b). However, height was the only variable that was consistently different between AA and NF (Heagle et al., 1988a). Krupa et al. (1994) demonstrated that of 73 comparisons between NF and AA plants (NCLAN data), 56 showed no statistical significance, due either to lack of chamber effect or to random compensation. A more relevant question, whether OTCs change plant response to O_3, has been addressed. A comparison of plant growth and plant response to O_3 exposure in OTCs, closed-top chambers, and air-exclusion systems has been carried out (Olszyk et al., 1986a). The authors discovered that there was interaction between plant response to O_3 and type of exposure system for less than 10% of the growth parameters measured in California, suggesting that plant response to O_3 was the same regardless of exposure system. Plants from exclusion systems were shorter than those grown in OTCs and generally weighed more. Of the three groups of plants, those in the control plots of the exclusion system (i.e., receiving ambient O_3exposure) were most similar in size to plants grown in field plots. Although this and another study (Olszyk et al., 1992) indicate that environmental modification caused by chambers will affect plant growth and yield, there is no evidence that there is a large effect of chambers on plant response to O_3. It is assumed that, because of the decreasing relative effects on plant environment caused by controlled environment, greenhouse, closed-top field chambers, OTCs, open-air systems, and ambient gradients, the system effects on plant response to O_3 will decrease in the same order. Microclimatic differences within an OTC can cause significant differences in yield, but rarely were there significant interactions between position effect and plant response to O_3 (Heagle et al., 1989a).

Considerable concern has been raised about plant response to trace pollutants in OTCs, specifically nitrogen pentoxide (N_2O_5) and nitric oxide (NO) in chambers receiving O_3 generated from dry air, and NO_2 in chambers receiving AA. These trace pollutants may have a direct effect (positive or negative) on plant processes or may change how plants respond to O_3, and, without careful evaluation, these effects may go undistinguished from those of O_3. A comparison of alfalfa (*Medicago sativa*) response to the same O_3 exposure, generated either electrostatically from air or through nonfiltration of AA, indicated that the generated O_3 treatment was more phytotoxic than the ambient O_3 treatment, probably due to the co-generation of N_2O_5 and NO, along with O_3 from dry air (Olszyk et al., 1990a). Open-top chamber studies that use filtered versus NF ambient O_3 have been proposed to avoid the problems of generating O_3. The drawback of this or any two treatment approaches is that such plant responses to low ambient levels of O_3, such as might occur in many years, is quite subtle. To detect statistically significant differences between filtered- and NF-chamber-grown plants when responses are subtle requires a high number of replications (Rawlings et al., 1988a). This fact is illustrated in Heagle's own two-chamber work; as described in Heagle (1989), some of the two-chamber studies had differences between AA and NF of greater than 10%. Such large differences reduce the number of replications needed to detect a significant difference at $p = 0.05$. In any event, the differences either were not treated nor tested, or were tested but were not significant, except in one case at

Beltsville, MD, with soybean (*Glycine max*). Heagle (1989) discussed the calculation of power and reviewed two-chamber studies in great detail.

Limited use (for O_3 studies) has been made of chamberless field exposure systems, which rely on ambient wind conditions to move O_3 across an open-field canopy. The O_3 is emitted from vertical pipes, which are spaced in a circle around the experimental plot of plants. The amount of O_3 emitted from each vertical pipe, as well as the number and compass direction of emitting pipes, depends on the wind direction and speed; this whole process is usually being computer controlled.

5.2.1.2 Methodologies Referenced Since the Air Quality Criteria for Ozone and Other Photochemical Oxidants (U.S. Environmental Protection Agency, 1986)

Branch and Leaf Chambers

Most of the developments in exposure systems since 1986 have been modifications of existing systems. The tremendous interest in evaluation of mature tree response to O_3 has prompted the development of large branch chambers for estimating O_3 flux to trees. These branch chambers share many of the design characteristics of a CSTR. The chamber walls are transparent film spread over a supporting frame. There is a fan to reduce boundary layer resistance across the foliar surface, and an air inlet and outlet so that differential O_3, CO_2 (photosynthesis), and water vapor (leaf diffusive resistance) measurements can be taken (Ennis et al., 1990; Houpis et al., 1991; Teskey et al., 1991). The advantages of this system include the ease with which the Teflon® bag can be replaced; uniform light transmission can be maintained; and the branch chamber can be moved from plant-to-plant, can be used in situ, and can be modified for different sized branches. One of the disadvantages of the branch chamber, and indeed of any such cuvette that isolates one part of the plant under different environmental conditions than the rest of the plant, is that the isolation may lead to a response different from that which would have been observed if the branch was under the same environmental conditions as the rest of the plant. In addition, total tree growth cannot be estimated using branch chambers because only part of the plant is treated with O_3.

Flux Measurement

Estimation of O_3 flux to foliage can be made directly by measuring the difference in O_3 concentration between air going into a leaf chamber and the same air stream exiting the chamber after passing over the leaf. This estimation also can be inferred from measurements of leaf diffusive resistance during exposure of a leaf to O_3. The former method requires a chamber or cuvette fumigation system with uptake of O_3 that is quite small or extremely nonvariable relative to the amount being taken up by the leaf. Otherwise, it is difficult to detect O_3 flux to a leaf with good precision. Such cuvettes can be adapted from those commercially available for portable photosynthesis meters (Graham and Ormrod, 1989) or constructed from a novel design, such as that developed by Fuentes and Gillespie (1992) to estimate the effect of leaf surface wetness on O_3 uptake of maple leaves. The criteria for flux cuvette design include good light transmissibility, ease of leaf manipulation, minimal reaction of chamber wall surface with O_3, and good air mixing within the chamber. Good mixing of air is necessary to avoid a gradient in pollutant concentration and to maintain a boundary layer resistance, which is much less than stomatal resistance. Maintenance of leaf temperature close to that of the surrounding air, so that transpiration rates are not abnormally high, is another benefit of good air mixing. The physical design of the Fuentes and Gillespie chamber was simple, consisting of two glass hemispheres that were clamped together and

separated by a Teflon® O-ring over the petiole of the leaf under investigation. Inlet and outlet air attachments were on opposite sides of the cuvette. Other cuvette designs have been used to estimate leaf gas-exchange responses to O_3; their principals of operation are similar, but there are differences in materials and design (Amiro et al., 1984; Freer-Smith and Dobson, 1989; Laisk et al., 1989; Moldau et al., 1991a; Skarby et al., 1987).

Compared to the CSTR, which has been used for mass balance measurement of gas flux by whole plants during fumigation (Le Sueur-Brymer and Ormrod, 1984), cuvette systems usually determine flux to one leaf at a time. This results in a more precise understanding of the interaction among leaf age, diffusive resistance, illumination and O_3 flux. However, these data are not particularly well adapted to estimating flux of O_3 to a large vegetated surface. Finally, regardless of the methodology used to determine O_3 flux to foliage, there exist only very sketchy mechanistic-process models that would link O_3 fluxes to decreases in growth and productivity of plants. These data primarily are useful for developing a relationship between *internal* O_3 dose and plant response and in estimating the strength of vegetation as sinks for O_3 flux on a large scale. Recent studies have estimated fluxes of O_3 to plant canopies by indirect methods. Ozone flux to oat (*Avena sativa*) in OTCs (using mass balance principles and a resistance analogue model) was compared to that for oat growing in the field, using an aerodynamic gradient method (Pleijel et al., 1994). Vertical flux density calculations for O_3 uptake by grassland vegetation (O_3 based on radiometric measurements) estimated exchange between the atmosphere close to the ground and the ecosystem (Grünhage et al., 1994; Dämmgen et al., 1994). Although fluxes of O_3 to vegetation cannot imply growth or O_3 physiological responses, techniques such as these can suggest whether plant responses to O_3 in OTCs might differ from those in ambient field culture because of micrometeorological-induced differences in O_3 flux.

Pollutant-Dispensing Systems

Although exposure chambers have changed little in design in the last several years, the profile characteristics and method of dispensing pollutant profiles have. Whereas early studies utilized static or square-wave exposures, usually controlled by hand-set flowmeters, many more recent systems expose plants with so-called dynamic exposures during which the O_3 concentration gradually reaches a maximum, thus simulating diurnal variation in O_3 concentration (Hogsett et al., 1985a). These profiles may be achieved by mass flow controllers that are themselves computer controlled. Proportional-add systems such as that used in NCLAN usually achieve ambient type profiles using rotameters instead of mass flow controllers. The O_3 concentration in each of the chambers is logged at preset intervals, so that the integrated exposure for the entire fumigation period can be calculated. Deviations from the planned O_3 episode can occur, due to failure in dispensing or monitoring equipment, as well as incursions of air through the tops of the chambers. The length of the interval between determinations of O_3 concentration in the chambers can be an important contribution to the control of O_3 profile. In general, longer intervals lead to less well-controlled and well-characterized O_3 exposure profiles (Lefohn et al., 1993). These deviations from the expected profiles can be mathematically quantified and monitored among treatments and replications (Hale-Marie et al., 1991).

Open-Air Field-Fumigation Systems

Open-air field-fumigation systems have the potential to estimate most closely field losses due to O_3, as the plants are grown and exposed under ambient field environmental

conditions. However, of all the fumigation systems, this is the least controllable and repeatable. It has been used in the past to expose plants to "static" concentrations (i.e., desired concentration is the same throughout the exposure period) of such pollutants as sulphur dioxide (SO_2) or hydrogen fluoride (HF) (Hogsett et al., 1987a). The Zonal Air Pollution System (ZAPS) has been modified vastly and improved on to enable fumigation of plants with a diurnally varying pattern of concentration (Runeckles et al., 1990). The system represents a significant advancement over earlier open-air field fumigation systems in that 12 discrete seasonal treatments that simulate ambient patterns are achieved, rather than the usual two or three. Ozone was supplied to 4-m plots, which were laid out in groups of four, through a manifold suspended over the plant canopy. The wind speed and direction determined the actual seasonal O_3 exposures, although the O_3 was released in concentrations proportional to that observed at the time in the ambient environment. Although the 12 treatments are not repeatable over time, a regression relationship between pollutant exposure and plant response can be established for each growing season.

The Liphook study in England of long-term responses of *Picea sitchensis*, *Picea abies*, and *Pinus sylvestris* to SO_2 and O_3 in combination consisted of seven growth plots, 50 m in diameter, five of which were surrounded by 64 vertical pipes from which pollutant gasses were emitted (McLeod et al., 1992). The 64 pipes were divided into four quadrants of 16 adjacent pipes, and each quadrant had diluted pollutant gases supplied to it from a computer controlled mass flow controller. The emitting quadrants, as well as the rate at which the gases were supplied to the quadrants, depended on wind speed and direction. The gases were emitted from the vertical pipes into the plant canopy at two heights, 0.5 and 2.5 m above a reference height, which was approximately two-thirds of tree height. This pattern of gas dispersion resulted in a uniform horizontal distribution of hourly mean gas concentration across each central 25-m diameter experimental plot. This exposure system, like all open-air exposure systems, clearly simulates field plant growth conditions far better than open- or closed-top chambers, and, with five enclosures and two nonenclosed ambient plots, this is by far the largest of the very few of these systems that are in operation. Measured over a winter wheat canopy, SO_2 concentration differed by less than 1 $nL.L^{-1}$ over a 5-h period of measurement; measurement of consecutive 2-min mean values at five locations across the plots demonstrated high uniformity (McLeod et al., 1985). The usefulness of the data is limited, however, by the low number of treatments and lack of replication of those treatments.

Field Chamber Exposure Systems

Open-top field chambers are used in most field studies of plant response to gaseous pollutants. The OTCs first were designed for studies on annual herbaceous crop plants (Mandl et al., 1973), but enlarged versions also have been used successfully in tree seedling and sapling studies (Adams et al., 1990a,b; Chappelka et al., 1990; Qiu et al., 1992; Kress et al., 1992; Hogsett et al., 1989; Andersen et al., 1991; Karnosky et al., 1992a,b; Wang et al., 1986a,b; Temple et al., 1992). Because the results from these studies using tree species are extrapolated to predict the effects of O_3 on forests, these studies require good exposure control in order to replicate ambient O_3 profiles characteristic of many low-elevation, rural areas of eastern North America. This condition could have been met using an open-field exposure system. Open-top chambers large enough for mature trees have been developed but are expensive (Mandl et al., 1989; Albaugh et al., 1992).

Microclimatic modification by OTCs, as well as O_3 exposure schedules that are disconnected from typical O_3 episode meteorology, have been addressed in a seasonal study of tree response to O_3 in the United Kingdom (Wiltshire et al., 1992). This study uses OTCs with roll-up sides, but, except for fumigation days, the plants are maintained in ambient climatic conditions. The exposure episodes number between 27 and 30 throughout the growing season and occur on days with ambient meteorology associated with naturally occurring O_3 episodes (i.e., high incident radiation and temperature, with little air movement) (Wiltshire et al., 1992). The maintenance of near-ambient meteorological conditions during both growth and exposure periods is an effort to make this study better represent field-grown plant responses to O_3, while maintaining control of O_3 exposure.

Several designs of field fumigation chambers have been developed to overcome some of the disadvantages of the OTCs, namely small plot size and incursion of ambient air. Closed-top chambers first were developed in the 1950s; generally, their use diminished in favor of OTCs. However, closed-top chambers smaller in dimension than the open-top design have been constructed more recently in California to assess crop loss to O_3. Closed-top chambers were chosen because the authors wished to characterize the pollutant dose to the plants very precisely; pollutant gradients within the chamber were minimal (Musselman et al., 1986a). The chambers were octagonal in shape and covered with Teflon® film; the soil was completely replaced with standard greenhouse mix. Temperatures in the chamber were higher (2 to 4 °C at midday, 1 to 2 °C at night) than in the ambient air, and light levels were reduced by 11% (spectral quality of the light in the chambers was not reported). The authors concluded that, although the chambers were not suitable for studies destined for extrapolation to plant response under field conditions, the chambers were very useful when tight control over soil moisture and pollutant concentration was needed.

Closed-top chambers were constructed and installed in the United Kingdom to study responses of shrubs and large herbaceous species to long-term, low (chronic) concentrations of SO_2, NO_2, and O_3 (Rafarel and Ashenden, 1991). These chambers were a smaller version of an earlier design, because the larger chambers required pure gas sources of NO_2 and SO_2 to be diluted into the ventilating air stream, which resulted in highly variable exposure concentrations. The flow rate of the smaller chambers meant that premixed gases were sufficient to maintain steady control of treatment concentrations. Because the gases were discharged from the source at constant concentrations, different treatments were achieved by placing one or more pollutant supply tubes in the fumigation chambers. Good air circulation and moderate ambient temperatures maintained the chambers at near ambient conditions; however, results cannot be extrapolated to predict plant response to O_3 under ambient air conditions.

Ambient Gradients for Evaluation of Plant Response to Ozone

The exposure system that utilizes ambient conditions of O_3 exposure, temperature, humidity, soils, and soil moisture is the ambient gradient system. By this method, plants are grown along a transect of known differential pollutant concentrations, usually downwind of a major point source or urban area. The concentration of pollutants is diluted as distance from the source increases. The most well-defined O_3 gradients exist in the Southern California Air Basin and have been used in studies by Oshima et al. (1976, 1977a,b); unfortunately, outside this region, few suitable gradients exist. A study using four different cultivars of red clover (*Trifolium praetense*) and spring barley (*Hordeum vulgare*), each differing in sensitivity to SO_2, NO_2, and O_3, was conducted along such a transect of gradient SO_2, NO_2,

and O_3 concentrations in the United Kingdom (Ashmore et al., 1988). Ozone concentration was inferred from injury to Bel W3 and Bel B cultivars of tobacco but was found to have very little relationship to cultivar performance. The authors cautioned that these results must be interpreted with an understanding that differences among sites in other environmental parameters could contribute to the detection of (or the failure to detect) O_3 effects on the crops. For ambient gradient studies to be interpretable, good characterization of site parameters (rainfall, temperature, radiation, and soil type) is needed. Additionally, the modeler needs to know how these factors should be used to adjust the apparent plant response. In order to know that, a good knowledge base is needed of how all of these factors modify plant response to O_3.

Although Manning and Krupa (1992) assert that natural gradients are the "ideal way to conduct O_3/plant response studies in ambient air in field plots," they concede that few gradients that meet statistical requirements for intermediate O_3 concentrations exist outside Southern California. It is possible, however, that more gradients will be identified as rural air monitoring increases. They also concede that, although using artificial soils removes a significant source of variation in plant response to O_3, pot-grown plants do not closely simulate the rooting environment found in the field (Manning and Krupa, 1992). Although plants using gradients are commonly considered to be easily replicable in large numbers, they should probably be considered as "repeats" rather than "replicates" in the conventional sense. If treatments are replicated by locating them very close together at the same location in the gradient, then they may better be considered as "sub-samples" of one replicate, if the climatic and edaphic conditions are very similar, or as repeats of a study, if the conditions are not. This argument is not just semantic; in data analysis, repeats and replicates should be handled differently, because the sum of squares for repeats is likely much larger than for replicates and may be composed significantly of plant response factors other than O_3 concentration.

At this time, although some information is available, the relationships still are incompletely understood. Many investigators consider that ambient gradients are impossible to find without major differences in environmental conditions that may affect plant response to O_3 and, therefore, confound interpretation of the results.

Cultivar Comparisons

The comparison of isogenic lines of a particular species that differ only in their tolerance to O_3 is "the ideal way to determine the effect of ambient O_3 on plants in the field ...." (Manning and Krupa, 1992). Heagle et al. (1994) report on the use of a white clover (*Trifolium repens* L.) system to estimate the effects of O_3 on plants. A field experiment conducted in 1984 and 1985 using white clover revealed a wide range of sensitivity among the genotypes present in the commercial line "Regal" (Heagle et al., 1991a, 1993). Plants were screened for relative sensitivity to O_3. Two clones were selected: one ozone-sensitive (NC-S) and another ozone-resistant (NC-R). Subsequent studies suggested that these clones could be useful as indicators of O_3 sensitivity, if they routinely displayed measurable differences in response to O_3, while responding similarly to other factors (e.g., biotic, climatic, soil, chemical, and other pollutants). Experimentation indicated that the white clover system can be used to indicate where and when ambient O_3 concentrations cause foliar injury and decrease growth. Hence, it can be inferred that other plant species sensitive to O_3 also may be affected (Heagle et al., 1994).

Protective Chemicals

Chemicals that protect plants from O_3 have been in use since the 1970s to evaluate plant response to O_3. Ethylene diurea (EDU) has been used in studies to modify the O_3 sensitivity of several species (see Section 5.4.7). Ethylene diurea (and perhaps other undetermined chemicals) has potential as a tool to evaluate field crop losses to O_3 in the absence of chambers, with their inherent modification of microclimate. A low-cost, simple technique, EDU can be applied to larger plot sizes than currently are possible with OTCs, thus reducing some of the uncertainty of extrapolating experimental results to a large scale. Field protocols for the use of EDU have not been well established. Frequency and rate of application that protects plants vary with species and edaphic and atmospheric conditions. Depending on the method of application, EDU may have little effect on field-grown plant response to O_3 (Kostka-Rick and Manning, 1993). The basis for the year-to-year variation in degree of protection of plants by EDU is not well understood, so drawing conclusions from multi-year studies, which is the situation most relevant to evaluation of plant community responses to ambient O_3, is difficult. Two-treatment studies of EDU and plant response to O_3 (Kostka-Rick and Manning, 1992a,b) indicate that protection is variable, suggesting that the experimental system under investigation (soil, plant, and climate) would have to be extremely well characterized and understood for interpretation of EDU studies to be complete. Manning and Krupa (1992) point out that EDU is probably more useful in conjunction with OTCs so that a factorial range of O_3 can be administered to the plants. It is not clear that EDU protection can be fine-tuned sufficiently into a range of discrete levels suitable for regression analysis (Kostka-Rick and Manning, 1993). The mechanism by which EDU protects plants, beyond being a systemic antioxidant, is unknown; understanding this mechanism has the potential to contribute to the broader understanding of the mechanisms of O_3 injury at the cellular/metabolic level of the plant.

5.2.2 Experimental Design and Data Analysis

Experimental design strategies, including the number, kind, and levels of pollution exposure; patterns of randomization; number of replicates; and experimental protocol are crucial to the ability of the statistical approaches to test and model the effects of O_3 on plant response and to extrapolate experimental results to real world conditions. The experimental design focuses an experiment on the specific objectives of the study and, so, may limit the application of the data to other research goals. The various experimental design and analyses for exposure-response data from controlled exposure studies have been well reviewed in the 1986 criteria document (U.S. Environmental Protection Agency, 1986) and will not be repeated here. In summary, most field studies involving OTCs have used randomized block or split-plot designs and pollution levels appropriate for regression analysis. These exposure-response relationships generalize the mathematical relationship between the plant parameter of interest and O_3 exposure. Plant response to concentrations other than those used in the experiment can be interpolated from these relationships, and thresholds of plant response can be determined (Ormrod et al., 1988). In the latter half of the NCLAN program, the Weibull model was chosen to characterize yield response to O_3 because of its flexibility to describe a wide range of data patterns (Rawlings and Cure, 1985) and, consequently, to allow a common model to be fit when pooling data across years and sites (Lesser et al., 1990).

Experimental designs for exposure-response relationships can be expanded easily so that plant response to O_3 and another factor at multiple levels can be determined. Because

of the need to contain each O_3 treatment by a chamber, incomplete factorial designs are more efficient approaches to multi-factor studies, leading to exposure-response surfaces (Allen et al., 1987). Choosing the appropriate incomplete factorial design for a response surface study requires forethought on whether all areas of the surface are of equal interest. For many O_3 plant response studies, this is not so because extremely high concentrations, although increasing the precision with which plant response to lower concentrations is estimated, are not as likely to occur in the ambient environment (see Chapter 4).

Because the U.S. Environmental Protection Agency (1986) decided to place greater emphasis on damage (i.e., effects that reduce the intended human use of the plant) than on injury, studies more frequently have used experimental designs that generate data suitable for regression and treatment mean separation analyses for the purposes of modeling and testing the impact of O_3 on plant response. Although the impact at current O_3 levels is of primary interest and can be studied effectively using two O_3 levels generated by CF and NF treatments, the development of exposure-response models necessitates the use of additional treatments at above ambient concentrations (Heagle et al., 1989a; Rawlings et al., 1988a). The optimal number, range, and spacing of treatment levels depends on the anticipated exposure-response model, but, in the case of the Weibull and polynomial models, greater precision for estimation of relative yield loss at ambient O_3 concentration is obtained when the lowest treatment level is near zero and the highest treatment level is well above the ambient concentration. For the Weibull model, the highest treatment should correspond to a concentration for which yield loss is at least 63% of the yield at zero exposure (Dassel and Rawlings, 1988; Rawlings et al., 1988b).

When studying the impact of mixtures of pollutants on plant processes in chambers, response surfaces can be generated from complete or incomplete factorial designs. These designs have been shown to increase the precision and efficiency of estimating relative yield loss at ambient concentrations (Allen et al., 1987). The optimal design cannot be specified a priori and necessitates the use of treatment levels from near zero to well above the ambient concentration for each pollutant. However, response surface designs have not been used widely in pollutant mixture studies, nor have these designs been used extensively to study the interaction between pollutant exposure and quantitative environmental parameters, such as light, temperature, and soil moisture. The interaction between O_3 and phytotoxic concentrations of other pollutants, in particular SO_2, has not been studied extensively because instances of co-occurrence of O_3 and other pollutants are not common in the United States. An analysis of pollution monitoring data showed fewer than 10 periods of co-occurrence between O_3 and phytotoxic concentrations of SO_2 during the growing season at the sites where the two pollutants were monitored (Lefohn and Tingey, 1984; U.S. Environmental Protection Agency, 1986).

Design and analysis of pollutant effects studies have used various characterizations of exposure to determine optimum spacings of treatment levels and to relate exposure to response. Most notably, the daytime mean concentration index (i.e., either M7 or M12) was adopted by the NCLAN program to determine the effects of O_3 on plant response. However, there has been considerable debate over the use of the mean index in exposure-response modeling; the variety of ways to compute the characterizations of plant exposure will be discussed in Section 5.5. When plant yield is considered, plant response is affected by the concentration of exposure and by other exposure-dynamic factors (e.g., duration, frequency, threshold, respite time), in combination with physiological, biochemical, and environmental factors that may mask treatment effects over the growing period. Research goals to

understand the importance of exposure dynamic factors have utilized experimental designs that apply two or more different patterns of exposure that are equal on some scaling (e.g., total exposure). Experiments designed specifically to address the importance of components of exposure may require the use of exposure regimes that are not typical of the ambient environment.

The majority of chambered field studies use regression-based designs that focus on developing exposure-response models but have limited application for testing the importance of exposure dynamics (e.g., exposure duration) for evaluating exposure indices based on statistical fit. When data from replicate studies of equal or varying duration are available, the ability to test for duration effects on plant response may be enhanced using regression analysis to combine data. The regression approach has been used to fit a common model to combined data from replicate studies of the same species when it is reasonable to assume that the primary cause of biological response is pollutant exposure, and that differences in environmental, edaphic, or agronomic conditions among sites do not significantly change the shape of the regression relationships. When pooling data across sites and years, additional terms for site and year effects often are included in the model as either fixed or random components, depending on the population of interest. Inferences over random environments implies that the environments sampled by the experiments are representative of the population of regions of interest under a variety of environmental conditions. In this case, site and year effects are incorporated as random components when fitting a common model. The appropriate analysis is to use a mixed model to fit an exposure response model with variance components. This analysis has been used recently to combine data from replicate studies of varying durations to test the importance of length of exposure in influencing plant response (see Section 5.5).

5.2.3 Mechanistic Process Models

In addition to regression type models of plant response to O_3, which are empirical and statistical in nature, there are mechanistic-process models (Luxmoore, 1988; Kickert and Krupa, 1991; Weinstein et al., 1991). The key difference between these two types of models is how the changes are handled in the dependent variable over time. Empirical models treat a time period (e.g., a growing season) as a single point and report the response of the dependent variable as a single point as well. Regression models also may oversimplify the characteristics of an O_3 exposure, in that the description of the O_3 exposure is compressed over time to a single number. The variety of ways to compute this single number will be discussed in Section 5.5.

Mechanistic-process models on the other hand describe the rate of change of a variable in response to the treatment (such as O_3) with change in time. The latter type of model has the potential to capture the interaction among plant age or stage of development, variability of ambient exposure concentrations, and plant response to O_3. For this reason, mechanistic-process models have been rated much more highly than regression models for their realism, scientific value, and applicability to other locations (Kickert and Krupa, 1991). However, compared to regression models, mechanistic-process models require more input data, and the input data are less accessible. The mechanistic-process models are more complex than regression models, requiring more computer time and memory to develop. The precision of the output regression models is greater than mechanistic-process models (for interpolative examinations only), as is their ability to estimate response probabilities. The

authors conclude that the popularity of single-equation, time-lumped models is related to the fact that the studies of plant responses to O_3 are oriented more to air quality standard setting as an endpoint, rather than the physiological processes underlying plant responses. The problems with process-based models are the necessity for some large assumptions (in place of real data) and the lack of validation. Without validation, using estimates from these models is questionable; if the estimations are used, then the uncertainties associated with them must be identified and quantified.

5.2.4 Summary

Each type of fumigation system is suited particularly well to certain types of studies of plant response to O_3; no one system is appropriate for all types of studies of plant responses to O_3 (Table 5-1). Each system has advantages and limitations that must be evaluated in terms of the research objectives that it was designed to meet. Table 5-1 lists the characteristics of the various exposure systems as they relate to experimental objectives, including simulation of field conditions, replication, range of treatment levels possible, and the ability to control extraneous environmental factors that may influence plant growth. Controlled-environment chambers are well suited for mechanistic type studies at the molecular or cellular level. Most plant cellular processes, as well as the equipment that measures them, are quite sensitive to temperature and light, so good control and definition of these factors are needed. Growth responses to O_3 determined from controlled-environment chamber studies cannot be extrapolated to the prediction of field losses to O_3 because the culture conditions in the two systems are just too dissimilar. Open-top chamber systems, although a compromise in ability to simulate field conditions, have major advantages over other fumigation systems for developing exposure-response functions (to develop a statistically robust surface requires at least three or, better yet, five treatment levels) because (1) a range of pollution levels at near-ambient environmental conditions can be generated to optimize the precision in empirical modeling; (2) extrapolation of experimental results to probable field responses to ambient exposure is possible to a certain extent because OTCs, although modifying microclimate, appear not to affect relative plant response to O_3; and (3) a semi-controlled environment is created for plant growth with only O_3 exposure level varied, thus it is valid to assume that the primary cause of response is due to O_3 exposure. Exclusion methods, particularly those using chemicals such as EDU, are the least disruptive of ambient culture conditions in the field, so these approaches most closely estimate real crop losses to O_3. However, their application is limited by the availability of ambient O_3 in any particular year or location, as well as by confounding by climatic and edaphic conditions. They are not well suited for establishing exposure-response relationships because it is difficult to quantify the degree of protection actually offered by the exclusion method in the field (Ashmore and Bell, 1994). In general, open-field exposure systems or natural gradients are not replicable, nor can a range of treatments be imposed to enable construction of a response function, which is necessary for interpolation of O_3 concentrations that cause plant response.

At the current time, OTCs represent the best technology for determination of crop yield responses to O_3; concentration and duration of the gas are well controlled, and the plants are grown under near-field-culture conditions. There are several limitations and uncertainties associated with the collected data: (1) the plot size is small relative to a field, (2) microclimate differences may influence plant sensitivity to O_3, and (3) air quality after

Table 5-1. Comparison of Fumigation Systems for Ozone Exposure-Plant Response Studies

Fumigation System	Simulation of Field Losses	Replication of Experimental Unit	Range of Treatment Levels	Likelihood of Extraneous Factors Affecting Response
Controlled-environment chambers	Low	Low	High	Medium
Greenhouse chambers	Low	Medium	High	Medium
Closed-top field chambers	Medium	High	High	Medium
Open-top field chambers	Medium to high	High	High	Medium
Mechanical field exclusion	High	Low to medium	Low	Medium
Open-field fumigation	High	Low	Low	High
Natural gradients	High	Low	Low	High

passage through a charcoal filter has not been widely characterized. These uncertainties are not quantified, although there are preliminary data establishing their existence. There is concern that these uncertainties are forgotten in the scaling of the plant response data to national yields and their integration into larger cost-benefit models. However, because the uncertainties are not yet quantified, they cannot be incorporated into the national estimates of losses to O_3. There is an urgent need to estimate these uncertainties so that the OTC data can be used fully, with little doubt as to how well the data represents real crop losses. Further comparisons of OTC and chemical exclusion plant responses, expanding the range of environmental conditions and species for which they are compared, would help determine the extent of the role of microclimate in modifying plant response to O_3. Large scale exclusion studies also could contribute to quantifying the uncertainty of extrapolating plot response to field scale. Analysis of the atmospheric chemistry inside OTCs under various scenarios of light, temperature, and humidity would address the question of what additional pollutants may influence plant growth or plant responses to O_3. Once these uncertainties are fully characterized and quantified, existing models of crop loss can be constructed more precisely and then incorporated into the national scale models with greater confidence.

5.3 Species Response/Mode of Action

5.3.1 Introduction

Plant adaptation to changing environmental factors or to stresses involves both short-term physiological responses and long-term physiological, structural, and morphological modifications. These changes help plants to minimize stress and to maximize the use of internal and external resources. A great deal of information is available on the physiology of single leaves; however, relatively little is known about whole-plant systems and whether the physiological mechanisms involved are initiated wholly within the leaf or are the result of whole-plant interactions (Dickson and Isebrands, 1991).

The many regulatory systems contained in leaves change both as a function of leaf development and in response to different environmental stresses. Leaves function as the major regulators of anatomical and morphological development of the shoot and control the allocation of carbohydrates to the whole plant (Dickson and Isebrands, 1991). This section discusses the movement of O_3 into plant leaves and what is known about their biochemical and physiological responses.

Movement of O_3 into plant leaves involves both a gas and a liquid phase. The phytotoxic effects of air pollution on plants appear only when sufficient concentrations of the gas diffuse into the leaf interior and pass into the liquid phase within the cells. Therefore, to modify or degrade cellular function, O_3 must diffuse in the gas-phase from the atmosphere surrounding the leaves through the stomata into the air spaces and enter into the cells after becoming dissolved in the water coating the cell walls (U.S. Environmental Protection Agency, 1986). The exact site or sites of action are not known. Biochemical pathways are closely interrelated, and sufficient knowledge of all the control and regulatory mechanisms does not exist (Heath, 1988). The previous criteria document summarized the overall processes controlling plant response to O_3.

> "The response of vascular plants to O_3 may be viewed as the culmination of a sequence of physical, biochemical, and physiological events. Ozone in the ambient air does not impair processes or performance, only the O_3 that

diffuses into the plant. An effect will occur only if a sufficient amount of O_3 reaches the sensitive cellular sites within the leaf. The O_3 diffuses from the ambient air into the leaf through the stomata, which can exert some control on O_3 uptake to the active sites within the leaf. Ozone injury will not occur if (1) the rate of O_3 uptake is sufficiently small that the plant is able to detoxify or metabolize O_3 or its metabolites; or (2) the plant is able to repair or compensate for the O_3 impacts (Tingey and Taylor, 1982). The uptake and movement of O_3 to the sensitive cellular sites are subject to various physiological and biochemical controls" (U.S. Environmental Protection Agency, 1986).

Responses to O_3 exposure that have been measured include reduced net CO_2 exchange rate (photosynthesis minus respiration), increased leaf/needle senescence, increased production of ethylene, and changes in allocation patterns. Overall understanding of the response of plants to O_3 has been refined since the last criteria document (U.S. Environmental Protection Agency, 1986). Increased emphasis has been placed on the response of the process of photosynthesis to O_3, on identification of detoxification mechanisms, and on changes in biomass (sugar and carbohydrate) allocation.

As indicated above, entry of O_3 into leaves involves the gas-phase external to the plant and the liquid-phase within the leaf cells. A precondition for O_3 to affect plant function is that it be absorbed into the tissues. Ozone uptake will be divided into two components: adsorption to surfaces and absorption into tissues. Adsorption will affect surface materials (e.g., cuticles) but have little direct affect on physiological processes, whereas O_3 absorption can affect physiological function if O_3 is not detoxified. In the following section, the processes that control movement of O_3 into the plant canopy and then into the leaf will be examined.

5.3.2 Ozone Uptake

Uptake of O_3 in a plant canopy is a complex process involving adsorption of O_3 to surfaces (stems, leaves, and soil) and absorption into tissues, primarily in the leaves (Figure 5-2). Movement of O_3 from the atmosphere to the leaf involves micrometeorological processes (especially wind) and the architecture of the canopy (including the leaves). Within the canopy, O_3 can be scavenged by chemicals in the atmosphere (Kotzias et al., 1990; Gäb et al., 1985; Becker et al., 1990; Yokouchi and Ambe, 1985; Bors et al., 1989; Hewitt et al., 1990); however, the products of these reactions themselves may be phytotoxic (Kotzias et al., 1990; Gäb et al., 1985; Becker et al., 1990; Hewitt et al., 1990). The extent to which these scavenging processes affect O_3 absorption by leaves is not well known. Uptake of O_3 by leaves is controlled, in large part, by the complex of microclimate and canopy architecture, which control movement of O_3 from the atmosphere to the leaf. Leaf conductance is determined by leaf boundary layer conductance and stomatal conductance. In this section, the theoretical and empirical studies on O_3 uptake at the canopy and leaf levels will be examined.

5.3.2.1 Ozone Uptake by Plant Canopies

Integration of O_3 uptake at the stand level requires attention to several levels of organization (Enders et al., 1992; Hosker and Lindberg, 1982) because uptake at this level

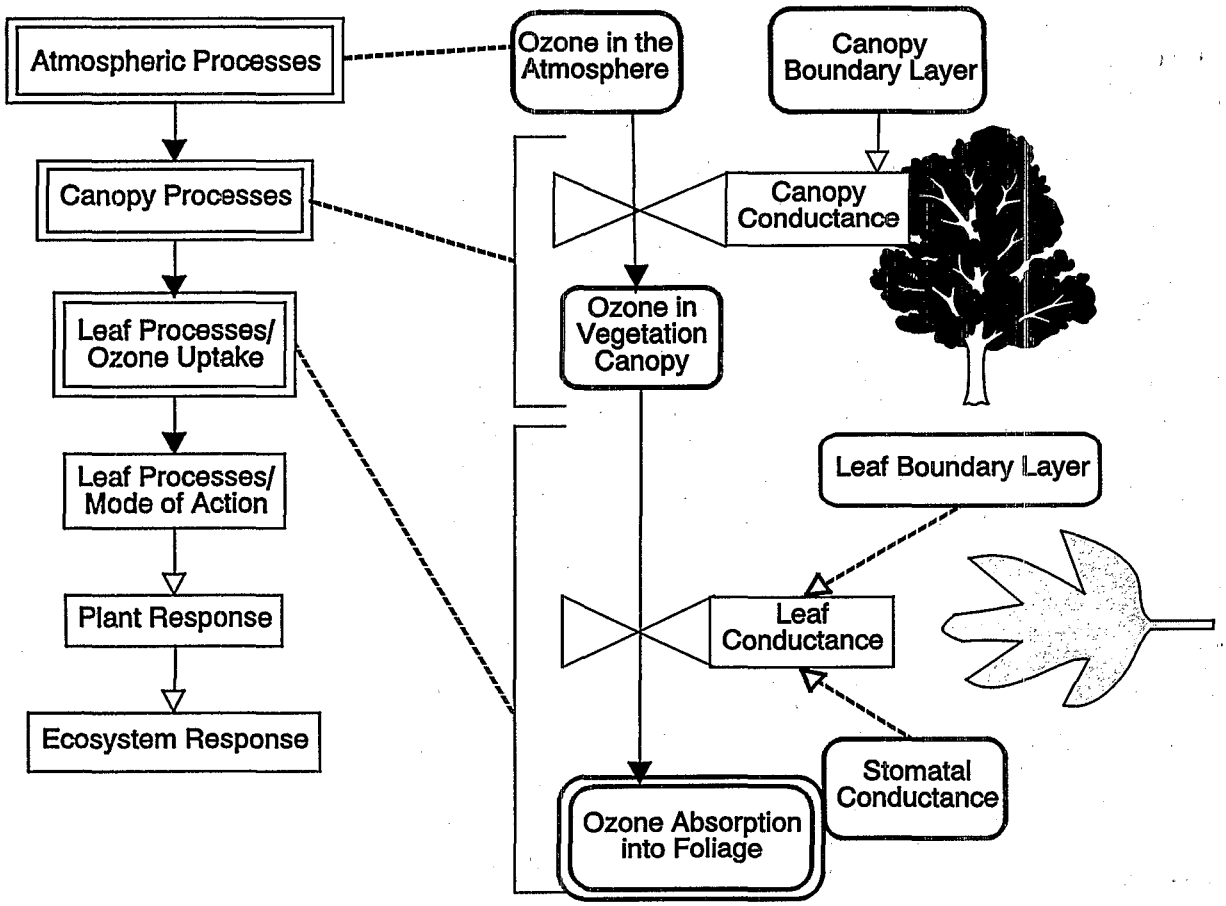

Figure 5-2. ***Uptake of ozone (O_3) from the atmosphere. Ozone is moved from the atmosphere above the canopy boundary layer into the canopy primarily by turbulent flow of air. Canopy conductance, controlled by the complexity of the canopy architecture and the wide distribution within the canopy, is a measure of the ease with which gases move into the canopy. Within the canopy, O_3 can be adsorbed by surfaces as well as being absorbed into the foliage. Foliage absorption is controlled by two conductances, leaf boundary layer and stomatal, which together determine leaf conductance. The solid black arrows denote O_3 flow; dotted arrows indicate processes affecting uptake or response to O_3. Boxes at the left with double borders are those processes described in the figure. The rounded box with a double border is the end of pathway on this figure.***

includes not only uptake by leaves but also adsorption by stems, the soil, and other structures with which O_3 can react. Although the actual pathway, and therefore conductance, will vary within the canopy, depending on position and wind profile, an integrated average conductance is frequently used to describe canopy conductance (Monteith and Unsworth, 1990). For most tree species, canopy conductance tends toward high values, whereas, for crops, it tends to be low.

Two general approaches have been used to estimate O_3 uptake by a plant stand: (1) measurement of gradients over the canopy using micrometeorological methods and (2) simulation of canopy conductance. The results of the two methods generally are different because the micrometeorological techniques include O_3 uptake by all surfaces, whereas simulation accounts only for O_3 absorbed by the surfaces simulated, primarily the foliage.

Two micrometeorological methods, (1) Bowen ratio and (2) eddy correlation, have been used to calculate canopy O_3 uptake. The Bowen ratio assumes a constant relationship between heat and water vapor fluxes (i.e., sensible and latent heat), then calculates O_3 uptake assuming a constant relation between water vapor and O_3 fluxes (Leuning et al., 1979a). The eddy correlation technique requires more elaborate instrumentation for measurement of variation in temperature, water vapor, and O_3 concentration over time and has stringent site requirements (Wesely et al., 1978).

Wesely et al. (1978), using eddy correlation, found a strong diurnal variation in the deposition velocity (the inverse of canopy conductance) and O_3 flux over a corn canopy. They also found evidence that 20 to 50% of the flux was to the soil and to the surface of the canopy. Ozone flux to a dead corn canopy also had a diurnal variation, but a lower magnitude, probably reflecting the absence of uptake through the stomata. Single time measures of deposition velocity, or canopy resistance, have been taken in a Gulf Coast pine forest (54 s m^{-1}; Lenschow et al., 1982) and in a New Jersey pine forest (120 and 300 s cm^{-1}; Greenhut, 1983). Ozone uptake in a maple forest varied diurnally in a pattern explainable by variation in leaf conductance and O_3 concentration (Fuentes et al., 1992). Ozone flux below the tree canopy at 10 m was about 10% of the flux above the canopy at 33 m. Measurements in specially constructed chambers showed that O_3 uptake, as well as photosynthesis, could occur when the foliage was wet (Fuentes and Gillespie, 1992). The fact that wet leaves could take up significant CO_2 is evidence that the stomata were not blocked by the water on the leaf surface. This result is counter to assumptions made in earlier work (Baldocchi et al., 1987) in which water on the surface of the leaf was presumed to interfere with O_3 uptake.

Simulation of canopy conductance requires scaling uptake from individual leaves to individual trees to that of a stand using a combination of canopy models (one for each species) and a stand model to handle interactions among individuals. Several assumptions are required for this approach: the primary sink for O_3 is the foliage, variation in stomatal conductance can be simulated through the canopy using either direct measurements or models, and canopy and plant models adequately simulate response when competition is occurring.

Leuning et al. (1979a,b) used a simple model to estimate canopy uptake in corn (*Zea mays*) and tobacco. Comparison of the results of these simulations with estimates using the Bowen ratio technique indicated that about 50% of the O_3 absorbed by the stands entered the leaves. Baldocchi et al. (1987) presented a model for canopy uptake of O_3 that incorporated stomatal function, some aspects of canopy architecture, and soil uptake. The results of the simulation of O_3 uptake by a corn canopy correlated well with estimations using the Bowen ratio, but tended to overestimate the magnitude. These authors point out that results of model simulation are quite sensitive to the assumptions used. As part of a series of simulations, Reich et al. (1990) explored the effects of different O_3 exposures (daily average O_3 concentrations of 0.035, 0.05, 0.065, and 0.080 ppm) on canopy carbon gain in a mixed oak-maple forest. Depending on the response function and O_3 exposure used, reductions in carbon gain were between 5 and 60%. An important result of these simulations

is that the effect of O_3 was strongest in the upper layer of the canopy, where most of the photosynthesis occurred. Although all these simulations provide some interesting insights into how O_3 uptake (and response) varies with time and exposure, data for validating the models are still needed.

Grünhage and Jäger (1994a,b), using information gathered from a micrometeorological study of O_3 flux observations above a natural grassland in Germany, developed a mathematical model to describe the flux and to estimate the potential injury to the grassland. The aim of the paper was to explain how both vertical flux and stomatal conductance changed during the day and influenced the uptake of air pollutants. For this reason, under ambient conditions, exposures cannot be expressed as a simple function of the pollutant concentration in air.

In summary, O_3 uptake (absorption to surfaces and absorption by tissues) by plant canopies has been measured only a few times. The results are consistent with the hypothesis that stomatal conductance plays a major role in the process. Modeling of O_3 absorption by leaves provides a means of assessing the understanding of the processes controlling O_3 absorption. Combining direct measurements over canopies with modeling will provide a means for assessing the dynamics of O_3 uptake in a canopy.

5.3.2.2 Ozone Absorption by Leaves

The importance of stomatal conductance for the regulation of O_3 uptake by a canopy has been hypothesized for some time (Heck et al., 1966; Rich et al., 1970). Uptake of O_3 by leaves is controlled primarily by stomatal conductance, which varies as a function of stomatal aperture (Figure 5-3). Kerstiens and Lendzian (1989) found that the permeability of cuticles by O_3 from several species was about 0.00001 that of open stomata. Movement of guard cells, which control stomatal opening, are affected by a variety of environmental and internal factors, including light, humidity, CO_2 concentration, and water status of the plant (Zeiger et al., 1987; Kearns and Assmann, 1993). Air pollutants, including O_3, also may affect stomatal function (U.S. Environmental Protection Agency, 1986). The pattern of diurnal stomatal conductance is produced by the integrated response of guard cells to a variety of factors.

As the primary "gate keepers" for gas exchange between the atmosphere and the leaf, stomata perform the vital function of controlling the movement of gases, including air pollutants such as O_3, to and from the leaf. The complexity of the response of stomata to environmental (microclimatic and edaphic) factors is indicated by the large amount of research on stomatal physiology and response to changing conditions (for reviews, see Zeiger et al., 1987; Schulze and Hall, 1982) and on developing models to simulate stomatal response (Avissar et al., 1985; Ball et al., 1987; Collatz et al., 1991; Eamus and Murray, 1991; Friend, 1991; Gross et al., 1991; Johnson et al., 1991; Küppers and Schulze, 1985). The magnitude and diurnal pattern of stomatal conductance depends on both internal, species-specific factors and on the external environment, including soil fertility and nutrient availability, as well as microclimate (Schulze and Hall, 1982; Beadle et al., 1985a,b). Mid-day stomatal closure is observed frequently under conditions of high temperature and low water availability (Helms, 1970; Tenhunen et al., 1980; Weber and Gates, 1990). As an example of the variability in diurnal gas exchange, Tenhunen et al. (1980) present nine graphs of diurnal photosynthesis for apricot (*Prunus armeniaca*) measured from July to September 1976. Although there is a general pattern of increase in the morning and of decline in the evening, the path of photosynthesis and conductance are quite different among

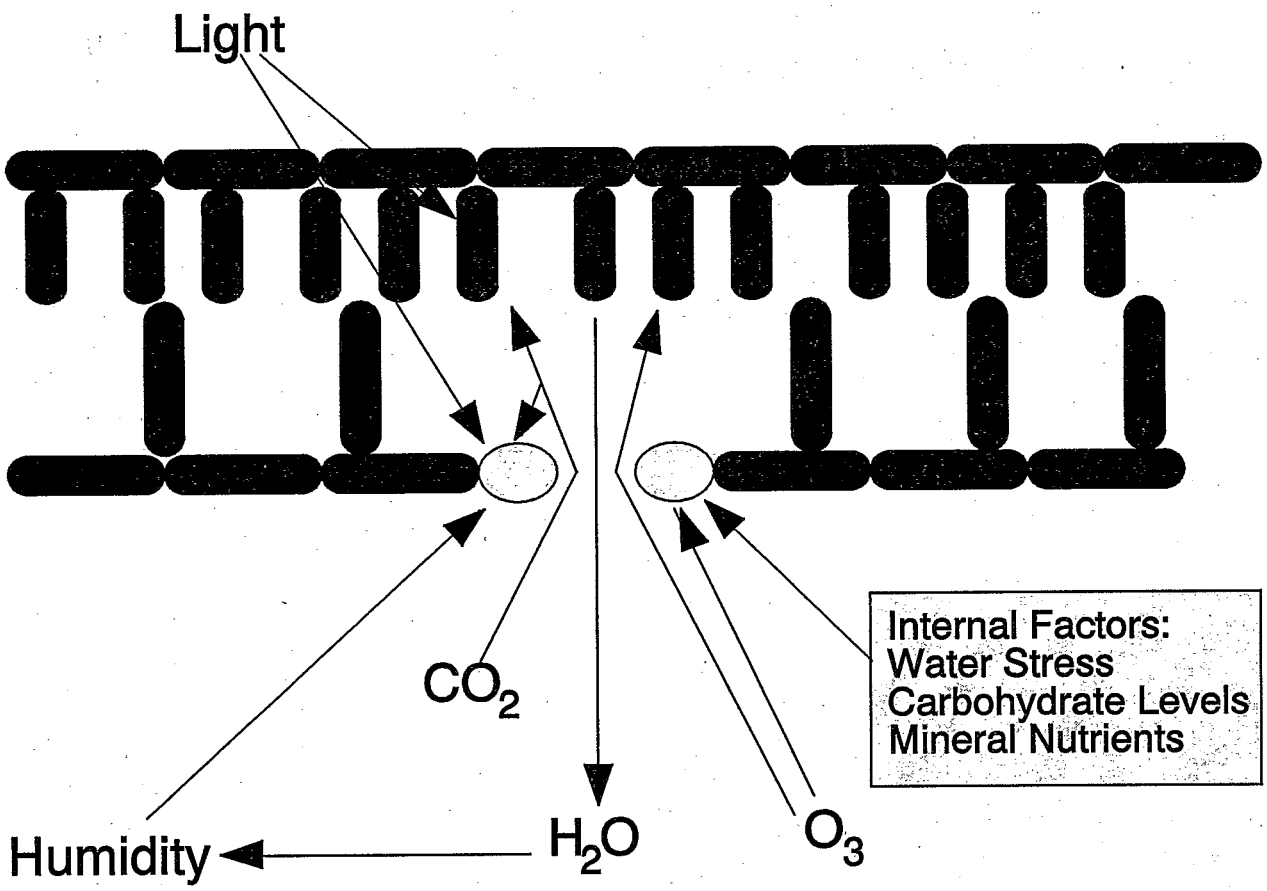

Figure 5-3. Movement of gases into and out of leaves is controlled primarily by the stomata (small openings in the leaf surface whose aperture is controlled by two guard cells). Guard cells respond to a number of external and internal factors, including light, humidity, carbon dioxide (CO_2), and water stress. In general, the stomata open in response to light and increasing temperature and close in response to decreasing humidity, increased CO_2, and increasing water stress. They also may close in response to air pollutants, such as ozone.

of days. The inherent variability in stomatal opening makes using a set time period for O_3 exposure problematic. This variability makes determining the effects of a given diurnal O_3 exposure pattern difficult without reference to physiological, meteorological, and edaphic information, as well as to the sensitivity of individual species exposed.

To be absorbed, O_3 must be present in the atmosphere surrounding the leaf and the stomata must be open. Any factor that affects stomatal opening affects O_3 absorption (Figure 5-3). Under drought conditions, when stomatal conductance is reduced, the relative effect of O_3 is less when compared with well-watered controls (Tingey and Hogsett, 1985; Flagler et al., 1987; Temple et al., 1993, also see Section 5.4). Low humidity has been

shown to modify plant response to O_3 (McLaughlin and Taylor, 1981), presumably due to reduced O_3 absorption (Wieser and Havranek, 1993).

To calculate O_3 absorption, some estimate of the internal O_3 concentration must be made. In earlier work, a finite O_3 concentration was assumed to exist in the intercellular air space of the leaf (Bennett et al., 1973; Tingey and Taylor, 1982; Lange et al., 1989). Estimating this concentration is difficult because the rate of O_3 absorption into the leaf must be known. Recently Laisk et al. (1989) presented evidence that this concentration is near zero, a result that is consistent with the highly reactive nature of O_3. Further studies on other species must be made to test the hypothesis that internal O_3 concentration is negligible in leaves.

The other component of absorption, O_3 concentration outside the leaf, may vary greatly with time of day and season (Chapter 4). Data on the effect of variations in O_3 profile (from constant concentrations to equal daily peaks to variable [episodic] peaks), based on greenhouse and OTC chamber studies using simulated exposures, suggest that those profiles that have periodic high concentrations have a greater effect than those with low peaks even though the exposure is equivalent (Hogsett et al., 1985a; Musselman et al., 1986b; see Section 5.6). Taylor and Hanson (1992) show how variations in conductance can affect O_3 absorption and conclude that conductances in and near the leaf surface have a major influence on absorption of O_3. Figure 5-4 shows a simulation of the effect of diurnal variation in stomatal conductance and O_3 concentration on the O_3 absorbed into the leaf. Amiro and Gillespie (1985) found that cumulative O_3 absorption correlated with visible injury in soybean. Weber et al. (1993) found that rate of uptake may play an important role in the response of ponderosa pine (*Pinus ponderosa*). The roles of cumulative uptake versus uptake rate have not been clarified and need further study.

Absorption of O_3 by leaves depends on variations in both stomatal conductance and O_3 concentration. The highly reactive nature of O_3 makes measuring its absorption difficult; therefore, models of stomatal conductance are used, along with O_3 concentrations, to estimate O_3 absorption. The relative importance of absorption rate versus cumulative absorption is not known at present.

5.3.3 Resistance Mechanisms

Resistance mechanisms can be divided into two types: (1) exclusion from sensitive tissue and (2) detoxification near or in sensitive tissue. For leaves, the former involve response and cuticles, and the latter involve various potential chemical and biochemical reactions that chemically reduce O_3 in a controlled manner. Although these systems potentially provide protection against O_3 injury to tissue physiology, they come at some cost, either in the reduction in photosynthesis, in the case of stomatal closure, or in carbohydrate used to produce detoxification systems.

Injury to leaf and needle cuticles does not appear to have a major effect on leaf function, based on the inconsistent data. Barnes et al. (1988a) found that O_3 exposure could damage leaf cuticles; however, Lütz et al. (1990) found no consistent changes in cuticle structure in Norway spruce (*Picea abies*).

5.3.3.1 Stomatal Limitation

As noted above, stomata can be affected by a wide variety of environmental factors (Section 5.3.2.2), by occurrences of stress (Section 5.4), and by age. In addition,

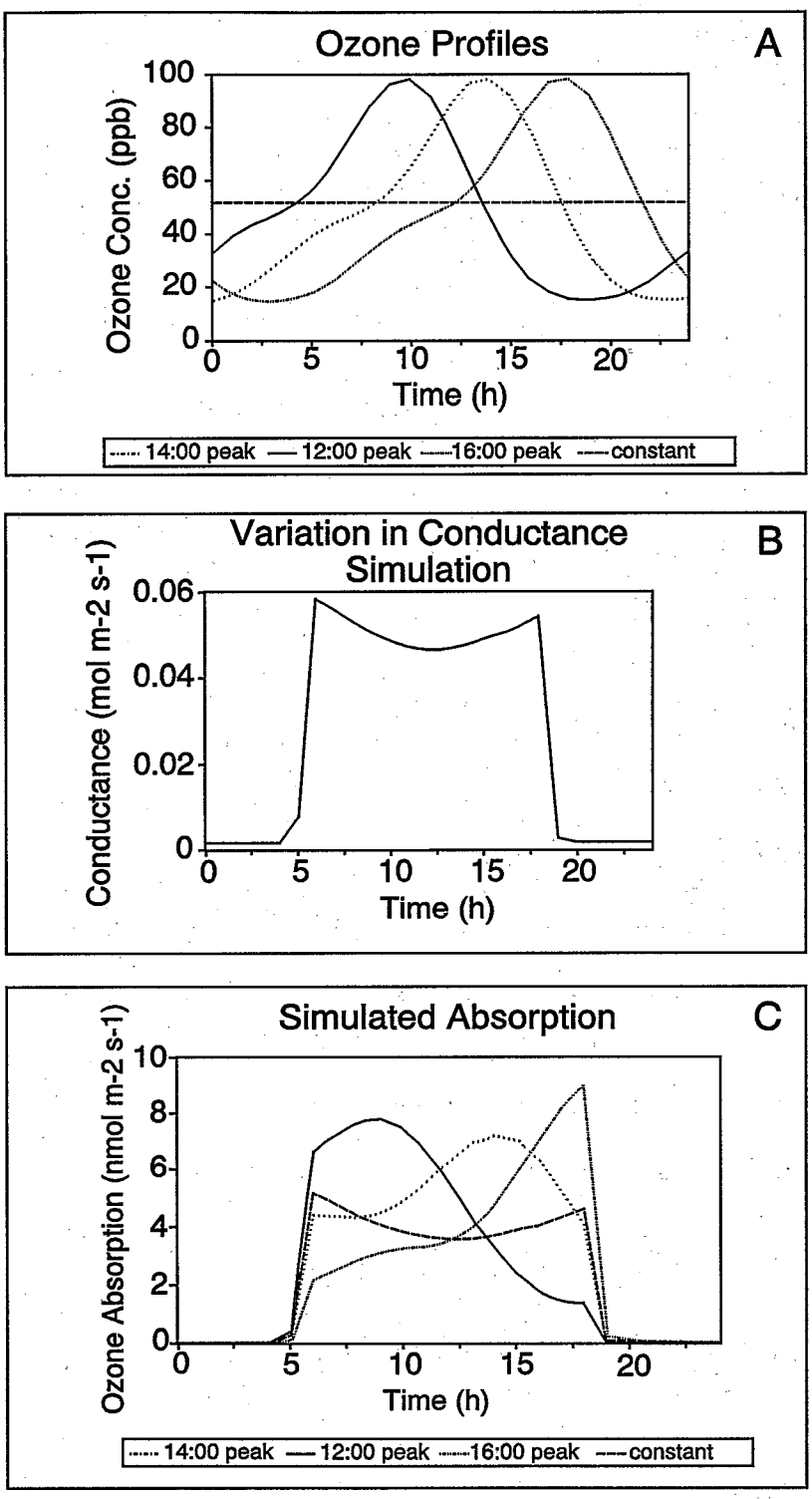

Figure 5-4. Simulation of the effects of diurnal variation in stomatal aperture and in ozone (O_3) concentration on O_3 uptake: (A) diurnal O_3 concentrations, (B) simulated conductance, and (C) O_3 uptake.

stomatal response can vary among species. These multiple interactions make accurate prediction of uptake under field conditions difficult. Some early research showed a decrease in leaf conductance (Figure 5-3), with O_3 exposure implying a direct effect of O_3 on stomatal conductance (U.S. Environmental Protection Agency, 1986). In studies at high O_3 concentrations (>0.3 ppm), stomatal response was rapid (Moldau et al., 1990). In other studies, reduction in conductance in response to O_3 required hours to days of exposure (Dann and Pell, 1989; Weber et al., 1993). Several studies have shown that discrimination against C_{13} in C_3 plants decreases with O_3 fumigation (Okano et al., 1985; Martin et al., 1988; Greitner and Winner, 1988; Saurer et al., 1991; Matyssek et al., 1992). These data are consistent with an increased restriction of diffusion of CO_2 into the leaf (Farquhar et al., 1989). However, Matyssek et al. (1992) and Saurer et al. (1991) found that internal CO_2 increased with O_3 exposure, and that water-use efficiency decreased, both the opposite of expectation, indicating that photosynthesis decreased relatively more than conductance. Although stomata limit O_3 uptake and may respond directly to high O_3 concentrations (e.g., >0.2 ppm, U.S. Environmental Protection Agency, 1986; Moldau et al., 1990), the relative importance of this response, compared to indirect effects induced by reductions in photosynthetic performance, has not been fully assessed.

5.3.3.2 Detoxification

When O_3 enters a cell, several highly reactive compounds can be produced (e.g., superoxide, free radicals, peroxides) (Heath, 1988). The effects of these compounds depends on their reactivity, mobility, and half-life. For detoxification to occur, oxidant and antioxidant must occur proximately. In addition, the rate of production of antioxidant must be a significant portion of the rate of oxidant entry into the system for effective detoxification to occur. Two general kinds of detoxification systems have been reported in plants: (1) those that utilize reductants (e.g., ascorbate) to reduce O_3 and (2) those that utilize enzymes (superoxide dismutase). In either case, excess oxidizing power is dissipated in a controlled manner, effectively protecting the tissue from damage. These protective systems probably developed in response to photooxidation, which can occur, for example, at low temperatures (Powles, 1984).

Several antioxidants have been reported, the most studied being ascorbate and glutathione (GSH). Much of this work has occurred since the 1986 criteria document (U.S. Environmental Protection Agency, 1986). Alscher and Amthor (1988) reviewed the literature in this area. In the chloroplast, the process requires dihydronicotinamide adenine dinucleotide phosphate and may be a cause for the transient reduction in photosynthesis observed in some studies (Alscher and Amthor, 1988).

Evidence for the participation of antioxidants in protecting cells from O_3 injury is primarily indirect (i.e., changes in levels of antioxidants or of associated enzymes). In red spruce (*Picea rubens*), GSH levels increased in year-old needles in response to O_3, but not in current-year needles (Hausladen et al., 1990; Madamanchi et al., 1991). Dohmen et al. (1990) found increased concentrations of reduced glutathione in Norway spruce in response to long-term O_3 fumigation. In a poplar hybrid (*Populus maximowiczii* x *P. trichocarpa*), total GSH increased with O_3 fumigation; however, the ratio of reduced forms to oxidized forms declined, indicating that oxidation of GSH possibly was stimulated by O_3 (Gupta et al., 1991). Mehlhorn et al. (1986) found that both GSH and ascorbic acid (AH_2) increased with O_3 fumigation in silver fir (*Abies alba*) and Norway spruce. The potential for AH_2 to protect cells from O_3 damage was explored by Chameides (1989), who concluded that such

protection was possible if AH_2 occurred in the apoplast at sufficient concentrations and production rates; however, experimental data are needed to test this hypothesis.

The response of enzymes involved in detoxification is not clear. Activities of enzymes involved in antioxidant production increased in response to O_3 in one study (Price et al., 1990); however, in several others, no effect was found (Madamanchi et al., 1992; Pitcher et al., 1991; Anderson et al., 1992; Nast et al., 1993). Activity of superoxide dismutase (SOD), an enzyme that can reduce one of the products of O_3 interaction with the cytoplasm, can be increased by O_3 fumigation (Alscher and Amthor, 1988; Gupta et al., 1991). There are both cytosolic and chloroplastic forms of this enzyme, but the role the different forms play in detoxification of O_3 is not clear. Teppermann and Dunsmuir (1990) and Pitcher et al. (1991) found that increased production of SOD had no effect on resistance to O_3 in tobacco.

The extent to which these detoxification systems can protect tissue from O_3 damage is unknown. However, "if plants have detoxification mechanisms which are kinetically limited, the rate of O_3 uptake may be important, so that even an integrated absorbed dose may be insufficient to account for observed responses" (Cape and Unsworth, 1988). Potential rates of detoxification for given tissues are needed to estimate the importance of these systems to overall O_3 response. In addition, the sites in which the detoxification systems occur need to be identified.

5.3.4 Physiological Effects of Ozone

The initial reactions of O_3 with cellular constituents are not known. The high reactivity and nonspecificity of O_3 reactions, coupled with the absence of a useful isotopic tag for O_3, make studies of the initial reactions difficult at best. The data on changes in biochemical function resulting from O_3 exposure probably represent effects one or more steps beyond the initial reactions. Nonetheless, data is available that indicate the wide range of cellular processes that can be affected by O_3.

Ozone that has not been neutralized by one of the detoxification systems (Figure 5-5) acts first at the biochemical level to impair the functioning of various cellular processes (Tingey and Taylor, 1982; U.S. Environmental Protection Agency, 1986). The result of these impairments are reflected in integrated changes in enzyme activities, membrane function, and energy utilization (Queiroz, 1988). Several related papers have shown that the activity of the primary carboxylating enzyme (RuBP-carboxylase) is reduced by O_3 exposures in the range of those measured at some sites (Dann and Pell, 1989; Enyedi et al., 1992; Pell et al., 1992; Landry and Pell, 1993). Membrane injury has been found in some experiments using acute levels of O_3 (Heath, 1988). Chronic exposure can lead to changes in lipid composition and in cold resistance (Brown et al., 1987; Davison et al., 1988; DeHayes et al., 1991; Lucas et al., 1988; Wolfenden and Wellburn, 1991). Recently, Floyd et al. (1989) have shown that O_3 can affect nuclear deoxyribonucleic acid (DNA).

Changes in the in vivo concentrations of various growth regulators in response to O_3 exposure could have important consequences for plant function. However, the effects of O_3 on levels and activities of growth regulators have not been studied extensively. Ozone has been shown to stimulate ethylene production, and inhibitors of ethylene production have been found to reduce the effects of O_3 in short-term experiments (Pell and Puente, 1986; Rodecap and Tingey, 1986; Taylor et al., 1988b; Mehlhorn et al., 1991; Telewski, 1992; Langebartels et al., 1991; Mehlhorn and Wellburn, 1987; Kargiolaki et al., 1991; Reddy

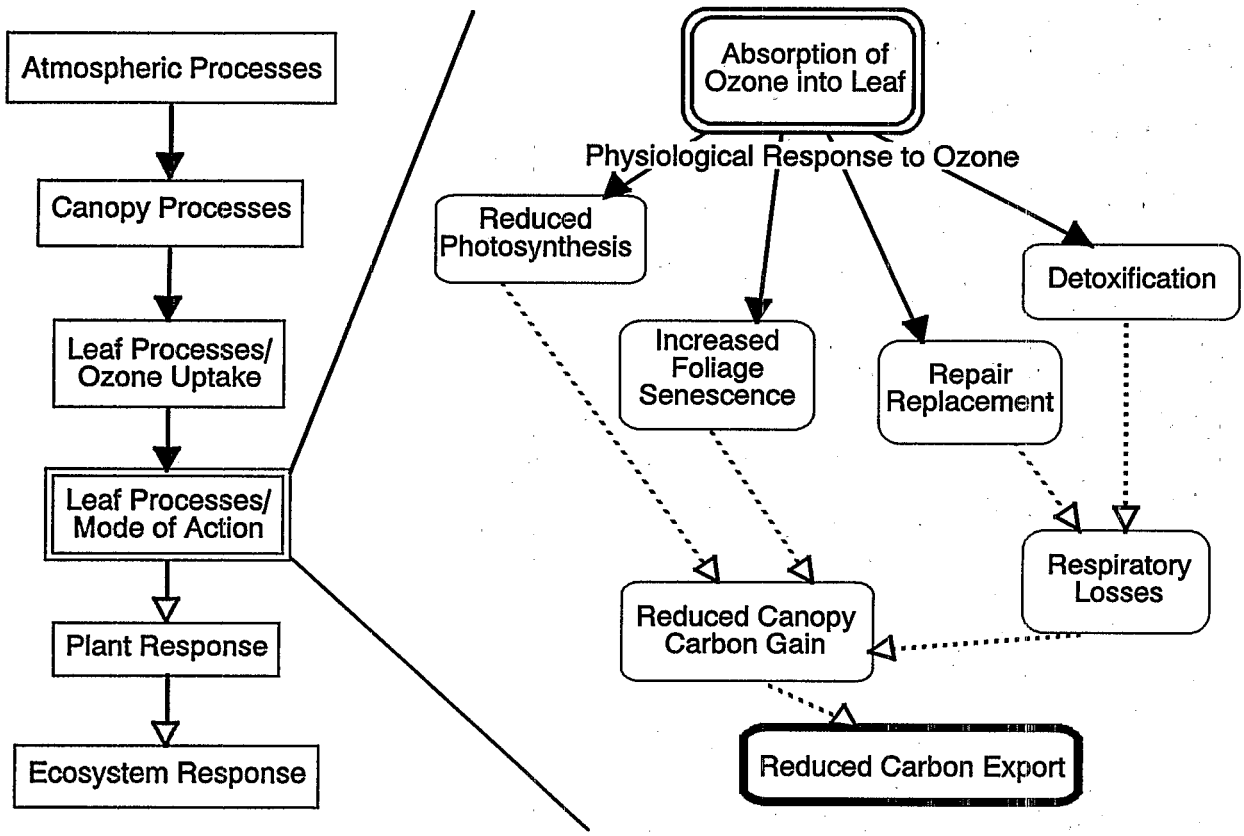

Figure 5-5. ***Effects of ozone (O_3) absorption into a leaf. Once inside the leaf, O_3 can have a number of effects, all of which affect carbohydrate production and utilization. Reduced photosynthesis, increased leaf senescence, production of detoxification systems, and increased respiration (both maintenance and growth) reduce the amount of carbohydrate available for allocation. Compensation through production of new leaves, for instance, can counter some or all of these effects, depending on the O_3 exposure, the physiological state of the plant, and the species. Integration of these processes leads to changes in the amount of carbohydrate available for allocation from the canopy. Solid black arrows denote O_3 flow, and dotted arrows show the cascade of effects of O_3 absorption on leaf function. Boxes at the left and at the top with double borders indicate leaf processes; the box at the bottom with a dark border indicates the impact.***

et al., 1993). Ethylene is produced during ripening of fruit, during periods of stress, and during senescence (Abeles et al., 1992). Increased levels of ethylene in the leaves could play a role in the early senescence of foliage. In some cases, there is a correlation between ethylene production and O_3 sensitivity; however, the relationship is complex and makes use of ethylene production as an index of sensitivity problematic (Pell, 1988).

Abscisic acid (ABA) plays an important role in stomatal function (Davies et al., 1980). Atkinson et al. (1991) found that stomata from O_3 fumigated leaves were less sensitive to ABA than control leaves. Maier-Maercker and Koch (1991a,b; 1992a,b) found that exposure to ambient pollutants, including O_3 and SO_2, caused histological changes in guard cells and resulted in some loss in stomatal control. Results from studies on European white birch (*Betula pendula*) also indicate some change in stomatal function (Matyssek et al., 1992). These data could explain the observation that stomatal function may be impaired by long-term O_3 exposure (Walmsley et al., 1980). Kobriger et al. (1984) found no effect of O_3 on whole-leaf content of ABA, but changes in compartmentation could not be ruled out.

Physiological effects of O_3 uptake are manifest in two ways: (1) reduced net photosynthesis and (2) increased senescence (Figure 5-5). Both decreased photosynthesis and increased leaf senescence result in the loss of capacity for plants to form carbohydrates, thereby potentially having a major impact on the growth of the plant (Figure 5-6). The exact response of a given individual will depend on its ability to compensate for O_3 injury.

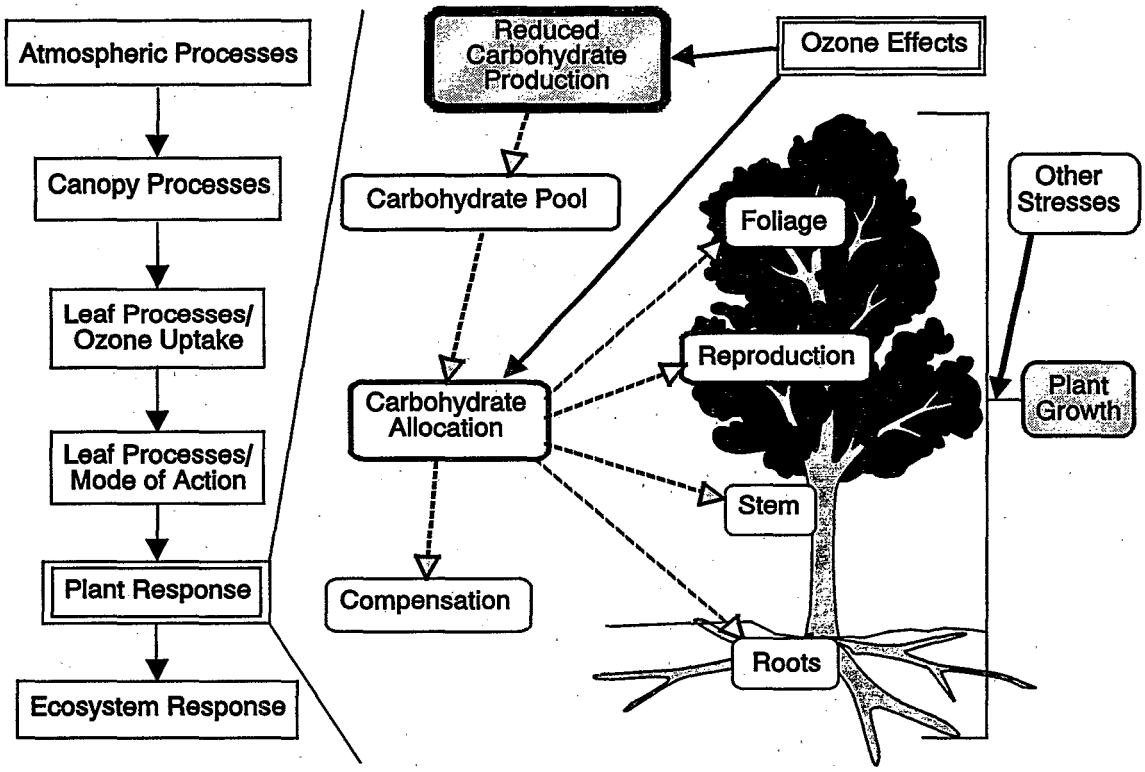

Figure 5-6. Effect of ozone (O_3) on plant function and growth. Reduction in carbohydrate allocation affects the pool of carbohydrates available for growth. Changes in relative growth rate of various organs as a function of O_3 exposure suggest that allocation patterns of carbohydrate are affected. Solid black arrows denote where O_3 absorption affects the allocation processes of the plant; dotted arrows show the cascade to plant growth. Boxes with dark borders indicate site of impact. The box with a double border, at left, indicates the location of response.

Ozone-induced reduction in net photosynthesis has been known for some time (U.S. Environmental Protection Agency, 1986). Changes in stomatal conductance, photosynthetic capacity, carbohydrate allocation, and respiration have been documented. The relationship between O_3 exposure and photosynthesis is not well known. Photosynthesis provides plants with the energy and structural building blocks necessary for their existence. The photosynthetic capacity of a plant is an important aspect of plant response to stresses in natural environments and is strongly associated with leaf nitrogen content and with water movement. Both resources are essential if the process is to occur and involves the allocation of carbohydrates from the leaves to the roots for nitrogen acquisition and water uptake. Leaf photosynthetic capacity is also age dependent. As the plant grows, the canopy structure changes altering the amount and angle of light hitting a leaf. Allocation of carbohydrates and nutrients to new leaves is especially important in stimulating growth production (Pearcy et al., 1987). Reductions in photosynthesis are likely to be accompanied by a shift in growth pattern that favors shoots and by an increase or decrease in leaf life span (Winner and Atkinson, 1986). Therefore, alteration of the processes of photosynthesis and carbohydrate allocation affects plant response to stresses such as O_3. Reduction in photosynthesis (reduced carbohydrate formation and allocation to leaf repair or to new leaf formation decreases the availability of carbohydrates) potentially alters the normal allocation pattern and, therefore, all aspects of plant growth and reproduction (Figure 5-6). The effects of a reduction in photosynthesis on growth and reproduction was discussed in the previous criteria document (U.S. Environmental Protection Agency, 1986).

Carbohydrate production by a single plant is controlled not only by photosynthetic capacity of the foliage but also by the amount and distribution of that foliage. Stow et al. (1992) and Kress et al. (1992) found that O_3 exposure affected needle retention in loblolly pine (*Pinus taeda*). Similar data have been reported for slash pine (*Pinus elliotti*) (Byres et al., 1992a). Keller (1988) and Matyssek et al. (1993a,b) reported increased senescence with increased O_3 exposure in trembling aspen, as did Wiltshire et al. (1993) in apple (*Malus spp*). Replacement of injured leaf tissue has been reported for some species when they are exposed to low O_3 concentrations (Held et al., 1991; Temple et al., 1993). Temple et al. (1993) also found increased photosynthetic capacity of new needles in O_3 treatments compared to controls.

Few direct effects of O_3 have been found outside leaves. Kargiolaki et al. (1991) found that intumescences (lesions) appear on stems of three species of poplar (*Populus*) after 72 days of O_3 fumigation (70 to 80 ppb). Ozone probably enters the stem through the lenticles that occur on the surface of the stem and allow direct exchange of gases between the stem and the air. The consequence of this response to O_3 is not clear; however, it may be related to the reduction in phloem transport rate observed in loblolly pine (Spence et al., 1990).

5.3.4.1 Carbohydrate Production and Allocation

The importance of photosynthesis and carbohydrate allocation in plant growth and reproduction has been pointed out previously. The patterns of carbohydrate allocation directly affect growth rate. Plants require a balance of resources to maintain optimal growth; however, in natural environments optimal conditions seldom occur. Therefore, some plants compensate for differences in resource availability and for environmental stresses. They do this by changing the way they allocate carbohydrates (Chapin et al., 1987). Each response to stress affects the availability of carbohydrates for allocation from the leaves (Figure 5-5).

The carbohydrate pool is affected both by a reduction in the carbohydrate produced and by a shift of carbohydrate to repair and replacement processes. The effect is particularly noticeable in the roots where O_3 exposure significantly reduces available carbohydrate (Andersen et al., 1991; Andersen and Rygiewicz, 1991). Effects on leaf and needle carbohydrate content have varied from a reduction (Barnes et al., 1990b; Miller et al., 1989c) to no effect (Alscher et al., 1989) to an increase (Luethy-Krause and Landolt, 1990). Cooley and Manning (1987) reviewed the literature on carbohydrate partitioning and noted that "storage organs ... are most affected by O_3-induced partitioning changes when O_3 concentrations are in the range commonly observed in polluted ambient air." Friend and Tomlinson (1992) found that O_3 exposure increased retention of ^{14}C-labeled photosynthate in needles of loblolly pine, and modified the distribution of labels among starch, lipids, and organic acids (Edwards et al., 1992b; Friend et al., 1992).

The above discussion supports the information in the previous criteria document (U.S. Environmental Protection Agency, 1986), which pointed out that roots usually were affected more by O_3 exposures than were the shoots. Studies by Miller et al. (1969), Tingey et al. (1976b), McLaughlin et al. (1982), and Price and Treshow (1972) were cited in support of this view. Miller et al. (1969) noted that reduction in photosynthesis was accompanied by decreases in sugar and polysaccharide fraction in injured needles of ponderosa pine seedlings, as well as by altered allocation of carbohydrates. Exposures were for 30 days, 9 h/day, to concentrations of 0.15, 0.30, or 0.40 ppm. These exposures reduced photosynthesis by 10, 70, and 85%, respectively. The observations of Tingey et al. (1976a) indicated that O_3 exposures differentially affected metabolic pools in the roots and tops of ponderosa pine seedlings grown in OTCs. Further, this study indicated that the amounts of soluble sugars, starches, and phenols tended to increase in the tops and decrease in the roots of ponderosa pine seedlings exposed to 0.10 ppm O_3 for 6 h/day for 20 weeks. The sugars and starches stored in the tree roots were significantly less than those in the roots of controls. In another study cited in the 1986 document, McLaughlin et al. (1982) also observed the reduced availability of carbohydrate for allocation to the roots and stated that the result was reduced vigor and enhanced susceptibility of trees to root diseases. Loss of vigor was due to a sequence of events that was associated with exposure to O_3, including premature senescence, loss of older needles, lower gross photosynthetic productivity, and reduced photosynthate (carbohydrates) available for growth and maintenance. Carbon-14 transport patterns also indicated changes in carbon allocation. Older needles were found to be the source of photosynthate for new needle growth in the spring and were storage sinks in the fall. Retention of ^{14}C-photosynthate by foliage and branches of sensitive trees indicated that allocation to the trunks and roots was reduced.

Lost carbohydrate production has effects throughout the plant (Figure 5-6). The roots and associated mycorrhizal fungi are especially susceptible to reduced carbohydrate availability and, quite frequently, show the greatest decline in growth (Adams and O'Neill, 1991; Edwards and Kelly, 1992; McQuattie and Schier, 1992; Meier et al., 1990; Taylor and Davies, 1990). However, in some cases, increased mycorrhizal formation has been reported (Gorissen et al., 1991b; Reich et al., 1985). It might be expected that reduced allocation to roots would affect shoot growth through increased susceptibility to water stress, reduced nutrient availability (Flagler et al., 1987), and reduced production of growth factors (Davies and Zhang, 1991; Letham and Palni, 1983). Effects on production and retention of leaves and needles were described above. Effects on stem growth have been found in tree species (Hogsett et al., 1985b; Mudano et al., 1992; Pathak et al., 1986; Matyssek et al., 1992;

Matyssek et al., 1993b). Changes in canopy density, root/shoot ratio, and stem growth will affect the functioning of the plant and may make plants more susceptible to environmental stresses, such as drought and nutrient limitation, that are characteristic of many ecosystems.

5.3.4.2 Compensation

Compensatory responses occur as plants attempt to minimize the effects of stress. Responses include adjustments to changes in physiological processes (e.g., photosynthetic performance and foliage production) that tend to counteract the effects of O_3 absorption by the leaves. Pell et al. (1994) have reviewed the extensive literature produced in the Response of Plants to Interacting Stresses (ROPIS) experiment (Goldstein and Ferson, 1994). A wide range of compensatory responses have been identified, especially reallocation of resources leading to increased relative growth in the shoot compared to the root (see above). Compensation can take the form of production of new tissue (e.g., leaves) to replace injured tissue or of biochemical shifts, including increased photosynthetic performance in new foliage.

Changes in respiratory rate have been attributed to such repair processes (U.S. Environmental Protection Agency, 1986). Recent studies have found stimulation of dark respiration in Norway spruce (Barnes et al., 1990b; Wallin et al., 1990) and pinto bean (Amthor, 1988; Amthor and Cumming, 1988; Moldau et al., 1991b). Repair of membranes (Sutton and Ting, 1977; Chevrier et al., 1988, 1990) and replacement of impaired enzymes are two probable reasons for increased respiration. Ozone has been shown to increase the adenosine triphosphate/adenosine diphosphate (ATP/ADP) ratio, which is consistent with increased respiratory activity (Weidmann et al., 1990; Hampp et al., 1990). As in the case of detoxification, the importance of repair processes in the overall carbohydrate budget of the plant and of their influence of apparent threshold is unknown.

Recovery of photosynthetic performance after O_3 exposure has been noted in some studies. Early work indicated that recovery of photosynthetic capacity could occur after exposure of high concentrations (>0.25 ppm) of O_3 (e.g., Botkin et al., 1971, 1972). Dann and Pell (1989) found that photosynthetic rate, but not Rubisco activity, recovered within a few days in potato (*Solanum tuberosum*) after exposure to 0.2 ppm O_3. In ponderosa pine, photosynthetic rates in O_3 treated needles recovered to that of controls within 40 to 50 days (Weber et al., 1993). To what extent this recovery can offset losses in carbohydrate gain is not known, nor is the mechanism.

Replacement of injured foliage (see Section 5.3.4) is another method to counteract the effects of O_3 exposure. The extent to which increased leaf and needle production and increased photosynthetic performance in the new foliage compensates for O_3 injury is not known.

The importance of various compensatory mechanisms is not sufficiently well known to allow an estimate of the degree to which they might mitigate the effect of O_3. The fact that increases in photosynthesis and in leaf production have been measured indicates that these processes, at least, may be important.

5.3.5 Role of Age and Size Influencing Response to Ozone

Plant age, physiological state, and frequency of exposure play important roles in plant response to O_3. In annual species, effects of O_3 on production will occur through changes in allocation of carbohydrates over the years, resulting in reduced seed production.

In perennial species, plant growth will be affected by reduction in storage of carbohydrates, which may limit growth the following year (carry-over effects). Carry-over effects have been documented in the growth of tree seedlings (Hogsett et al., 1989; Sasek et al., 1991; Temple et al., 1993) and roots (Andersen et al., 1991). Accumulation of these effects will affect survival and ability to reproduce. Data on cumulative effects of multiple years of O_3 exposures have been, for the most part, the result of 2- to 3-year studies.

A tacit assumption in much of the research on O_3 effects on trees is that seedling response to O_3 is a good predictor of large-tree response. This assumption has been necessitated by the difficulty in exposing large trees to O_3 for long periods. Pye (1988) reviewed the problems of extrapolation from seedling/sapling experiments to large trees and noted several areas of difference between seedling/saplings and large trees: (1) microclimate (especially radiation), (2) transport distances, (3) ratio of photosynthetic to respiratory tissue, and (4) potential for storage. Cregg et al. (1989) also argued that these differences in scale can affect growth responses seen. Some studies have indicated that seedlings may be more sensitive (i.e., greater visible injury) than large trees (Kozlowski et al., 1991); however, Samuelson and Edwards (1993) found that leaves on large red oak trees (*Quercus rubra*) are more sensitive than those on seedlings. It is likely that a variety of factors determines sensitivity to O_3, including stomatal function and presence of detoxification systems, so that, in some cases, seedlings will be more sensitive and, in others, large trees will be. Although each of the four differences between small and large trees mentioned above can be supported on theoretical grounds, little direct information is available to evaluate the importance of these differences, especially with respect to O_3.

The microclimate of the canopy of mature trees is quite different from that of seedlings, as is that of a stand of trees compared to a single tree in a field. Light intensity through the multilayer canopy can vary by an order of magnitude or more (Jones, 1992). In addition, gradients of other important microclimatic variables (temperature, humidity, and wind speed) exist within the canopy. These will all affect stomatal conductance, and some (e.g., wind speed) will affect canopy conductance. In addition, leaf development will be affected by these microclimatic variables (especially light intensity), leading to leaves with different physiological capacity and sensitivity to O_3 (Samuelson and Edwards, 1993; Waring and Schlesinger, 1985).

The effect of plant size on transport processes and the subsequent response to O_3 is unknown. The simple fact of greater distance over which transport must occur will affect the timing of response of organs distant from the primary site of O_3 impact, the foliage. Studies using methods that integrate functions over the whole tree could provide useful information. For example, combinations of porometer measurements on foliage and whole-plant water use measured (Schulze et al., 1985) on individuals of different sizes could provide very useful information on the coupling of leaf-level processes to whole-canopy and whole-plant response. Greater evaporative demand in large trees as the result of greater leaf area and different microclimate than in small trees could lead to transient water stress and stomatal closure because of insufficient water transport capacity.

As a tree grows from a seedling to a large tree, the ratio between photosynthesis and respiration declines as a greater portion of the plant tissue becomes nonphotosynthetic. It is reasonable to assume that such a change could result in less resource being available for detoxification and repair as the plant grows. How this change affects the ability of a plant to survive O_3 (or any other stress) is not known. Recently, Samuelson and Edwards (1993) presented data on northern red oak that show O_3 decreased photosynthetic performance more

on lower leaves within the canopy of large trees than on leaves near the top of the canopy (a result apparently counter to the model results of Reich et al., 1990). Seedling photosynthesis was not affected by the same O_3 exposure. A more interesting result of this work is the reduction in total canopy biomass found in large trees exposed to O_3. It is not possible to assess directly the relative importance of reduced photosynthesis versus loss of canopy from these data, but the data do show that differences may exist between large trees and seedlings in their response to O_3. These differences may be due to changes in carbon budgets, stomatal characteristics, microclimate, and flushing patterns that develop as seedlings become trees. The ability of northern red oak seedlings to produce three flushes and thus replace injured foliage may be an important defense mechanism in the seedling stage. The physiological basis of these findings need further investigation.

In evergreen perennial plants, foliage must be maintained from one year to the next, frequently through periods unfavorable to growth. In evergreen species that retain a few to several years of leaves, increased susceptibility to stress (e.g., frost) could further reduce potential canopy photosynthesis in subsequent years (Brown et al., 1987; Davison et al., 1988; DeHayes et al., 1991; Lucas et al., 1988). Fincher (1992) found that O_3 decreased frost tolerance in red spruce in both seedlings and trees; the consequences of this change in seedlings and large trees needs of further study.

The effect of O_3 on storage of carbohydrates in large compared to small trees is not known. Changes in storage could affect the ability of the plant to withstand other stresses or to produce adequate growth during each growing season.

Dendrochronology (tree-ring analysis) provides the opportunity to do retrospective studies over the life of large trees. Reduction in annual radial growth has been found in the southern Sierra Nevada for Jeffrey pine but not for ponderosa pine (Peterson et al., 1987, 1989, 1991; Peterson and Arbaugh, 1988). One difficulty with using tree-ring data to estimate O_3-related effects is that it is not always possible to separate reductions due to O_3 from other effects (e.g., drought).

Development of reliable methods for scaling from small to large trees is crucial to the prediction of the long-term effects of O_3 on forest function. Measurement of the response of different size trees to O_3 could provide useful data on the relative responses of small and large trees. However, problems exist in giving similar exposures to trees of widely different sizes. The most direct method is to fumigate trees over a significant portion of their life span. Time is the primary obstacle to these studies because they would require decades to complete. Whatever methods are used must be based on a good understanding of the physiological changes that occur as trees grow.

5.3.5.1 Summary

In the previous criteria document, it was concluded that the "critical effects, including reduction in photosynthesis and a shift in the assimilation of photosynthate, will lead to reduced biomass, growth, and yield" (U.S. Environmental Protection Agency, 1986). In addition, changes in carbohydrate allocation patterns and effects on foliage were noted as important. Since that report, additional information has been developed, especially on the effects of O_3 on photosynthetic performance. However, at present there is still no clear understanding of the initial biochemical changes resulting within the leaf cells after the entry of O_3 and how these changes interact to produce the observed responses. Much of the earlier research used very high ($\geq$0.25 ppm) O_3 concentrations, which produced what could be characterized as acute responses. More recent research has used lower concentrations,

usually including near ambient (0.04 to 0.06 ppm) O_3 levels, so that the observed responses may be more relevant to field conditions. One characteristic of these more recent data is that a longer exposure (days to weeks, instead of hours) is needed to show a response.

As a result of the research since the last criteria document (U.S. Environmental Protection Agency, 1986), the way in which O_3 exposure reduces photosynthesis, especially its effects on the central carboxylating enzyme (ribulose-6-P-carboxylase/oxygenase), is better understood. The rate of senescence of leaves has been shown to increase as a function of increasing O_3 exposure. At near-ambient exposures, leaf production has been shown to increase in some species, thereby off-setting the increased loss to due senescence. The mechanism of the increase in senescence is not known, hence deserves further study. Finally, the role that changes in allocation of resources play in plant response to O_3 is now better understood. Most studies have shown that allocation of photosynthate to roots is decreased by O_3. In some cases, allocation to leaf production has increased. Whether these changes are driven entirely by changes in carbohydrate availability or are controlled by other factors (e.g., hormones) is not known.

Some potentially significant processes have been investigated since the last criteria document, especially detoxification and compensatory processes. The role of detoxification in providing a level of resistance to O_3 has been investigated; however, it is still not clear to what degree these processes can provide protection against O_3 injury. Data are needed especially on the potential rates of antioxidant production and on the subcellular localization of the antioxidants. Potential rates of antioxidant production are needed to assess whether they are sufficient to detoxify the O_3 as it enters the cell. The localization is needed to assess whether the antioxidants are in a location (cell wall or plasmalemma) that permits contact with the O_3 before it has a chance to damage subcellular systems. Ozone exposure has been shown to decrease cold tolerance of foliage in some species. This response could have a major impact on long-lived evergreen species that retain leaves for several years. Various forms of compensation, especially stimulation of production of new leaves and higher photosynthetic performance of new leaves, have been reported. Although these processes divert resources away from other sinks, compensation may counteract the reduction in canopy carbon fixation caused by O_3. The quantitative importance of these processes is still in need of investigation.

The major problem facing researchers trying to predict long-term O_3 effects on plants is how the plant integrates all of the response to O_3 into the overall response to the environment, including naturally occurring stresses. Little is now known about how response to O_3 changes with increasing age and size. This information is crucial to predicting the long-term consequence of O_3 exposure in forested ecosystems.

5.4 Factors That Modify Plant Response

5.4.1 Modification of Functional and Growth Responses

Plant response to oxidants may be modified by various biological, physical, and chemical factors. Biological factors that modify plant response include those within the plant, as well as those external to the plant. The genetic makeup and the developmental stage play critical roles in the way individual plants respond to O_3 and other external stresses. For example, different varieties or cultivars of a particular species are known to differ greatly in their responses to a given exposure to O_3, whereas the magnitude of the

response of a particular variety, in turn, depends on environmental factors such as temperature and humidity, soil moisture and nutrition, the presence of pests or pathogens, and exposure to other pollutants or agricultural spray chemicals. In other words, response will be dictated by the plant's present and past environmental milieu, which also includes the temporal pattern of exposure and the plant's stage of development. The corollary is also true: exposure to oxidants can modify response to other environmental variables. For example, exposure to O_3 reduces the ability of trees to withstand winter injury caused by exposure to freezing temperatures (Davison et al., 1988) and influences the success of pest infestations (Hain, 1987; Lechowicz, 1987). Hence, both the impact of environmental factors on response to oxidants and the effects of oxidants on responses to environmental factors have to be considered in determining the impact of oxidants on vegetation in the field. These interactions are summarized as the involvement of "other stresses" in the scheme shown in Figure 5-6 (Section 5.3). In the following review, the environmental factors are grouped into three categories: (1) biological (including genetic and developmental components), (2) physical, and (3) chemical.

Runeckles and Chevone (1992) have provided a general review of the interactive effects of environmental factors and O_3. The subject also is treated in a National Acid Precipitation Assessment Program (NAPAP) report (Shriner et al., 1991). The numbers of publications that have appeared since the previous criteria document and supplement vary widely among the different environmental factors reviewed. As a result, in several sections, material covered in these earlier documents has been repeated in order to provide comprehensive coverage and to place new findings into context.

5.4.2 Genetics

The response of an individual plant within a species and at a given age is affected both by its genetic makeup and the environment in which it grows. This section examines the role of genetics in plant response to O_3 and its implication for both managed and natural ecosystems. In addition, major knowledge gaps in the understanding of genetic aspects of O_3 responses are pointed out.

The responses of plants to O_3 are strongly influenced by genetics, as was summarized in the air quality criteria document for O_3 (U.S. Environmental Protection Agency, 1986). Thus, the plants of a given population or family will not respond to O_3 in the same way, even if they are grown in a homogenous environment. This has been demonstrated amply through intraspecific comparisons of O_3 sensitivity as determined by foliar sensitivity of ornamental plants, the aesthetic value of which is decreased by visible foliar injury, and of woody plants that are important components of natural ecosystems (Table 5-2). Ornamental plants and plants growing in wilderness areas, for example, have an intrinsic worth, apart from any economic value related to growth (Tingey et al., 1990). Considerable genetic variation in O_3 sensitivity also has been demonstrated for growth responses of crop plants (Table 5-3). The range of responses displayed for visible foliar injury and growth, biomass, or yield vary from species to species and from study to study. However, it is not uncommon to have genotypes varying from no response to well over 50% leaf area injured or 50% growth or yield reductions in the same study. Additional examples of genetic variation in O_3 response are shown in Figure 5-7 for visible foliar injury and in Figure 5-8 for growth. From Figure 5-7, one can see that, depending on what population has been examined, white ash (*Fraxinus americana*) and green ash (*F. pennsylvanica*) could

Table 5-2. Examples of Intraspecific Variation of Foliar Symptoms in Ozone Response

Species	Genetic Unit[a]	Ozone Concentration	Duration	Range of Response[b]	Reference
Ornamental, Non-woody Plants					
Petunia sp. (Petunia)	Cultivars	400 ppb 4 h/day	4 days	20 to 60% (3)	Elkiey and Ormrod (1979a,b), Elkiey et al. (1979)
Poa pratensis L. (Kentucky bluegrass)	Cultivars	400 ppb 300 ppb	2 h 4 h	0 to 90% (3) 30 to 60% (3)	Murray et al. (1975), Wilton et al. (1972)
Trees					
Acer rubrum L. (Red maple)	Populations	750 ppb 7 h/day	3 days	19 to 34% (2)	Townsend and Dochinger (1974)
Fraxinus americana L. (White ash)	Half-sib families	500 ppb 250 ppb	7.5 h 6 h	0 to 50% (3) 2 to 33% (2)	Karnosky and Steiner (1981), Steiner and Davis (1979)
Fraxinus pennsylvanica Marsh. (Green ash)	Half-sib families	500 ppb 250 ppb	7.5 h 6 h	0 to 40% (3) 2 to 39% (2)	Karnosky and Steiner (1981), Steiner and Davis (1979)
Gleditsia triacanthos L. (Honeylocust)	Cultivars	Ambient	1 growing season	0 to 34% (3)	Karnosky (1981a)
Pinus ponderosa Dougl. ex P. Laws and C. Laws (Ponderosa pine)	Half-sib families	1.5 × ambient	3 growing seasons	0 to 28% (2)	Temple et al. (1992)
Pinus strobus L. (Eastern white pine)	Clones	300 ppb	6 h	0 to 60% (3)	Houston (1974)
Pinus taeda L. (Loblolly pine)	Half-sib families	250 ppb ambient + 60 ppb	8 h 1 growing season	3 to 29% (2) 1 to 42% (1)	Kress et al. (1982a), Adams et al. (1988)
Populus tremuloides Michx. (Trembling aspen)	Clones	200 ppb 150 ppb	3 h 6 h	7 to 56% (1) 10 to 91% (1)	Karnosky (1977), Berrang et al. (1991)

[a]Cultivars = a variety of agricultural or horticultural crops produced by selective breeding or a vegetatively propagated tree selection; Half-sib = seedlings with one parent in common; Clones = vegetatively propagated individual genotypes; and Populations = seedlings derived from a common gene pool.
[b]Range of response is expressed as (1) percentage of leaves showing visible symptoms, (2) percentage of leaf area injured, or (3) percentage from a leaf injury rating scheme.

Table 5-3. Examples of Intraspecific Variation in Growth Responses Following Ozone Exposures

Species	Genetic Unit[a]	Ozone Concentration	Duration	Range of Response[b]	Reference
Crops and Non-woody Plants					
Agrostis capillaris L. (Bentgrass)	Populations	60 ppb	4 weeks	−45 to +20% (2)	Dueck et al. (1987)
Begonia semperflorens Hort. (Bedding begonia)	Cultivars	500 ppb 4 h/day 250 ppb 4 h/day	2 days 4 days	−59 to 0% (2) −16 to +10% (2)	Reinert and Nelson (1979), Reinert and Nelson (1980)
Festuca arundinacea Schreb. (Fescue)	Cultivars	400 ppb 6 h/day	7 days	−53 to −35% (2)	Flagler and Younger (1982)
Lycopersicon esculentum L. (Tomato)	Cultivars	400 ppb 1.5 × ambient	2 h 1 growing season	−50 to −4% (2) −54 to −17% (3)	Reinert and Henderson (1980), Temple (1990a)
Phaseolus vulgaris L. (Snapbean)	Cultivars	60 ppb 7 h 72 ppb 7 h 80 ppb 7 h/day	mean - 44 days mean - 54 days 42 days	−26 to −2% (3) −73 to −44% (3) −68 to −50% (3)	Heck et al. (1988), Temple (1991), Eason and Reinert (1991)
Plantago major L. (Common plantago)	Populations	70 nL/ 1-7 h/day	2 weeks	−24 to 0% (1)	Reiling and Davison (1992a)
Raphanns sativus L. (Radish)	Within cultivar	0.1 μL/ 1-4 h/day 3 days/week	3 weeks	−40 to −5% (2)	Gillespie and Winner (1989)
Silene cucabalus (Bladder campion)	Populations	35 ppb 12 h/day	4 weeks	−75 to −48% (2)	Ernst et al. (1985)
Solanum tuberosum L. (Potato)	Cultivars	150 ppb 6 h/day	8 days	−10 to 0% (2) −40 to 0% (2)	Pell and Pearson (1984), Ormrod et al. (1971)
Spinacia oleracea L. (Spinach)	Cultivars	130 ppb 7 h/day	38 days	−56 to −28% (2)	Heagle et al. (1979a)
Trees and Other Woody Plants					
Acer rubrum L. (Red maple)	Populations	750 ppb 7 h/day	3 days	−36 to −17% (1)	Townsend and Dochinger (1974)
Abies alba Mill. (Silver fir)	Populations	250 ppb 7 h/day	10 days	−18 to +3% (1)	Larsen et al. (1990)
Pinus elliottii Engelm. (Slash pine)	Half-sib families	3 × ambient	3 growing seasons	−20 to 0% (1)	Dean and Johnson (1992)

Table 5-3 (cont'd). Examples of Intraspecific Variation in Growth Responses Following Ozone Exposures

Species	Genetic Unit[a]	Ozone Concentration	Duration	Range of Response[b]	Reference
Pinus taeda L. (Loblolly pine)	Full-sib families	50 ppb 6 h/day 1.9 × ambient	28 days 2 growing seasons	−18 to 0% (1) −19 to 0% (2)	Kress et al. (1982b), Shafer and Heagle (1989)
Pinus taeda L. (Loblolly pine)	Half-sib families	Ambient + 60 ppb 2.5 × ambient 250 ppb	1 growing season 1 growing season 8 h	−27.5 to +3% (2) −19 to −2% (2) −22 to +30% (2)	Adams et al. (1988), Qiu et al. (1992), Winner et al. (1987)
Populus tremuloides Michx. (Trembling aspen)	Clones	26.4 ppm-h ambient	92 days 3 growing seasons	−74 to −5% (2) −24 to −12% (2)	Karnosky et al. (1992a), Wang et al. (1986a,b)
Rhododendron obtusum (Lindl) Planch. (Azalea)	Cultivars	250 ppb - 3 h/day	6 days	−43% to 0% (2)	Sanders and Reinert (1982)

[a]Cultivars = a variety of agricultural or horticultural crops produced by selective breeding or a vegetatively propagated tree selection; Half-sib = seedlings with one parent in common; Clones = vegetatively propagated individual genotypes; and Populations = seedlings derived from a common gene pool.
[b]Range of response is expressed as (1) decrease compared to charcoal-filtered-air control plants in terms of growth, (2) biomass, or (3) yield.

have been classified as either O_3 sensitive or O_3 tolerant. Also noticeable from this figure is the large amount of variation in O_3 tolerance of individual half-sib (one parent in common) families from a given population. From Figure 5-8, the heterogeneity within a given loblolly pine half-sib family in terms of growth is displayed. This variability has some interesting implications. First, because plants of a given species vary widely in their response to O_3 exposure, response relationships generated for a single genotype or small group of genotypes may not represent adequately the responses of the species as a whole (Temple, 1990a). Second, because of the genetic variability and differential fitness extant among different genotypes in a population of plants, O_3 imposes a selective force favoring tolerant genotypes over sensitive ones (Roose et al., 1982; Treshow, 1980). Each of these implications will be discussed in this section.

Mechanisms and Gene Numbers

Little is known about the genetic bases for O_3 resistance mechanisms or about the numbers of genes involved in these mechanisms (Pitelka, 1988). Most O_3 resistance mechanisms involve a physiological cost that will result in decreased growth and productivity of resistant plants grown under O_3 stress. Partial or complete stomatal closure in the presence of O_3 is an example of a mechanism of resistance that has been demonstrated for several plants (Engle and Gabelman, 1966; Thorne and Hanson, 1976; Reich, 1987;

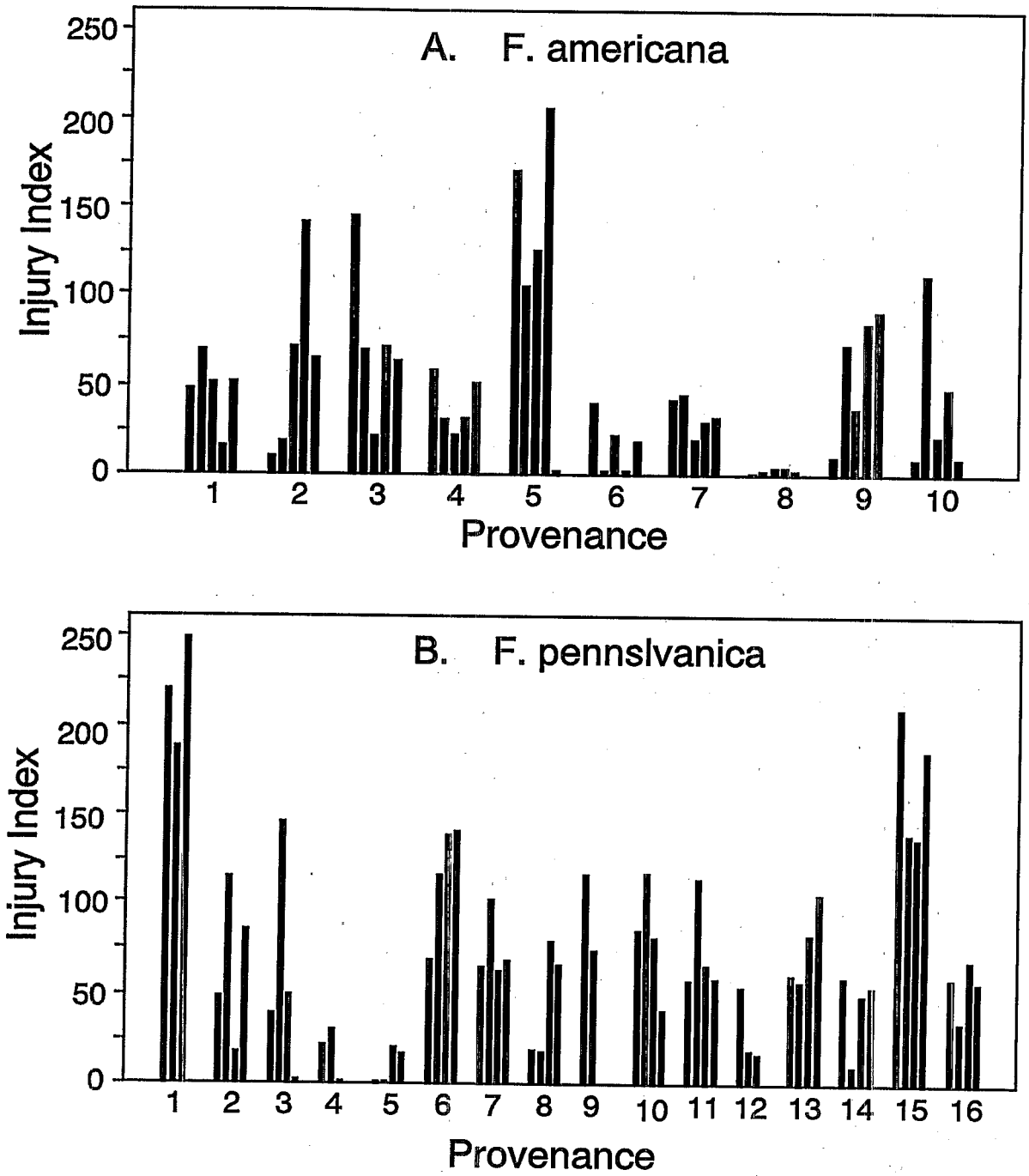

*Figure 5-7. The average injury index for visible foliar injury after exposure of 1-year-old seedlings to 50 pphm ozone for 7.5 h. Each mean shown represents the average of five trees per family. There were either four or five half-sib families for each white ash (*Fraxinus americana *L.) provenance (geographic location) and either three or four families for each green ash (*F. pennsylvanica *Marsh.) provenance. The specifics of the experimental design are reported in Karnosky and Steiner (1981).*

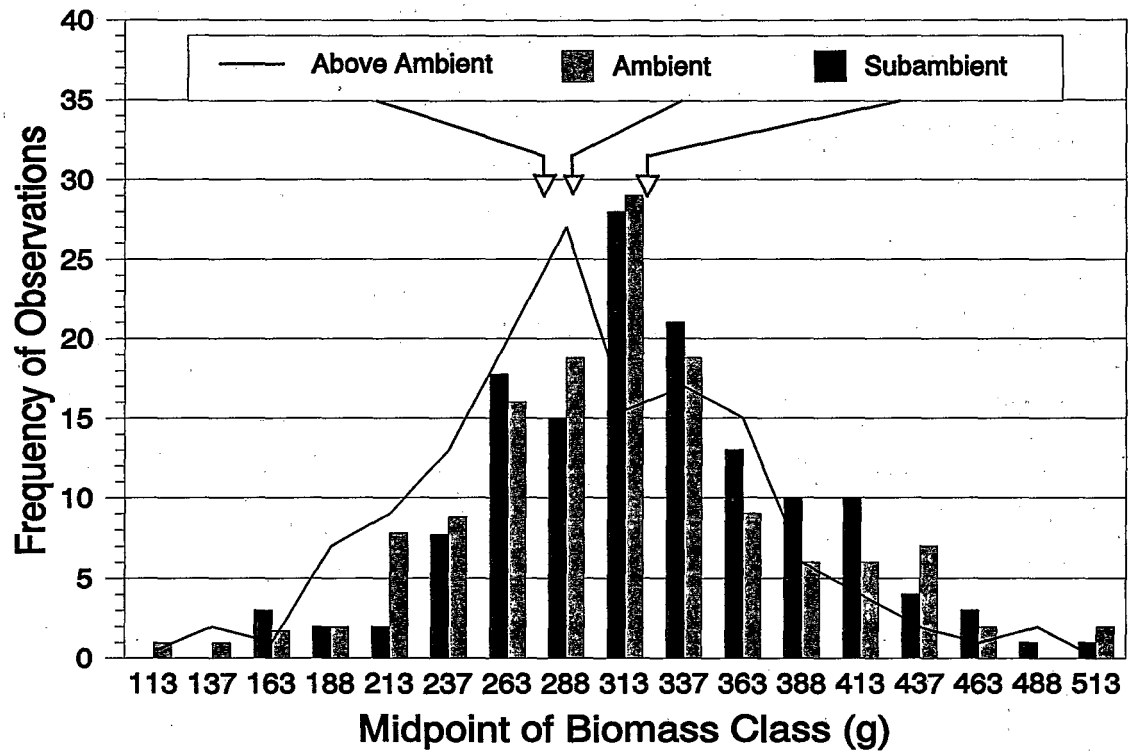

Figure 5-8. Frequency distribution showing the variability in ozone (O_3) response (midpoint of whole-plant biomass) within one half-sib family of loblolly pine (P. taeda L.) exposed to increasing levels of O_3 under chronic-level field conditions over several growing seasons (Adams et al., 1988). The arrows show the mean response for each of the three O_3 treatments (subambient, ambient, and above-ambient O_3). The specifics of the experimental design are reported by Adams et al. (1988). This figure was developed by Taylor (1994).

Sumizono and Inoue, 1986; Tingey and Taylor, 1982) and that involves a high physiological cost because plants that have reduced stomatal conductivity also will have reduced carbon assimilation for growth (Ehleringer, 1991). Tolerance of internal leaf tissues to O_3 may involve the production of antioxidant defense compounds (Lee and Bennett, 1982; Gupta et al., 1991) or other types of biochemical defense systems. The extent to which these internal tolerance mechanisms have physiological costs associated with them is not yet understood, but it is likely that increased defense compound production, triggered by O_3, will impact the amount of carbon available for growth (Ehleringer, 1991). The genetic regulation of these or other O_3 resistance mechanisms has not yet been characterized thoroughly.

Whether or not O_3 resistance is due to single gene or multi-gene control will affect the rate and extent of resistance development (Roose, 1991). Rapid stomatal closing in the presence of O_3 appears to be under the control of either a single gene or a few genes in onion (*Allium cepa*) (Engle and Gabelman, 1966), some bean (*Phaseolus vulgaris*) cultivars (cultivated varieties) (Knudson-Butler and Tibbitts, 1979), soybean (Damicone et al., 1987b),

and petunia (*Petunia* spp.) (Elkiey and Ormrod, 1979a,b; Elkiey et al., 1979). Generally, resistance mechanisms appear to be more complex (Karnosky, 1989a) and seem to involve multiple gene control as has been demonstrated in tobacco (Aycock, 1972; Huang et al., 1975; Povilaitis, 1967), some bean cultivars (Mebrahtu et al., 1990a,b,c), corn (Cameron, 1975), tall fescue (*Festuca arundinacea*) (Johnston et al., 1983), potato (De Vos et al., 1982; Dragoescu et al., 1987), and loblolly pine (Weir, 1977; Taylor, 1994).

Genetic Implications of Ozone Effects: Managed Ecosystems

Because of the high cost involved in conducting long-term growth studies to determine O_3 effects on plants, only a small proportion of the total number of commercial crop cultivars and important tree seed sources, families, clones, and cultivars have been examined adequately for O_3 sensitivity. Still, a tremendous amount of variation has been found, as was described in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986) and in Tables 5-2 and 5-3.

Plant breeders and nurserymen working in locations with high O_3 concentrations have inadvertently developed selections more tolerant to O_3 than those developed in locations with low O_3 exposures (Reinert et al., 1982; Roose et al., 1982). The cultivars Team alfalfa and Kennebec, Pungo, and Katahdin potatoes were developed at the U.S. Department of Agriculture Research Center at Beltsville, where 0.120 ppm O_3 frequently is exceeded (Lefohn and Pinkerton, 1988; Ludwig and Shelar, 1980). These cultivars have proven to be more O_3 tolerant than cultivars developed elsewhere (Reinert et al., 1982). Similarly, cotton (*Gossypium* spp.) and sugar beet (*Beta* spp.) cultivars developed in Southern California, where O_3 levels are among the highest in the country, are more O_3 tolerant than cultivars developed in low O_3 areas (Reinert et al., 1982).

Nurserymen, Christmas tree growers, and seed orchard managers all routinely have discarded pollution-sensitive chlorotic dwarf and tip-burned white pine trees because of their slow growth in areas with high O_3 exposures (Umbach and Davis, 1984). Thus, they have contributed to the selection of more O_3-tolerant commercial forests.

Although these examples suggest that selection of O_3-tolerant genotypes is possible, the general consensus of the scientific community is that top priority should be given to solving pollution problems at their source (Karnosky et al., 1989) and not in selecting pollution-tolerant cultivars.

An interesting set of experiments by Barnes et al. (1990c) and Velissariou et al. (1992) have described a concern about the modern crop varieties that have been developed in clean-air environments but are being grown routinely in areas with elevated O_3 exposures. These authors speculated that breeders of spring wheat (*Triticum aestivum*) grown in Greece inadvertently had selected varieties with increased O_3 sensitivity due to their higher rates of stomatal conductivity (Velissariou et al., 1992). Velissariou et al. (1992) found a significant correlation between year of introduction and stomatal conductance, with stomatal conductance, increasing with the more modern introductions. The authors suggested that the selection for higher yields had resulted in a higher O_3 uptake for the modern spring wheat cultivars, contributing to their increased O_3 sensitivity. When they compared the relative growth rates of spring wheat cultivars released over the period from 1932 to 1980, the modern cultivars had more foliar injury and more growth decrease when grown in the presence of O_3 (Barnes et al., 1990c; Velissariou et al., 1992).

Genetic Implications of Ozone Effects: Natural Ecosystems and Biodiversity

Air pollutants can affect the genetics of plant populations in two ways: (1) they may increase mutation rates or (2) they may apply selection pressures that eventually may lead to adaptive responses (Cook and Wood, 1976). The issue of O_3-induced changes in mutation rate has not been studied adequately, but recent evidence by Floyd et al. (1989) suggests that DNA may be affected by O_3 to induce mutation in plants. However, there is evidence that O_3 may be affecting plant populations via natural selection. According to Bradshaw and McNeilly (1991), there are three stages of selection-driven population change: Stage I, elimination of the most sensitive genotypes; Stage II, elimination of all genotypes except the most resistant; and Stage III, interbreeding of the survivors.

The first report of O_3 as a selective force in plant populations was that involving lupine (*Lupinus bicolor*) populations in the greater Los Angeles area (Dunn, 1959). Local Los Angeles area populations were more O_3 resistant than populations originating from cleaner-air areas. Berrang et al. (1986, 1989, 1991) have presented evidence for population change in trembling aspen (*Populus tremuloides*). Aspen clones from across the United States were sampled randomly from populations in polluted and nonpolluted areas. Aspen from areas with high ambient O_3 concentrations were injured visibly to a lesser extent by experimental O_3 exposures than clones from areas with low O_3 concentrations (Berrang et al., 1986, 1991). Similar results were seen for field trials of O_3 injury (Berrang et al., 1989). More recently, growth rate and biomass differences have been reported for aspen clones differing in O_3 tolerance (Karnosky et al., 1992b). Berrang et al. (1989) suggest that sensitive genotypes are not killed directly by O_3, but are eliminated through intraspecific competition for light, nutrients, and water with their resistant neighbors. Spatial (population) variation in O_3 resistance that is related to background O_3 pollution also has been demonstrated in British populations of plantago (*Plantago major*) (Reiling and Davison, 1992a,b).

There have been three concerns raised regarding the spatial variation studies of O_3 resistance. First, because O_3 generally does not show steep concentration gradients, spatial studies must involve populations that are great distances from one another, so it is difficult to determine whether geographical differences in O_3 resistance are related primarily to local O_3 exposures or to other environmental factors (Reiling and Davison, 1992a). Second, spatial studies are limited by the general absence of historical records of ambient O_3 concentrations at the sites where the populations were sampled (Bell et al., 1991). Third, no O_3 study has collected plants from the same population over time to demonstrate O_3-induced population change over time (Bell et al., 1991), as has been demonstrated for other pollutants. However, Karnosky (1981b, 1989b) studied the O_3 symptom expression and survival of over 1,500 eastern white pine trees growing in southern Wisconsin and found that O_3-sensitive genotypes had a 10-times-higher rate of mortality than did the O_3-resistant genotypes over a 15-year study (Table 5-4). This is direct evidence of the occurrence of Stage I natural selection. Further evidence of this type was presented by Heagle et al. (1991a), who found a population change in O_3 sensitivity over 2 years with white clover (*Trifolium repens*) exposed to O_3 in OTCs. A high O_3 dose at the end of the study caused significantly less foliar injury in plants that survived two seasons of exposure to high O_3 concentrations than in plants that had survived low O_3 concentrations.

The rate of evolution is dependent on the selection pressure, the magnitude of the genetically controlled variability, and the number of genes involved (Roose, 1991). Long-lived species, such as trees, will evolve more slowly than annuals or biennials

Table 5-4. Mortality of Three Ozone Sensitivity Classes of Eastern White Pine (*Pinus Strobus* L.) Trees During 1971 to 1986

Sensitivity Class[a]	Number Trees	Number Trees Dead	Percent Mortality
Resistant	1,386	34	2.4%
Intermediate	98	3	3.1%
Sensitive	57	14	24.6%

[a]Resistant = not showing visible foliar injury during the study; Intermediate = showing visible injury, including foliar tip burn during 1 or 2 years; Sensitive = showing visible injury, including foliar tip burn, short needles, and poor needle retention for 3 or more years of the study.

Source: Karnosky (1989b).

(Barrett and Bush, 1991). Gillespie and Winner (1989) found O_3 to be a strong and rapid selective force with radish (*Raphanus sativus*). Ozone resistance was expressed within one generation following a series of artificial pollinations with various populations from the radish cultivar "Cherry Belle".

Whether or not the loss of some genotypes from plant populations is important is a debatable question. However, it is likely that sensitive genotypes are being lost from natural ecosystems with current O_3 exposures. Field studies documenting differential growth rates of O_3-sensitive and tolerant genotypes of eastern white pine in natural ecosystems influenced by O_3 were summarized in the previous air quality criteria document for O_3 (U.S. Environmental Protection Agency, 1986). Similar findings subsequently have been reported for O_3-sensitive and tolerant Jeffrey pine trees in California (Peterson et al., 1987). It is likely that these growth-rate differences affect the competitive ability of O_3-sensitive genotypes and increase their mortality rate (Karnosky, 1989b).

Although some loss of rare alleles (one of a series of genes that are alternative in inheritance) and change in gene frequency is likely with loss of sensitive genotypes, the significance of these effects on biodiversity is unknown (Barrett and Bush, 1991). If the remaining population of O_3-resistant plants is less adaptable to subsequent change due to a reduced redundancy, as has been predicted by Gregorius (1989), or if O_3 sensitivity is linked to other traits such as rapid growth or high productivity, as has been suggested because of the inherently higher gas-exchange rates of some O_3-sensitive genotypes (Barnes et al., 1990c; Thorne and Hanson, 1976; Turner et al., 1972; Velissariou et al., 1992), then losing these sensitive genotypes is both biologically and economically important. This remains a point of scientific debate. Although the evolution of resistance to air pollution is hypothesized to contribute to the loss of genetic variability (Scholz et al., 1989; Karnosky, 1991), other scientists suggest that there is little experimental evidence for concluding that genetic diversity is actually threatened by air pollution and that air pollution has less important implications for plant populations than do factors such as global climate change and habitat fragmentation (Parsons and Pitelka, 1991; Taylor and Pitelka, 1992). Clearly, there is a need for additional research in this area of O_3 effects in plant biodiversity (Karnosky et al., 1989).

Reproductive Aspects and Related Genetic Implications

In the previous discussion in this section, only natural selection at the whole-plant level has been mentioned. This type of selection occurs as plants compete with their neighbors for survival and the ability to reproduce. Selection is thought to occur also during the reproductive process (Feder and Sullivan, 1969; Krause et al., 1975), and this is referred to as gametophytic selection (Mulcahey, 1979; Wolters and Martens, 1987) or fertility selection (Venne et al., 1989). The ability of gametophyte (haploid part of the plant-life cycle) selection to modify the sporophytic generation depends on two critical issues: (1) pollen genes should be expressed after meiosis (cell divisions leading to production of gametes), and (2) those same genes also should be expressed in the sporophytes (diploid part of the plant-life cycle) (Mulcahey and Mulcahy, 1983). This genetic overlap has been demonstrated in some species (Mulcahy, 1979; Searcy and Mulcahy, 1985; Walsh and Charlesworth, 1992). Indirect evidence for O_3-induced gametic selection was presented for Scot's pine (*Pinus sylvestris*) by Venne et al. (1989). Based on their studies of the effects of O_3 on the pollen germination and tube elongation of some 30 Scots pine clones, they found that O_3 could change markedly the relative male contribution to successful fertilization. However, this study did not actually examine offspring, as would be needed to positively prove O_3-induced gametophytic selection.

Studies of O_3 effects on pollen germination and tube elongation generally have found a negative impact of O_3 on this critical element of reproduction (Table 5-5). Whether or not selection is occurring at the pollen level because of a selective disadvantage of the pollen from sensitive genotypes is a debatable issue. Feder (1986) and Krause et al. (1975) found that the pollen from O_3-sensitive genotypes of petunia and tomato (*Lycopersicon esculentum*) was more severely affected by O_3 than pollen from tolerant genotypes, suggesting that gametophytic selection could be occurring. Similar results were found for Scots pine clones by Venne et al. (1989). These authors found that the relative male contribution for charcoal-filtered air versus O_3-treated conditions was very different and potentially could lead to a strong gametophytic selection response caused by O_3. However, Hanson and Addis (1975) did not see any differences in the effect of O_3 on the pollen from sensitive and tolerant petunia (*Petunia hybrida*) genotypes, and Benoit et al. (1983) found no apparent differences in the susceptibility of eastern white pine pollen from O_3-sensitive or tolerant genotypes. Clearly, the question of whether O_3-induced gametophytic selection is occurring has not been resolved.

Reduced flowering as the result of prolonged fumigation with O_3 has been shown in bladder campion (*Silene cucabalus*) (Ernst et al., 1985). Decreased floral initiation and decreased floral productivity under long-term O_3 exposures also have been reported in geranium (*Pelargonium* spp.) and carnation (*Dianthus caryophyllus*) (Feder, 1970). Ozone-induced impairment of flowering will reduce the fitness of the affected genotypes, populations or species and may result in the eventual loss of these genetic units from the O_3-stressed ecosystem. Reduced eastern white pine fecundity in air-pollution-stressed ecosystems has been reported by Houston and Dochinger (1977).

Genetic Summary

Plant species, cultivars, populations, and individuals within populations display variable responses to O_3. Variability in O_3 responses among and within species was described in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986). An important component of this variation is genetically controlled. The specific

Table 5-5. Examples of Ozone Effects on Pollen Germination and Tube Elongation

Species	Pollen Germination	Pollen Tube Elongation	Reference
Nicotiana tobacum L. (Tobacco)	Decrease	Decrease	Feder (1968) Feder and Shrier (1990)
Petunia hybrida (Petunia)	Not tested	Decrease	Feder and Shrier (1990)
Pinus strobus L. (Eastern white pine)	No effect	Decrease	Benoit et al. (1983)
Zea mays L. (Corn)	Decrease	Not tested	Mumford et al. (1972)

genes controlling O_3 response and involved in mechanisms of O_3 tolerance are largely unknown. However, control of stomatal conductance and internal biochemical defense systems are among the most commonly described tolerance mechanisms. Ozone tolerance is generally thought to be controlled by multiple genes.

There are implications of genetic variation in O_3 response, both for managed and natural ecosystems. These are summarized below along with the relative degree of uncertainty attached to each.

It is known, with a great deal of certainty, that plants have a high degree of genetic variation in O_3 response. Thus, exposure-response equations and yield-loss equations developed for a single or small number of cultivars, genotypes, families, or populations may not represent adequately the response of the species as a whole.

The issue of O_3 effects on biodiversity via natural selection is a topic of debate within the scientific community. The potential for natural selection for O_3 tolerance and associated loss of sensitive genotypes is regional in nature, unlike well-known, point-source pollution impacts that occur on local plant populations. However, the intensity of O_3 selection generally is thought to be quite low, 0.3 or less (Taylor and Pitelka, 1992), in the majority of the United States. The extent that germplasm has been, or continues to be, affected, in terms of allele loss or gene frequency changes by O_3, and how this might be impacting the genetic adaptability of populations, are open and important research questions.

Although it is well known that individual plants within a species vary in their O_3 tolerance, the physiological costs to tolerant plants are not known in terms of carbon assimilation and allocation. Tolerance mechanisms based on reduced stomatal conductivity in the presence of O_3 would likely reduce growth of tolerant plants. Similarly, tolerance mechanisms based on the productivity of antioxidant compounds likely will shunt plant resources away from growth to the production of the defense compounds. The characterization of the extent and types of physiological costs involved in O_3 tolerance remains an important research question.

5.4.3 Environmental Biological Factors

The previous criteria document (U.S. Environmental Protection Agency, 1986) discussed pollutant-plant-pest and pollutant-plant-pathogen interactions together, and provided a tabular summary of pathogen effects. However, in light of the numerous studies of insect and pathogen interactions that have appeared in recent years, the topics are dealt with separately below. Nevertheless, it is worth reiterating several points made in the previous criteria document.

- Pests and diseases are natural components of managed and natural ecosystems.
- Significant crop and timber losses result from pests and pathogens.
- The establishment of disease and pest infestations and their subsequent development involve complex interactions among the host plant, the environment, and the causal organism.
- The generalized disease (or pest infestation) cycle involves the arrival of the pathogen or pest on the host plant surface or its introduction into the host plant tissues through wounds or as a result of insect feeding activity.
- Growth and development or propagation of the pathogen or pest only occurs if all environmental conditions are favorable.
- Such development leads to various degrees of host tissue destruction or malfunction, and usually culminates in the causal organism entering a reproductive stage and producing propagules (e.g., spores or eggs) that facilitate its spread.

Ozone may modify any stage of the disease cycle directly, by affecting the causal organism itself, or indirectly, by effects on the host plant (Lechowicz, 1987). Conversely, the plant-pest interaction may modify the sensitivity of the host plant to O_3.

The roots of many members of the pea family (including many important crops such as soybeans, beans, and peas [*Pisum sativum*]) are infected by symbiotic nitrogen-fixing bacteria (*Rhizobium spp.*), leading to the formation of bacteria-rich nodules that contribute to the nitrogen economy of the plant through their ability to fix and convert atmospheric nitrogen to biologically useful forms. Other nitrogen-fixing microorganisms are associated with the roots of several species, and, in many cases, roots are invaded by species of soil fungi to form mycorrhizal symbioses that assist in root functioning. These symbioses constitute micro-ecosystems and are discussed more fully in Section 5.7 as they relate to forest tree species.

Biological interactions also affect the growth of plants in populations (pure stands) and communities (mixtures of species) through the individual plants' competition for available resources (light, CO_2, water, and nutrients). Such plant-plant interactions are features of all managed and natural ecosystems, but they operate at the individual plant level. Hence, the effects of oxidants on these interactions are discussed in this section, as well as in Section 5.7, which deals with ecosystem responses.

5.4.3.1 Oxidant-Plant-Insect Interactions

The previous criteria document (U.S. Environmental Protection Agency, 1986) concluded that little was known at that time about O_3-insect interactions. Since then, the topic has been covered in several reviews: Fluckiger et al. (1988), Hughes (1988), Manning and Keane (1988), and Hain (1987). Relevant studies of the effects of O_3 on the feeding preference of herbivorous insects and on their growth, fecundity, and survival are presented

in Table 5-6. As can be seen readily in this summary, the information is scattered widely among a wide range of host plants and pests. Nevertheless, there appears to be a general trend in the observations suggesting that O_3-induced changes in the host plants frequently result in increased feeding preference of a range of insect species, although this may or may not be reflected in effects on the growth of the insect.

However, in most studies, the effects have been far from clear-cut. For example, variable responses were observed with the aphid, *Aphis fabae*, on broad bean (*Vicia faba*) (Brown et al., 1992); with the aphids, *Acyrthosiphon pisum* and *Aphis rumicis*, on pea and dock (*Rumex obtusifolius*), respectively; with the beetle, *Gastrophysa viridula*, on dock (Whittaker et al., 1989), with the Mexican bean beetle, *Epilachna varivestis*, on Corsoy soybean (Endress and Post, 1985); and with the gypsy moth, *Lymantria dispar*, on white oak (*Quercus alba*) (Jeffords and Endress, 1984). Although statistically significant effects were observed frequently, they did not provide any consistent pattern of insect growth response to different levels or patterns of exposure.

Brown et al. (1992) observed that the response of *Aphis fabae* depended on the dynamics of exposure: growth was stimulated in short-term (<24 h) continuous exposures or in episodic exposures over several days, whereas longer continuous exposures caused decreased growth. Chappelka et al. (1988c) found that O_3 consistently enhanced the feeding preference and larval growth of the Mexican bean beetle on soybean, leading to increased defoliation. Although the cultivar Forrest was significantly more sensitive to O_3 than Essex, this difference did not lead to any differences in insect behavior and development. Similarly, clear stimulatory responses were observed with pinworm, *Keiferia lycopersicella*, on tomato (*Lycopercicon esculentum*) (Trumble et al., 1987); with an aphid, *Phyllaphis fagi*, and a weevil, *Rhynchaenus fagi*, on European beech (*Fagus sylvatica*) (Braun and Fluckiger, 1989; Hiltbrunner and Fluckiger, 1992); with the monarch butterfly, *Danaus plexippus*, on milkweed (*Asclepias syriaca*) (Bolsinger et al., 1991, 1992); and with infestation by the willow leaf beetle, *Plagiodera versicolora*, on cottonwood (*Populus deltoides*) (Coleman and Jones, 1988). However, there was less egg-laying by *Plagiodera* on O_3-treated foliage, and treatment had no effect on beetle growth rates and survival (Jones and Coleman, 1989).

In view of previous experiments in which it was demonstrated clearly that aphid growth was stimulated significantly by ambient pollutant mixtures containing O_3, SO_2, and NO_2 and, in light of other reports of O_3-induced stimulations of insect growth, the inhibitory effects of O_3 on the growth of *Aphis fabae* on broad bean (Dohmen, 1988) or kidney bean (Braun and Fluckiger, 1989) may be anomalous. The inhibitory effects on broad bean were observed only at low O_3 levels; exposure to higher concentrations resulted in a stimulation of aphid growth, which Dohmen (1988) attributed to the increased rate of leaf senescence of the host plant. The effects observed on kidney bean could not be accounted for by differences in the amino acid composition of the plant sap, although differences in other constituents or direct effects of O_3 on the pea aphid itself could not be ruled out (Braun and Fluckiger, 1989).

A well-established indirect stimulatory effect is the predisposition to bark beetle attack of ponderosa pine injured by exposure to O_3. However, the infested trees do not favor good brood production; O_3 injury results in a more susceptible but less suitable host (Hain, 1987).

In all of these studies, the focus was on direct or indirect effects of O_3 on the insect. With the exception of the work of Braun and Fluckiger (1989), any effects on the

Table 5-6. Ozone Effects on Insect Pests

Host Plant/Insect	Exposure[a]	Experimental Conditions[b]	Effect of Ozone on Insect	Reference
CROP SPECIES				
Broad bean/aphid	3 day, 0.085 ppm <24 h, 0.1 ppm >24 h, 0.1 ppm 8 h/day, episodic	Chamber, whole plant Chamber, whole plant Chamber, whole plant Chamber, whole plant	3-13% decreased growth rate 17% increased growth rate 12% decreased growth rate 15% increased growth rate	Dohmen (1988), Brown et al. (1992)
Pea/aphid	4-8 day, var.	Chamber, whole plant	Variable effects on growth	Whittaker et al. (1989)
Kidney bean/aphid	14 day, var.	OTC	15-50% reduction in growth of insect	Braun and Fluckiger (1989)
Soybean/beetle (cv. Corsoy) (cvs. Essex, Forrest)	16 day, var. 21 day, 7 h/day, var.	Chamber OTC	Variable feeding preference 0.11>0.0>0.05>0.03 ppm; feeding preference increased and greater larval growth	Endress and Post (1985), Chappelka et al. (1988c)
Tomato/pinworm	2-4 day, 3 h/day 0.28 ppm	Chamber, detached leaf Chamber, whole plant	80% increase in larval development; no effect on fecundity	Trumble et al. (1987)
NATURAL VEGETATION				
Milkweed/monarch butterfly	17-19 day, 7 h/day 0.150-0.178 ppm	Chamber, whole plant	No feeding preference but greater larval growth rate	Bolsinger et al. (1991, 1992)
Dock/aphid	15 day, var.	Chamber, whole plant	10% increased growth rate	Whittaker et al. (1989)
Dock/beetle	15 day, var.	Chamber, whole plant	10% larger egg batches; fourfold greater larval survival	Whittaker et al. (1989)
TREES SPECIES				
European beech/aphid	2 mo, var.	OTC	75% increase in number	Braun and Fluckiger (1989)
European beech/weevil	72 h, var.	OTC	Twofold increase in feeding preference	Hiltbrunner and Fluckiger (1992)

Table 5-6 (cont'd). Ozone Effects on Insect Pests

Host Plant/Insect	Exposure[a]	Experimental Conditions[b]	Effect of Ozone on Insect	Reference
TREE SPECIES (cont'd)				
Cottonwood/beetle	5 h, 0.2 ppm	OTC	22-60% greater consumption of foliage but decreased fecundity	Jones and Coleman (1988), Coleman and Jones (1988)
Ponderosa pine/bark beetle	Natural	None, field	Increased infestation but decreased survival	Hain (1987)
White oak/gypsy moth	11 day, 7 h/day, var.	Chamber, leaf disks	Variable feeding preference 0.15 > 0.03 > 0.09 ppm	Jeffords and Endress (1984)

[a]var. indicates a range of exposures.
[b]Chamber indicates closed chamber; OTC indicates open-top field chamber.

host plant that were reported were confined to observations on visible symptoms of foliar injury. The only report of an O_3-insect interaction affecting the response of the host plant appears to be that of Rosen and Runeckles (1976). This study showed that exposure to subacute levels of O_3 and infestation with the greenhouse whitefly, *Trialeurodes vaporariorum*, acted synergistically (i.e., more than additively) in causing leaf injury and accelerated senescence of kidney bean. However, the extent to which other insects with sucking mouthparts, such as aphids, might be involved in similar interactive responses is unknown, as is the nature of any interactions that involve pests that ultimately invade and develop within the host plant, such as those that cause the formation of galls.

The reports of O_3-insect-plant interactions are thus scattered among a wide range of host plant and insect species, and represent only a minute fraction of the plant-insect interactions that involve crop and native species. Although there appears to be a trend in the limited data available that suggests that exposures to moderate O_3 levels may increase the likelihood of insect attack and its consequences, there is insufficient information to decide whether extrapolation of this generalization is warranted or not. Even if the generalization is valid, it is not possible to generate any quantitative measure of response. Before such estimates will be possible on a broad scale, studies of a much wider range of plant insect-systems will be needed, together with systematic, in-depth studies of individual systems, aimed at determining the long-term effects on both the host plant and the insect. Such studies should include investigations of biological control systems employing beneficial insects, which are used increasingly as alternatives to chemical insecticides and herbicides.

5.4.3.2 Oxidant-Plant-Pathogen Interactions

Plant disease is the result of infection by fungi, bacteria, mycoplasmas, viruses, and nematodes. Recent reviews of pathogen-plant-O_3 interactions have been published by Dowding (1988) and Manning and Keane (1988) and extend the coverage of the previous criteria document (U.S. Environmental Protection Agency, 1986), in which the results of published studies of the effects of O_3 on disease development were summarized in tabular form. Interactions involving fungal pathogens occupied most of that review, and more recent studies have maintained this emphasis.

The previous criteria document concluded that it was "impossible to generalize and predict effects in particular situations" (U.S. Environmental Protection Agency, 1986). However, Dowding (1988) since has concluded that pathogens that can benefit from injured host cells or from disordered transport mechanisms are enhanced by pollution insult to their hosts, whereas those that require a healthy mature host for successful invasion and development are depressed by pollutant stress to their host.

This conclusion is supported by evidence that the development of diseases caused by obligate parasites such as the rust fungi and bacterial pathogens usually is reduced by O_3. As shown by the observations summarized in Table 5-7, reductions in disease development were observed in five of the nine studies of obligate fungal parasites listed, whereas increases were observed in all but four of the studies of facultative fungal pathogens. Similarly, in four of the five bacterial systems, O_3 reduced infection or disease development. It should be noted that, in three of the four studies of obligate fungi on which exposure to O_3 either had no effect or that resulted in stimulated fungal growth, the pathogen was a powdery mildew *(Erysiphe, Microsphaera)*. As discussed by Tiedemann et al. (1991), these species constitute a special case because they are ectoparasites whose hyphae merely penetrate the surface epidermal cells of the host plant's leaves rather than the mesophyll

Table 5-7. Ozone-Plant-Pathogen Interactions[a]

Host Plant	Pathogen	Effect of O_3 on Disease	Effect of Disease on O_3 Response	Reference
OBLIGATE FUNGI				
Kidney bean	*Uromyces phaseoli*	Increased number of smaller pustules	Reduced injury on severely diseased leaves	Resh and Runeckles (1973)
Barley	*Erysiphe graminis*	Reduced infection but greater spore production	Not reported	Heagle and Strickland (1972)
Cottonwood	*Melampsora medusae*	Reduced infection and development	Not reported	Coleman et al. (1987)
Lilac	*Microsphaera alni*	No effect	Not reported	Hibben and Taylor (1975)
Oats	*Puccinia coronata*	Reduced infection and development	No effect	Heagle (1970)
Wheat	*Erysiphe graminis*	Increased infection and development	Not reported	Tiedemann et al. (1991)
	Puccinia graminis	Reduced infection and development	Reduced leaf injury	Heagle and Key (1973a,b)
	Puccinia graminis	Reduced development	Not reported	Heagle (1975)
	Puccinia recondita	Reduced infection and development	Not reported	Dohmen (1987)
FACULTATIVE FUNGI				
Barley	*Drechslera teres*	Increased infection	Not reported	Tiedemann et al. (1990)
	Gerlachia nivalis	Increased infection	Not reported	Tiedemann et al. (1990)
	Helminthosporium sativum	No effect	Not reported	Tiedemann et al. (1990)
Cabbage	*Fusarium oxysporum*	Decreased development	Not reported	Manning et al. (1971a)
Corn	*Helminthosporium maydis*	Increased development	Not reported	Heagle (1977)

Table 5-7 (cont'd). Ozone-Plant-Pathogen Interactions[a]

Host Plant	Pathogen	Effect of O_3 on Disease	Effect of Disease on O_3 Response	Reference
FACULTATIVE FUNGI (cont'd)				
Cottonwood	*Marssonina brunnea*	Increased infection	Not reported	Coleman et al. (1988)
Geranium	*Botrytis cinerea*	Decreased infection	Not reported	Krause and Weidensaul (1978)
Onion	*Botrytis* (3 spp.)	Increased infection and development	Not reported	Wukasch and Hofstra (1977a,b)
Potato	*Botrytis cinerea*	Increased infection and development	Not reported	Manning et al. (1969)
	Alternaria solani	Increased infection	Not reported	Holley et al. (1985)
	Alternaria solani	Increased infection	Not reported	Bisessar (1982)
Soybean	*Fusarium oxysporum*	Increased infection	Increased leaf injury	Damicone et al. (1987a)
Wheat	*Gerlachia nivalis*	Increased infection	Not reported	Tiedemann et al. (1990)
	Helminthosporium sativum	No effect	Not reported	Tiedemann et al. (1990)
	Helminthosporium sativum	Increased infection	Not reported	Tiedemann et al. (1991)
	Septoria (2 spp.)	Increased infection	Not reported	Tiedemann et al. (1990)
	Septoria (2 spp.)	Increased infection	Not reported	Tiedemann et al. (1991)
Jeffrey pine	*Heterobasidium annosum*	Increased development	Not reported	James et al. (1980a)
Ponderosa pine	*Heterobasidium annosum*	Increased development	Not reported	James et al. (1980b)
White pine	*Vertcicladiella procera*	Slightly increased incidence	Not reported	Costonis and Sinclair (1972)
	Lophodermium pinastre	Slightly increased incidence	Not reported	Costonis and Sinclair (1972)

Table 5-7 (cont'd). Ozone-Plant-Pathogen Interactions[a]

Host Plant	Pathogen	Effect of O_3 on Disease	Effect of Disease on O_3 Response	Reference
BACTERIA				
Alfalfa	*Xanthomonas alfalfae*	Reduced development	Reduced leaf injury	Howell and Graham (1977)
Soybean	*Pseudomonas glycinea*	Reduced incidence	No effect	Laurence and Wood (1978a)
	Pseudomonas spp.	Reduced infection	Reduced leaf injury	Pell et al. (1977)
White bean	*Xanthomonas phaseoli*	No effect	Reduced leaf injury	Temple and Bisessar (1979)
Wild strawberry	*Xanthomonas fragariae*	Reduced incidence	No effect	Laurence and Wood (1978b)
NEMATODES				
Begonia	*Aphelenchoides fragariae*	Reduced nematode reproduction	Reduced leaf injury	Weber et al. (1979)
Soybean	*Belonolaimus longicaudatus*	Stimulation or no effect	Not reported	Weber et al. (1979)
	Heterodera glycines	Reduced nematode reproduction	Not reported	Weber et al. (1979)
	Paratrichodorus minor	Reduced nematode reproduction	Reduced leaf injury	Weber et al. (1979)
	Pratylenchus penetrans	No effect	Not reported	Weber et al. (1979)
Tobacco	*Meloidogyne hapla*	Possible stimulation[b]	Increased leaf injury	Bisessar and Palmer (1984)

[a]See Appendix A for abbreviations and acronyms.
[b]Based on studies using the protectant EDU (see Section 5.2.1.2).

tissues within the leaves. They noted that Heagle and Strickland (1972) observed greater pustule development of *Erysiphe* on exposed barley once infection was established, although the pathogen was sensitive during the early stages of infection. Tiedemann et al. (1991) suggest that the observed stimulations result from a differential weakening of the host's resistance response to the pathogen.

In a few of the studies summarized in Table 5-7, effects of disease development on the sensitivity of the host plant to O_3 were noted. Heagle and Key (1973b) and Resh and Runeckles (1973) confirmed the earlier observation of Yarwood and Middleton (1954) that infection with obligate rust fungi could reduce the severity of acute injury caused by exposure to O_3. However, with *Uromyces* on bean, the "protection" was noted only on severely infected leaves (Resh and Runeckles, 1973), and Heagle (1970) observed no such effect with crown rust, *Puccinia coronata*, on oats.

Infection with bacterial pathogens and nematodes also tends to reduce the impact of O_3, and almost all studies of the interactions of O_3 with virus infections appear to do so. The previous criteria document (U.S. Environmental Protection Agency, 1986) reviewed the supporting evidence from numerous studies with a range of host plants and viruses, and noted only two studies in which O_3 injury was apparently increased by virus infection (Ormrod and Kemp, 1979; Reinert and Gooding, 1978). However, with tomato infected by mosaic viruses, injury was reduced in the leaves of plants in which viral infection was well established (Ormrod and Kemp, 1979). Two more recent studies have indicated either no effect or variety-dependent increased sensitivity to relatively high O_3 levels. Heagle et al. (1991a, 1992) found no effects of infection with several viruses on the response of two clonal strains of white clover. On the other hand, Reinert et al. (1988) reported that three cultivars of burley tobacco responded differently to O_3 when infected with either tobacco etch virus or tobacco vein mottling virus (TVMV). Although tobacco etch virus infection resulted in the protection of cultivars from O_3-induced growth suppression, TVMV infection enhanced the suppression of the growth of two cultivars, Burley 21 and Greenville 131, but had no effect on the third, Burley 49.

With the exception of one field study demonstrating the suppression of O_3 injury on tobacco infected with tobacco mosaic virus (Bisessar and Temple, 1977), the other investigations of virus interactions all have been conducted in laboratory or greenhouse chambers, which raises the question of the relevance of these investigations to field conditions. As noted in the previous criteria document (U.S. Environmental Protection Agency, 1986), with few exceptions, the reports of viral protection are probably of little commercial significance but may provide information at the mechanistic level of plant response. The same caveat is equally applicable to the significance of protective effects of other obligate pathogens.

No studies appear to have been conducted of interactions involving disease-causing mycoplasmas.

As in the case of plant-insect interactions, much more systematic study is needed before it will be possible to provide any quantitative estimates of the magnitude of the interactive effects. The patterns of pollutant modification of plant-pathogen relations suggested by Dowding (1988) are supported partly by the limited evidence available for O_3, but studies of a wider range of plant-pathogen systems will be needed before it will be possible to provide quantitative generalizations.

5.4.3.3 Oxidant-Plant-Symbiont Interactions

Exposure to O_3 can modify the symbiotic relationships between plants and microorganisms. In the case of *Rhizobium*, the important nitrogen-fixing symbiont of many leguminous species, the adverse effects of exposure of the host plant reviewed in the previous criteria document (U.S. Environmental Protection Agency, 1986) all were observed at O_3 levels of 0.3 ppm or greater. However, Flagler et al. (1987) observed a consistent decline in total nitrogen-fixing activity of nodulated soybean roots with increasing O_3 concentrations up to 0.107 ppm (7-h/day seasonal average), with no effect on specific nodule activity. In a greenhouse study of soybean plants exposed at three different growth stages to a 12-h treatment in which the peak O_3 concentration (at 6 h) was 0.2 ppm, Smith et al. (1990) observed a 40% decrease in specific nodule activity. Hence, there is limited evidence to indicate adverse effects on Rhizobial nitrogen-fixation at O_3 levels experienced in polluted air.

The effects of O_3 on mycorrhizal fungal symbioses have been reviewed by Manning and Keane (1988) and McCool (1988). Seasonal exposures averaging 0.079 ppm O_3 resulted in a 40% reduction in the growth of the vesicular-arbuscular endomycorrhizal fungus, *Glomus fasciculatus*, on soybean roots; however, mycorrhizal infection lowered the O_3-induced reduction in pod yield from 48 to 25% (Brewer and Heagle, 1983). Once-weekly exposures of tomato plants to 0.3 ppm for 3 h retarded the early development of the same fungus on tomato seedling roots, leading to reduced seedling growth (McCool et al., 1982). Greitner and Winner (1989) reported that the increased availability of nitrogen to alder (*Alnus serrulata*) seedlings resulting from the presence of root nodules containing the nitrogen-fixing actinomycete, *Frankia*, enabled plants to recover their photosynthetic integrity rapidly after exposure to O_3; however, they did not investigate effects on symbiont.

In spite of the inconsistencies in the available evidence, it appears that rhizobial and mycorrhizal growth is likely to be impaired as a consequence of long-term exposure to oxidant stress, probably because of reduced allocation of photosynthate to the root system (Chapter 7, U.S. Environmental Protection Agency, 1986). However, the implications of such effects on mycorrhizae are particularly difficult to predict because of an inadequate understanding of the functioning of the tree root-mycorrhiza-soil system.

5.4.3.4 Oxidant-Plant-Plant Interactions—Competition

In the field, the growth of any plant is to some extent dependent on its ability to compete for resources with its neighbors. Some are better competitors than others for light, water, nutrients, and space. Grime (1979) characterized as "competitors" those with a rapid growth rate associated with a capacity to adjust to rapidly changing conditions. Factors such as light or soil nutrients are not available *ad libitum*, because of the mutual shading of leaves within the canopy and root competition. Competition may be either intra- or interspecific, (i.e., the interference may be caused by neighboring members of the same or other species). The planting densities and row spacings adopted for agricultural crops represent compromises between maximizing the number of plants per unit area and the adverse effects of intraspecific competition. Weeds are typical interspecific competitors; interspecific competition also occurs in mixed plantings, such as grass-clover forage and pasture plantings and is an important feature of natural ecosystems.

Although competition from weeds may contribute more to crop losses on a global scale than any other factor, no studies appear to have been conducted on the effects of oxidant pollution on such competition. On the other hand, a few crop mixtures have been

studied. A consistent finding with grass-clover mixtures has been a significant shift in the mixture biomass in favor of the grass species (Bennett and Runeckles, 1977; Blum et al., 1983; Kohut et al., 1988a; Rebbeck et al., 1988; Heagle et al., 1989b).

As the number of competing species increases, the interactions more appropriately are dealt with at the ecological level, but, as demonstrated by the work of Evans and Ashmore (1992), it is important to recognize that, because of the differential stresses imposed by competition, the impact of O_3 on the components of a mixture may not be predictable on the basis of knowledge of the responses of the individual species grown in isolation. A similar caution must be stated about extrapolating to field conditions the results obtained in laboratory studies in which competition may be minimal. However, the development and use of field exposure systems have permitted many recent studies of crop species to be conducted at normal planting densities and, hence, have incorporated interspecific competition as an environmental factor. On the other hand, most forest tree studies have tended to be "artificial" in their use of individual seedlings or saplings or spaced trees, even when exposed in open-air systems (McLeod et al., 1992).

The significance of the effects of competitive interactions on the O_3 response of the competing species is thus largely unknown except for a few cases involving grass-legume mixtures. However, these are far from typical because they only involve two species, one of which is a legume with unique nitrogen nutrition conferred by the nitrogen-fixing capabilities of Rhizobial symbionts. Hence, the lack of knowledge of the effects of O_3 on competitive interactions leads to considerable uncertainty in attempting to assess the impact of O_3 on both managed and natural ecosystems by extrapolation from effects on individual species.

5.4.4 Physical Factors

The physical components of the plant's aerial environment are light, temperature, humidity, air turbulence, and surface wetness, whereas the physical, edaphic components affecting the plant roots are temperature, soil moisture, and soil salinity. The previous criteria document (U.S. Environmental Protection Agency, 1986) also included soil fertility under this heading; in the present review, this topic is dealt with separately in Section 5.4.5, which deals with chemical factors. The effects of the physical climatic factors (light, temperature, atmospheric turbulence, and the availability of water) on plant growth and survival are major determinants of the geographic distribution of the earth's natural vegetation and of the distribution of agricultural lands and the suitability of the crops grown on them. Because of the control that these factors exert over plant growth, their variation, especially in the short term, can be expected to influence the magnitude of plant responses to oxidants. As in the previous criteria document, the factors are discussed individually, although their actions on plant growth and sensitivity are interrelated closely. Ozone uptake and the effect of air turbulence on boundary layer processes is discussed in Section 5.3.2. A brief integration of their effects is presented in Section 5.4.8, which discusses the effects of global climate change.

At the time of the previous criteria document, much of the knowledge of the effects of these factors came from laboratory and greenhouse experimentation that focused the foliar injury response of high exposures to O_3, which exceeded those likely to be encountered in ambient air. Since then, more information has become available on growth effects, especially with regard to the key area of the interactions involving drought stress.

5.4.4.1 Light

Light influences plant growth through its intensity, quality (i.e., the distribution of wavelengths), and duration (i.e., daylength or photoperiod). Much of the early work on light-oxidant interactions is largely of academic interest because light intensity and daylength are uncontrolled in natural field situations. However, reduced intensities are needed for the production of shade-grown cigar wrapper tobacco and in many commercial greenhouse floriculture operations, in which photoperiod also may be controlled in order to induce flowering. The general conclusion reported previously (U.S. Environmental Protection Agency, 1986) is that susceptibility to foliar injury is increased by low intensities and short photoperiods, although unpredictable responses had been observed when plants were subjected to increased or decreased intensities during and after exposure to O_3. One aspect of increased susceptibility to low light intensities that needs to be emphasized concerns the fact that many studies of oxidant effects have been conducted in controlled-environment chambers in which the light intensities used have rarely approached those of natural sunlight and, hence, may have magnified the observed responses. Significant differences in the amounts of foliar injury were observed on soybean plants grown in a growth chamber, a shaded greenhouse, or in an OTC in the field, when subsequently treated with a standard O_3 exposure, although the growing conditions other than light intensity and quality were comparable (Lewis and Brennan, 1977). Factors other than light intensity must have contributed to the observed differences because the descending order of sensitivity was greenhouse-growth chamber-field chamber, although the average light intensities in the greenhouse and growth chamber were 81 and 18%, respectively, of those in the field chamber.

Reduced light intensities have been measured in OTCs in the field, resulting from the build-up of dust on the walls. However, Heagle and Letchworth (1982) could detect no significant effects on soybean growth and yield in a comparison of plants grown in unshaded OTCs and chambers to which shading cloth was applied.

At the mechanistic level, Darrall (1989) has reviewed the effects of light intensity and suggests that, at high intensities, the potential for endogenous oxyradical production is greatest, and that this, combined with the production of oxyradicals from O_3, might exceed the leaf's detoxification ability. However, at lower intensities, decreased carbon assimilation would limit the availability of energy for use in cellular repair.

In most species, light indirectly plays a major role in the opening and closing of stomata. Because stomata, therefore, tend to close at night and open during the day, light duration, to some extent, dictates whether or not O_3 can be taken up by foliage from the ambient air.

5.4.4.2 Temperature

Temperature affects almost all physical processes and chemical reactions within the plant. Hence, it is the temperature within the plant tissues that is important. Although air temperature dictates the overall heat balance in the surrounding air, the temperature of the leaf also is determined by the absorption of infrared radiation during the photoperiod (which increases the leaf temperature) and the loss of water vapor through transpiration (which provides evaporative cooling). Hence, the effects of air temperature per se must be viewed in the context of these other physical factors. It therefore is not surprising that the few early studies of the effects of air temperature alone, using controlled environment chambers, led to variable and conflicting results, as noted in the previous criteria document (U.S.

Environmental Protection Agency, 1986). In most of these studies, the RH and light intensity were held constant. In water-saturated air with a RH of 100%, the absolute humidity (or water vapor pressure) increases with temperature. Such increases occur at all RHs. Therefore, at constant RH, the increase in absolute humidity, or vapor pressure with temperature, in turn, increases the vapor-pressure deficit (VPD) (i.e., the difference between the absolute humidity, or vapor pressure) and that of completely saturated air at the same temperature. Because VPD controls the rate of evaporation of water, at constant RH, the effects of temperature are unavoidably confounded with effects on VPD. In a recent study with tomato seedlings, in which differences in VPD at different temperatures were minimized, Todd et al. (1991) showed that, out of 11 growth variables measured, the only significant modifications of the effects of O_3 caused by temperature were on stem fresh weight and specific leaf area (leaf area/leaf dry weight). The authors suggest that VPD probably plays a more important role in determining sensitivity to O_3 than temperature.

Although transpiration rate is dependent on VPD, it also is regulated by the opening and closing of stomata on the leaf surface, vertical wind velocities, and factors, such as O_3, that cause stomatal closure indirectly will cause leaf temperature to rise. Such stomatal and temperature changes have been observed during exposure to O_3 (Matsushima et al., 1985; Temple and Benoit, 1988).

An important O_3-temperature interaction affecting trees and other woody perennials is winter hardiness. Several studies have shown that exposures to O_3 at realistic levels may reduce the cold- or frost-hardiness of plants, as reviewed by Davison et al. (1988). Using the pea plant as a laboratory model, Barnes et al. (1988b) showed that daily 7-h exposures to 0.075 or 0.09 ppm O_3 for 7 days significantly reduced plant survival after exposure to night-time temperatures that fell from 2 to −4 °C over a 2-h period and then were held at −4 °C for a further 4 h.

Various responses of coniferous trees to the exposure to O_3 during the growing season and freezing temperatures during the following winter have been reported. With Norway spruce, Eamus and Murray (1991) found that the recovery of photosynthetic rates after freezing was slower in O_3-treated seedlings. Brown et al. (1987) and Barnes and Davison (1988) observed severe necrosis of the older needle classes of seedlings of some Norway spruce clonal saplings exposed to O_3 and then to freezing temperatures, although other clones showed no effect. Increased winter injury on plants exposed to O_3 also was observed with Sitka spruce (*Picea sitchensis*) (Lucas et al., 1988) and red spruce (Fincher et al., 1989). With loblolly pine, Edwards et al. (1990a) observed variable results, but Chappelka et al. (1990) reported that a late winter frost resulted in severe tip die-back of the youngest needles of seedling trees exposed to 1.7 (350 ppm·h) and 2.5 (433 ppm·h) times the ambient (272 ppm·h) O_3 concentration during the previous growing season (in contrast to the effects observed on Norway spruce). The response also varied with plant genotype.
A reason for the difference may be that, in the study with Norway spruce, the freezing period occurred soon after exposure to elevated O_3 levels, whereas in the loblolly pine study, the frost occurred in late winter. The diversity of results led Eamus and Murray (1991) to develop a conceptual framework that recognizes that, even in severe winters, there are brief periods of mild temperatures that induce partial dehardening. Ozone decreases frost hardiness, per se, and it also increases the trees' predisposition to dehardening during winter; such dehardening puts O_3-exposed trees at greater risk from subsequent low temperatures.

In a greenhouse study with 1-year-old red spruce seedlings, Neighbour et al. (1990) reported that decreasing the level of NO at the time of exposure to O_3 prevented the

appearance of O_3-induced frost injury. They suggest that the effects attributed to O_3 are probably due to the combination of O_3 with traces of NO above a critical level. However, this effect apparently has not been investigated further.

In a study of the subtropical trees, Volkamer lemon (*Citrus volkamericana*) and avocado (*Persea americana*), in Florida, Eissenstat et al. (1991a) found that, although O_3 could reduce frost hardiness, the effects were subtle, and the authors concluded that the likelihood that frost resistance is adversely affected by current O_3 levels is slight.

The general consequences of global warming on O_3 responses are discussed in Section 5.4.8.

5.4.4.3 Humidity and Surface Wetness

A review of early investigations led to the conclusion that, in general, high RH tends to sensitize plants to O_3 (U.S. Environmental Protection Agency, 1986). Such a conclusion is supported on mechanistic grounds. A study by McLaughlin and Taylor (1981) indicated that measured O_3 uptake by bush bean plants (*Phaseolus vulgaris*) increased with RH, and there are several reports that, at high RH, the rapid decrease in stomatal conductance caused by O_3 at lower RHs is inhibited (Otto and Daines, 1969; Rich and Turner, 1972; Elkiey and Ormrod, 1979a; Elkiey et al., 1979). However, stomatal responses to O_3 show considerable variability among species and even among cultivars of the same species (Elkiey and Ormrod, 1979a; Elkiey et al., 1979), and, hence, it is to be expected that the patterns of the O_3-RH interaction may not always be as clear. Thus, with yellow poplar (*Liriodendron tulipifera*), five consecutive daily exposures to 0.15 ppm for 7 h at either 40 or 80% RH revealed considerable variation in stomatal conductance (Jensen and Roberts, 1986). At 40% RH, there was a tendency for O_3 to cause a decrease in conductance during the later exposures. Nevertheless, at 80% RH, the conductances generally were greater and tended to increase during the later exposures.

Surface wetness also influences the foliar uptake of O_3, although there appear to have been no studies undertaken to investigate the consequences of such uptake. Until recently, it has been suggested that O_3 uptake is reduced when foliage is wet because the stomata may be covered with water (Hicks et al., 1987). However, Fuentes and Gillespie (1992) reported that both wetness from dew or raindrops on the upper surface of red maple leaves can increase O_3 uptake significantly. Although this may be due partly to a stomatal response to resulting increases in RH, the fact that increased uptake occurred in darkness, when the stomata largely were closed led the investigators to suggest that direct uptake into the surface water is the more important mechanism. However, no information is available as to the consequences of such deposition.

5.4.4.4 Drought and Salinity

Short- and long-term variations in the availability of soil water have a profound influence on plant growth. In some agricultural situations, the use of irrigation may eliminate drought stress. However, the growth of crops and natural vegetation in many areas will be affected adversely by the varying degrees of water shortage that occur, both during a growing season and from year to year. The previous criteria document (U.S. Environmental Protection Agency, 1986) summarized earlier studies and concluded that drought stress reduced the magnitude of adverse effects of O_3, including injury and growth and yield reductions. The effect was attributed to an increased rate of stomatal closure in drought-stressed plants in response to O_3 that effectively reduced uptake of the pollutant.

These conclusions were based almost exclusively on studies with crop species. Since then, a number of studies with tree seedlings and further studies with crops species have shown that the interaction between drought and O_3 is more complex and variable than originally thought.

Heagle et al. (1988a) summarized the results of investigations into the drought-O_3 interaction in six soybean studies, three cotton studies, one study each of alfalfa and a clover-fescue mixture. These studies were undertaken as part of NCLAN (Heck et al., 1984). The results of these investigations are included in Table 5-8. Significant interactions between O_3 and drought stress (soil moisture deficit, [SMD]) were reported only in three soybean studies, two cotton studies, and the alfalfa study. The interaction was usually revealed by the fact that the clear negative relationships between yield and O_3 exposure observed with watered plants were either much reduced or could not be demonstrated with drought-stressed plants, bearing in mind that, in most of these situations, the yields already were depressed by the SMD. As a result, the lack of any significant response to O_3 in some cases with such stressed plants reflects the decreased range of yield responses within which an O_3 effect could operate. However, as shown in Table 5-8, Heggestad et al. (1988) found with Forrest soybean that SMD significantly enhanced the effects of low O_3 exposures. Heagle et al. (1988a), therefore, concluded that the suppression of the response to O_3 caused by drought appeared to be dependent on the severity of the SMD-induced stress.

Brennan et al. (1987) suggested that the normal experimental protocols used in most NCLAN studies, which called for the use of irrigation to avoid possible complications due to drought, might have biased the yield loss data for soybean because it increased plant sensitivity to O_3. However, Heggestad and Lesser (1990) found no evidence to support this suggestion, in view of the comparable estimates of yield losses predicted by the O_3-response curves.

Bytnerowicz et al. (1988) found no interaction between SMD and O_3 in 18 desert annual species. However, moderate SMD rendered the tropical fiber plant, kenaf (*Hibiscus cannabinus*), less sensitive to O_3, although sensitivity was enhanced by severe water stress (Kasana, 1992). A field survey of milkweed plants in two areas in the mid-Ohio River Valley revealed much less foliar injury attributable to O_3 in 1988, a dry year in which the maximum concentration recorded nearby reached 0.2 ppm, than in 1989, a year with ample precipitation and a nearby maximum of 0.12 ppm (Showman, 1991).

Although there have been several recent studies of the effects of O_3 exposure and drought stress on tree species, they have little in common with respect to the treatments applied or the measurements made. However, clear demonstrations of significant interactions have been obtained with beech, poplar, and loblolly pine seedlings. Davidson et al. (1992) found that, although O_3 reduced root growth in well-watered plants, SMD reversed this inhibition and led to slight O_3-induced stimulations. Drought reduced foliar injury caused by O_3 to poplar (Harkov and Brennan, 1980), ponderosa pine (Temple et al., 1992), and loblolly pine (Meier et al., 1990). In poplar, the effect was attributed to the reduced stomatal conductance observed, which reduced O_3 uptake. Similar effects on stomatal conductance were observed in Norway spruce and sitka spruce (Dobson et al., 1990). In ponderosa pine, SMD also countered the inhibitory effects of O_3 on needle growth and retention (Temple et al., 1993). Tseng et al. (1988), however, observed no effects of O_3 on Fraser fir (*Abies balsamea*) grown under three levels of SMD. No consistent patterns were found with various physiological measurements made on red spruce seedlings subjected to both O_3 and drought (Roberts and Cannon, 1992). Lee et al. (1990b) observed reduced root conductivity in the second drought cycle following exposure to O_3. Thus, there is some

Table 5-8. Field Studies of Ozone-Drought Stress Interactions in Crop Species[a] (Adapted in part from Heagle et al., 1988a)

Crop/Cultivar	Year	Response	Significant Interaction[c]	Estimated Yield Loss (%) per Seasonal Mean O_3 Concentration (ppm)[b] 0.04	0.05	0.06	0.07	0.08	Reference
Soybean									
Williams	1982	Yield	No	7	13	19	24	30	Heggestad et al. (1985) Heggestad and Lesser (1990)
Williams and	1983	Yield	WW	7	13	18	24	30	
Corsoy 79		Yield	DS	6	11	15	19	23	Heggestad and Lesser (1990)
Williams		Root length	WW		No significant O_3 effect				Heggestad et al. (1988)
		Root length	DS		[33	36	52][d]		
Forrest	1982	Yield	WW	3	9	21	39	60	Heggestad et al. (1985)
		Yield	DS	13	21	28	35	41	Heggestad and Lesser (1990)
Davis	1983	Yield DS	WW	4	7	12	16	21	Heagle et al. (1987a)
		Yield DS			No significant O_3 effect				
Davis	1984	Yield	No	4	7	12	18	24	Heagle et al. (1987a)
Corsoy 79	1986	Yield	WW	2	4	8	13	21	Irving et al. (1988)
		Yield	DS	0	0	0	0	1	
Young	1986	Yield	No	6	11	17	25	34	Miller et al. (1989b)
Cotton									
Acala SJ-2	1981	Yield	WW	3	7	13	21	30	Temple et al. (1985)
		Yield	DS	1	2	3	7	12	
Acala SJ-2	1982	Yield	No	6	15	26	40	55	Temple et al. (1985)
McNair 235	1985	Yield	No	7	13	21	30	40	Heagle et al. (1988b)
Acala SJ-2	1986	Shoot dry mass	WW[e]		[22	26	42][f]		Temple et al. (1988b)
		Shoot dry mass	DS[e]		[20	37	44][f]		
		Shoot dry mass	DS (severe)[e]		[+14	+22	27][f]		

Table 5-8 (cont'd). Field Studies of Ozone-Drought Stress Interactions in Crop Species[a] (Adapted in part from Heagle et al., 1988a)

Crop/Cultivar	Year	Response	Significant Interaction[c]	Estimated Yield Loss (%) per Seasonal Mean O_3 Concentration (ppm)[b] 0.04	0.05	0.06	0.07	0.08	Reference
Alfalfa									
WL-514	1984	Yield	No	6	9	13	17	20	Temple et al. (1988a)
WL-514	1985	Yield	No[g]	4	7	10	14	18	
Tall Fescue-Ladino Clover									
Kentucky 31	1984	Yield	No	5	8	12	17	22	Heagle et al. (1989b)
and Regal	1984	Yield	No	6	11	17	24	32	

[a]See Appendix A for abbreviations and acronyms.
[b]Where a significant interaction was observed, separate responses are listed for well-watered (WW) and drought-stressed (DS) plants; otherwise, the pooled response is listed.
[c]Based on Weibull model estimates (Heagle et al., 1988a)
[d]Data presented are percent reductions in root length per soil core at seasonal mean O_3 exposures of 0.074, 0.107, and 0.132 ppm relative to 0.052 ppm. Increased root lengths in DS treatments ranged from 136 to 11% with increasing O_3 exposure.
[e]Interaction not significant by analysis of variance, but significant suppression of O_3 response in DS (severe).
[f]Weibull model data not available. Data presented are actual percent yield losses at seasonal mean O_3 exposures of 0.074, 0.094, and 0.111 ppm relative to 0.015 ppm.
[g]Polynomial regression analysis showed slightly greater response in WW than DS plots.

evidence from tree species to support the view that drought stress may reduce the impact of O_3. However, the work with trees provides no additional information to help in resolving the quantitative nature of the drought-O_3 interaction.

Although drought stress may be the result of insufficient rainfall, conditions of effective SMD also may be induced by excessive soil salinity. Laboratory studies reviewed in the previous criteria document (U.S. Environmental Protection Agency, 1986) showed that increased salinity could reduce the impact of O_3 on injury and yield of various crops. However, in a more recent field study with alfalfa, Olszyk et al. (1988) found no overall interaction between O_3 and salinity on growth or yield. Although salinity decreased the number of empty nodes caused by exposure to above-ambient levels of O_3, the effect was statistically significant only for the second of four harvests. In general, salinity was found to be more harmful to alfalfa growth than exposure to O_3, but, as pointed out by Olszyk et al. (1988), the amount of information available is insufficient to permit the development of models for estimating losses due to O_3-salinity combinations.

The bulk of the available evidence supports the view that drought stress may reduce the impact of O_3 on plants. However, it must be emphasized that, in terms of growth and productivity, any "protective" benefit will be offset by the effects of SMD per se, as noted in the previous criteria document (U.S. Environmental Protection Agency, 1986).

The O_3-water interaction is not confined to the effects of SMD on direct plant response to O_3. Numerous studies have shown that O_3 may affect various aspects of plant water status, including water-use efficiency (WUE), the ratio of the rates of photosynthetic carbon gain and transpirational water loss. For example, Reich et al. (1985) observed that daily exposures to 0.13 ppm O_3 for 6.8 h resulted in a 25% reduction in WUE in well-watered Hodgson soybean, when compared to exposure to 0.01 ppm. Similar findings have been reported for alfalfa (Temple and Benoit, 1988) and radish (Barnes and Pfirrmann, 1992). However, WUE is a complex resultant of both stomatal conductance and the activity of the photosynthetic system, both of which may be independently affected by O_3. Genetic or environmentally induced difference in the relative sensitivities of the stomatal and photosynthetic components will dictate the nature and magnitude of any effect of O_3 on WUE. Thus, with radish and soybean, Greitner and Winner (1988) observed effects on stomatal conductance and photosynthetic CO_2 assimilation that translated into O_3-induced increases in WUE; however, they point out, that this advantageous increase far outweighed the adverse effects of O_3 on growth.

However, these reports concern herbaceous weedy species, and there appears to be only one report concerning tree species. Johnson and Taylor (1989) reported that exposure to higher than ambient levels of O_3 results in adaptation to a more efficient use of water by the foliage of loblolly pine seedlings. The corollary to this observation is that trees exposed continuously to low O_3 levels may be more sensitive to recurrent drought stress than are those grown under higher exposure levels. As with most studies of tree species, these observations were made on tree seedlings, and the relevance to mature trees is still to be established.

It therefore is clear that not only does drought have a pronounced effect on the response of most species to O_3, but that O_3 also may modify plant water relations, including conferring drought tolerance. However, more study will be needed before it will be possible to generalize about the implications of the latter effect and its importance to forest ecosystems.

5.4.5 Nutritional Factors

All land plants require an adequate supply of essential mineral elements from the soil in order to avoid adverse effects on growth and survival resulting from mineral deficiencies. Two of the essential elements needed for growth are nitrogen and sulfur, and although these are normally obtained from the soil through the root system, the plant's needs, at least in part, also can be met by the uptake of pollutant gases such as NO_2 and SO_2. Other nutrients such as phosphorus, potassium, magnesium, and calcium generally are available only from the soil.

A supply of elements such as nitrogen, potassium, phosphorus, sulfur, magnesium, and calcium is essential for plant growth, but optimal growth requires that the supply be balanced; with insufficiency (or excess) of any of them, growth will be suboptimal. Not surprisingly, therefore, nutrient imbalance has been shown to affect response to O_3, although the previous criteria document (U.S. Environmental Protection Agency, 1986) concluded that work to that date had not clarified the relationship between soil fertility and sensitivity to O_3, largely because of the differences in nutrients and species selected for study and the experimental conditions used. This conclusion is still valid, in spite of the results of a limited number of more recent studies, and is not surprising in view of the vast number of possible permutations and combinations of nutrient elements and their levels that may exert effects on O_3 response. A comprehensive summary of the relevant studies is presented in Table 5-9.

Most information concerns nitrogen. However, inspection of Table 5-8 shows that, in four of the 13 studies, increased nitrogen supply increased susceptibility to foliar injury or enhanced adverse effects on growth; two of the studies showed opposite effects; in three studies, injury was greatest at normal nitrogen levels and less at higher or lower levels; and, in one study, injury was least at normal nitrogen levels. No interactions were observed with soil nitrogen in three studies. Knowledge of the tissue nitrogen levels resulting from the fertilizer treatments, as recommended by Harkov and Brennan (1980), might resolve these contradictions, but these were not reported in most studies. The contradictory evidence for tobacco may reflect different responses of different cultivars, as suggested by Menser and Hodges (1967).

The possibilities of response to O_3 being modified as a result of significant dry deposition of nitric acid (HNO_3) vapor or of wet deposition of nitrate ion in acid precipitation are discussed in Sections 5.4.6.3 and 5.4.6.5, respectively.

The limited evidence for phosphorus, potassium, and sulfur consistently indicated a decrease in sensitivity with increased nutrient level. With respect to general fertility, both studies listed in Table 5-8 revealed decreased sensitivity to O_3 at high levels of nutrient supply, although, with soybean, nutrient-deficient plants also showed decreased sensitivity. Heagle (1979) found that, although injury and growth reductions tended to be greatest at normal levels of fertility, the effects were dependent on the rooting medium used; in media containing peat, the impact of O_3 on growth was least at the lowest fertility level.

Cowling and Koziol (1982) have suggested that, in spite of the apparent contradictory evidence regarding the effects of nutrition on O_3 response, there is evidence to support the hypothesis that differences in sensitivity are ultimately linked to changes in the status of soluble carbohydrates in the plant tissues (Dugger et al., 1962). However, this hypothesis has yet to be tested systematically.

Table 5-9. Ozone-Soil Nutrient Interactions (Based in part on Cowling and Koziol, 1982)[a]

Species	Response to Increase in Nutrient Level	Reference
Nitrogen		
Loblolly pine	Decreased reduction of growth due to O_3	Tjoelker and Luxmoore (1991)
Ponderosa pine	No injury or growth interactions	Bytnerowicz et al. (1990)
Poplar	Maximum injury in mid-range but no growth interaction	Harkov and Brennan (1980)
Yellow poplar	No growth interaction	Tjoelker and Luxmoore (1991)
Ladino clover/tall fescue	No growth interaction	Montes et al. (1982)
Mangel	Increased injury	Brewer et al. (1961)
Radish	Increased reduction of growth due to O_3	Ormrod et al. (1973)
	Increased reduction of growth due to O_3	Pell et al. (1990)
Spinach	Increased injury	Brewer et al. (1961)
Tobacco	Decreased injury	Menser and Street (1962)
	Minimum injury in mid-range	MacDowall (1965)
	Maximum injury in mid-range	Leone et al. (1966)
	Maximum injury in mid-range	Menser and Hodges (1967)
Phosphorus		
Radish	No growth interaction	Ormrod et al. (1973)
Tomato	Increased injury	Leone and Brennan (1970)
Potassium		
Norway spruce	Decreased reduction of CO_2 assimilation due to O_3	Keller and Matyssek (1990)
Pinto bean	Decreased injury	Dunning et al. (1974)
Soybean	Decreased injury	Dunning et al. (1974)
Sulfur		
Bush bean	Decreased injury	Adedipe et al. (1972)
Magnesium		
Loblolly pine	No growth interaction	Edwards et al. (1992b)
General Fertility (nitrogen, phosphorus, and potassium)		
Bush bean	Decreased injury	Heck et al. (1965)
Soybean	Maximum injury and growth reduction in mid-range	Heagle (1979)

[a]See Appendix A for abbreviations and acronyms.

Nutritional nitrogen and sulfur also can be supplied directly to foliage in the form of nitrogen and sulfur oxides. The interactions of these gaseous pollutants with O_3, dealt with in the next section, focus on toxic rather than nutritional effects. However, one example of a beneficial effect concerns N_2O_5. Because N_2O_5 is produced in trace amounts by high-voltage, corona-discharge O_3 generators, it may contaminate O_3 produced from air by such generators for use in studies of effects of O_3 on vegetation, unless the O_3 stream is passed first through a water scrubber. Brown and Roberts (1988) reported that deposition of the nitrate formed by hydration of trace amounts of N_2O_5 in unscrubbed O_3 significantly increased the nitrogen status of the exposed plants, which may have confounded the effects attributed to O_3.

5.4.6 Interactions with Other Pollutants

The concurrent or sequential exposure of vegetation to different gaseous air pollutants has been found to modify the magnitude and nature of the response to individual pollutants. Some of the early work reviewed in the previous criteria document (U.S. Environmental Protection Agency, 1986) on the effects of gaseous pollutant combinations is of academic interest, with little relevance to the present review because of the levels of exposure and the exposure profiles used and the fact that the experimental regimes usually involved concurrent exposures to two or more pollutants repeated daily. Lefohn and Tingey (1984) and Lefohn et al. (1987b) reviewed the patterns of co-occurrence of O_3, SO_2, and NO_2 in urban, rural, and remote sites in the United States for the years 1978 to 1982 and found that co-occurrences were usually of short duration and occurred infrequently. They noted that the most frequent types of co-occurrence were either purely sequential or a combination of sequential and overlapping exposures of short duration. Accordingly, the present review will focus on the evidence from experiments that simulated these naturally occurring patterns of combined exposure or, at least, that used exposure levels in the ranges of those occurring in polluted air. An exception is the co-occurrence of O_3 and PAN, which are both components of photochemical oxidant.

Over the past decade, the effects of pollutant mixtures have been reviewed by Wolfenden et al. (1992), Shriner et al. (1991), Mansfield and McCune (1988), Torn et al. (1987), Lefohn and Ormrod (1984), Reinert (1984), and Runeckles (1984).

5.4.6.1 Oxidant Mixtures

Because of their photochemical origins, elevated levels of O_3 and PAN can occur simultaneously. There appear to have been no further investigations of the effects of simultaneous or sequential exposures since the limited number of studies reviewed in the previous criteria document (U.S. Environmental Protection Agency, 1986). Hence, there is no reason to question the general conclusion, based on the work of Tonneijck (1984) and Nouchi et al. (1984), that the two gases tend to act antagonistically in both concurrent and sequential exposures. Hydrogen peroxide (H_2O_2) is also a component of photochemically polluted atmospheres. Although Ennis et al. (1990) reported reduced stomatal conductances in red spruce needles exposed to a mixture of O_3, SO_2, and H_2O_2, no studies have been made of O_3/H_2O_2 interactions.

5.4.6.2 Sulfur Dioxide

Because SO_2 originates from point sources of combustion, the occurrence of high ambient concentrations at a given location is usually episodic because of its dependence on wind speed and direction and the distance from the source. However, aggregations of point sources can lead to more widespread but less marked increases in ambient SO_2 levels. Thus, the potential exists for elevated O_3 exposures to be superimposed on patterns of SO_2, ranging from severe fluctuations to almost steady low-level concentrations. Concern over the importance of O_3-SO_2 interactions dates from the observations of Menser and Heggestad (1966) that simultaneous exposures of tobacco to SO_2 and O_3 acted synergistically (i.e., the effects of the mixture were greater than the sum of the responses to either pollutant alone). Indeed, in the Menser and Heggestad study, foliar injury was found to result from exposure to mixtures, although exposures to either gas alone at the same concentrations as in the mixtures did not result in injury.

Although much of the early work was concerned with foliar injury responses to simultaneous exposures to high levels of O_3 and SO_2, more recent studies have tended to focus on the consequences of growth and yield of repeated exposures to lower level mixtures or sequences. Several studies have been aimed at obtaining statistical evidence for the existence of interactions. For example, Ashmore and Onal (1984), studying six cultivars of barley, found that SO_2 at 0.065 ppm for 6 h, an exposure that induced no adverse effects, acted antagonistically to a 6-h exposure to 0.18 ppm O_3, causing significant decreases in foliar injury, ranging from 46% to as much as 95%. However, only one cultivar, Golden Promise, showed a significant interaction on yield, with SO_2 completely reversing the decrease caused by O_3 alone. The results could not be explained by effects on stomatal uptake because stomatal conductances were found to be highest in the mixture. In contrast, with pea, Olszyk and Tibbitts (1981) reported that O_3 + SO_2 caused the same degree of stomatal closure as SO_2 alone. An antagonism similar to that observed on Golden Promise also was observed in field studies of Arena barley (Adaros et al., 1991a) and spring rape (*Brassica napus*) (Adaros et al., 1991b). However, with Tempo spring wheat, a synergistic interaction was observed: the adverse effect of O_3 on yield (−26%) was increased to −38% by SO_2, which, by itself, only reduced yield by 7% (Adaros et al., 1991a). On the other hand, neither Amundson et al. (1987) nor Kohut et al. (1987) observed any interaction in a field study with Vona winter wheat. Irving et al. (1988) observed no interaction on field corn.

In a series of experiments in which exposure to O_3 or an O_3/SO_2 mixture was preceded by exposures to SO_2 alone, an antagonistic response was observed on foliar injury to white bean (Hofstra and Beckerson, 1981). In contrast, the responses of cucumber (*Cucumis sativus*) and radish were synergistic, whereas there was no interaction on soybean or tomato. However, when followed by exposure to an O_3/SO_2 mixture, SO_2 pretreatment resulted in an increase in injury to white bean, decreases in cucumber and tomato, and no effect on soybean and radish.

Field studies with soybean using an air-exclusion system to provide a range of exposures to O_3 and SO_2 at ambient and subambient levels revealed an antagonistic interaction on yield at low concentrations (Jones et al., 1988). However, Kress et al. (1986) found no interaction in a soybean field study using OTCs. No interactions were found with potato (Pell et al., 1988) or with a red clover-timothy (*Phleum pratense*) forage mixture (Kohut et al., 1988b).

From the foregoing, it is apparent that no clearer pattern of the interactive effects of O_3 and SO_2 on crops has emerged since the previous criteria document (U.S. Environmental Protection Agency, 1986). The same is true for the responses of tree species.

With tree seedlings, Chappelka et al. (1988a) observed no interaction on white ash. Although a synergistic interaction was found on root growth of yellow poplar (Chappelka et al., 1985), only additive interactions were found on the growth of other parts of the plant. In a unique study, Kargiolaki et al. (1991) noted that SO_2 reduced the accelerated leaf senescence caused by O_3 on two poplar clones, but had no effect on other clones. They also observed additive or less than additive interactions on the formation of intumescences, due to hypertrophy of the stems and bark cracking. They attributed the differences in clonal response to differences in the levels of pollutant-induced ethylene evolution.

Sulfur dioxide reversed the inhibition of photosynthesis caused by exposure to O_3 in two lichen species, *Flavoparmelia caperata* and *Umbilicaria mammulata* (Eversman and Sigal, 1987).

Several studies have attempted to quantify the magnitudes of joint responses to O_3 and SO_2. The earliest (Macdowall and Cole, 1971) showed that the synergistic injury response of tobacco occurred at concentrations of SO_2 less than the threshold for SO_2 injury, but not less than the O_3 threshold. Oshima (1978), working with kidney bean, found that the synergistic reduction due to intermittent exposures to O_3 was linear through a range of O_3 concentrations achieved by varying degrees of filtration of ambient air (expressed as 10 to 90 ppm-h of concentrations greater than zero), although the threshold for an O_3 response was approximately 47 ppm-h.

A selection of statistical models of injury- or yield responses to O_3/SO_2 is listed in Table 5-10. It is immediately apparent that the models reveal no consistent patterns of response. In part, this is because they were developed on the basis of individual experiments conducted under different environmental conditions at different locations in different years. Although each model was statistically significant, it was based on a unique data set. One study with soybean indicated an antagonistic interaction (Heagle et al., 1983b), but another indicated no interaction (Kress et al., 1986). Cucumber (Hofstra et al., 1985) and snap bean (Heggestad and Bennett, 1981) were reported to respond synergistically, whereas white bean responded antagonistically (Hofstra et al., 1985).

All that can be concluded from these studies is that the type of interaction, and whether or not one exists, is probably highly dependent on species and cultivar, and possibly dependent on other environmental variables. The available evidence is insufficient to be able to decide in which way, and to what extent, SO_2 exposure will influence the effects of O_3 on a particular species or cultivar at a particular location. The synergism originally observed (Menser and Heggestad, 1966) is not a general response.

5.4.6.3 Nitrogen Oxides, Nitric Acid Vapor, and Ammonia

As discussed in Chapter 3, the photochemical formation of O_3 involves a complex series of reactions in which NO, NO_2, and HNO_3 participate as intermediates or reaction products. Of these, the limited number of reports of interactive effects with O_3 is confined to NO_2. Some of the few studies of O_3/NO_2 interactions that have utilized realistic concentrations have involved mixtures of the pollutants. Adaros et al. (1991a) found in a 2-year study of two cultivars each of barley and spring wheat that significant interactions could be detected only on wheat yield in one growing season. With both cultivars, the

Table 5-10. Some Statistical Models of Combined Ozone and Sulfur Dioxide Responses[a]

Species	Type of Interaction	Model	Reference
Corn			
Golden Jubilee	Synergistic	Injury = $-11.39 + 5.471\ln(IHT) - 9.59[O_3] + 11.81[SO_2] - 86.63[SO_2]^2 + 428.95[O_3][SO_2]$ (IHT = initial plant height, used as a covariate; $[O_3]$ and $[SO_2]$, ppm)	Deveau et al. (1987)[b]
Cucumber			
National Pickling	Synergistic	Injury = 2.70 + 1.95 n; at $[SO_2]$ = 0.10 ppm Injury = 2.40 + 0.21 n; at $[SO_2]$ = 0.05 ppm Injury = 2.39 + 0.39 n; at $[SO_2]$ = 0.03 ppm Injury = 1.86 + 0.166 n; at $[SO_2]$ = 0.02 ppm (n = number of daily 8-h SO_2 exposures; O_3 exposure, 0.15 ppm, 6 h)	Hofstra et al. (1985)
Snap Bean			
Maple Arrow	Additive; no interaction	Injury = $4.44 + 34.19[O_3] + 19.98[SO_2]$ ($[O_3]$ and $[SO_2]$, ppm)	Deveau et al. (1987)[b]
White Bean			
Seafarer	Antagonistic	Injury = 6.31 − 0.90 n; at $[SO_2]$ = 0.10 ppm Injury = 5.95 − 0.45 n; at $[SO_2]$ = 0.05 ppm (n = number of daily 8-h SO_2 exposures; O_3 exposure: 0.15 ppm, 6 h)	Hofstra et al. (1985)
Potato			
Norchip	Additive; no interaction	Yield = $1.27 - 0.0037[O_3] + 0.00092[SO_2]$ (Yield = number of Grade No. 1 tubers per plant; $[O_3]$: ppm, 10 h/day seasonal mean; $[SO_2]$, ppm, 3 h/day)	Pell et al. (1988)
Soybean			
Davis	Antagonistic	Polynomial model: Yield = $534.5 - 3988.6[O_3] - 479.7[SO_2] + 2661.0[O_3][SO_2] + 1{,}0960[O_3]^2$ Weibull model: Yield = $531 \times \exp[-([O_3]/0.133)] \times \exp[-([SO_2]/0.892)]$ (Yield = g/m of row; $[O_3]$: ppm, seasonal 7 h/day mean; $[SO_2]$: ppm, seasonal 4 h/day mean)	Heagle et al. (1983b)
Amsoy-71 and Corsoy-79 (pooled)	No interaction	Yield = $1934.4*\exp[-([O_3]/0.124)^{2.666}]* \exp[-([SO_2]/1.511)^{1.044}]$ (Yield = kg/ha; $[O_3]$: ppm, seasonal 7 h/day mean; $[SO_2]$: ppm, seasonal 4 h/day mean)	Kress et al. (1986)

Table 5-10 (cont'd). Some Statistical Models of Combined Ozone and Sulfur Dioxide Responses[a]

Species	Type of Interaction	Model	Reference
Tomato			
New Yorker		Injury = $-75.78 + 20.48\ln[PI] - 29.16[O_3] + 1{,}016[O_3]^2 + 9.02[SO_2] - 17.29[SO_2]^2 + 258.76\,[O_3][SO_2]$ (PI = plastochron index, used as a covariate; $[O_3]$ and $[SO_2]$: ppm)	Deveau et al. (1987)[b]

[a]See Appendix A for abbreviations and acronyms.
[b]Report includes models for other growth variables.

interaction was antagonistic. Nitrogen dioxide also reduced the adverse effect of O_3 on the yield of spring rape (Adaros et al., 1991b). Foliar injury to sunflower (*Helianthus annuus*) caused by daily exposures to O_3 (0.1 ppm, 8 h) was increased by continuous exposure to 0.1 ppm NO_2 (Shimizu et al., 1984). Plant dry weight was decreased by O_3 + NO_2 relative to growth in O_3 alone, but because O_3 exposure resulted in a slight increase in dry weight relative to the controls, the growth in the mixture and in the controls did not differ significantly.

The results of a study of seven tree species exposed to 0.1 ppm O_3 and/or 0.1 ppm NO_2 for 6 h/day for 28 days (Kress and Skelly, 1982) were reported in detail in the previous criteria document (U.S. Environmental Protection Agency, 1986). However, although several growth interactions were noted in the review, the only statistically significant effect was on top growth of pitch pine (*Pinus rigida*), in which NO_2 reversed a growth stimulation caused by exposure to O_3. In contrast, although Yang et al. (1982) also observed an antagonistic interaction on the needle dry weights of two eastern white pine clones, in these cases NO_2, reversed the adverse effect of O_3.

There appear to have been only three studies using sequential exposures of O_3 and NO_2. Runeckles and Palmer (1987) exposed radish, wheat, bush bean, and mint (*Mentha piperita*) daily to 0.08 to 0.1 ppm NO_2 for 3 h (0900 to 1200 hours), to 0.08 to 0.1 ppm O_3 for 6 h (1200 to 1800 hours), or to the two gases in sequence. With each species except mint, pretreatment with NO_2 significantly modified the growth responses to O_3. In radish and wheat, the two gases acted conjointly to reduce growth more than O_3 alone, whereas in bean NO_2 was antagonistic. In studies with tomato, Goodyear and Ormrod (1988) found that sequential exposure to 0.08 ppm O_3 for 1 h, followed by 0.21 ppm NO_2 for 1 h, significantly reduced growth. No significant effects were found when the sequence was reversed or the two gases were used as a mixture. However, because the study did not include a treatment with O_3 alone, no information was obtained as to how NO_2 may have influenced the response to O_3. Bender et al. (1991) exposed kidney beans in OTCs in the field to the sequence: O_3 (0800 to 1600 hours, ambient + 0.50 ppm) followed by NO_2 (1600 to 0800 hours, ambient + 0.3 ppm), during two growing seasons. No significant treatment effects on growth were observed in 1988, but in 1989 a significant interaction on total plant biomass was noted after 48 days; the overnight NO_2 exposures negated the inhibition caused by O_3 with a change from −32 to +14%, relative to the controls. This type of response is similar to that observed on bean by Runeckles and Palmer (1987).

With such limited information, it is not possible to generalize, particularly because antagonistic and additive responses have been reported even for individual species. However, because, on a daily basis, changes in NO_2 levels tend to lead to maxima at times when O_3 levels are lowest, the evidence is sufficiently compelling to indicate that modifications of the O_3 response, as a result of increased NO_2, are highly probable. Direct interactive effects of O_3 and NO virtually are precluded because of their rapid reaction to form NO_2.

In Southern California, O_3 levels have been correlated with levels of HNO_3 vapor (Fenn and Bytnerowicz, 1993). No studies of possible interactive effects between O_3 and HNO_3 have been reported. However, Taylor et al. (1988a) suggest that HNO_3 is largely deposited on foliar surfaces and, hence, may be leached to the soil by rainfall. Such leaching, together with rates of dry deposition to soil that have been conservatively estimated to range between 5.7 and 29.1 kg nitrogen ha^{-1} $year^{-1}$, would lead to nitrogen additions to the soil at rates considerably less than agricultural rates of nitrogen-application to crops.

However, such additions to forest soils could increase nitrogen levels significantly and lead to interactive effects with O_3 via changes in soil fertility, as discussed in Section 5.4.5.

Ammonia (NH_3) can contribute significantly to total nitrogen deposition in some locations. However, virtually nothing is known of its interactive effects with O_3. Tonneijck and van Dijk (1994) reported that, although NH_3 and O_3 showed a significant antagonism with regard to foliar injury of O_3-sensitive bean cv. Pros, no interactions occurred with regard to growth effects.

5.4.6.4 Hydrogen Fluoride and Other Gaseous Pollutants

The adverse effects of HF released from the aluminum smelting process and superphosphate fertilizer manufacture are well documented, but information about possible HF/O_3 interactions are limited to a single study. MacLean (1990) reported that exposures of corn plants on alternate days to 4 h at $1 \mu g/m^3$ fluorine as HF or 0.06 ppm O_3 showed reduced rates of senescence, compared with plants exposed only to O_3.

5.4.6.5 Acid Deposition

Any impact that acid deposition has on crops or natural ecosystems occurs either through direct effects on foliage or indirectly through the soil. Soil effects may result from a change in pH or to the deposition of sulfate or nitrate onto the soil. The effects of acidic deposition have been reviewed extensively by Shriner et al. (1991). Although concerns over the possible role of exposures to acid rain or acid fog and O_3 in the forest-decline syndrome led to several studies with forest tree species, studies also have been conducted on crops. Of over 80 recent reports of studies on over 30 species, more than 75% of the reports indicated no significant interactions between O_3 and acidity of simulated acid rain (SAR) or acid fog. The reports are summarized in Table 5-10. In 63 studies, there was either no effect of one or other of the pollutants (usually acid rain) or the effects of both pollutant stresses were simply additive.

However, in other studies, statistically significant interactions have been reported for several species, as also shown in Table 5-11. For example, although a large number of studies of loblolly pine revealed no interaction, Qiu et al. (1992) reported significant interactions on foliar and stem and root biomass with seedling trees of an O_3-sensitive family. However, because the study failed to show a significant main effect of acidity of the SAR, the authors question whether the interaction is meaningful.

With Norway spruce, antagonistic interactions were noted on stomatal conductance (Barnes et al., 1990a) and dark respiration (Barnes et al., 1990b). In contrast, Eamus and Murray (1991) reported greater than additive effects of O_3 and acid mist on photosynthetic rates. However, no interactions were noted in nine other investigations (Table 5-11).

Kohut et al. (1990) observed significant interactions on needle and shoot growth of red spruce. In both cases the inhibition caused by O_3 and SAR at pH 5.1 was reversed by more acidic rain at pH 3.1. However, there were unexplained inconsistencies in the trends because the combination of intermediate O_3 levels and low pH resulted in the greatest reductions in dry matter. Percy et al. (1992), also working with red spruce, observed an unexplained statistically significant interaction on the thickness of the needle epidermal cell cuticular membrane: at intermediate O_3 exposures, increased acidity led to reduced membrane thickness, whereas lower or higher O_3 levels led to thicker membranes.

Shelburne et al. (1993) reported that, in two growing seasons, needle biomass of shortleaf pine (*Pinus echinata*) was reduced significantly in tree seedlings receiving the

Table 5-11. References to Reports of Interaction or No Interaction Between Ozone and Acid Rain or Acid Fog

Species	No.	Interaction References	No.	No Interaction References
Tree Species				
CONIFERS				
Jeffrey pine	0	—	1	62
Loblolly pine	1	41	13	1, 10, 16-21, 26, 32, 43, 47, 49, 55
Ponderosa pine	0	—	2	65, 66
Shortleaf pine	1	52	1	8
Slash pine	2	9, 13	0	—
White pine	1	47	3	44, 46, 56
Douglas fir	0	—	1	25
Norway spruce	1	3, 4	9	2, 5-7, 15, 24, 30, 36, 50
Red spruce	2	31, 39	5	33, 34, 38, 40, 62
Sequoia	1	63	0	—
Totals	9		35	
HARDWOODS				
Green ash	0	—	1	23
White ash	0	—	1	23
European beech	1	14	1	35
Paper birch	1	29	0	—
Sugar maple	0	—	2	44, 45
Red oak	0		2	44, 45
Yellow poplar	3	11, 12, 27	1	48
Totals	5		8	
Crop Species				
FORAGES AND FIELD CROPS				
Alfalfa	1	59	4	42, 53, 59, 64
Sorghum	1	51	0	—
Soybean	1	67	4	28, 37, 53, 57
Wheat	0	—	1	53
Totals	2		5	
HORTICULTURAL CROPS				
Snap bean	0	—	1	53
Celery	0	—	1	60
Corn	0	—	1	60
Pepper	0	—	2	58, 60

Table 5-11 (cont'd). References to Reports of Interaction or No Interaction Between Ozone and Acid Rain or Acid Fog

Species	No.	Interaction References	No.	No Interaction References
HORTICULTURAL CROPS (cont'd)				
Strawberry	0	—	2	60, 61
Tomato	0	—	2	53, 60
Avocado	1	22	0	—
Citrus	1	22	0	—
Totals	2		9	
Others				
Ivy	0	—	1	30
Lichen (Lobaria)	0	—	1	54
Totals	0		2	
TOTALS	19		63	

References:
1. Adams and O'Neill (1991). 2. Barnes and Brown (1990). 3. Barnes et al. (1990a). 4. Barnes et al. (1990b). 5. Blank et al. (1990a). 6. Blank et al. (1990b). 7. Blaschke and Weiss (1990). 8. Boutton and Flagler (1990). 9. Byres et al. (1992a,b). 10. Carter et al. (1992). 11. Chappelka et al. (1985). 12. Chappelka et al. (1988b). 13. Dean and Johnson (1992). 14. Eamus and Murray (1991). 15. Ebel et al. (1990). 16. Edwards and Kelly (1992). 17. Edwards et al. (1990b). 18. Edwards et al. (1991). 19. Edwards et al. (1992a). 20. Edwards et al. (1992b). 21. Edwards et al. (1992c). 22. Eissenstat et al. (1991b). 23. Elliott et al. (1987). 24. Führer et al. (1990). 25. Gorissen et al. (1991b). 26. Hanson et al. (1988). 27. Jensen and Patton (1990). 28. Johnston and Shriner (1986). 29. Keane and Manning (1988). 30. Kerfourn and Garrec (1992). 31. Kohut et al. (1990). 32. Kress et al. (1988). 33. Laurence et al. (1989). 34. Lee et al. (1990b). 35. Leonardi and Langebartels (1990). 36. Magel et al. (1990). 37. Norby et al. (1986). 38. Patton et al. (1991). 39. Percy et al. (1992). 40. Pier et al. (1992). 41. Qiu et al. (1992). 42. Rebbeck and Brennan (1984). 43. Reddy et al. (1991a,b). 44. Reich and Amundson (1985). 45. Reich et al. (1986b). 46. Reich et al. (1987). 47. Reich et al. (1988). 48. Roberts (1990). 49. Sasek et al. (1991). 50. Senser (1990). 51. Shafer (1988). 52. Shelburne et al. (1993). 53. Shriner and Johnson (1987). 54. Sigal and Johnston (1986). 55. Somerville et al. (1992). 56. Stroo et al. (1988). 57. Takemoto et al. (1987). 58. Takemoto et al. (1988a). 59. Takemoto et al. (1988b). 60. Takemoto et al. (1988c). 61. Takemoto et al. (1989). 62. Taylor et al. (1986). 63. Temple (1988). 64. Temple et al. (1987). 65. Temple et al. (1992). 66. Temple et al. (1993). 67. Troiano et al. (1983).

highest O_3 exposures (2.5 × ambient) and SAR at pH 3.3. However, there were no effects at lower O_3 exposure levels or at higher pHs.

A 3-year study of slash pine revealed a significant interaction on stem volume increment in each year (Dean and Johnson, 1992). This was attributed to a high rate of increase observed with increasing acidity in trees exposed to an intermediate O_3 level (2 × ambient). In contrast, at higher or lower O_3 exposures, acidity of the SAR applied had little effect. Although another study with slash pine indicated a significant interaction on photosynthetic rates, no information was provided about its nature (Byres et al., 1992b).

The mineral status (potassium, calcium, and manganese) of white pine showed antagonistic interactions between O_3 and SAR (Reich et al., 1988). Increased acidity

nullified the increase in foliar potassium and the decreases in root calcium caused by O_3, whereas increased O_3 nullified the increase in root manganese that resulted from increased acidity.

Temple (1988) reported a synergistic response to O_3 and SAR of root growth of giant sequoia. Yellow poplar showed no interactions in one study (Table 5-11), but a greater than additive response of root growth was observed by Chappelka et al. (1985). Chappelka et al. (1988b) found that, although neither O_3 nor the pH of SAR caused any significant effects on growth, at intermediate O_3 levels, increased acidity caused significant decreases in stem and leaf biomass. Jensen and Patton (1990), on the other hand, reported significant antagonistic interactions on yellow poplar leaf and shoot growth. Based on estimates from growth models derived from experimental data, increased acidity (pH 5.5 to 3.0) of SAR reduced the decreases caused by O_3 by almost 50%.

Adverse effects of O_3 on the leaf area and shoot, leaf, and root biomass of paper birch (*Betula papyrifera*) were reversed by increased acidity of SAR (Keane and Manning, 1988). Similarly, in both avocado and lemon (*Citrus volkameriana*) trees, Eissentstat et al. (1991b) found that increased acidity offset the negative effects of O_3 on leaf growth.

Although there are four reports of no interactions on alfalfa, Takemoto et al. (1988b) observed significant interactions on leaf drop. In charcoal-filtered air, leaf drop increased by a factor of 6 as the pH of the fog treatment changed from 7.24 to an extremely acid pH 1.68, the lowest level recorded in the field in Southern California. In unfiltered air, in contrast, leaf drop increased only 20%.

Several studies with soybean revealed no significant interactions. However, Troiano et al. (1983) reported a 42% reduction in seed yield between CF and unfiltered air with SAR at pH 2.8 versus a 6% reduction at pH 4.0. Increased acidity thus multiplied the effect of O_3, due largely to a stimulation of seed yield caused by increased acidity. Shafer (1988) observed a stimulation of shoot growth of sorghum at pH 2.5 of SAR over growth at pH 5.5, as a result of which, greater growth occurred at low O_3 exposure levels, although there was no effect of acidity at the highest O_3 level (0.3 ppm).

In summary, although the majority of studies have not demonstrated the existence of interactions between O_3 and SAR, where statistically significant interactions on growth or physiology have been reported, the interactions were mostly antagonistic. The only synergistic interactions reported are in two studies of yellow poplar and single studies of sequoia and shortleaf and slash pines. In most cases where significant interactions were noted, the authors have had difficulty in providing any mechanistic explanation. It appears that, although the effects may have passed normally accepted tests of statistical significance, they may nevertheless have been spurious findings. Overall, it appears that exposure to acidic precipitation is unlikely to result in significant enhancement of the adverse effects of O_3 in most species. In the few cases of antagonistic interactions, the suggestion was made that these may have reflected a beneficial fertilizer effect due to the nitrate and sulfate present in the SAR applied.

The preceding review has focused on interactive effects of O_3 and wet hydrogen ion deposition. With regard to the anionic constituents of acid deposition, studies with SAR have tended to use dilute mixtures of nitric and sulfuric acids, together with other anions and cations, to achieve the desired pH levels. However, no studies appear to have been undertaken to separate any interactive effects of the individual cations (nitrates or sulfates) from those involving hydrogen ions. However, given the limited and variable information on interactive responses of O_3 and nitrogen and sulfur as soil nutrients, it is not possible to

predict the nature of any possible interactions of O_3 with the wet deposition of these elements.

5.4.6.6 Heavy Metals

Interactions of O_3 with several heavy metal pollutants were reviewed in the previous criteria document (U.S. Environmental Protection Agency, 1986). The limited data for pollutants such as cadmium, nickel, and zinc almost invariably showed that they enhanced the adverse effects of O_3, usually additively, but occasionally more than additively. To the results with cadmium, nickel, and zinc on garden cress (*Lepidium sativum*), lettuce (*Lactuca sativa*), pea, tomato, and aspen, reviewed at that time, should be added similar findings with zinc on pinto bean (McIlveen et al., 1975); increased zinc results in significantly increased foliar injury and decreased mycorrhizal infection. However, in a study of the effects of O_3, nickel, and copper on tomato, Prokipcak and Ormrod (1986) found that, as the levels of both O_3 and nickel increased, the interaction changed from additive to less than additive. Complex interactions were observed when the treatments included both nickel and copper.

No information appears to be available about possible interactions with lead. Although qualitatively heavy metals appear to increase plant sensitivity to O_3, the limited information available precludes defining any quantitative relationships.

5.4.6.7 Mixtures of Ozone with Two or More Pollutants

Pollutant-pollutant interactions are not limited to mixtures or sequences of two pollutants. Several studies have been made of interactions of O_3 with various combinations of SO_2, NO_2, and acid rain. However, in some of these investigations, no treatment with O_3 was included in the experimental design, and, therefore, no information was obtained on effects in response to O_3. Some studies using only repeated daily exposures to high levels (>0.3 ppm) of one or more pollutants are excluded from this review.

Adaros et al. (1991b), in a field study of spring rape using open-top chambers, found no significant interactions between O_3 and NO_2 (sequential exposures) and SO_2 (continuous exposures). In a 2-year study on spring barley and spring wheat, some statistically significant interactions were noted, but they were scattered through the different growth measurements, cultivars, and years with no consistent pattern (Adaros et al., 1991c). Additive effects with no interactions were observed in studies of shore juniper (*Juniperus conferta*) (Fravel et al., 1984), radish (Reinert and Gray, 1981), and azalea (*Rhododendron* spp.) (Sanders and Reinert, 1982). Yang et al. (1982) reported a less than additive interaction on injury to white pine.

No significant three-way interactions were found in studies of soybean (Norby et al., 1985), yellow poplar (Chappelka et al., 1985, 1988b), or any other hardwood species (Davis and Skelly, 1992a; Jensen and Dochinger, 1989; Reich et al., 1985) exposed to O_3, SO_2, and SAR.

No information was collected on interactions in the few published studies involving O_3, SO_2, NO_2, and SAR.

The limited data make it difficult to draw any firm conclusions, but, in general, the consequences of such exposures appear to be dictated largely by the dominant individual two-way interaction.

5.4.7 Interactions with Agricultural Chemicals

Agricultural chemicals are used for the control of insect pests, diseases, and weeds and for the control of growth in specialized situations, such as the selective thinning of fruit on orchard trees. The potential for some agricultural chemicals to modify plant response to O_3, first noted with certain fungicides on pinto beans (Kendrick et al., 1954), led to numerous field and laboratory studies. As noted in the previous criteria document (U.S. Environmental Protection Agency, 1986), protection against O_3 injury was found to be conferred by applications of numerous commercial fungicides, herbicides, and growth regulators.

The available information is derived from studies involving a number of different commercial chemicals and species. No comprehensive and systematic studies have been reported, but the weight of evidence indicates that certain fungicides are consistent in providing protection. In particular, there have numerous reports of protection conferred by applications of benomyl (benlate; methyl-1-[butylcarbamoyl]-2-benzimidazolecarbamate). In addition to the studies reviewed in the previous criteria document (U.S. Environmental Protection Agency, 1986), benomyl protection of grape (Musselman and Taschenberg, 1985) and bean cultivars (Pell, 1976; Pellisier et al., 1972) also has been reported. It is of interest to note that, although several nematocides were found to increase sensitivity of tobacco and pinto bean to O_3, applications of benomyl overcame this response and conferred resistance (Miller et al., 1976). However, benomyl was found to increase the injury caused by PAN (Pell and Gardner, 1979). It also should be noted that many of the effective fungicides are carbamates and have been used as antioxidants in other applications, such as rubber formulations.

The need to distinguish between protective action against O_3 injury and fungicidal activity per se is shown by a study of fentin hydroxide (Du-Ter; tetraphenyltin hydroxide) on potato (Holley et al., 1985). The fungicide reduced foliar injury in the field and also the colonization of injured leaf tissue by the early blight fungus, *Alternaria solani*. However, yield increases appeared to result from the reduction of disease rather than from diminished O_3 injury.

The triazoles are a family of compounds with both fungicidal and plant growth regulating properties. Fletcher and Hofstra (1985) reported on the protective action of triadimefon [1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazo-1-yl)-2-butanone], and Musselman and Taschenberg (1985) found that triadimefon and the triazole, etaconazole (1-[(2,4-dichlorophenyl)-4-ethyl-1,3-dioxolan-2-yl]methyl-1H-1,2,4-triazole), were as effective as benomyl in protecting grape from oxidant injury; cultivar differences were noted, with the fungicides being more effective on Concord than on Ives foliage. Seed treatment with triazole S-3307 ([E]-1-[4-chlorophenoxy]-3,3-dimethyl-2-[1,2,4-triazol-1-yl]-1-penten-3-ol) resulted in a 50% reduction in the size of wheat plants but provided complete protection from an excessive exposure to 0.5 ppm O_3 for 6 h that resulted in severe necrosis on the leaves of untreated plants (Mackay et al., 1987).

A range of commercial plant growth regulating compounds was studied by Cathey and Heggestad (1972). The plant growth retardants, CBBP (Phosfon-D; 2,4-dichlorobenzyltributyl phosphonium chloride) and SADH (Alar®; succinic acid, 2,2-dimethylhydrazide) and several of its analogs, were found to be more effective than benomyl in reducing O_3 injury on petunia.

Conflicting reports of the effects of herbicide-O_3 interactions were reviewed in the previous criteria document (U.S. Environmental Protection Agency, 1986). Recent studies of metolachlor (2-chloro-*N*-[2-ethyl-6-methlphenyl]-*N*-[2-methoxy-1-methylethyl] acetamide) (Mersie et al., 1989) and atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) (Mersie et al., 1990) revealed species-dependent effects: metolachlor sensitized corn to O_3 but offered protection to bean and soybean. The effects of atrazine on corn were additive to those induced by exposure to 0.2 ppm O_3 for 6 h/day, twice weekly, for three weeks, but antagonistic to exposures to 0.3 ppm. Mersie et al. (1990) also observed a protective action of the commercial antioxidant, *n*-propyl gallate, on corn.

In spite of reports to the contrary (Teso et al., 1979), Rebbeck and Brennan (1984) found that the insecticide, diazinon (O,O-diethyl-O-[2-isopropyl-4-methyl-6-pyrimidinyl] phosphorothioate), did not protect alfalfa from O_3 injury in a greenhouse study.

The knowledge of the interactions of these different types of agricultural chemicals with O_3 is still too fragmentary to enable any general conclusions to be drawn, other than to note the general efficacy of the carbamate fungicides. As noted in the previous criteria document (U.S. Environmental Protection Agency, 1986), it is premature to recommend their use specifically for protecting crops from the adverse effects of O_3, rather than for their primary purpose.

5.4.8 Factors Associated with Global Climate Change

This section focuses solely on the ways in which features of global climate change may be expected to affect the impact of oxidants on vegetation. It is not intended to provide a comprehensive review of the issues and components of climate change per se.

The magnitudes and causes of some of the changes in features of the global climate that have been observed or are predicted to occur are currently the subject of controversy. However, there is clear evidence of increases in mean CO_2 levels (Keeling et al., 1989), which, together with other anthropogenic emissions of radiatively active gases, may contribute to the upward trend in mean surface-level temperatures observed over the past century (Jones, 1989) and to changes in precipitation patterns throughout the world (Diaz et al., 1989). In addition, depletion of the stratospheric O_3 layer in the polar regions, caused by halofluorocarbons, results in increased penetration of the atmosphere by solar ultraviolet-B (UV-B) radiation (280- to 320-nm wavelengths). However, the intensity of UV-B radiation reaching the earth's surface may be attenuated by O_3-pollution in the lower troposphere (Brühl and Crutzen, 1989). Differences in the degree of this attenuation probably contribute to the discrepancies between recently observed trends in surface-level UV-B intensities (Scotto et al., 1988; Blumenthaler and Ambach, 1990).

Independent of any effects of ambient temperature, CO_2 level affects plant-water relations through effects on stomatal aperture and conductance, leading to effects on leaf and canopy temperature and the uptake of gaseous pollutants. The effects of UV-B on numerous growth processes have been reviewed by Tevini and Teramura (1989) and Runeckles and Krupa (1994). Individual interactive effects of O_3 and several effects of global climate change have been reviewed in the previous sections. However, it is important to recognize that, because of the interactions among the different components of climate change themselves, a holistic approach is essential, which includes the potential of interactions for modifying plant response to oxidants. Overall reviews of the interactions involving the

factors of climate change and O_3 have been presented by Krupa and Kickert (1989) and Ashmore and Bell (1991).

The effect of increased CO_2 in stimulating photosynthetic rates also may lead to increased leaf area, biomass, and yield (Allen, 1990). Increased CO_2 also leads to stomatal closure. However, with regard to water use, the result of decreased stomatal conductance in reducing transpiration is offset partly by the increase in leaf and canopy temperature, resulting from reduced evaporative cooling, and the increase in leaf area. The net result is that increased CO_2 may lead to only slight increases in water-use efficiency, which are attributable more to increased photosynthetic activity than to reduced transpiration (Allen, 1990). On the other hand, because the primary route of entry into the leaf of a gaseous pollutant such as O_3 is through the stomata, increased CO_2 levels would be expected to decrease the impact of O_3 by reducing uptake as a consequence of reduced stomatal conductance. The effects of increasing CO_2 levels discussed above relate to plants with the C_3 pathway of carbon fixation. These include the following major broad-leaved crops: wheat, rice (*Oryza sativa*), legumes, potato, and cole crops. Plants with the C_4 pathway tend to have greater water-use efficiencies (WUEs) than C_3 plants, but show less response to increased CO_2 levels. Major C_4 crops are corn and sorghum. However, no studies appear to have been conducted on O_3/CO_2 interactions in C_4 species.

Allen (1990) provides a simulation of the effect of doubling the average ambient CO_2 level from 340 to 680 ppm on soybean yield, based on the Weibull response model to O_3 and SO_2 of Heagle et al. (1983b) and the model of stomatal conductance developed for soybean by Rogers et al. (1983):

$$g_s = 0.0485 - 7.00 \times 10^{-5}[CO_2] + 3.40 \times 10^{-8}[CO_2]^2,$$

where g_s is stomatal conductance (in meters per second), and $[CO_2]$ is CO_2 concentration (in parts per million). According to this model, a doubling of the CO_2 level would reduce g_s by a factor of 0.69, effectively reducing the O_3 and SO_2 concentrations to 0.038 and 0.018 ppm, respectively. At the current 340 ppm CO_2 level, the Weibull model predicts a yield of 340.5 g/m of row. Reduced pollutant entry at 680 ppm CO_2 gives a predicted yield of 390.6 g/m of row, an increase of 14.7%. This is a conservative estimate because it ignores the direct effect of the increased CO_2 level on soybean growth.

Although the calculation makes numerous assumptions, it is supported qualitatively by evidence from the few studies published to date on CO_2/O_3 interactions. Barnes and Pfirrmann (1992) reported that an increased CO_2 level of 765 ppm countered the adverse effects of O_3 on photosynthesis, shoot growth rate, leaf area, and water-use efficiency of radish. Protection against the adverse effects of O_3 on soybean by elevated CO_2 also was reported by Kramer et al. (1991). The yield loss due to O_3 at ambient CO_2 was 11.9%, whereas, in the presence of ambient + 150 ppm CO_2, the loss was only 6.7%.

Although these studies support the prediction of Allen (1990), they were conducted in growth chambers (Barnes and Pfirrmann, 1992) or OTCs (Kramer et al., 1991; Mulchi et al., 1992), as were the studies on which Allen's model was based. Hence, the plants would not have been subjected to the environmental conditions typical of the open field, particularly with respect to wind speed and its effects on transpiration and temperature. Nevertheless, these studies support the view that increased CO_2 levels will reduce adverse effects of O_3 on crops.

It is unclear as to whether such CO_2-induced reductions of the impact of O_3 also apply to the long-term growth of trees, and it is equally unclear as to how increased CO_2 will affect the impact of O_3 on ecosystems. These uncertainties arise because of the numerous compensatory feedback mechanisms that play important roles in both long-term perennial growth and in the behavior of ecosystems. Such feedback includes changing demands for nutrients, increased leaf area and potential water loss, and changes in litter quality and quantity. For example, in terms of the effects of increased CO_2 alone, long-term studies of several species suggest that, although photosynthesis may be demonstrably stimulated, there may be little or no net response at the ecosystem level (Bazzaz, 1990).

The consequences of global warming as a feature of climate change are difficult to assess because, as discussed in Section 5.4.4, the information on the effects of temperature on O_3-response is conflicting. However, as Ashmore and Bell (1991) point out, concerns over the effects of O_3 on sensitivity to freezing temperatures will become increasing unimportant as warming occurs.

Various models of climate change scenarios have indicated that changed precipitation patterns will lead to increased drought in some mid-latitude regions of the world. The bulk of the evidence reviewed in Section 5.4.4 suggests that this would reduce the impact of O_3. However, because of the major direct impact of drought per se, such protection would be of little practical significance.

Greater certainty surrounds the likelihood that global warming will increase the incidence and severity of losses caused by pests and diseases. Concurrent increases also may favor the competitiveness of many weed species. At present, it is not possible to quantify such changes or to determine how they would influence the interactions discussed in Section 5.4.3.

With regard to possible interactions of O_3 and UV-B, Runeckles and Krupa (1994) point out that, because of the episodic nature of O_3 pollution, including its typical diurnal pattern, surface-level exposures to UV-B also will be episodic. They have described various possible O_3/UV-B scenarios that need to be considered. With low surface-O_3 levels and increased UV-B irradiation due to stratospheric O_3 depletion, effects of UV-B will predominate. On the other hand, elevated surface-O_3 levels will cause increased attenuation of UV-B resulting in reduced surface intensities. With no stratospheric O_3 depletion, this condition implies that surface effects of O_3 will predominate over the effects of UV-B; with stratospheric O_3 depletion, the resulting surface level irradiation will be dependent on the concentration and thickness of the surface O_3 layer, and both O_3 and UV-B effects may occur.

To date, there have been no experiments conducted specifically to simulate these different scenarios. However, Miller et al. (1994) exposed soybean in field OTCs, within which lamps were suspended to provide increased intensities of UV-B. The O_3 treatments were ambient and 1.5 $\times$ ambient. No significant O_3/UV-B interactions were noted; the effects on growth were solely attributable to the O_3 exposure. However, increased UV-B irradiation resulted in increases in the foliar content of UV-absorbing constituents.
In contrast, Miller and Pursley (1990) reported that a preliminary experiment revealed a less than additive interaction of O_3 and UV-B on soybean growth.

It is clear overall that the effects of O_3 on vegetation will be modified to some degree by various components of the complex mix of factors that constitute climate change. Considerably more research will need to be undertaken before quantitative assessments of the magnitudes of the changes will be possible.

5.4.9 Summary—Environmental Factors

Since the previous criteria document (U.S. Environmental Protection Agency, 1986), additional studies have been published on a wide range of biological, physical, and chemical factors in the environment that interact with plant response to O_3.

Biological components of the environment of individual plants include pests, pathogens, and plants of the same or other species in competition. With regard to insect pests, although only a very limited number of plant-insect systems have been studied, there is a general trend in the observations that suggests that some pests have a preference for and grow better when feeding on plants that have been impacted by O_3. Unfortunately, because there is no knowledge of how the vast majority of plant-insect systems will be affected by O_3, it is not possible to offer any quantitative overall assessment of the consequences of such interactions on the growth of crops and natural vegetation. At best, there is a reasonable likelihood that some insect pest problems will increase as a result of increased ambient O_3 levels, but there is no evidence to suggest that O_3 may trigger pest outbreaks.

Plant-pathogen systems also are affected by O_3, but, here too, the available evidence is far from representative of the wide spectrum of plant diseases. Nevertheless, the suggestion of Dowding (1988) that diseases caused by obligate pathogens tend to be diminished by O_3, whereas those caused by facultative pathogens tend to be favored, generally is supported by the limited evidence available. In terms of its broader implications, this suggests that continued exposure to O_3 may lead to a change in the overall pattern of the incidence and severity of specific plant diseases affecting crops and forest trees. However, it is not possible, with the limited evidence currently available, to predict whether the net consequences of O_3 exposure would be more or less harmful.

A major level of uncertainty concerns the effects of O_3 at the population and community levels within natural ecosystems. Very few studies have been conducted on multi-species systems, and Woodward (1992) has pointed out the hazards of attempting to extrapolate from responses of the individual plant to responses of a population of such plants. This is borne out by the observations of Evans and Ashmore (1992) who showed that the behavior to O_3-exposure of a species growing in mixture with other species is not predictable from its behavior when grown in isolation. This has serious implications with regard to complex natural ecosystems and identifies a serious gap in the knowledge of the effects of O_3 that can be filled only by a substantial research effort.

With regard to the physical environment, the combination of light, temperature, air turbulence, and water availability largely determines the success of plant growth because of the influence of these factors on the processes of photosynthesis, respiration, and transpiration. Air turbulence plays an important role in O_3 uptake because it determines the amount of O_3 to which a plant is exposed, as well as when exposure will occur. For agricultural crops, perhaps the most important of these potential interactions with O_3 concerns water availability and use. There is consistent evidence that drought conditions tend to reduce the direct adverse effects of O_3 on growth and yield. Conversely, the ready availability of soil water tends to increase the susceptibility of plants to O_3 injury. However, a lack of water should not be viewed as a potentially protective condition, because of the adverse effects of drought per se. The combination of drought conditions and exposure to O_3 is likely to result in adverse effects on growth and yield that are largely the result of lack of water. However, with perennial trees, there is evidence that prolonged exposures to

O_3 may lead to greater water use efficiency, which would enable such trees to be better able to survive drought conditions.

In contrast with crop species, with tree species, the relative roles of light, temperature, and water are shifted somewhat because of the differences in plant form. In particular, the photosynthetic function of the leaves is carried out by a much smaller proportion of the plant's biomass. Conversely, a larger demand is placed on temperature-dependent respiratory processes to maintain and support the tissues of the stem and root systems. In addition, in temperate regions, the perennial habit brings with it the requirement for storage of carbohydrates and other reserves, in order to permit survival during the winter season and to facilitate renewed spring growth. Hence, with tree species it becomes important to distinguish between the immediate effects of exposure to O_3 and the longer term consequences of these effects.

Of particular importance in northern latitudes and at higher elevations is the demonstrated role of O_3 in adversely affecting cold hardiness by reducing carbohydrate storage. Independent of effects on winter hardiness, there is also evidence to indicate that adverse effects on storage also may be a component of changes in growth occurring in subsequent seasons (Hogsett et al., 1989; Andersen et al., 1991; Sasek et al., 1991). However, it is not yet possible to assemble these observations into a general quantitative model.

The plant's environment also contains numerous chemical components, ranging from soil nutrients and other air pollutants to agricultural chemicals used for pest, disease, and weed control. With regards to plant nutrients and their influence on plant response to O_3, the available evidence is highly fragmentary and frequently contradictory and, hence, does not permit the drawing of any general conclusions. A large number of studies have been conducted on the effects of O_3 in conjunction with other gaseous air pollutants such as SO_2 and NO_2, although the information obtained in several of the studies is of no more than academic interest because of the unrealistic exposure conditions used. Although there is clear evidence to show that O_3 and SO_2 may act synergistically in increasing foliar injury in some species, the available evidence indicates that this type of response is not universal. Several empirical models of the O_3-SO_2 interaction have been developed, but they have little in common and are highly specific to the crop and exposure conditions used. Furthermore, the frequently observed lack of interaction implies that in many cases the impact of O_3 is probably best assessed on its own. The same is true of the situation with regard to combinations of O_3 and acid rain or acid fog and of O_3 and NO_2.

Numerous agricultural chemicals have been found to influence the responses of plants to O_3. In particular, several fungicides have been shown to provide protection against visible injury, although none has been adopted for commercial application for this purpose. On the other hand, the experimental chemical EDU has been found consistently to provide protection of a wide range of species, both in the laboratory and in the field.

Because increased tropospheric O_3 is a component of global climate change, results from studies on the interactions of O_3 with increased levels of CO_2 and UV-B radiation are beginning to appear. Initial work with CO_2 suggests that increased CO_2 levels may ameliorate the effects of O_3. However, it is too soon to be able to generalize on the outcome of this interaction. At the present time, no investigations of the compound interactions involving O_3, CO_2, UV-B, increased temperature, and changed soil-moisture status have been reported.

In conclusion, in spite of the amount of work carried out on the interactions of O_3 with environmental factors, there exists only a very fragmented understanding from which to draw conclusions. This is probably inevitable in view of the vast scope of the possible interactions between O_3 and all the other environmental variables. It is also a result of the fact that most of the published work consists of studies resulting from personal interests of the investigators, rather than from coordinated programs of research that focus on systematic investigations. The consequence is that, although information has been reported about magnitudes of many interactions of O_3 with environmental variables (or the lack thereof), the fragmented and nonsystematic nature of the information prevents the drawing of general conclusions and of defensible estimates of the uncertainties associated with these interactions.

5.5 Effects-Based Air Quality Exposure Indices

5.5.1 Introduction

5.5.1.1 Biological Support for Identifying Relevant Exposure Indices

The effects of O_3 on individual plants and the factors that modify plant response to O_3 are complex and vary with species, environmental conditions, and soil and nutrient conditions. Because of the complex effect of O_3 and its interactions with physical and genetic factors that influence response, the development of exposure indices to characterize plant exposure and to quantify the relationship between O_3 exposure and ensuing plant response has been, and continues to be, a major problem. At best, experimental evidence of the effect of O_3 on biomass production can refine the knowledge of those factors of O_3 exposure that affect the ability to predict plant response using exposure indices. The impacts of measured O_3 concentrations on plant response are discussed and evaluated to determine the key factors of exposure that account for the variations in plant response and, if possible, to develop measures of pollutant exposure that relate well with plant response.

Considerable evidence of the primary mode of action of O_3 on plants (e.g., injury to proteins and membranes, reduction in photosynthesis, changes in allocation of carbohydrate, early senescence), which eventually impacts biomass production, identifies O_3 uptake as the measurement of plant exposure (Section 5.3). Ozone uptake is controlled by canopy and stomatal conductance and by ambient O_3 outside the leaf (see Figure 5-3). Any factor that will affect stomatal conductance (e.g., light, temperature, humidity, soil and atmospheric chemistry, air turbulence, nutrients, time of day, phenology, biological agents) will affect O_3 uptake and, consequently, plant response (i.e., yield or biomass). Biochemical mechanisms describe the mode of action of O_3 on plants as the culmination of a series of physical, biochemical, and physiological events leading to alterations in plant metabolism. Ozone-induced injury is cumulative, resulting in net reductions in photosynthesis, changes in allocation of carbohydrate, and early senescence, which ultimately lead to reductions in biomass production. In most cases, increasing the duration of exposure increases the effect of O_3 on plant response. Peak concentrations, when they occur during daylight (when stomatal conductance is high), can have more influence in determining the impact of O_3 on plant response than lower concentrations or night concentrations because of a greater likelihood of intracellular impairment.

From a toxicological perspective, duration and peak concentrations above some level have value in determining plant response but interact with other factors such as respite time, temporal variation, phenology, canopy structure, physiological processes,

environmental conditions, and soil and nutrient conditions in different fashions, depending on species. Effects occur on vegetation when the amount of pollutant absorbed exceeds the ability of the plant to detoxify O_3 or to repair the initial impact (Tingey and Taylor, 1982).

Although O_3 uptake integrates the above factors with atmospheric conditions and relates well with plant response, it is difficult to measure. Several empirical models to predict stomatal conductance have been developed for particular species (Lösch and Tenhunen, 1981) but have not been used to estimate O_3 uptake or to develop exposure indices. Based on atmospheric measurements of deposition and diurnal patterns of O_3 and gas exchange in a natural grassland ecosystem, Grünhage and Jäger (1994a,b) and Grünhage et al. (1993a,b) proposed an ambient O_3 exposure potential for characterizing O_3 uptake and related it to the damaged-leaf area (DLA) of leaf No. 4 of Bel W3 tobacco (Grünhage et al., 1993a,b).

5.5.1.2 Historical Perspective on Developing Exposure Indices

For almost 70 years, air pollution specialists have explored alternative mathematical approaches for summarizing ambient air quality information in biologically meaningful forms that can serve as surrogates for dose for vegetation effects purposes. Some of the indices introduced have attempted to incorporate some of the factors (directly or indirectly) described above. Recognizing the importance of duration and peak concentrations in conjunction with stomatal conductance, the optimum exposure index can be written as

$$\text{Index} = \sum_{i=1}^{n} w_i \times f(C_i), \qquad (5\text{-}1)$$

where C_i is the hourly mean concentration, $f(C_i)$ is some function of C_i, and w_i is some weighting scheme that relates ambient condition and internal O_3 flux. The optimal weights are difficult to develop because of the complex relationship among exposure, environmental condition, and species.

Equation 5-1 represents a taxonomy of exposure indices that have been proposed as surrogates of dose in the literature. The exposure indices differ in the ways in which the values are assigned to w_i. Based on the weighting function, the exposure indices can be arranged into the categories described below (description from Lee et al., 1989).

- One Event: $w_i = 0$ for all C_i, except for the few concentrations where $w_i = 1$. Examples of such indices are the second highest daily maximum 1-h concentration (2HDM), the maximum of 7-h (P7) and 1-h (P1) maximum daily averages, and the 90th or higher percentiles of hourly distribution.
- Mean: $w_i = 0$ for all C_i outside the period of interest (P) and $w_i = v_i/\Sigma_{i=1}^{n} v_i$ for all C_i inside the period P, where v_i is a function of C_i or some environmental variable. Examples are the seasonal mean of 7-h daily means (M7) (Heagle et al., 1979b); the effective mean (m_e), where m_e^v is the index in Equation 5-1 with $f(C_i) = C_i^{-1/v}$ and $w_i = 1$ for some parameter v (Larsen and Heck, 1984); the solar-weighted mean where v_i is the hourly solar radiation value (Rawlings et al., 1988b).
- Cumulative: $w_i = 1$ for all C_i. An example is the seasonal sum of all hourly concentrations (i.e., total exposure, denoted as SUM00).
- Concentration Weighting: $w_i = g(C_i)$ where g() is a monotonically nondecreasing function. Examples are the seasonal sum of hourly

concentrations at or above a threshold level such as 0.06 ppm (SUM06) or 0.08 ppm (SUM08); the seasonal sum of the difference between an hourly concentration above a threshold level, less the threshold value, such as 0.08 ppm (AOT08); the total impact with $w_i = C_i^{(-1-1/v)}$ for some v (Larsen et al., 1983); the index with the allometric function, $g(C_i) = C_i^a$, $a > 0$; the index with sigmoidal weighting function, $g(C_i) = 1/[1 + M \times \exp(-AxC_i)]$, where M = 4,403 and A = 126, denoted as W126 by Lefohn et al. (1988a), and M = 500 and A = 100, denoted SIGMOID by Lee et al. (1989); total hours with concentrations at or above a threshold level, such as 0.08 ppm (HRS08), $g(C_i) = 0$ for $C_i < 0.08$ ppm and $w_i = 1/C_i$ for $C_i \geq 0.08$ ppm.

- Multicomponent: $w_i = g(C_i, i)$. Examples are indices that incorporate several characteristics of exposure and crop development stage, including the phenologically weighted cumulative impact indices (Lee et al., 1987).

Oshima (1975) and Oshima et al. (1976) proposed an exposure index, where the difference between the value above 0.10 and 0.10 ppm was summed. This is referred to as the AOT10 exposure index with $f(C_i) = C_i - 0.10$ and $w_i = 0$ for $C_i < 0.10$ ppm and $w_i = 1$ for $C_i \geq 0.10$ ppm in Equation 5-1. Alternatively, Lefohn and Benedict (1982) introduced an exposure index based on the hypothesis that, if the higher O_3 concentrations had greater value in predicting adverse effects on agricultural crops than did the lower values, then the higher hourly mean concentrations should be given more weight than the lower values. This index summed all hourly concentrations equal to and above a 0.10-ppm threshold level. This index is referred to as the SUM10 exposure index, with $f(C_i) = C_i$ and $w_i = 0$ for $C_i < 0.10$ ppm and $w_i = 1$ for $C_i \geq 0.10$ ppm. The SUM indices are not concentration weighting but threshold weighting, in that all concentrations at or above a threshold level have equal weight rather than increasing weight to higher concentrations.

A 6-h, long-term, seasonal mean, O_3 exposure index was used by Heagle et al. (1974). Also, Heagle et al. (1979b) reported the use of a 7-h experimental period mean. The 7-h (0900 to 1559 hours) mean, calculated over an experimental period, was adopted as the statistic of choice by the U.S. Environmental Protection Agency's (EPA's) NCLAN program (Heck et al., 1982). The 7-h daily daylight period was selected by NCLAN because the index was believed to correspond to the period of greatest plant susceptibility to O_3 pollution. In addition, the 7-h period of each day (0900 to 1559 hours) was assumed to correspond to the time that the highest hourly O_3 concentrations would occur. However, not all monitoring sites in the United States experience their highest O_3 exposures within the 0900 to 1559 hours 7-h time period (Lefohn and Jones, 1986; Lefohn and Irving, 1988; Logan, 1989). Toward the end of the program, NCLAN redesigned its experimental protocol and applied proportional additions of O_3 to its crops for 12-h periods. The expanded 12-h window reflected NCLAN's desire to capture more of the daily O_3 exposure. In the published literature, the majority of NCLAN's experiments were summarized using the 7-h experimental-period average.

Based on the concept that higher concentrations of O_3 should be given more weight than lower concentrations (summarized in U.S. Environmental Protection Agency, 1986), concerns about the use of a long-term average to summarize exposures of O_3 began appearing in the literature (Lefohn and Benedict, 1982; Tingey, 1984; Lefohn, 1984; Lefohn and Tingey, 1985; Smith et al., 1987). Specific concerns were focused on the fact that the use of a long-term average failed to consider the impact of peak concentrations. The 7-h seasonal mean contained all hourly concentrations between 0900 to 1559 hours; this

long-term average treated all concentrations within the fixed window in a similar manner. A large number of hourly distributions within the 0900- to 1559-hours window could be used to generate the same 7-h seasonal mean, ranging from those containing many peaks to those containing none. Larsen and Heck (1984) pointed out that it was possible for two air sampling sites with the same daytime arithmetic mean O_3 concentration to experience different estimated crop reductions.

In the late 1980s, the focus of attention turned from the use of long-term seasonal means to cumulative indices (i.e., exposure indices that sum the products of concentrations multiplied by time over an exposure period). As indicated previously, the cumulative index parameters proposed by Oshima (1975) and Lefohn and Benedict (1982) were similar. Both parameters gave equal weight to the higher hourly concentrations but ignored the concentrations below a subjectively defined minimum threshold (e.g., 0.10 ppm). Besides the cumulative indices proposed by Oshima (1975), Oshima et al. (1976), and Lefohn and Benedict (1982), other cumulative indices were suggested, including the number of occurrences of daily maximum hourly averaged concentrations greater than a threshold level (Ashmore, 1984) and the use of exponential functions (Nouchi and Aoki, 1979; Larsen and Heck, 1984) to assign unequal weighting to O_3 concentrations.

A possible disadvantage of applying an integrated exposure index, as defined by Oshima (1975) and Lefohn and Benedict (1982), is that the use of an artificial threshold concentration as a cutoff point eliminates any possible contribution of the lower concentrations to vegetation effects. Although this disadvantage may not be important when considering O_3 exposures that occur in the California South Coast Air Basin, where repeated high concentrations are experienced from day to day, and there are relatively short periods between episodes, it is important when assessing the typical exposures experienced in other parts of the United States.

Recognizing the disadvantage, Lefohn and Runeckles (1987) suggested a modification to the Lefohn and Benedict (1982) exposure index by weighting individual hourly mean concentrations of O_3 and summing over time. Lefohn and Runeckles (1987) proposed a sigmoidal weighting function that was used in developing a cumulative integrated exposure index. The index included the lower concentrations in the integrated exposure summation.

None of the exposure indices mentioned above fully characterize the potential for plant uptake of O_3 because the indices, being measures of ambient condition, ignore the biological processes controlling the transfer of O_3 from the atmosphere through the leaf and into the leaf interior (U.S. Environmental Protection Agency, 1986, 1992). Early studies with beans and tobacco, reviewed in the previous criteria document (U.S. Environmental Protection Agency, 1986), showed that short-term, higher peak exposures induced more visible injury than longer term, lower peak exposures of the same total exposure, indicating that concentration has more value than exposure duration in eliciting a response, at least for short-lived species. Other studies with soybean, tobacco, and bean, conducted prior to 1983 and described in U.S. Environmental Protection Agency (1986), showed that the foliar injury response to subsequent peak exposures varies with temporal pattern. Predisposition to low levels of O_3 for a few days increases plant sensitivity to subsequent peaks (Johnston and Heagle, 1982; Heagle and Heck, 1974; Runeckles and Rosen, 1977). Tobacco plants exposed to 2 consecutive days of peak exposures showed greater injury on the first day (Mukammal, 1965). Plants exposed to a series of successive short exposures suffered more

injury than did those plants that received a continuous uniform exposure, with all plants receiving equal total exposure (Stan and Schicker, 1982).

When yield or growth are considered, "not only are concentration and time important, but the dynamics of the O_3 exposure are also important" (U.S. Environmental Protection Agency, 1986). Musselman et al. (1983) were the first to demonstrate that plants exposed to variable concentrations showed greater effect on plant growth than those exposed to a fixed or daily peak concentration of equal total exposure but lower peak concentrations. The Hogsett et al. (1985b) study also reported a greater effect on plant growth of variable concentrations; however, no plant response data is presented in the paper. Musselman et al. (1986b), in a subsequent experiment, exposed kidney bean plants to either a simulated ambient or a uniform concentration that had equal total exposure and peak concentration (at two levels of 0.30 and 0.40 ppm) and found that the effects of the two distributions did not differ significantly. Consequently, when peak concentrations and total exposures are equal, the diurnal distribution of concentrations appears to be unimportant.

More recent studies with bean (Kohut et al., 1988b), soybean (Heagle et al., 1986b), and tobacco (Heagle et al., 1987b) (reviewed in U.S. Environmental Protection Agency, 1992) showed conflicting evidence of no significant differences in response to different exposure patterns of equal total exposure but varying peak concentrations. The value of peak concentrations in influencing response was inconclusive. For the study with beans, plants exposed to peak exposures showed significant impairment in the early harvests, but, at the final harvest, O_3 effects on growth and yield were not statistically significant. For the NCLAN studies with soybean and tobacco, differences in yield between the constant and proportional 7-h O_3-addition exposures were not significant, even though the proportional-addition treatments had greater peak concentrations. In reanalysis of the soybean and tobacco studies, Rawlings et al. (1988b) stated that the differences between the constant and proportional O_3 additions were relatively small, thus limiting the power of the comparison test. However, 12-h exposures caused greater effects than 7-h exposures, but the decrease in yield loss was not directly proportional to the increased length of exposure (Rawlings et al., 1988b).

Considerable research since the publication of the previous criteria document (U.S. Environmental Protection Agency, 1986) has been directed at developing measures of exposure that were consistent with then-current knowledge of the mode of action of O_3 on plants, as well as on factors such as concentration, duration, and temporal dynamics of exposure influencing response. A number of retrospective studies of existing data to evaluate and compare exposure indices based on statistical fit (Rawlings et al., 1988b; Adomait et al., 1987; Cure et al., 1986; McCool et al., 1986, 1987; Smith et al., 1987; Lee et al., 1987, 1988; Lefohn et al., 1988a; Tingey et al., 1989; Musselman et al., 1988) have been summarized in the literature between 1986 and 1988 and reviewed by the U.S. Environmental Protection Agency (1992). Studies using O_3 exposures in chambers suggest the following conclusions: O_3 effects are cumulative, peak concentrations may be more important than lower concentrations in eliciting a response, and plant sensitivity to O_3 varies with time of day and crop development stage. Exposure indices that cumulate the exposure and preferentially weight the peaks yield better statistical fits to response than do the mean and peak indices.

Because the mean exposure index treats all concentrations equally and does not specifically include an exposure duration component, the use of a mean exposure index for characterizing plant exposures appears to be inappropriate for relating exposure with

vegetation effects (U.S. Environmental Protection Agency, 1992). In particular, the weighting of the hourly O_3 concentrations of the mean is inconsistent with the weighting function of plant exposure to O_3 in Equation 5-1, which attempts to relate O_3 flux to ambient condition. The total exposure index includes an exposure duration component but does not adequately relate pollutant exposure with plant response because the index weights all concentrations equally and focuses on the lower concentrations.

Evidence supporting the use of peak-weighted, cumulative indices in relating O_3 exposure and plant response is based on statistical reanalyses of NCLAN data. However, it is unlikely that the empirical modeling of plant response will determine the optimal weighting function of hourly O_3 concentrations for use in characterizing plant exposure, which varies with environmental factors and species. The development and comparison of exposure indices based on statistical fits is difficult because only a limited number of experiments have been designed specifically to test and evaluate the various exposure indices.

Although much research has been conducted on O_3 effects on crops and trees since 1988, the overall understanding of the mode of action of O_3 on plants and factors that modify plant response remains unchanged since the previous criteria document (U.S. Environmental Protection Agency, 1986) and its supplement (U.S. Environmental Protection Agency, 1992). Additional studies further support the value of concentration, duration, and temporal pattern of exposure in describing plant exposure and its relation to plant response. Studies that applied two or more different exposure patterns of equal exposure but possibly different peak concentrations are reviewed in Section 5.5.2.2 to substantiate the value of exposure structure in influencing the magnitude of plant response. Recent papers that report results from replicate studies over time and space are summarized in Section 5.5.2.3 to test the value of duration and its relation to plant response. In addition, a few recent studies that provide additional insight to those factors that modify plant response are reviewed in Sections 5.5.2.4 and 5.5.2.5.

5.5.2 Developing Exposure Indices

5.5.2.1 Experimental Design and Statistical Analysis

Controlled and field exposure-response studies, where extraneous factors influencing response are controlled or monitored, allow the study of concentration, duration, respite time, and temporal fluctuations at various stages of crop development in influencing response. These studies provide insight on the efficacy of exposure indices in explaining variation of response. A small number of experiments have been designed specifically to study the components of exposure and have applied two or more different patterns of exposure that measure the same SUM00 values. These designs provide the best evidence to determine whether plants respond differentially to temporal variations in O_3 concentrations; however, they may have limited application in developing a statistical relationship between O_3 exposure and plant response. Other design considerations, including the number, kind, and levels of O_3 exposure; the patterns of randomization; the number of replicates used in the experiment; and experimental protocol, determine the strength of the statistical analysis that is applied to the treatment mean comparison tests and the range of ambient and environmental conditions over which generalizations may be made. These designs have been used successfully to test the value of components of exposure, particularly concentration, in influencing response (Musselman et al., 1983, 1986b, 1994; Hogsett et al., 1985b). Different approaches that include either a mean separation procedure or a regression

procedure have been used to identify those important components of exposure that influence response.

To identify the importance of exposure in contributing to variation of plant response, the majority of pollutant effects studies use regression-based designs that apply a single pattern of exposure at varying concentration levels. However, if these designs are used, the application of the results is limited; plant response (i.e., plant yield) with respect to exposure is unchanged with different measures of exposure. The relative position and spacing between exposure levels is a function of how the exposure index weights the hourly O_3 concentrations and governs the statistical fit to response. The regression approach has been used to compare and evaluate various exposure indices, but the ability to discriminate among indices is low for these studies. By their nature, those studies that have used regression-based designs that utilize data from single patterns of exposure cannot distinguish between mean exposure indices and sums constructed from means (i.e., mean $\times$ duration) and, consequently, cannot be used to test the value of duration in explaining the variation of response.

Evidence to substantiate the value of duration in explaining the experimental variation of plant response may be obtained when combining data from replicate studies of the same species and cultivar over time and space. Pooling of data from replicate studies of the same species to evaluate duration effects and to compare various exposure indices assumes that the primary cause of biological response is pollutant exposure. This assumption may or may not be valid, particularly when plants from replicate studies are grown under varying environmental, edaphic, and agronomic conditions that tend to mask the treatment effects during the growth of the plant (Section 5.3). Hence, it is more difficult to substantiate the importance of exposure-dynamic factors from retrospective analyses of combined data from replicate studies of the same species than from experiments designed specifically to address the components of exposure. The comparison of environmental conditions, as well as the yields of plants exposed to CF air over replicate studies, is a simple check of interaction but does not ensure that O_3 effects on response can be isolated. In addition, when the main effect of O_3 is insignificant, the data may be limited for determining the value of duration or other components of exposure in predicting response. Nonetheless, if an air pollutant is the primary source of variability in plant response, the relationship between exposure and response should be consistent when data sets for the same crop are combined over several years or locations.

Sets of replicate studies of equal and varying duration are readily available in the published literature, but only a few reports have combined the data to test specifically the value of duration in explaining variation of plant response or to evaluate exposure indices based on statistical fit. Lefohn et al. (1988a) were the first to fit a common response model to combined data from two replicate studies of varying duration using various exposure indices. Greater yield losses occurred when plants were exposed for the longer duration, indicating that the duration component of exposure was important in influencing response, and that a cumulative-type index was able to describe adequately the relationship between exposure and yield. More recent papers have reported results of the 2 years of replicate studies, and a few papers have used the regression approach, with and without variance components for sites and years, to evaluate various exposure indices based on the adequacy of fit of a common response model.

A number of the papers relevant to the study of components of exposure influencing plant response report only the mean and total exposure (SUM00) indices.

Because exposure indices weight hourly O_3 concentrations differently, it is almost impossible to convert one index to another. The original data, which in many cases are not available, would be necessary to generate alternative exposure indices. Therefore, unless adequate information is given to allow calculation of exposure indices, the analysis of reported results from individual and combined data to evaluate different exposure indices is not possible, although it may be possible to perform retrospective evaluation of the structure of exposure in altering plant response.

Another concern relates to the experimental design, particularly the number, kind, and levels of exposure used in the study. Generalization of experimental results is largely dependent on the degree to which atmospheric and biospheric conditions mimic those of the target population when growing under ambient conditions. However desirable the need to mimic the real world, understanding the relationship between exposure and the ensuing response (i.e., plant yield) and identifing those components of exposure that influence response may require the use of exposure regimes with temporal pattern, concentration, or structure that are not observed in nature. Such data requires the comparison of exposure levels between CF and near-NF conditions, but the mathematician who attempts to model an experiment requires higher than NF levels of O_3 to better determine the nature of plant response to O_3. A discussion of the advantages and disadvantages of OTCs and the types of NCLAN exposures is discussed in the section on methodologies (Section 5.2.1.1). The O_3 exposures utilized by the NCLAN program have been described as producing artificial regimes that do not mimic actual conditions.

In addition to the CF concentration regimes, Lefohn et al. (1988a) have reported that the highest treatments have a tendency to display bimodal distributions that are unrealistic (Figure 5-9). At this time, there is no evidence to suggest whether or not these higher NF exposures provide realistic information on the impact of O_3 on plant response.

Studies that utilize exposures with peak concentrations above 0.40 ppm may not provide realistic evidence of O_3 impact on plant response in the United States. These studies provide limited evidence for substantiating the value of peak concentrations in influencing response. Consequently, these studies are not included in this section.

5.5.2.2 Studies with Two or More Different Patterns of Exposure

Experiments using chambers that focused on the structure of exposure have shown that plant response is differential to temporal patterns of O_3 exposure. For crop species, there is evidence to suggest that plant response is influenced more by higher concentrations than by lower concentrations or exposure duration. Greater response to concentration occurred when plants were predisposed to low concentrations for a few days or when peaks occurred just prior to or at maximum leaf expansion (U.S. Environmental Protection Agency, 1978, 1986). Plants exposed to two (or more) different exposure patterns of equal exposure (i.e., same SUM00 value) showed greater foliar injury response to:

(1) the short-term, high-concentration exposure than to the longer term exposure with lower peak concentrations (Heck et al., 1966; Heck and Tingey, 1971; Bennett, 1979; Nouchi and Aoki, 1979; Amiro et al., 1984; Ashmore, 1984; Tonneijck, 1984); and

(2) the exposure that predisposes plants to low O_3 concentrations for a few days prior to a high O_3 concentration than to exposures that have a set diurnal pattern of O_3 concentrations or less than 2 days of respite time between high concentrations (Heck and Dunning, 1967; Johnston and Heagle, 1982; Heagle

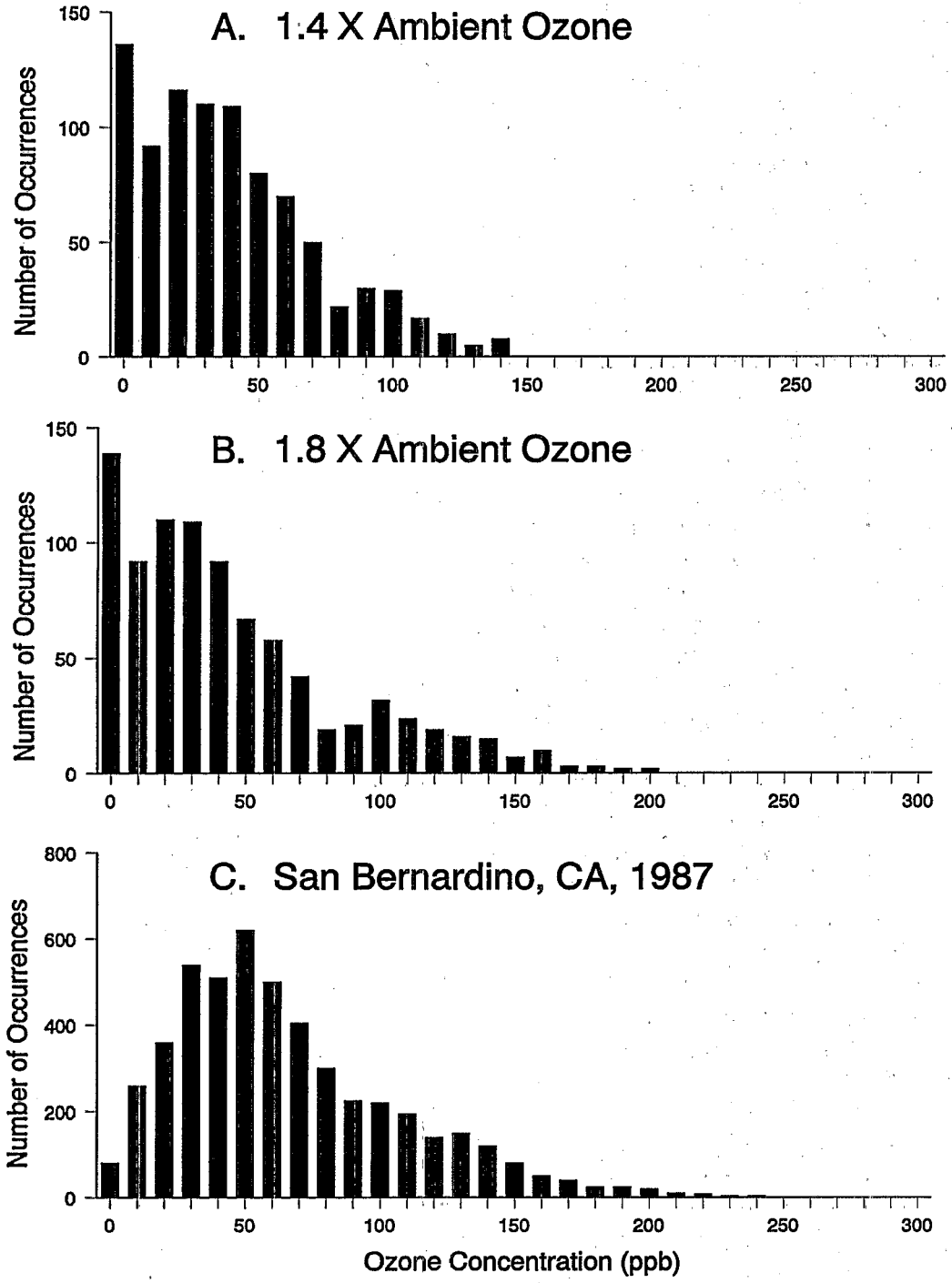

Figure 5-9. Distribution pattern showing the number of ozone concentrations within specified ranges for the 1983 winter wheat proportional-addition experiment for the (A) 1.4 × ambient air and (B) 1.8 × ambient air treatments and for (C) San Bernardino, CA, in 1987.

and Heck, 1974; Runeckles and Rosen, 1977; Mukammal, 1965; Stan and Schicker, 1982).

The studies that applied the same exposure but used different patterns of exposure have been reviewed in previous criteria documents (U.S. Environmental Protection Agency, 1978, 1986, 1992) and substantiate the role of concentration, temporal dynamics, respite time, and predisposition in influencing the magnitude of plant response to O_3.

Musselman et al. (1983) and Hogsett et al. (1985b) were among the first to demonstrate that variable concentrations produced greater effect on plant growth than did fixed or set diurnal patterns of exposure of equal total exposure but with lower peak concentrations (Table 5-12). Musselman et al. (1986a), in a subsequent experiment, exposed kidney bean plants to either a variable or a uniform concentration of equal total exposure and equal peaks (at two levels of 0.30 and 0.40 ppm) and found that the effects of the two distributions were not significantly different (Table 5-12). Musselman et al. (1994), in a third experiment, exposed kidney bean plants to four different patterns of equal total exposure and, like Musselman et al. (1983), found that patterns with higher peak concentrations or longer duration of high concentrations (>0.16 ppm) produced significantly greater effect on top dry weight than the square wave pattern. Cumulative, peak-weighted exposure indices with an allometric weighting parameter between 2 and 3.5 gave the best fit for dry weight, necrosis, and number of pods. These results provide evidence that: total exposure (i.e., SUM00), being unable to differentiate among the exposure patterns, is a poor predictor of plant response; the peak concentrations or sequence of peak concentrations (>0.16 ppm) are important in determining plant response; and greater weight should be given to higher concentrations when describing exposure. Consequently, when peak concentration and total exposure are equal, the diurnal distribution of concentrations (e.g., sequence of peak concentrations >0.16 ppm) *may be* an important factor.

One recent study exposed bean plants to two consecutive exposures of 0.30 ppm for 3 h/day in the rapid vegetative growth stage and showed greater reductions in total dry weight when exposures were 3 to 6 days apart (McCool et al., 1988) (Table 5-12); this finding is consistent with earlier results on the role of predisposition in influencing response (e.g., Hogsett et al., 1988). Predisposition to a high concentration above the level that causes visible injury may increase plant sensitivity over time (Mukammal, 1965). As a result, the subsequent response to a high concentration following recovery may be greater than experienced in prior exposures. In future modeling efforts, this phenomenon may have to be taken into consideration, by the weighting of hourly concentrations, for properly characterizing plant exposure.

Sensitivity of plants to O_3 is a function of stomatal conductance and varies with the cultivar, time of day, and phenology. To test the role of phenology, Heagle et al. (1991b) applied 16 patterns of exposure in combinations of either CF or NF air for each quarter of the experimental period (31 days/quarter) (Table 5-12). The authors concluded that the phenological stage of development played a role in plant response, and that exposures during mid- to late-growth stages caused greater yield losses than did exposures during earlier developmental stages. For crops, foliage appears to be most sensitive to O_3 just prior to or during maximum leaf expansion (U.S. Environmental Protection Agency, 1978). These results are consistent with earlier studies (Lee et al., 1987) that reported better statistical fits to response using exposure indices that preferentially weighted hourly O_3 concentrations during the period of anthesis to seed fill.

Table 5-12. A Summary of Studies Reporting the Effects of Ozone for Two or More Exposure Patterns on the Growth, Productivity, or Yield of Plants[a]

Species	Facility[b]	Total Number of Chambers	Exposure Patterns[c]	Exposure Duration	Concentration (ppm)/Exposure[d] (ppm-h)	Variable	Effect[e]	Reference
Glycine max L. Merrill cv. Davis, Forrest, Bragg, and Ransom	OTC in pots	24	16 combinations of CF or NF + over 4 quarters (31-days/quarter)	124 days	M7 (ppm): CF range from 0.016 to 0.038 over the 4 quarters, NF+range from 0.096 to 0.098 over the 4 quarters	Total seed weight	Forrest: greater effect in Q3 than in other quarters. Davis: no consistent effect Q1, significant but similar effects for Q2, Q3, and Q4. Ransom: no significant O_3 effects in Q1 or Q2, and equal responses in Q1, Q3, and Q4. Bragg: no significant O_3 effects in Q1 or Q2, significant decreases in Q3 and Q4.	Heagle et al. (1991b)
Medicago sativa L.	OTC in pots	8	E, DP	133 days	Equal SUM07 (ppm-h): DPH=113, DPL=63, EH=117, EL=72 Equal SUM00 (ppm-h): DPH=183, DPL=140, EH=193, EL=145 M7 (ppm): DPH=0.099, DPL=0.074, EH=0.084, EL=0.064	Shoot dry weight	91 and 67% reductions for EH and DPH. Significant difference between E and DP regimes. Treatment means are ordered CF<DPL<EL<DPH<EH.	Hogsett et al. (1985b)
Phaseolus vulgaris L. cv. Calif. Dark Red Kidney Bean	GC in pots	8	U, V	3 weeks (1 day/ week)	Equal SUM00 (ppm-h/day): UL=VL=0.69; UH=VH=0.92 Equal Max concentration (ppm): UL=VL=0.30, UH=VH=0.40	Pod and seed dry weights	6% less yield when exposed to variable (NS). Response to O_3 same in reproductive and vegetative growth stages.	Musselman et al. (1986b)
Phaseolus vulgaris L. cv. Calif. Dark Red Kidney Bean	GC in pots	8	U, V	3 weeks (1 day/ week)	Equal SUM00 (ppm-h/day): UL=VL=1.20; UH=VH=1.68 Max concentration. (ppm): UL=0.20, VL=0.50,UH=0.28, VH=0.715	Pod and seed dry weights	38% less yield when exposed to variable. No significant difference between low and high. Beans in reproductive growth stage when exposed.	Musselman et al. (1983)
Phaseolus vulgaris L. cv. Calif. Dark Red Kidney Bean	GC in pots	8	Square wave (SW), Triangular (T), Flattened triangular (FT), Rhomboid (R)	7 weeks (3 days/ week)	Equal SUM00 (ppm-h/day): SW=T=FT=R=0.60. Max concentration (ppm): SW=0.12, T=0.36, FT=R=0.24.	Top dry weight	SW had significantly higher yield than other three patterns. Treatment means are ordered R=T<FT<SW	Musselman et al. (1994)
Phaseolus vulgaris L. cv. Calif. Dark Red Kidney Bean	GC in pots	10	Initial exposure of 0.3 ppm for 3-h and second exposure of 0.3 ppm at 2-6 (or 1-5) days after initial exposure	2-6 days in 1984 and 1-5 days in 1985	Equal maximum concentration of 0.30 ppm.	Total dry weight	Reductions due to the second exposure were significant when exposures were 3-6 days apart in 1984 and 5 days apart in 1985.	McCool et al. (1988)

[a]See Appendix A for abbreviations and acronyms.
[b]GC = Controlled environmental growth chamber, or CSTR; OTC = Open-top chamber.
[c]CA = Constant addition, PA = Proportional addition, CF = Charcoal-filtered, NF = Nonfiltered, NF+ = Nonfiltered plus ozone, E = Episodic, DP = Daily peak, U = Uniform, V = Variable, HE = High elevation.
[d]H = High, L = low.
[e]Significant at the 0.05 level, NS = not significant.

There is very limited information on the effect of O_3 on mature trees. Most of the information available deals with the nature of seedling response to O_3 (see Section 5.6.4); however, much less is known about the role of exposure-dynamic factors (e.g., concentration, duration, respite time, temporal variation) in influencing biomass response in long-lived species.

When yield is considered, a number of exposure-dynamic factors, including concentration, temporal pattern, predisposition, and respite times, as well as phenological stage of plant development, have been shown to influence the impact of O_3 on plant response. Evidence from studies of kidney bean (Musselman et al., 1983, 1994), alfalfa (Hogsett et al., 1985b), tobacco (Heagle et al., 1987b), soybean (Heagle et al., 1986b), ponderosa pine, and aspen suggests that concentration and temporal variation of exposure are important factors in influencing biomass production and, consequently, become considerations in measures of exposure. Because the SUM00 index weights all concentrations equally, the SUM00 is inadequate for characterizing plant exposure to O_3 (Lefohn et al., 1989). Other factors, including predisposition time (McCool et al., 1988) and crop development stage (Heagle et al., 1991b), contribute to variations in biological response, which suggests the need for weighting O_3 concentrations to account for predisposition time and phenology. However, the roles of predisposition and phenology in influencing plant response vary with species and environmental conditions and are not understood well enough to allow specification of a weighting function for use in characterizing plant exposure.

5.5.2.3 Combinations of Years, Sites, or Species: Comparisons of Yield Losses with Different Exposure Durations

Duration has not been a focus in experimental designs of studies that applied two or more exposure regimes over the growing season. Several lines of evidence suggest that the ultimate yield depends on the cumulative impact of repeated peak concentrations (U.S. Environmental Protection Agency, 1986, 1992), and that O_3-induced reductions in growth are linked to reduced photosynthesis, which is impaired by the cumulative O_3 exposure (Reich and Amundsen, 1985; Reich, 1987; Pye, 1988). In EPA reviews of the literature (U.S. Environmental Protection Agency, 1986, 1992), EPA concluded that "When plant yield is considered, the ultimate impact of an air pollutant on yield depends on the integrated impact of the pollutant exposures during the growth of the plant." As a measure of plant exposure, the appropriate index should differentiate between exposures of the same concentration but of different duration. For example, a mean index calculated over an unspecified time cannot accomplish this (Lefohn et al., 1988a; Hogsett et al., 1988; Tingey et al., 1989, 1991; U.S. Environmental Protection Agency, 1986, 1992).

The paper by Lefohn et al. (1988a), reviewed previously in U.S. Environmental Protection Agency (1992), along with published criticisms and responses, was the first to fit a common response model to combined data from two replicate studies of unequal duration (71 and 36 days for the 1982 and 1983 wheat studies, respectively, conducted at Ithaca, NY) to test specifically for the importance of duration in influencing plant response. Greater yield losses occurred in 1982, which can be attributed partially to the longer duration. Because the mean index ignores the length of the exposure period, the year-to-year variation in plant response was minimized by the use of several cumulative indices rather than the mean. Lefohn (1988) and Lefohn et al. (1988b) concluded that duration has value in explaining

variation in plant response, and that a cumulative-type index was preferred over a mean or peak index based on statistical fit.

When O_3 effects are the primary cause of variation in plant response, plants from replicate studies of varying duration showed greater reductions in yield or growth when exposed for the longer duration (Lee et al., 1991; Olszyk et al., 1993; Adaros et al., 1991a) (Table 5-13, Part A). Using NCLAN data for wheat, cotton, kidney bean, and potato from replicate studies with markedly different exposure durations, Lee et al. (1991) showed that year-to-year variations in the magnitude of relative yield loss were minimized by the use of exposure indices that are cumulative and weight peak concentrations more than low concentrations, indicating that O_3 effects are cumulative (Figure 5-10). Olszyk et al. (1993), using the two NCLAN cotton studies summarized by Temple et al. (1985) and Lee et al. (1991), in addition to cotton studies replicated at four sites in California's San Joaquin Valley over 2 years, tested and compared various exposure indices (SIGMOID, SUM06, M7, and 2HDM) based on statistical fit of a common response model. A Weibull response model with variance components was fit to the combined data and used to test for a common response (Gumpertz and Rawlings, 1991, 1992; Gumpertz and Pantula, 1992). The likelihood ratio test of parallel exposure-response curves was statistically significant for M7 and 2HDM for at least one set of cotton data, indicating significant differences in the magnitude of response across years or sites. On the other hand, the SIGMOID and SUM06 indices resulted in consistent patterns of response for both sets of cotton data, as well as between sets of cotton data (Figure 5-11). The authors concluded that the peak-weighted, cumulative indices minimized the temporal and spatial variations in crop yield and better predicted cotton yield responses than the M7 or 2HDM indices. The mean and peak indices did not differentiate between exposure seasons of differing duration and could not account for year-to-year differences in response.

The results of European studies with wheat (Adaros et al., 1991a,c), spring rape (Adaros et al., 1991b), barley (Adaros et al., 1991c), and kidney beans (Bender et al., 1990), using data from replicate studies with varying duration, are less conclusive as to the role of duration in determining plant response (Table 5-13, Part A). Exposures are reported using a mean index. Adaros et al. (1991a) showed a greater reduction in above-ground dry weight when exposed for the longer duration for the wheat cultivar Star but not for the cultivar Turbo (Figure 5-12). Adaros et al. (1991c), in another 2-year study with barley (cv. Arena and Alexis) and wheat (cv. Star and Turbo), involving mixtures of O_3, SO_2, and NO_2, showed greater reductions in yield when exposed for the longer duration for all species and cultivars except barley cv. Alexis (Table 5-13, Part A). Ozone effects were insignificant in both years for barley cv. Alexis. The authors did not attribute the differential response in growth and yield to any single factor, but the data suggested that O_3 effects are cumulative. When O_3 exposure is the primary source of response, the mean exposure index of unspecified duration could not account for the year-to-year variation in response.

The role of duration in influencing growth or yield is unclear for the other studies because of the following limitations in the data:

(1) Treatment levels were below the levels necessary to induce injury or damage to kidney bean plants in 2 of the 3 years. None of the years produced a significant O_3 effect at or below 70 ppb concentration (Bender et al., 1990). Similarly, the study with barley showed no significant O_3 effects.

Table 5-13. A Summary of Studies Reporting the Effects of Ozone on the Growth, Productivity, or Yield of Plants for Two or More Replicate Studies Having Equal Total Exposures and Either Varying Durations (Part A) or Similar Durations (Part B)[a]

Species	Facility[b]	Total No. of Plots	Duration [dates and (days)]	Concentration (ppm)/ Exposure (ppm-h)[c]	Variable	Effect[d]	Reference
PART A							
Brassica napus L. var. Napus cv. Callypso	OTC in pots	1987: 18 1988: 24 1989: 16	1987: 05-13 to 08-10 (89) 1988: 05-02 to 08-24 (113) 1989: 05-08 to 08-01 (84)	1987: M24 (M8) in ppb range from 5 (9) to 16 (43). 1988: M24 (M8) in ppb range from 3 (5) to 16 (48). 1989: M24 (M8) in ppb range from 6 (5) to 22 (62).	Seed dry weight	1987: 27% reduction at M8 = 43 ppb (***). 1988: 18% reduction at M8 = 48 ppb (***). 1989: 11% reduction at M8 = 62 ppb (***).	Adaros et al. (1991b)
Gossypium hirsutum L. cv. Acala SJ2	OTC	1981: 12 1982: 12	1981: 07-06 to 09-15 (72) 1982: 06-04 to 09-09 (98)	1981: M7 (SUM06) range from 18 ppb (0 ppm-h) to 138 (68). 1982: M7 (SUM06) range from 12 ppb (0 ppm-h) to 111 (71).	Lint dry weight	45 and 66% reductions at M7 = 111 ppb. 57 and 60% reductions at SUM06 = 68 ppm-h.	Lee et al. (1991), Olszyk et al. (1993)
Hordeum vulgare L. cf. Arena and Alexis	OTC in pots	1988: 24 1989: 16	1988: 04-29 to 08-15 (108) 1989: 05-08 to 08-15 (99)	1988: M8 (max 8-h mean) in ppb range from 5 (15) to 48 (89). 1989: M8 (max 8-h mean) in ppb range from 11 (27) to 62 (101).	Seed dry weight	Arena: 14% (*) and 6% (NS) reductions at M8 = 48 ppb. Alexis: No reductions at M8 = 48 ppb (NS).	Adaros et al. (1991c)
Phaseoulus vulgaris L. cv. Calif. Dark Red Kidney Bean	OTC	1980: 20 1982: 20	1980: 08-20 to 09-10 (22) 1982: 08-11 to 10-06 (57)	1980: M7 (SUM06) range from 24 ppb (0 ppm-h) to 139 (19). 1982: M7 (SUM06) range from 19 ppb (0 ppm-h) to 110 (40).	Seed dry weight	13 and 59% reductions at M7 = 110 ppb. 28 and 8% reductions at SUM06 = 19 ppm-h.	Lee et al. (1991)
Phaseolus vulgaris L. cf. Rintintin	OTC in pots	1988 I: 4 1988 II: 6 1989 III: 8	1988 I: 06-15 to 08-04 (51) 1988 II: 07-24 to 08-29 (37) 1989 III: 06-04 to 07-25 (52)	I. M8 (max) in ppb range from 3 (19) to 48 (70). II. M8 (max) in ppb range from 2 (19) to 50 (105). III. M8 (max) in ppb range from 6 (26) to 109 (159).	Pod dry weight	I. 2% reduction at M8 = 48 ppb (NS). II. 0% reduction at M8 = 50 ppb (NS). III. 0% (NS) and 47% (*) reductions at M8 = 50 and 109 ppb.	Bender et al. (1990)
Solanum tuberosum L. cv. Norchip	OTC	1985: 15 1986: 39	1985: 06-14 to 08-22 (70) 1986: 06-20 to 08-20 (62)	1985: M7 (SUM06) range from 22 ppb (0 ppm-h) to 85 (47). 1986: M7 (SUM06) range from 24 ppb (0 ppm-h) to 88 (38).	Tuber weight	42 and 25% reductions at M7 = 85 ppb. 32 and 27% reductions at 12-h SUM06 = 38 ppm-h.	Lee et al. (1991)
Triticum aestivum L. cv. Vona	OTC	1982: 20 1983: 12	1982: 05-18 to 07-17 (61) 1983: 06-12 to 07-17 (36)	1982: M7 (SUM06) range from 21 ppb (0 ppm-h) to 95 (41). 1983: M7 (SUM06) range from 26 ppb (0 ppm-h) to 96 (22).	Seed dry weight	74 and 49% reductions at M7 = 95 ppb. 49 and 62% reductions at SUM08 = 21 ppm-h. 55 and 60% reductions at 7-h SUM06 = 22 ppm-h.	Lefohn et al. (1988a), Lee et al. (1991)

Table 5-13 (cont'd). A Summary of Studies Reporting the Effects of Ozone on the Growth, Productivity, or Yield of Plants for Two or More Replicate Studies Having Equal Total Exposures and Either Varying Durations (Part A) or Similar Durations (Part B)[a]

Species	Facility[b]	Total No. of Plots	Duration [dates and (days)]	Concentration (ppm)/ Exposure (ppm-h)[c]	Variable	Effect[d]	Reference
PART A (cont'd)							
Triticum aestivum L. cv. Star and Turbo	OTC in pots	1988: 6 1989: 10	1988: 04-27 to 08-23 (118) 1989: 05-09 to 08-15 (98)	1988: M8 (max,SUM06) in ppb range from 4 (58,0) to 51 (106,8.2). 1989: M8 (max,SUM06) in ppb range from 10 (34,0) to 113 (162,87).	Seed dry weight	Star: 20% (*) and 9% (NS) reductions at M8 = 51 ppb. Turbo: 25% (*) and 31% (*) reductions at M8 = 51 ppb.	Adaros et al. (1991a)
Triticum aestivum L., cv. Star and Turbo	OTC in pots	1988: 24 1989: 16	1988: 04-29 to 08-15 (108) 1989: 05-08 to 08-15 (99)	1988: M8 (max 8-h mean) in ppb range from 5 (15) to 48 (89). 1989: M8 (max 8-h mean) in ppb range from 11 (27) to 62 (101).	Seed dry weight	Star: 26% (*) and 12% (*) reductions at M8 = 48 ppb. Turbo: 34% (*) and 17% (*) reductions at M8 = 48 ppb.	Adaros et al. (1991c)
PART B							
Glycine max L. Merr. cv. Davis	OTC in pots	1977: 8 1978: 8	1977: 06-17 to 10-10 (116) 1978: 06-28 to 10-21 (116)	1977: M7 (max) in ppb range from 27 (78) to 154 (277). 1978: M7 (max) in ppb range from 28 (84) to 131 (241).	Seed dry weight	47 and 37% reductions at M7 = 131 ppb.	Cure et al. (1986), Heagle et al. (1983a)
Glycine max L. Merr. cv. Williams	OTC	1981: 31 1982: 31 1983: 31	1981: 07-20 to 09-22 (65) 1982: 07-14 to 09-22 (71) 1983: 07-23 to 09-23 (63)	1981: M7 in ppb range from 15 to 64. 1982: M7 in ppb range from 17 to 99. 1983: M7 in ppb range from 19 to 132.	Bean dry weight	28, 20, and 32% reductions at M7 = 64 ppb. 43 and 41% reductions at M7 = 99 ppb in 1982 and 1983	Heggestad and Lesser (1990), Heggestad et al. (1988)
Medicago sativa L. cv. WL-514	OTC	1984: 30 1985: 30	1984: 03-16 to 10-10 (209) 1985: 03-23 to 10-09 (201)	1984: M12 in ppb range from 16 to 109. 1985: M12 in ppb range from 10 to 94.	Top dry weight	29% (*) and 25% (*) reductions at M12 = 94 ppb.	Temple et al. (1988a)
Pinus rigida Mill.	OTC in pots	Exp. 1: 4 Exp. 2: 4	Exp. 1: 13 weeks Exp. 2: 13 weeks	1: M8 in ppb range from 0 to 200 (U). 2: M8 in ppb range from 0 to 200 (U).	Total dry weight	49 and 46% reductions at M8 = 200 ppb.	Schier et al. (1990)
Pinus taeda L.	GC in pots	1986: 15 1987: 15	1986: 09-15 to 12-04 (81) 1987: 07-27 to 10-15 (81)	1986: SUM00 in ppm-h range from 0 to 99 (U). 1987: SUM00 in ppm-h range from 0 to 99 (U).	Total dry weight	43 and 28% reductions at SUM00 = 99 ppm-h averaged across all families. Individual families show similar reductions (e.g., 35 and 33% reductions at SUM00 = 99 ppm-h for family 5.56, 14 and 12% reductions at SUM00 = 99 ppm-h for family 1.68).	Shafer et al. (1993)

Table 5-13 (cont'd). A Summary of Studies Reporting the Effects of Ozone on the Growth, Productivity, or Yield Of Plants for Two or More Replicate Studies Having Equal Total Exposures and Either Varying Durations (Part A) or Similar Durations (Part B)[a]

Species	Facility[b]	Total No. of Plots	Duration [dates and days]	Concentration (ppm)/ Exposure (ppm-h)[c]	Variable	Effect[d]	Reference
PART B (cont'd)							
Picea rubens Sarg.	OTC in pots	1987: 12 1988: 12	1987: 05-30 to 12-15 (199) 1988: 06-01 to 12-01 (184)	1987: SUM00 in ppm-h are 32, 61, 91, and 119. 1987: SUM00 in ppm-h are 36, 70, 101, and 135.	Total dry weight	0% (NS) reduction in biomass after first year, 8% (*) reduction at SUM00 = 135 ppm-h after second year of exposure.	Alscher et al. (1989) Amundson et al. (1991)
Pisum sativum L. cv. Puget	ZAPS	1986: 14 1987: 14	1986: last 58 days 1986: last 52 days	M12 and D25 (numbers of days with 1-h concentrations >25 ppb) used in simple linear regression.	Pea fresh weight	0% reductions at M12 = 100 ppb based on linear regression models.	Runeckles et al. (1990)
Populus tremuloides Michx clones	OTC in pots	1988: 18 1989: 18	1988: 07-19 to 09-27 (71) 1989: 07-20 to 09-20 (64)	1988: SUM00 in ppm-h are 5.0, 10.0, and 19.4 (U). 1989: SUM00 in ppm-h are 7.7, 15.4, and 26.4 (U).	Stem and leaf dry weights	36% (*) and 40% (*) reductions at SUM00 = 19.4 ppm-h.	Karnosky et al. (1992b)
Triticum aestivum L. cv. Albis	OTC	1986: 12 1987: 16 1988: 16	1986: 05-06 to 07-31 (86) 1987: 04-27 to 08-10 (92) 1988: 05-04 to 08-01 (89)	1986: M24 (max) in ppb range from 12 (61) to 47 (181). 1987: M24 (max) in ppb range from 12 (54) to 45 (175). 1988: M24 (max) in ppb range from 17 (65) to 45 (148).	Seed dry weight	1986: 61% reduction at M24 = 47 ppb. 1987: 27% reduction at M24 = 45 ppb. 1988: 65% reduction at M24 = 45 ppb.	Fuhrer et al. (1989)
Triticum aestivum L. cv. Albis	OTC	1989: 24 1990: 24	1989: 05-16 to 08-14 (91) 1990: 05-14 to 08-09 (88)	1989: M7 (SUM06) range from 18 ppb (0 ppm-h) to 62 (3.8). 1990: M7 (SUM06) range from 17 ppb (0 ppm-h) to 71 (5.6).	Seed dry weight	29 and 22% reduction at M7 = 62 ppb. 29 and 17% reduction at SUM06 = 3.8 ppm-h.	Fuhrer et al. (1992)
Triticum aestivum L. cv. Severn, Potomac, Oasis, MD5518308	OTC	1984: 20 1985: 20	1984: 05-14 to 06-22 (40) 1985: 05-06 to 06-15 (41)	1984: M4 (AOT03) in ppb (ppb-h) range from 32 (0) to 93 (10). 1985: M4 (AOT03) in ppb (ppb-h) range from 30 (0) to 86 (9).	Seed dry weight	31% (*) and 9% (NS) reductions at M4 = 86 ppb.	Slaughter et al. (1989)

[a]See Appendix A for abbreviations and acronyms.
[b]GC = Controlled environmental growth chamber, or CSTR; OTC = open-top chamber; ZAPS = zonal air pollution system.
[c]U = Uniform.
[d]* = Significant at the 0.05 level; NS = not significant.

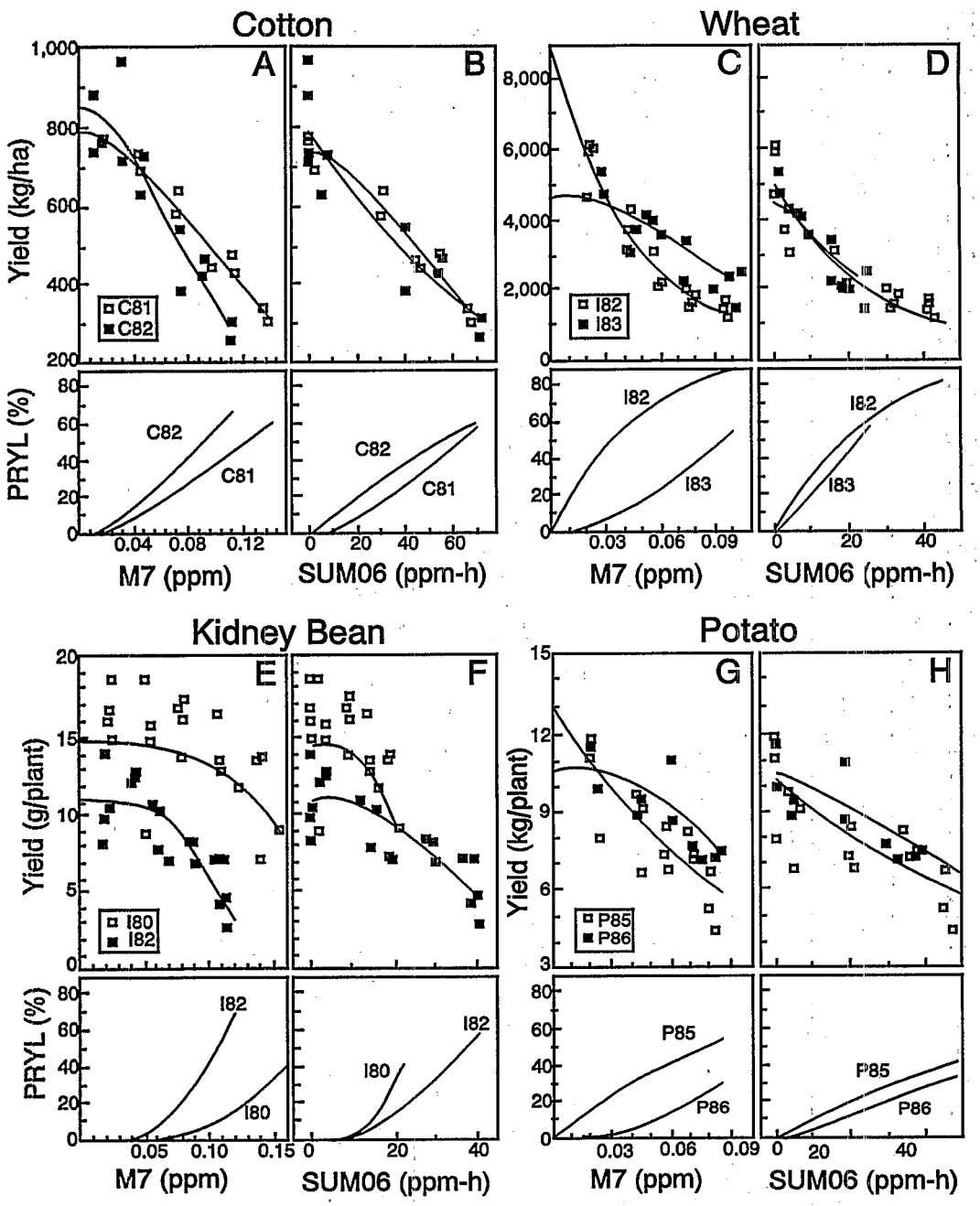

Figure 5-10. Comparison of the Weibull exposure-response functions and its predicted relative yield loss (PRYL) curves (relative to 0 ozone) using M7 and daytime SUM06 for replicate years of National Crop Loss Assessment Network Program's data for (A) and (B) cotton (var. Acala SJ-2), (C) and (D) wheat (var. Vona), (E) and (F) kidney bean (var. California light red), and (G) and (H) potato (var. Norchip), respectively. Mean dry weights and the Weibull exposure-response functions for replicate studies are given in the top portion of the graphs (Lee et al., 1991).

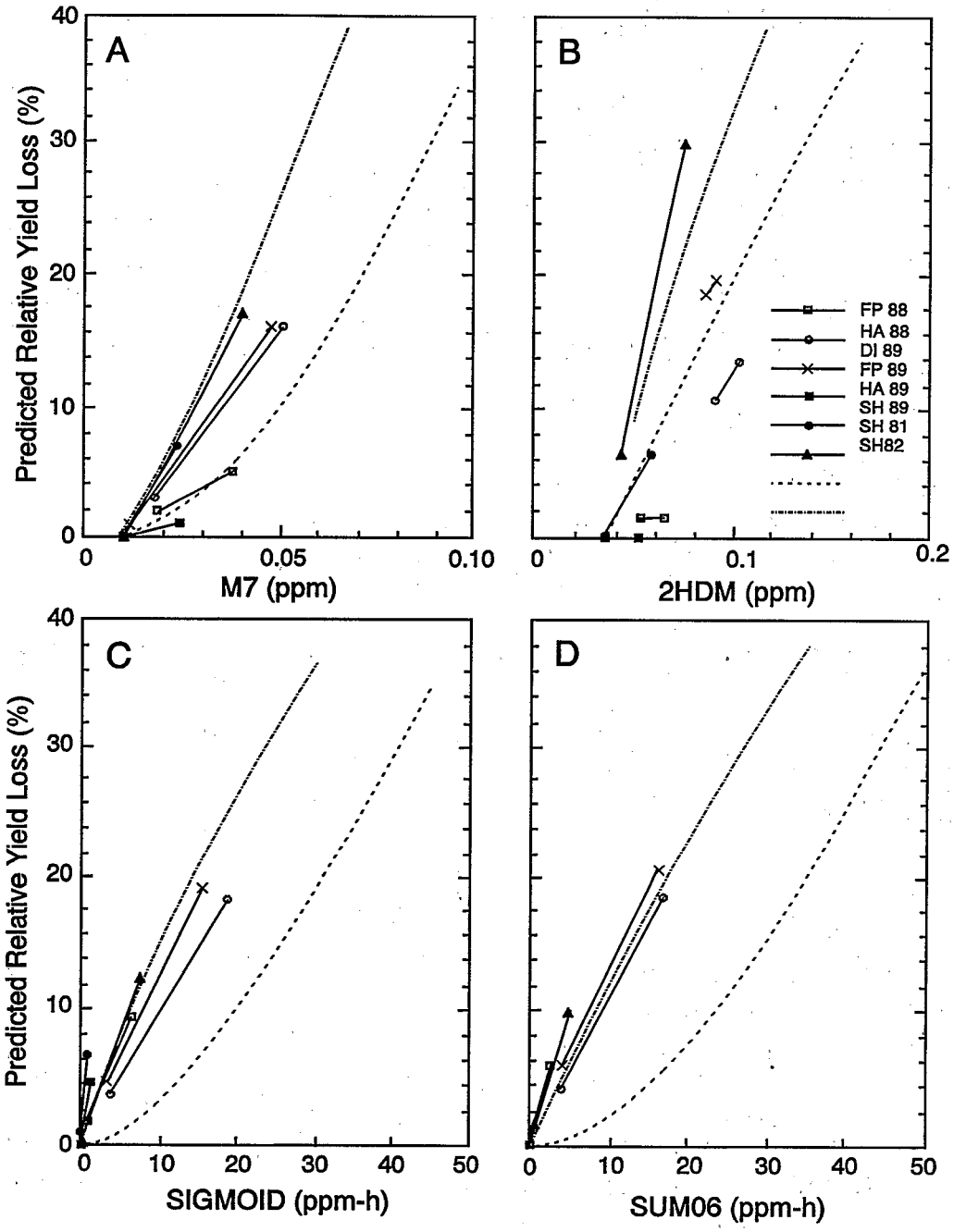

Figure 5-11. ***Predicted relative yield losses (lint weight) for Acala SJ-2 cotton for four sites and multiple years (1981, 1982, 1988, and 1989) relative to 0.01 ppm for M7, 0.035 ppm for 2HDM, 0 ppm-h for SIGMOID, and 0 ppm-h for SUM06, which correspond to typical levels in the charcoal-filtered chambers. Predicted losses are based on M7 (A), 2HDM (B), SIGMOID (C), and SUM06 (D) exposure indices. Abbreviations: DI = Dinuba, FP = Five Point, HA = Hanford, and SH = Shafter (Olszyk et al., 1993).***

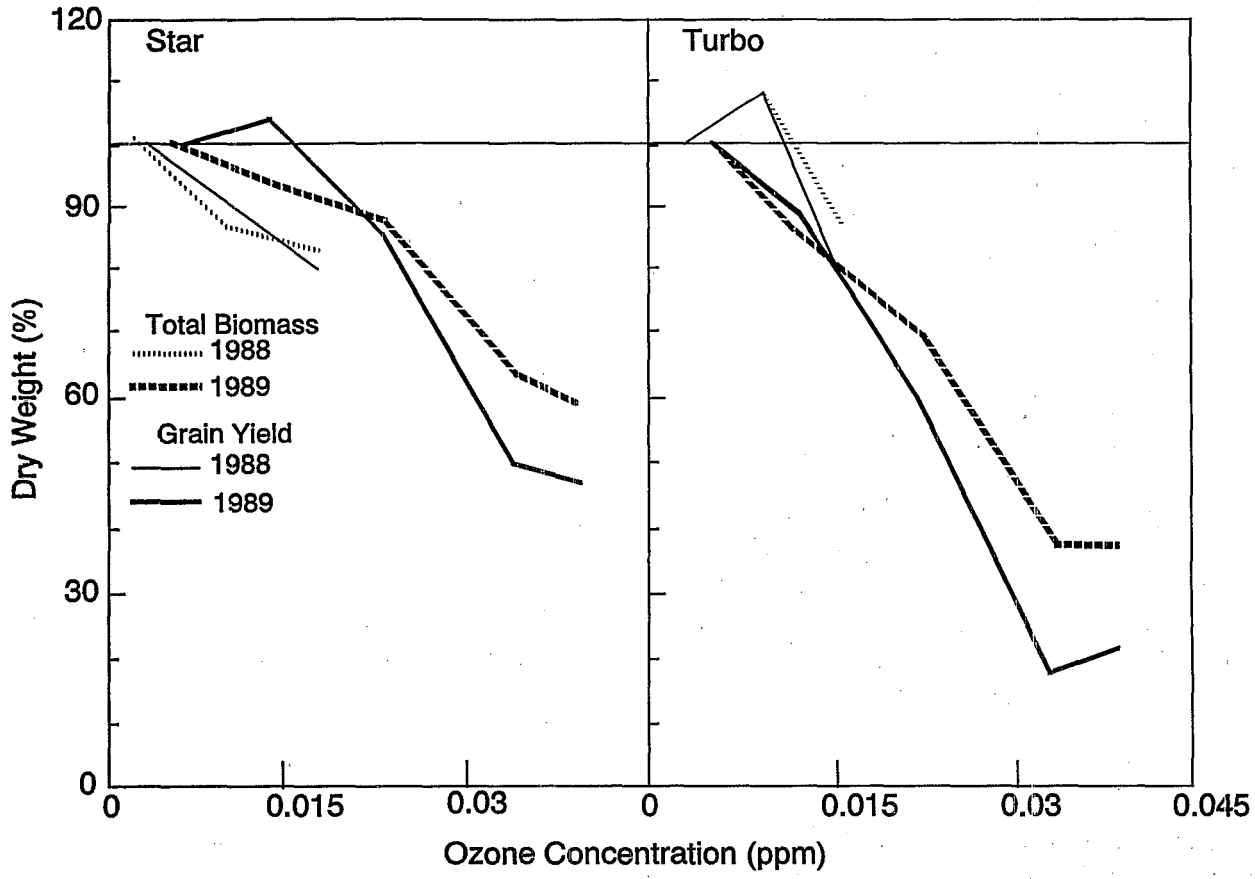

Figure 5-12. Relative effect of ozone on growth and yield of spring wheat cultivars (var. Star and Turbo) from two growing seasons (Adaros et al., 1991a).

(2) Differences in growing conditions and varying kinds of interactions among O_3, SO_2, and NO_2 resulted in different sizes of control plants of spring rape over years and affected the magnitude of response to O_3. Compared to 1987, yield of control plants increased by 32% in 1988 and by 94% in 1989 (Adaros et al., 1991b). Consequently, the evidence of duration as the primary cause of differences in response over years was difficult to substantiate.

When durations were nearly equal, plant response to O_3 were similar for 2- or 3-year studies with alfalfa (Temple et al., 1988a), pea (Runeckles et al., 1990), soybean (Heagle et al., 1983a; Heggestad and Lesser, 1990; Cure et al., 1986), wheat (Fuhrer et al., 1989, 1992), aspen clones (Karnosky et al., 1992b), loblolly pine (Shafer et al., 1993), and pitch pine (Schier et al., 1990) (Table 5-13, Part B). For example, year-to-year variations in wheat yield response to O_3 were small for the 3 years having durations between 86 and 92 days, allowing pooling of the data to fit a common Weibull model using Rawling's solar-radiation-weighted mean index (Fuhrer et al., 1989) (Figure 5-13). Different growing conditions were reported in studies of Shafer et al. (1993), Fuhrer et al. (1989), but no

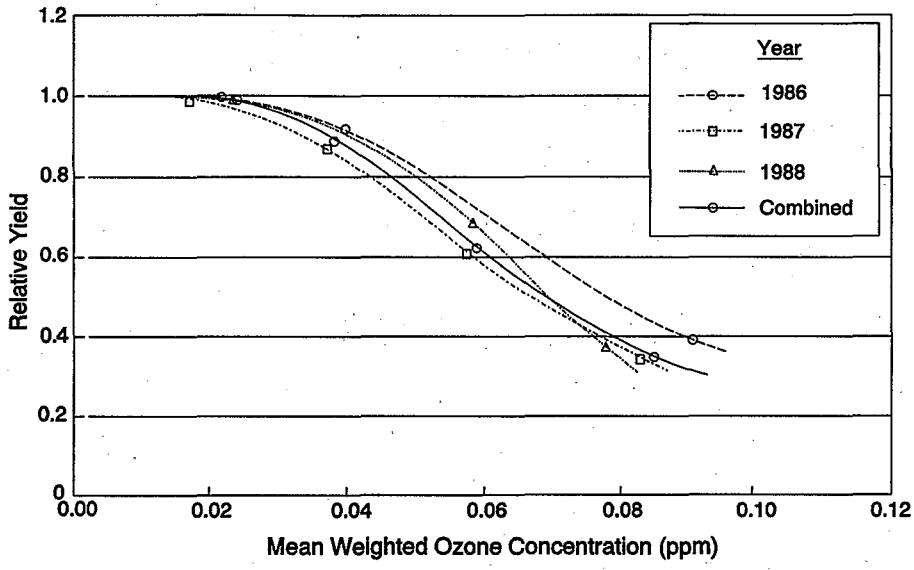

Figure 5-13. Weibull exposure-response curves for the relative effect of ozone on grain yield of spring wheat for 3 years, individually and combined (Fuhrer et al., 1989).

interaction between O_3 and climatic effects was found. On the other hand, Slaughter et al. (1989) reported reductions in wheat grain yield of 69 and 9% in a 2-year study having equal exposure durations, which the authors attribute to differences in rainfall and temperature. Environmental conditions in 1985 favored greater photosynthate partitioning for grain development rather than for vegetative growth, resulting in larger plants in 1985. Air pollution effects may not have been the primary source of variation in response, and, consequently, the data do not substantiate the role of duration in influencing response.

These studies report plant response as a function of a mean exposure index and do not evaluate or compare various exposure indices, based on statistical fit. In a series of papers that examined the response of spring wheat to O_3 at higher elevations, Grandjean Grimm and Fuhrer (1992a,b) and Fuhrer et al. (1992) conducted a 2-year study in which the flux of O_3 was determined in OTCs. Plants were exposed to O_3 for periods lasting 44 and 50 days in 1989 and 1990, respectively, and flux measurements were taken repeatedly over the experimental period. In addition to O_3 flux, exposures were characterized using M7, M24, SUM06, and the solar-radiation-weighted mean index (Rawlings et al., 1988b). The quadratic response curves relating the various indices with grain yield showed that year-to-year variations were minimized using the mean O_3 flux index (Figure 5-14). The other three exposure indices showed slightly greater yield losses in 1989 than in 1990, in contrast with longer exposure in 1990 and drier conditions in 1989. The authors concluded that the O_3 flux related well with yield because the mean flux incorporated environmental factors, canopy structure, and physiological processes, which affected the uptake of O_3 from the air to the leaf interior. The measurements of pollutant concentrations ignored these factors and, consequently, were unable to account for all of the year-to-year variability in wheat response. The authors suggested that O_3 flux was a surrogate of Fowler and Cape's

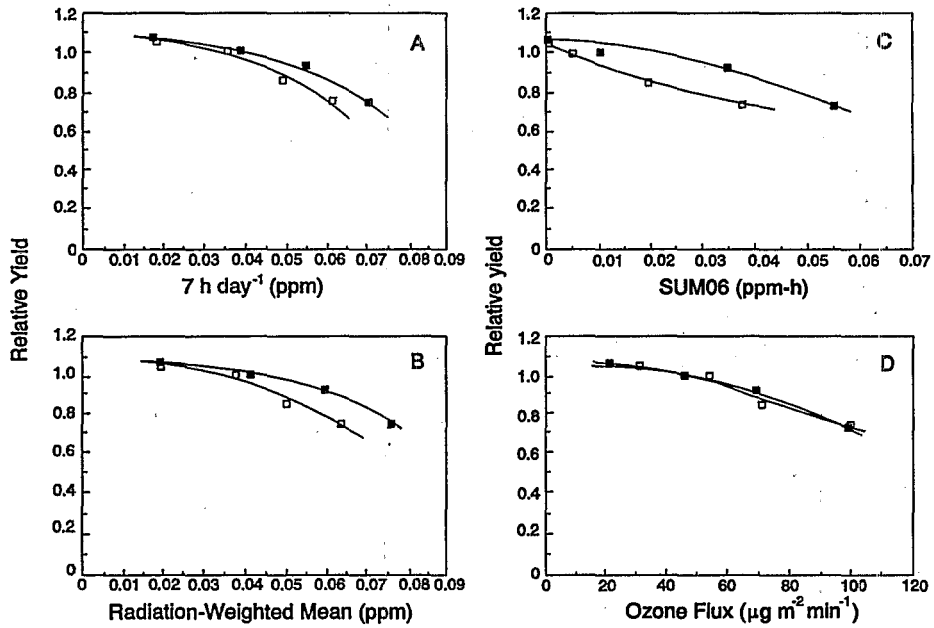

Figure 5-14. Quadratic exposure-response curves for the relative effect of ozone on grain yield of spring wheat in 1989 and 1990, using four different exposure indices (A through D).

Source: Modified from Fuhrer et al. (1992).

(1982) "pollutant absorbed dose" and appeared to be the relevant measure for use in relating exposure and plant response.

Alscher et al. (1989) and Amundson et al. (1991) report on the impact of O_3 on growth, injury, and biomass response of 2-year-old red spruce seedlings after 1 and 2 years of exposure, respectively. Exposures were characterized using the M12 (or M7), M24, and SUM00 indices. No significant O_3 effects on biomass were detected in 1987 (Alscher et al., 1989) because stomatal conductances in red spruce are inherently low and, consequently, result in low rates of pollutant uptake (Seiler and Cazell, 1990). However, in the second year, O_3 reduced leaf and root starch, increased foliar antioxidant content, and reduced biomass of 1988 fixed-growth foliage. However, O_3 effects on biomass were slight in the second year. The authors concluded O_3 effects are cumulative because the onset of damage occurred in the second year rather than the first year of exposure.

Plant response is influenced by exposure duration and O_3 concentration. Regardless of whether concentrations are above or below levels at which injury has been observed, plant responses are determined by the cumulative effects of the number of times exposures have occurred. The results of these studies are in general agreement that O_3 effects are cumulative, and the ultimate impact of long-term exposures to O_3 on crops and seedling biomass response depends on the integration of repeated peak concentrations during the growth of the plant. Consequently, the mean or peak indices are inappropriate because the length of exposure is unspecified, and these indices cannot differentiate among exposures of the same concentration but of various durations. These results support the conclusion that

an appropriate O_3 index should cumulate all hourly concentrations in some fashion to reflect the nature of O_3 on plant response. Fuhrer et al. (1992) suggested that the weighting function should reflect the relationship between ambient pollutant concentration and internal O_3 flux, consistent with the mode of action of O_3 on plants and with earlier findings that peak-weighted, cumulative indices give better predictions of plant response than mean or peak indices.

5.5.2.4 Comparisons of Measures of Exposure Based on Reanalysis of Single-Year, Single-Species Studies

Studies cited in previous sections focused on the role of the structure of exposure in influencing plant response but do not identify specifically the weighting function for use in characterizing plant exposure to O_3. In addition to these types of studies, other studies have focused on comparison of measures of exposure based on reanalysis of single-year, single-species studies. The variety of statistical approaches used to relate exposure and plant response range from informal description of the distributions of O_3 concentrations associated with response to more formal regression-based procedures.

The regression approach is designed to select those exposure indices that properly order and space the treatment means along the horizontal axis to optimize the fit of a linear or curvilinear model. However, because the experimental designs are not intended to evaluate various indices, the power of the regression approach to identify the important exposure-dynamic factors influencing plant response is less desirable (Lefohn et al., 1992a). Consequently, these retrospective studies provide less substantiating evidence of the role of exposure-dynamic factors (e.g., concentration, duration, temporal pattern, respite time) than do those studies with experimental designs and analyses that focus on specific components of exposure.

Most of the early retrospective studies reporting regression results using data from the NCLAN program or from Corvallis, OR (Lee et al., 1987, 1988; Lefohn et al., 1988a; Tingey et al., 1989), or using data collected by Oshima (U.S. Environmental Protection Agency, 1986; Musselman et al., 1988) were in general agreement and consistently favored the use of cumulative peak-weighted exposure indices. These studies have been reviewed previously by EPA (U.S. Environmental Protection Agency, 1992). Lee et al. (1987) suggested that exposure indices that included all the data (24 h) performed better than those that used only 7 h of data; this is consistent with the conclusions of Heagle et al. (1987b) that plants receiving exposures for an additional 5 h/day showed 10% greater yield loss than those exposed for 7-h/day. In a subsequent analysis using more of the NCLAN data, Lee et al. (1988) found the "best" exposure index was a general phenologically weighted, cumulative-impact index, with sigmoid weighting on concentration and a gamma weighting function as surrogate of time of increased plant sensitivity to O_3. For most cases, Lee et al. (1987) computed their exposure indices based on the daylight exposure periods used by the NCLAN investigators. The exposure indices with minimum residual sum of squares were those indices that cumulated hourly O_3 concentrations over the growth of the plant, gave preferential weighting to peak concentrations, and phenologically weighted the exposures to emphasize concentrations during the plant growth stage. The paper by Tingey et al. (1989) is a summarization of the results in Lee et al. (1988) and shows the limitations of the mean index.

Lefohn and Foley (1992) characterized the NCLAN exposures that had a SUM06 level closest to those that predicted a 20% yield loss, using the exposure-response equations

as reported in Lee et al. (1991) and Tingey et al. (1991). Lefohn and Foley (1992) characterized the hourly average concentrations using percentiles, HRS06, HRS10, SUM06, and W126 for each of 22 NCLAN studies. The authors noted that the frequent occurrence, in many cases, of high hourly concentrations ($\geq$0.10 ppm) may have been partly responsible for the 20% yield loss. The number of hourly average concentrations ranged from 0 to 515 with only one of the 22 NCLAN experiments experiencing no hourly average concentrations $\geq$0.10 ppm, whereas the remaining experiments experienced multiple occurrences $\geq$0.10 ppm. The repeated occurrences of high hourly average concentrations were a result of the NCLAN protocol (Table 5-14). As a result of their analysis, Lefohn and Foley (1992) and Lefohn et al. (1992b) stressed that, because the NCLAN experiments contained peak hourly average concentrations, it is important that any index selected to characterize those regimes responsible for growth reduction adequately capture the presence of these peak concentrations when attempting to predict biological responses using actual ambient air quality data.

For example, Tingey et al. (1991), using mostly NCLAN data, identified 24.4 ppm-h as the SUM06 value, calculated over a 3-mo period, that would protect 50% of the NCLAN crops analyzed at the 10% yield reduction level. These predicted relative yield loss (PRYL) calculations assume that the crops being protected will be grown using NCLAN protocol. There are monitoring sites in the United States that experience 3-mo cumulative SUM06 values greater than 24.4 ppm-h, but do not experience frequent occurrences of hourly average concentrations $>$0.10 ppm. For example, 24% (1987), 10% (1988), 30% (1989), 25% (1990), and 31% (1991) of the rural agricultural sites listed in the EPA Aerometric Information Retrieval System (AIRS) database experienced 3-mo cumulative SUM06 values greater than 24.4 ppm-h but experienced fewer than 11 hourly average concentrations equal to or greater than 0.10 ppm. Lefohn and Foley (1992) noted that agricultural crops grown at a site experiencing a 3-mo cumulative SUM06 value greater than 24.4 ppm-h, but with infrequent high hourly average concentrations (e.g., $\geq$0.10 ppm), might experience less yield reduction than predicted using NCLAN experimental results. For rural forest sites, 21% (1987), 23% (1988), 54% (1989), 50% (1990), and 52% (1991) of the sites exhibited 3-mo cumulative SUM06 values greater than 24.4 ppm-h, but fewer than 11 hourly average concentrations equal to or greater than 0.10 ppm. Tables 5-15 and 5-16 illustrate that sites that experience 3-mo SUM06 values $\geq$24.4 ppm do not necessarily have peaks, whereas sites that experience values $<$24.4 ppm-h do have peaks.

Reich (1987) reviewed 44 studies on 45 species to study the effects of O_3 on net photosynthesis (Pn) and growth of crops and tree species. Plants responded differently to equivalent total exposures (i.e., SUM00), when peak concentrations differed widely, with greater loss of Pn for increasing concentrations (Figure 5-15). Short-term, high concentrations above 0.40 ppm (e.g., 0.50 ppm for 8 h) caused rapid and significant reduction in Pn. Longer term exposures (for weeks) to lower concentrations had a significant effect on Pn; the observed reductions were less severe than at the higher concentrations. Based on short-term, high concentration studies, SUM00 alone was an inadequate descriptor of exposure for predicting response. However, for assessing the effects of long-term, low concentrations typical of ambient condition, SUM00 may be adequate, because the response of field-grown plants to SUM00 was roughly linear. SUM00 explained much, although not all, of the variation in Pn and the growth of conifers, hardwood trees, and agricultural crops (Figures 5-16 through 5-18). Unexplained variation can be attributed to biological variation, inherent experimental error, experimental conditions, and differences

Table 5-14. Summary of Ozone Exposures That Are Closest to Those Predicted for 20% Yield Reduction per SUM06 Exposure Response Models Used by Lee et al. (1991) in Selected National Crop Loss Assessment Network Experiments[a] (Concentrations are in parts per million.)

Experiment[b]	Chamber	Min.	10	30	50	70	90	95	99	Max	Number of Obs.	≥0.06	≥0.08	≥0.10	SUM 06 (ppm-h)	SUM 08 (ppm-h)	W126 (ppm-h)
						Percentiles						Number of Occurrences					
SOYBEAN																	
A80S0 - Corsoy	NF+0.03-1	0.000	0.000	0.011	0.026	0.045	0.077	0.090	0.111	0.123	1,344	263	113	35	21.1	10.7	17.7
A83SO - Amsoy	NF+0.03-1	0.000	0.001	0.014	0.028	0.049	0.083	0.098	0.123	0.168	1,992	467	223	90	39.1	22.1	33.2
A83SO - Corsoy	NF+0.03-1	0.000	0.001	0.014	0.028	0.049	0.083	0.098	0.123	0.168	1,992	467	223	90	39.1	22.1	33.2
A85SO - Corsoy-79 D	NF×2.00-1D	0.000	0.000	0.008	0.023	0.051	0.110	0.129	0.160	0.194	2,352	657	495	319	67.5	56.2	63.0
A85SO - Corsoy-79 W	NF×2.00-1W	0.000	0.000	0.011	0.026	0.063	0.114	0.134	0.162	0.199	2,352	729	547	358	75.1	62.5	70.0
A86SO - Corsoy-79 D	NF×2.5-1D	0.000	0.002	0.016	0.035	0.085	0.137	0.161	0.207	0.279	2,040	784	654	515	92.1	83.2	88.6
A86SO - Corsoy-79 W	NF×2.0-1W	0.000	0.002	0.015	0.033	0.065	0.105	0.124	0.161	0.242	2,040	719	510	271	69.6	55.1	63.7
B83SO - Corsoy-79 D	NF-1D	0.000	0.002	0.006	0.018	0.037	0.063	0.074	0.087	0.111	1,512	184	51	5	13.5	4.4	10.6
B83SO - Corsoy-79 W	NF+0.03-1W	0.000	0.002	0.006	0.019	0.049	0.084	0.097	0.118	0.135	1,512	359	198	70	30.1	18.9	25.8
B83SO - Williams D	NF+0.03-1D	0.000	0.002	0.006	0.019	0.049	0.084	0.098	0.118	0.137	1,512	364	204	66	30.5	19.5	26.0
B83SO - Williams W	NF+0.03-1W	0.000	0.002	0.006	0.019	0.049	0.084	0.097	0.118	0.135	1,512	359	198	70	30.1	18.9	25.8
I81SO - Hodgson	NF+0.06-1	0.000	0.004	0.007	0.015	0.031	0.083	0.090	0.105	0.132	1,680	323	191	29	26.7	17.4	22.9
R81SO - Davis	NF-1	0.000	0.003	0.015	0.026	0.043	0.066	0.075	0.088	0.145	2,664	421	79	6	30.2	7.0	22.6
R82SO - Davis	NF+0.02-1	0.000	0.001	0.013	0.026	0.047	0.080	0.091	0.123	0.203	2,160	471	218	56	39.0	21.4	33.1
R83SO - Davis Dry	NF+0.02-1D	0.000	0.002	0.015	0.030	0.055	0.089	0.104	0.126	0.155	2,640	721	378	163	61.6	37.7	53.1
R83SO - Davis Wet	NF+0.02-1W	0.000	0.002	0.015	0.030	0.054	0.087	0.101	0.119	0.138	2,640	698	359	140	58.7	35.0	50.7
R84SO - Davis Dry	NF+0.015-1D	0.000	0.006	0.018	0.030	0.047	0.077	0.089	0.113	0.140	2,496	512	208	59	41.2	19.9	34.8
R84SO - Davis Wet	NF+0.015-1W	0.000	0.006	0.018	0.029	0.046	0.075	0.089	0.110	0.159	2,496	486	193	62	38.9	18.6	32.6
R86SO - Young Dry	NF×1.3-1D	0.000	0.003	0.013	0.024	0.047	0.089	0.107	0.137	0.206	2,568	597	345	175	53.7	36.2	47.7
R86SO - Young Wet	NF×1.3-1W	0.000	0.003	0.013	0.023	0.046	0.087	0.101	0.129	0.198	2,568	573	323	136	50.2	32.8	44.1
SORGHUM																	
A82SG - Dekalb	NF+0.10-1	0.000	0.001	0.010	0.023	0.055	0.145	0.160	0.185	0.223	2,040	599	557	516	79.1	76.3	78.2
WHEAT																	
A82WH - Abe	NF+0.03-1	0.000	0.002	0.015	0.027	0.047	0.079	0.094	0.113	0.149	1,344	300	130	43	24.1	12.5	19.8
A82WH - Arthur-71	NF+0.06-1	0.000	0.002	0.015	0.027	0.053	0.109	0.121	0.144	0.170	1,344	373	293	186	37.4	31.8	35.3
A83WH - Abe	NF+0.06-1	0.000	0.004	0.019	0.032	0.054	0.108	0.123	0.159	0.186	1,296	365	295	186	37.4	32.5	35.6
A83WH - Arthur-71	NF+0.06-1	0.000	0.004	0.019	0.032	0.054	0.108	0.123	0.159	0.186	1,296	365	295	186	37.4	32.5	35.6
BTI82WH - VONA	NF-1	0.000	0.011	0.025	0.034	0.042	0.057	0.064	0.072	0.098	1,464	114	2	0	7.6	0.2	6.2
BTI83WH - VONA	NF-1	0.000	0.006	0.021	0.036	0.049	0.071	0.083	0.097	0.116	864	165	51	4	12.4	4.7	9.8

Table 5-14 (cont'd). Summary of Ozone Exposures That Are Closest to Those Predicted for 20% Yield Reduction per SUM06 Exposure Response Models Used by Lee et al. (1991) in Selected National Crop Loss Assessment Network Experiments[a] (Concentrations are in parts per million.)

Experiment[b]	Chamber	Min.	Percentiles 10	30	50	70	90	95	99	Max.	Number of Obs.	Number of Occurrences ≥0.06	≥0.08	≥0.10	SUM 06 (ppm-h)	SUM 08 (ppm-h)	W126 (ppm-h)
CORN																	
A81MA - PAG 397	NF+0.06-2	0.000	0.000	0.008	0.020	0.052	0.111	0.126	0.150	0.187	1,968	552	461	306	57.5	51.0	55.1
A81MA - Pioneer	NF+0.06-2	0.000	0.000	0.008	0.020	0.052	0.111	0.126	0.150	0.187	1,968	552	461	306	57.5	51.0	55.1
COTTON																	
R82CO - Stoneville	NF-1	0.000	0.003	0.018	0.029	0.044	0.065	0.074	0.087	0.152	2,856	390	64	7	28.2	5.8	22.7
R85CO - McNair Dry	NF×1.99-1D	0.000	0.003	0.012	0.024	0.052	0.117	0.154	0.221	0.291	3,000	810	609	407	92.9	78.9	88.2
R85CO - McNair Wet	NF×1.33-1W	0.000	0.003	0.012	0.024	0.041	0.073	0.091	0.129	0.166	3,000	487	226	118	41.4	23.5	35.9
PEANUT																	
R80PN - NC-6	NF+0.015-1	0.000	0.004	0.017	0.029	0.043	0.066	0.076	0.091	0.112	2,688	369	101	5	27.2	8.8	22.0
TOBACCO																	
R83TO - McNair 944	NF+0.020-1	0.000	0.003	0.018	0.037	0.061	0.089	0.104	0.121	0.155	1,968	611	288	117	50.7	28.4	42.6

[a]See Appendix A for abbreviations and acronyms.
[b]Separate analyses were performed for each water stress level, dry (D) and well-watered (W).

Table 5-15. Summary of Percentiles for Ozone Monitoring Sites in 1989 (April through October) with a Maximum Three-Month SUM06 Value <24.4 ppm-h but with a Second Hourly Maximum Concentration ≥0.125 ppm

AIRS Site	Name	Min.	10	30	50	70	90	95	99	Max	Maximum Uncorrected SUM06 (ppm-h)	Number of Observ. Over 7-mo Period
						Percentiles						
060010003	Livermore, CA	0.000	0.000	0.010	0.030	0.040	0.050	0.060	0.090	0.140	17.0	5,067
060371301	Lynwood, CA	0.000	0.000	0.010	0.020	0.030	0.050	0.070	0.100	0.140	18.1	4,793
060374002	Long Beach, CA	0.000	0.010	0.020	0.020	0.030	0.050	0.060	0.080	0.160	13.6	4,876
060375001	Hawthorne, CA	0.000	0.000	0.020	0.030	0.040	0.060	0.060	0.080	0.190	18.1	4,894
060830008	Santa Barbara, CA	0.000	0.010	0.020	0.030	0.040	0.050	0.060	0.080	0.190	17.1	4,823
060830010	Santa Barbara, CA	0.000	0.010	0.020	0.030	0.040	0.050	0.060	0.080	0.220	13.3	4,663
060833001	Santa Barbara County, CA	0.000	0.010	0.020	0.030	0.040	0.050	0.060	0.080	0.140	12.3	5,077
090010113	Bridgeport, CT	0.000	0.002	0.011	0.022	0.033	0.048	0.059	0.091	0.156	16.5	4,865
090091123	New Haven, CT	0.000	0.003	0.010	0.019	0.029	0.045	0.056	0.091	0.156	12.9	4,502
220191003	Westlake, LA	0.000	0.003	0.013	0.022	0.033	0.052	0.061	0.082	0.137	12.2	4,811
220330003	Baton Rouge, LA	0.000	0.001	0.009	0.021	0.034	0.059	0.069	0.094	0.168	17.4	4,964
220330004	Baton Rouge, LA	0.000	0.002	0.008	0.016	0.028	0.047	0.057	0.078	0.138	8.4	4,791
220331001	East Baton Rouge, LA	0.000	0.003	0.012	0.022	0.034	0.056	0.066	0.092	0.171	14.4	4,890
220470002	Iberville Parish, LA	0.000	0.005	0.014	0.023	0.034	0.057	0.068	0.093	0.149	15.9	5,040
220770001	New Roads, LA	0.000	0.001	0.011	0.021	0.033	0.052	0.062	0.083	0.141	12.0	4,964
230052003	Cape Elizabeth, ME	0.001	0.017	0.027	0.034	0.042	0.055	0.064	0.093	0.146	16.7	4,627
471630009	Kingsport, TN	0.001	0.001	0.005	0.017	0.032	0.054	0.062	0.078	0.125	13.4	4,252
481410027	El Paso, TX	0.000	0.010	0.020	0.030	0.040	0.050	0.060	0.080	0.260	14.9	4,484
481990002	Kountze, TX	0.000	0.000	0.010	0.020	0.030	0.050	0.060	0.080	0.130	10.6	4,630
482010024	Harris County, TX	0.000	0.000	0.010	0.020	0.030	0.060	0.070	0.110	0.230	19.2	4,728
482010062	Houston, TX	0.000	0.000	0.010	0.020	0.030	0.050	0.070	0.110	0.170	16.8	4,600
482011034	Houston, TX	0.000	0.000	0.010	0.010	0.030	0.050	0.060	0.100	0.220	14.0	4,595
482011037	Houston, TX	0.000	0.000	0.010	0.010	0.030	0.050	0.060	0.110	0.250	16.3	4,729
490350003	Salt Lake County, UT	0.000	0.001	0.008	0.029	0.042	0.056	0.062	0.083	0.125	17.4	4,585
490353001	Salt Lake City, UT	0.000	0.002	0.014	0.029	0.041	0.053	0.061	0.079	0.140	13.0	4,544

Table 5-16. Summary of Percentiles for Ozone Monitoring Sites in 1989 (April Through October) with a Maximum Three-Month SUM06 Value ≥ 24.4 ppm-h but with a Second Hourly Maximum Concentration <0.125 ppm

AIRS Site	Name	Min.	10	30	50	70	90	95	99	Max	Maximum Uncorrected SUM06 (ppm-h)	Number of Observ. Over 7-mo Period
						Percentiles						
040132004	Scottsdale, AZ	0.000	0.006	0.018	0.031	0.045	0.062	0.071	0.084	0.107	31.7	5,070
060070002	Chico, CA	0.000	0.010	0.020	0.030	0.040	0.060	0.070	0.080	0.100	33.5	4,690
060170009	South Lake Tahoe, CA	0.000	0.020	0.030	0.040	0.050	0.060	0.070	0.080	0.100	44.8	4,768
060430004	Yosemite National Park, CA	0.000	0.008	0.022	0.035	0.049	0.065	0.072	0.083	0.111	37.6	4,853
060710006	San Bernardino County, CA	0.000	0.020	0.040	0.050	0.060	0.070	0.080	0.090	0.100	70.5	4,856
061011002	Yuba City, CA	0.000	0.000	0.020	0.030	0.040	0.060	0.070	0.080	0.100	29.0	4,623
120094001	Cocoa Beach, FL	0.002	0.017	0.024	0.032	0.042	0.059	0.068	0.077	0.094	28.7	5,012
170190004	Champaign, IL	0.000	0.008	0.020	0.029	0.039	0.065	0.072	0.078	0.088	32.0	5,091
170491001	Effingham County, IL	0.000	0.009	0.023	0.036	0.046	0.063	0.070	0.081	0.104	25.3	4,600
180970042	Indianapolis, IN	0.001	0.006	0.021	0.034	0.046	0.063	0.072	0.085	0.103	25.4	4,592
240030014	Anne Arundel, MD	0.000	0.006	0.021	0.032	0.045	0.064	0.073	0.090	0.120	25.5	4,360
240053001	Essex, MD	0.000	0.002	0.010	0.024	0.038	0.059	0.069	0.089	0.121	25.2	5,028
310550032	Omaha, NE	0.002	0.021	0.030	0.037	0.047	0.062	0.067	0.075	0.098	24.9	4,160
350431001	Sandoval County, NM	0.000	0.010	0.020	0.030	0.040	0.060	0.060	0.070	0.090	25.1	5,059
360310002	Essex County, NY	0.016	0.033	0.042	0.050	0.056	0.067	0.073	0.086	0.106	45.6	4,070
370270003	Lenoir, NC	0.000	0.007	0.019	0.032	0.045	0.062	0.067	0.078	0.092	25.8	4,806
370810011	Guilford County, NC	0.004	0.010	0.023	0.034	0.046	0.063	0.070	0.083	0.113	27.7	4,853
371470099	Farmville, NC	0.000	0.010	0.023	0.034	0.044	0.062	0.070	0.083	0.100	26.4	4,833
390030002	Allen County, OH	0.000	0.007	0.022	0.032	0.043	0.060	0.068	0.086	0.107	24.5	4,854
391510016	Canton, OH	0.000	0.008	0.019	0.030	0.042	0.060	0.070	0.088	0.110	26.3	4,875
420070003	New Brighton, PA	0.000	0.008	0.021	0.032	0.043	0.062	0.070	0.087	0.102	29.4	5,055
420770004	Allentown, PA	0.000	0.003	0.016	0.028	0.039	0.060	0.070	0.087	0.102	25.1	5,040
470090101	Smoky Mountain National Park, TN	0.000	0.025	0.036	0.044	0.053	0.065	0.070	0.081	0.098	35.9	4,764
510130020	Arlington County, VA	0.000	0.001	0.010	0.023	0.037	0.059	0.071	0.088	0.116	25.7	5,029
510610002	Fauquier County, VA	0.000	0.009	0.021	0.033	0.045	0.061	0.069	0.084	0.122	24.6	5,050
511870002	Shenandoah National Park (Dickey Ridge), VA	0.004	0.027	0.037	0.045	0.054	0.065	0.071	0.082	0.100	59.0	4,454
550270001	Horicon, WI	0.002	0.019	0.029	0.037	0.047	0.062	0.070	0.088	0.111	24.6	4,142
551390007	Oshkosh, WI	0.002	0.016	0.028	0.038	0.048	0.063	0.070	0.084	0.121	27.9	4,206

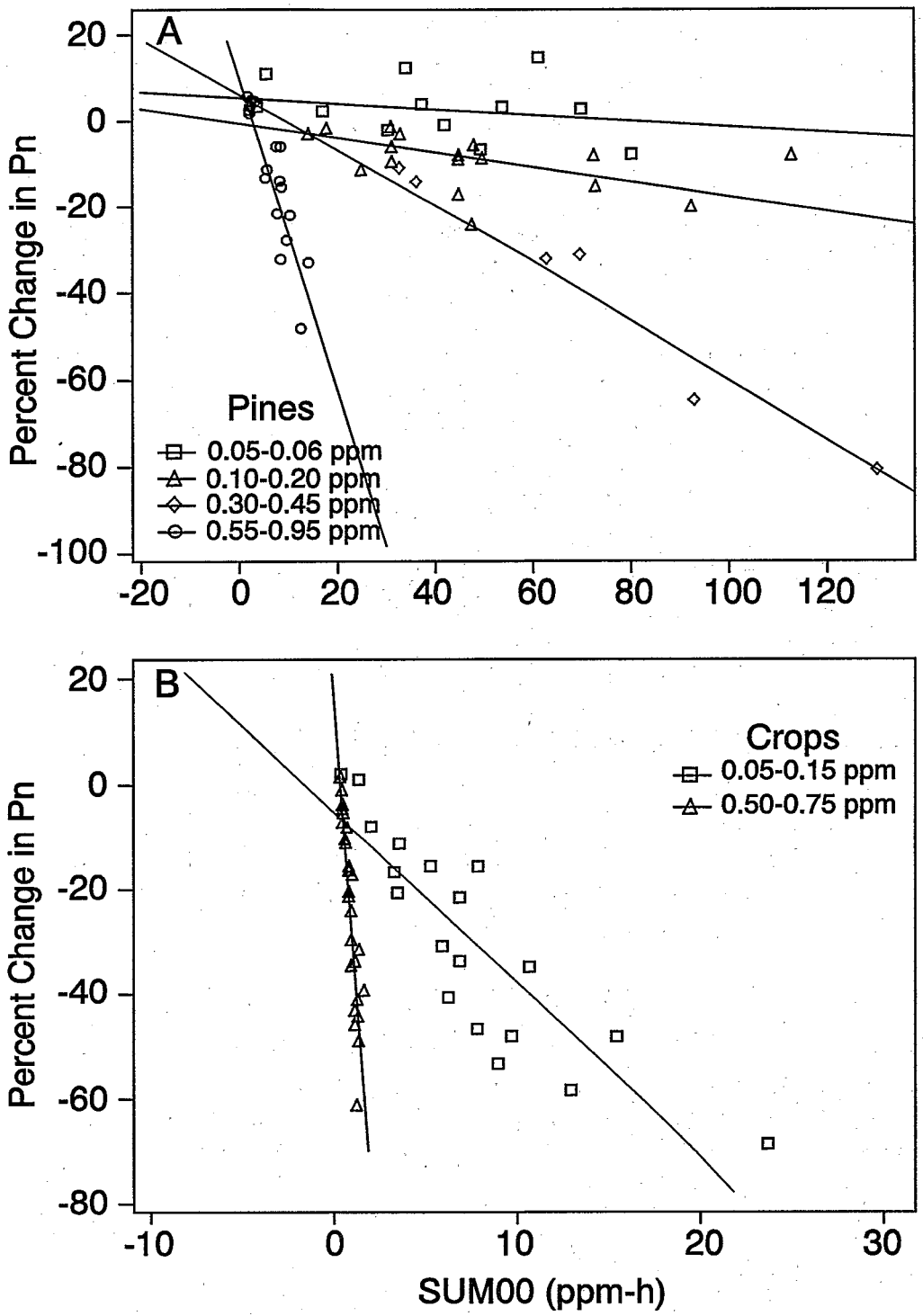

Figure 5-15. ***Percent reduction in net photosynthesis (Pn) of (A) pines (including one point for red spruce) and (B) agricultural crops in relation to total ozone exposure (SUM00), for several ranges of peak concentrations (Reich, 1987).***

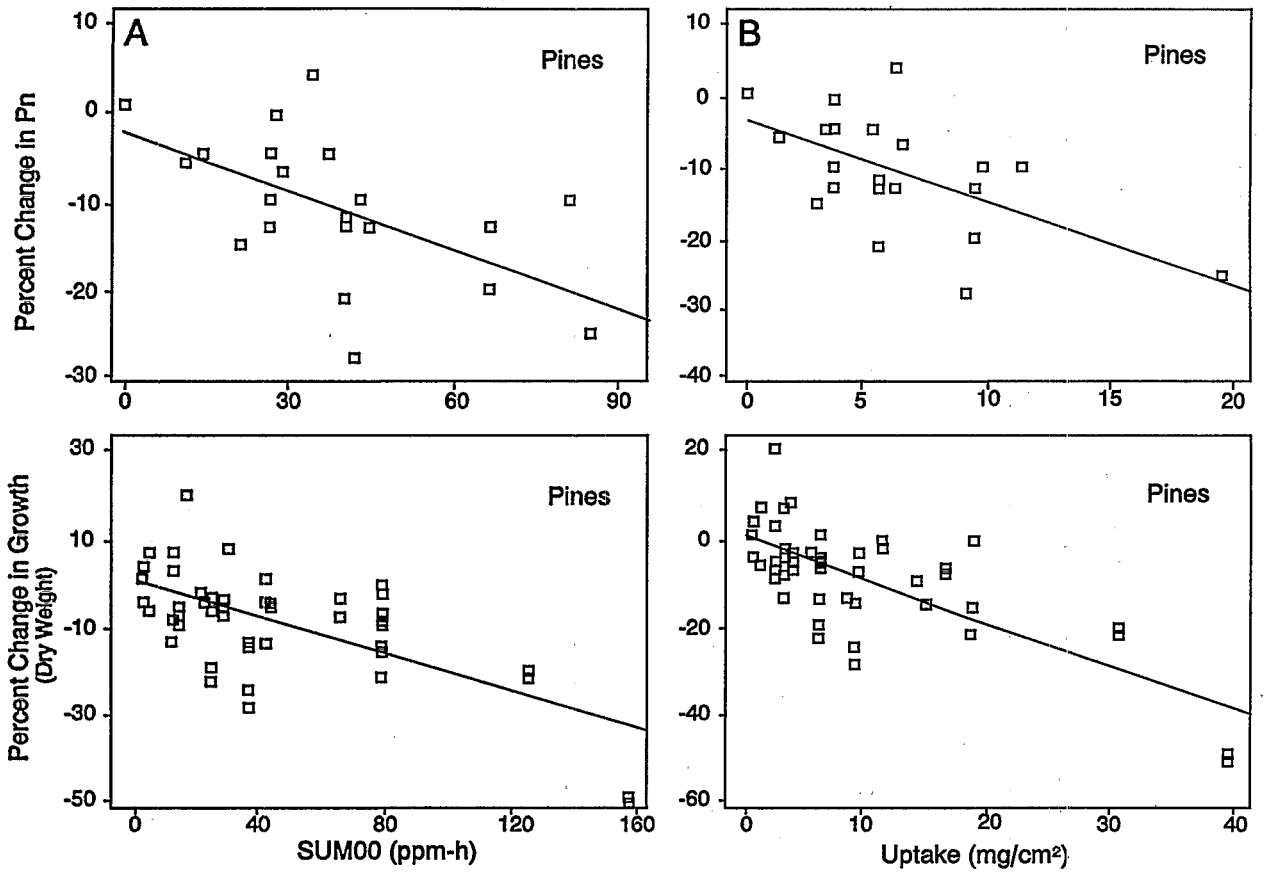

Figure 5-16. Percent reduction in net photosynthesis (Pn) and biomass growth of coniferous species in relation to (A) total exposure (SUM00) and (B) estimated total ozone uptake (Reich, 1987).

in O_3 uptake. Imputed O_3 uptake calculated as the product of SUM00 and mean diffusive conductance (ks) for each species better correlated with Pn and growth than did SUM00.

Kickert and Krupa (1991) criticized Reich's (1987) findings on the basis of insufficient reporting of statistical model parameters, possible nonnormality of Pn and growth variables, exclusion of ks terms for imputing O_3 uptake for each species, and the absence of implication for any individual plant species. However, Reich's synthesis of Pn and growth, using the SUM00 index, would not necessarily be invalidated by nonnormality of the variables. Reich's use of a mean diffusive conductance to impute O_3 uptake is questionable because leaf diffusive conductance measurements vary with time of day, season, and environmental condition. In addition, the timing of an O_3 exposure and stomatal conductance is of utmost importance because they determine whether a plant will respond to O_3 exposure or not. Consequently, numerous measurements of conductance are required to weight hourly O_3 concentrations to calculate O_3 uptake over the growth of a plant.

Pye (1988) reviewed 15 studies on 26 seedling species and found reductions in biomass response increased with SUM00 (Figure 5-19). Seasonal sum of hourly

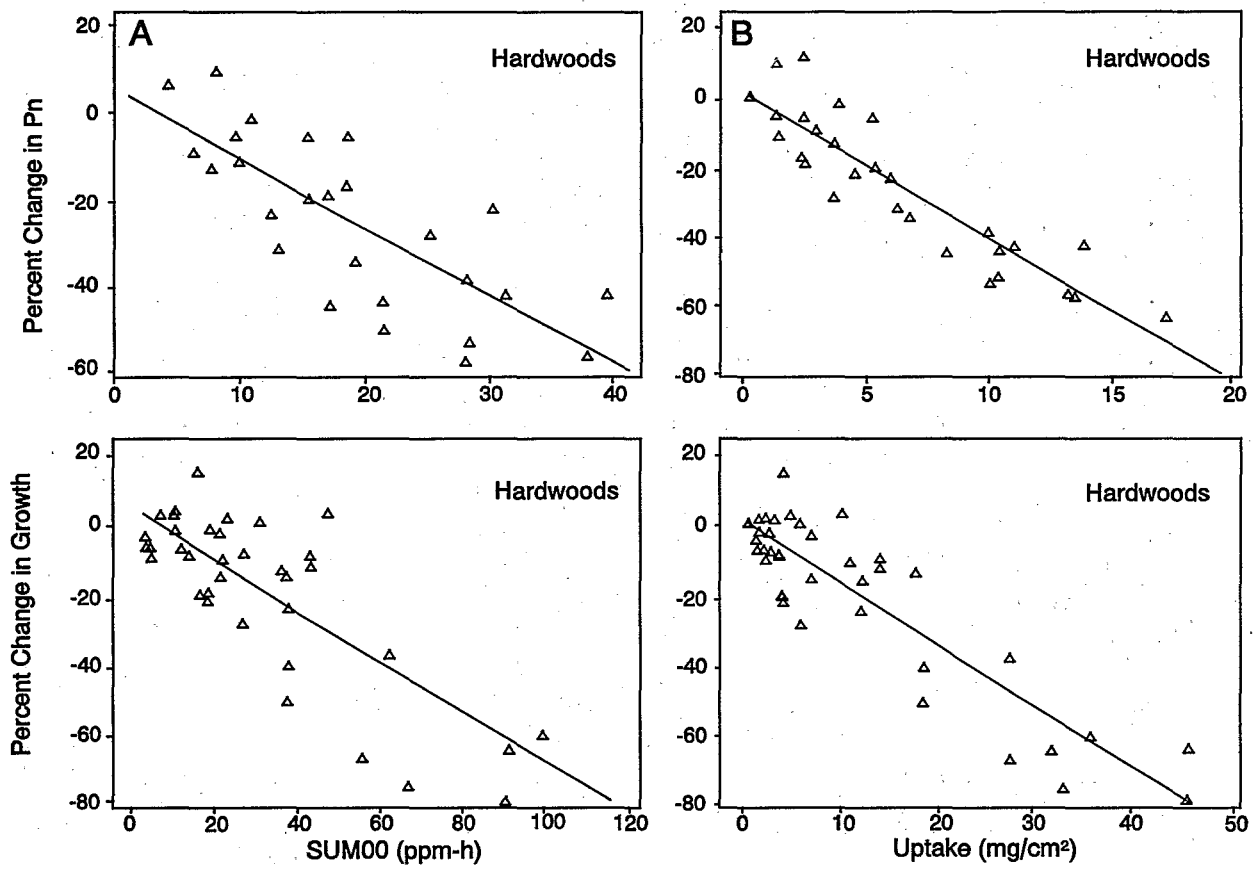

Figure 5-17. Percent reduction in net photosynthesis (Pn) and biomass growth of hardwood species in relation to (A) total exposure (SUM00) and (B) estimated total ozone uptake (Reich, 1987).

concentrations values ranged from 4 to 297 ppm-h. However, there was substantial variation in response. Pines, poplars, sycamore (*Platanus occidentalis*), ash, and maple (*Acer saccharum*) are all relatively sensitive. Both concentration and duration are important factors governing impact on growth and photosynthesis, but they probably are not equally important. The biomass data suggest a nonlinear response to fumigation, and the presence of convexity of response implies that for similar mean O_3 exposures, damage will be greater when O_3 concentrations are more variable.

There is limited information for assessing the relative performance of exposure indices for relating to vegetation effects. Lefohn et al. (1992a) reported that it was not possible to differentiate among the SUM00, SUM06, SUM08, and W126 exposure indices because the indices were highly correlated with one another in the experiment (Figure 5-20). However, results based on biological experiments, reported by Musselman et al. (1983, 1994) and Hogsett et al. (1985b) have shown that different exposure regimes with similar SUM00 values resulted in those exposures experiencing peak concentrations exhibiting the greater effects. The authors demonstrated that plants exposed to variable O_3 concentrations

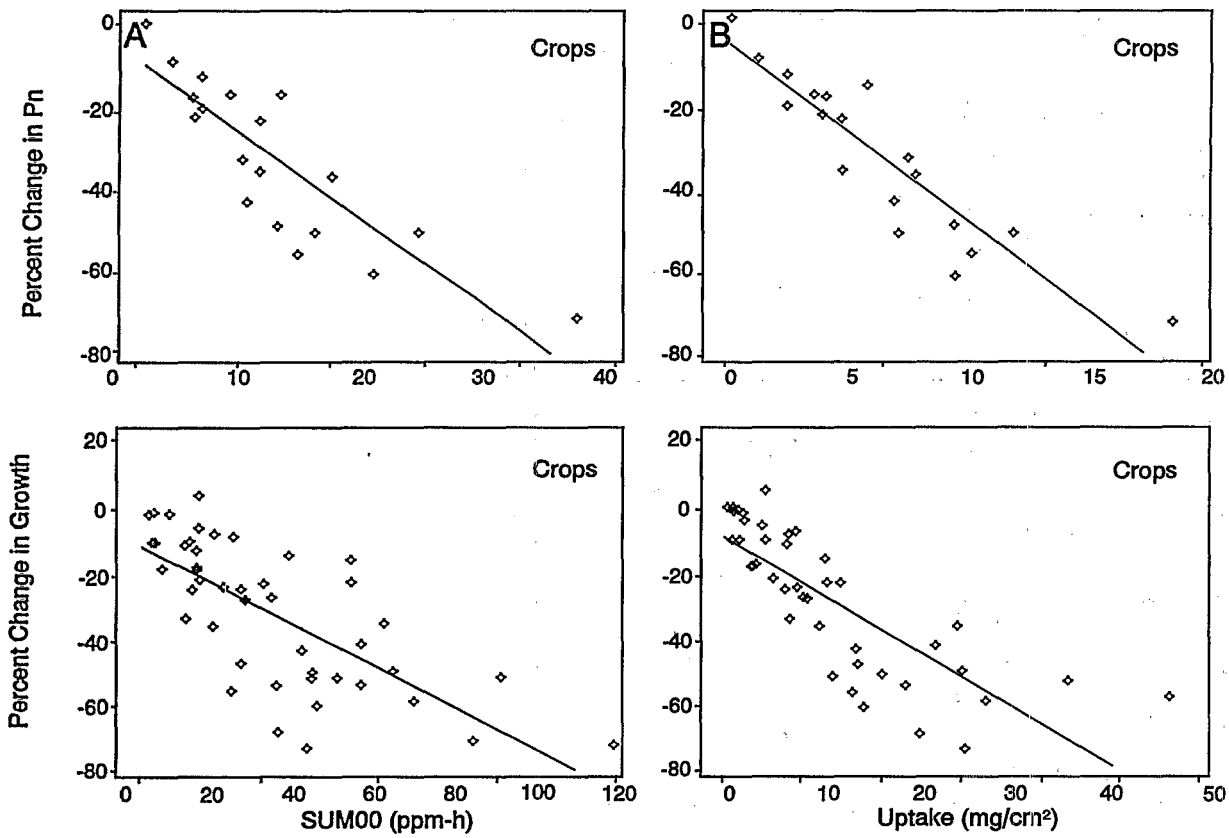

Figure 5-18. Percent reduction in net photosynthesis (Pn) and biomass growth of agricultural crops in relation to (A) total exposure (SUM00) and (B) estimated total ozone uptake (Reich, 1987).

in chambers showed greater effect on plant growth than did those exposed to a fixed or daily peak concentration of equal SUM00, but with lower peak concentrations.

Building on the above cited results of chamber studies that indicated a greater biological response to the higher hourly average concentrations, Lefohn et al. (1989) concluded that the SUM00 index did not appear to perform adequately. Using air quality data, Lefohn et al. (1989) showed that the magnitude of the SUM00 exposure index was largely determined by the lower hourly average concentrations (Figure 5-21). Figure 5-21 illustrates that the slope of the curve that described the cumulative frequency for the SUM00 index (referred to as TOTDOSE) was greater than the slope of the curve for the W126 index until approximately 0.06 ppm; thereafter, the reverse was true. This occurred because the W126 index weighted the higher concentrations more heavily than the lower ones, whereas the TOTDOSE index did not.

Supplementing the results in Lefohn et al. (1989), Lefohn et al. (1992a), using loblolly pine data exposed at Auburn, AL, to varying levels of O_3 over 555 days (Lefohn et al., 1992a) reported that the magnitude of the SUM00 values in the CF chamber, although

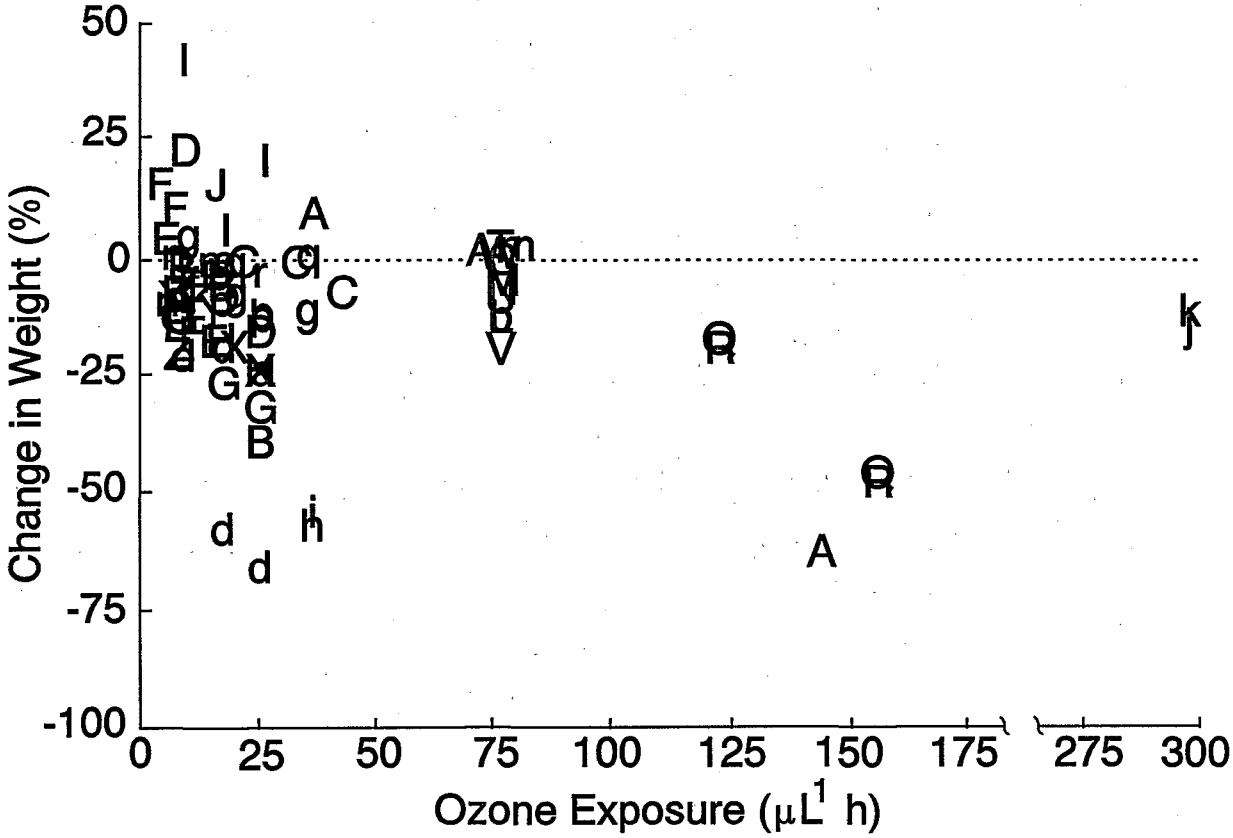

Figure 5-19. Percent reduction in biomass growth of tree seedlings in relation to total exposure (Pye, 1988).

experiencing hourly average values greater than those at the South Pole or Pt. Barrow, AK, was about 50% less than the SUM00 values experienced at the South Pole and Pt. Barrow.

In a similar analysis using ambient data, Lefohn et al. (1992a) identified a separate set of ambient sites that experienced SUM00 values similar to those of the ambient treatments at Auburn; these ambient sites experienced fewer hourly concentrations above 0.07 ppm than did the ambient chambers. Similar to the results cited above, the authors noted that the magnitude of the SUM00 index was unable to capture the occurrence of the higher hourly average concentrations in the ambient treatments. The authors indicated that the SUM00 index was inadequate because of the observed inconsistencies of the SUM00 value between chambers and selected monitoring sites.

When taken by themselves, the importance of these findings may be debatable because the clean sites are not representative of loblolly growing regions, and there is no substantiating evidence of differing effects at these levels. However, the coupling of the air quality considerations, as described by Lefohn et al. (1989, 1992a), with the biological findings reported by Musselman et al. (1983, 1994) and Hogsett et al. (1985b), builds a consistent picture that the SUM00 index does not describe properly the occurrence of the higher hourly average concentrations.

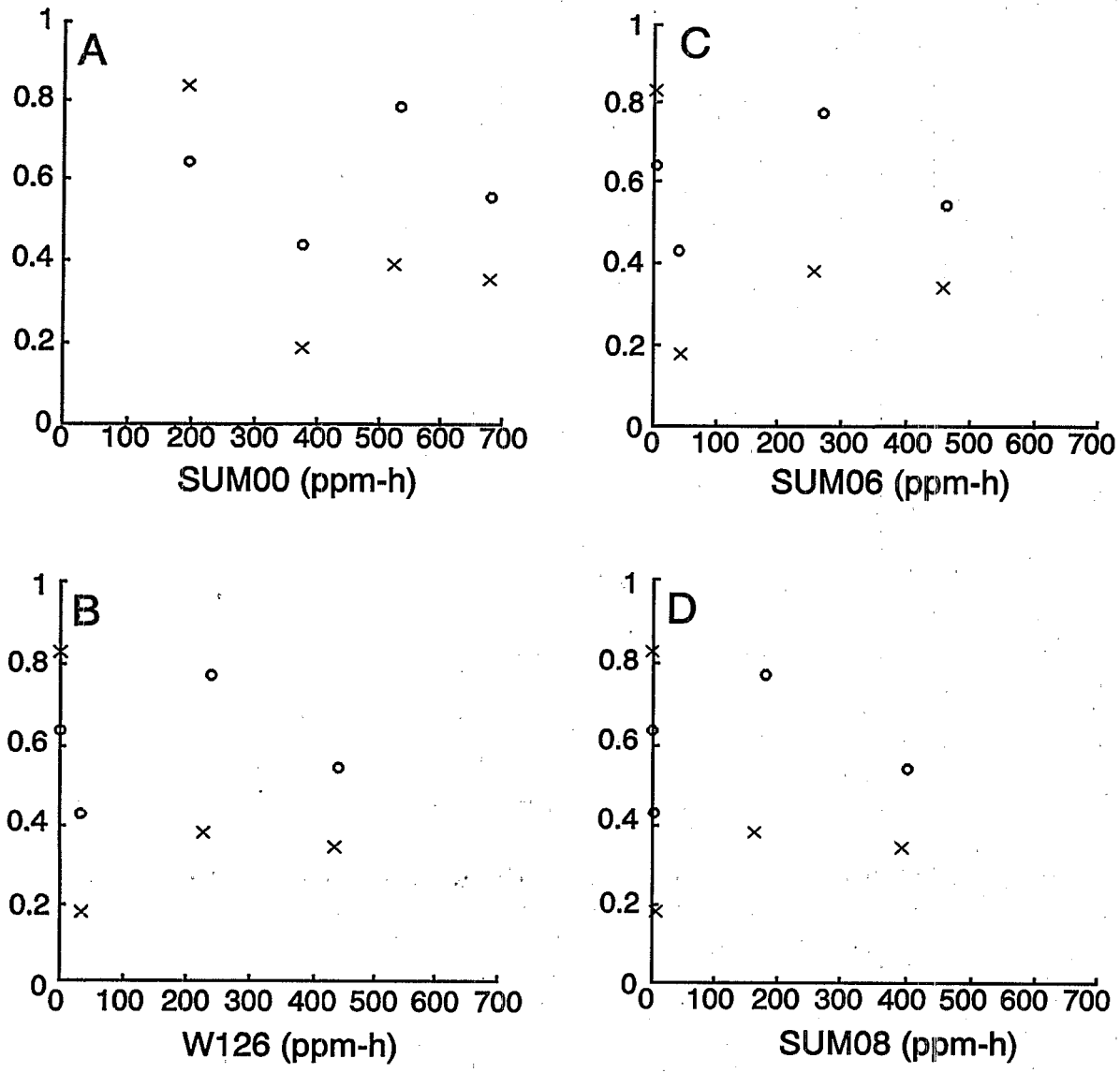

Figure 5-20. Reduction in volume production of loblolly pine seedlings (family 91) in relation to four exposure indices (A through D) (Lefohn et al., 1992a).

As noted earlier in this section (see also Section 5.4), the sensitivity of vegetation at time of exposure varies with species and is a function of several factors (e.g., soil moisture, light conditions, humidity, air turbulence). Assuming all factors are held constant (a condition not found in the ambient atmosphere), the results reported by Musselman et al. (1983, 1994), and Hogsett et al. (1985b), imply that, given any distribution of hourly average concentrations, higher hourly average concentrations should be given greater weight than lower hourly average concentrations. This statement provides only guidance concerning the potential of each hourly average concentration to affect one type of vegetation relative to

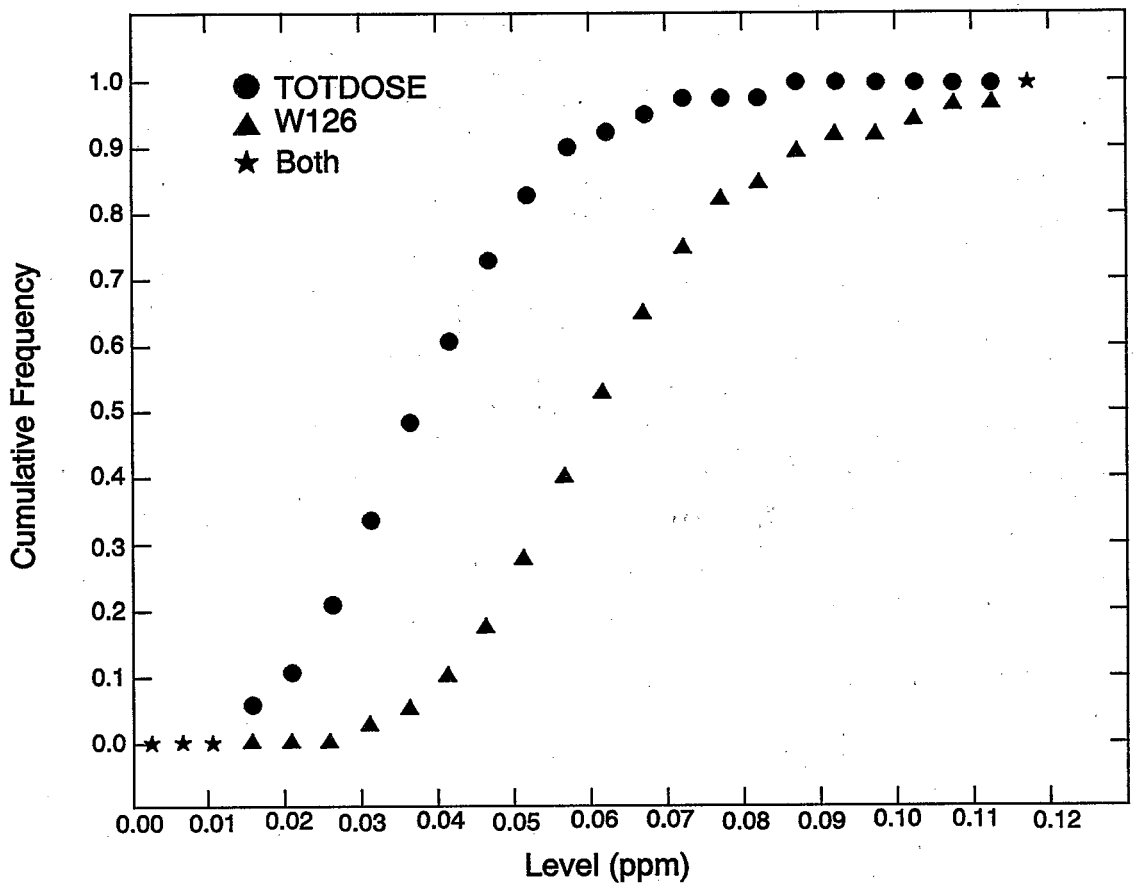

Figure 5-21. A comparison between the resulting cumulative frequencies for the exposure parameters, sum of all hourly average concentrations (SUM00) and the sigmoidally weighted integrated exposure index, W126. The ozone data were collected in 1981 at a site located in the Mark Twain National Forest, MO, EPA AIRS site number 291230001 (Lefohn et al., 1989).

another. This statement does not provide any insight concerning whether the magnitude of a SUM06 or a W126 value, calculated using monitoring data collected at a specific site over a specified time interval (i.e., months and hours of the day), is associated more with mid-level than high hourly average concentrations. The contribution of each range of hourly average concentrations to the magnitude of the cumulative index value is related to the distribution of the hourly average concentrations measured at the site.

5.5.2.5 Comparison of Effects on Vegetation of Cumulative "Peak" Versus "Mid-Level" Ozone Exposures

Not all studies dealing with the response of crop plants to O_3 exposures agree with the conclusions emphasized in the foregoing pages of this section that "higher" hourly concentrations should be given greater weight than "lower" concentrations. Based on their

studies, Tonneijck (1994), Krupa et al., (1993, 1994, 1995), Grünhage et al. (1993b), and Grünhage and Jäger (1994b) concluded that mid-level hourly average O_3 concentrations of 0.05 to 0.09 ppm are of greater importance than are higher hourly average concentrations in affecting vegetation.

It is clear from the studies over the years that the cumulative effects of exposure to all concentrations, peak and mid-range included, can play an important role in producing plant growth responses. The apparent difference in viewpoints is based on whether cumulative peak concentrations play a greater role in producing growth responses than do cumulative mid-range concentrations. As emphasized later, these views are based on experimental results that are not comparable. The studies that support the importance of peaks are chamber studies primarily using peak exposures, whereas the majority of the studies emphasizing that mid-range concentrations must be considered in plant response base their conclusions on both OTC and ambient field data. The key to plant response is timing because peak and mid-range concentrations do not occur at the same time. The greatest potential effect of O_3 on plants will occur when stomatal conductance is highest. If peaks do occur when stomatal conductance is greatest, the contribution of mid-range exposures will not be observable because they are masked. Associated with this is the importance of atmospheric conductivity (i.e., the O_3 concentration must reach the leaf surface if it is to be taken up by a plant).

Many studies over the years, depending on the duration of exposures and sensitivity of the plants have shown that injury to crops and other vegetation could occur when exposed to O_3 concentrations that ranged from 0.04 to either 0.4 or 0.5 ppm, with the higher concentration usually causing injury in the shortest period of time (Table 5-17; U.S. Environmental Protection Agency, 1978, 1986). This range encompasses both peaks and mid-range concentrations reported in the studies with the differing viewpoints cited above (Musselman et al., 1983, 1986b, 1994; Hogsett et al.,1985b; Tonneijck and Bugter, 1991; Tonneijck, 1994; Krupa et al., 1993, 1994, 1995; Grünhage et al., 1993b; Grünhage and Jäger, 1994b).

Unfortunately, the terms "high" and "low" concentrations and "peak" and "cumulative peak" concentrations are often used in publications (e.g., the majority of those cited above) without any explanation or the concentration being specified or, when specified, varying terminology has been applied with regard to what constitutes high concentrations or categories of lower values. For example, in an early paper discussing the development of vegetation effects exposure indices, Hogsett et al. (1988) termed 0.05 to 0.09 ppm as "mid-range", whereas >0.10 was considered as being "relatively high". In a recent paper, Krupa et al. (1995) term the concentrations of 0.05 to 0.09 as "moderately enhanced" and those >0.09 ppm as high. For consistency within this present review, concentrations ranging from 0.05 to 0.09 ppm are termed mid-range and those above 0.10 ppm as high or peaks.

When evaluating the results of the studies cited above, most attention has been focused on the concentrations used in the experiments (whether peaks or mid-range) by those espousing a particular viewpoint, whereas little mention has been accorded to duration of exposure, number of peaks during the exposure, whether or not there were peaks, and whether the experiments were conducted in chambers in the greenhouse, in the field, or in OTCs in the field. In the introduction to their paper, Musselman et al., (1983) describe the major problem plant scientists have encountered when attempting to relate exposures to plant responses in stating: "Pollutant dose, a quantitative description of pollutant exposure, has been defined as a product of concentration and exposure duration. The components of

Table 5-17. Ozone Concentrations for Short-Term Exposures That Produce 5 or 20% Injury to Vegetation Grown Under Sensitive Conditions[a]

	Ozone Concentrations That May Produce 5% (20%) Injury (ppm):		
Exposure time (h)	Sensitive Plants[b]	Intermediate Plants[c]	Less Sensitive Plants[d]
0.5	0.35 - 0.50 (0.45 - 0.60)	0.55 - 0.70 (0.65 - 0.85)	≥0.70 (0.85)
1.0	0.15 - 0.25 (0.20 - 0.35)	0.25 - 0.40 (0.35 - 0.55)	≥0.40 (0.55)
2.0	0.09 - 0.15 (0.13 - 0.25)	0.15 - 0.25 (0.25 - 0.35)	≥0.30 (0.40)
4.0	0.04 - 0.09 (0.10 - 0.15)	0.10 - 0.15 (0.15 - 0.30)	≥0.25 (0.35)
8.0	0.02 - 0.04 0.06 - 0.12	0.07 - 0.12 0.13 - 0.25	≥0.20 (0.30)

[a]The concentrations in parenthesis are for the 20% injury level.
[b]Examples of sensitive plants: oat, bean, and tobacco.
[c]Examples of intermediate plants: legumes, clover, and wheat.
[d]Examples of less sensitive plants: vegetables, woody plants, and cucumber.

Source: U.S. Environmental Protection Agency (1978, 1986).

pollutant dose are now recognized to be much more complex. Exposure concentration should consider distribution, peaks, and means, whereas exposure duration includes length of time exposed to zero concentration to indicate time intervals between exposures as well as the duration of individual exposures. Sequence and patterns of intermittent pollutant exposures also are involved when describing dose."

The papers on which the differing viewpoints are based represent attempts by the various scientists to address the problems noted in the preceding paragraph. When reading these papers, it soon becomes clear that each study is unique, some exposures were conducted in chambers in the greenhouse or in the field on plants growing in pots, and others were conducted in ambient air with plants grown in pots (See Table 5-18). None of the studies, even those in which the same scientists exposed the same plant species or cultivar, replicates a previous study. No two of the studies have exposed plants in the same manner or under similar conditions (Table 5-18). The O_3 concentrations, the duration, the conditions under which exposures were made, and the medium in which the plants were grown all vary. When similar exposure methods have been used, the exposures (concentration × duration [C × T]) and the plant species exposed have been different, and, when the same species or cultivar has been used, the exposure methods have been different, and plants were grown in a different medium. Therefore, the data presented in each paper were obtained under the particular set of circumstances applicable to that given study. Attempting to extrapolate the data from these studies to a broader scale causes many problems. Several of the authors of the above papers have recognized this fact (Musselman et al., 1983, 1986b, 1994; Tonneijck and Bugter, 1991; Krupa et al.,1993) and state that their studies have limited applicability,

Table 5-18. A Summary of Studies Reporting Effects of Peaks or Mid-Range Concentrations[a]

Species	Concentration (ppm)	Exposure Pattern	Exposure Duration	Methodology	Response	Reference
Kidney Bean cv. California Dark Red *Phaseolus vulgaris* L.	0.28 0.2 0.1-0.5 0.14-0.7	UH[b] UL[c] Simulated ambient: diurnal, variable diurnal, variable	One 6-h (0915-1515 h) exposure/week 1/3 plants: at 6 weeks; 1/3 plants: at 6 and 7 weeks; 1/3 at 6, 7, and 8 weeks plants harvested at end of exposure period	8 CSTR, in pots in soil	Greatest injury at 6 and 7 weeks; senescence at 8 weeks	Musselman et al. (1983)
Kidney Bean cv. California Dark Red *Phaseolus vulgaris* L.	0.3 0.4 0.06-0.3 0.08-0.4	UL "square wave" UH "square wave" Ambient, variable Ambient, variable	One 2-h (1051-1309 h) exposure/week for 6, 7, or 8 weeks One 6-h (900-1500) exposure/week 1/3 plants: at 6 weeks; 1/3 plants: at 6 and 7 weeks; 1/3 at 6, 7, and 8 weeks plants harvested at end of exposure period	8 negative pressure chambers, in pots in soil	Square wave vs. ambient: no difference in response if total dose equivalent	Mussleman et al. (1986b)
Kidney Bean cv. California Dark Red *Phaseolus vulgaris* L.	1. 0.12 2. 0.36 peak, max 1-h avg = 0.28 3. 0.24 4. 0.24 1-h peak	Uniform Narrow-based triangle Broad based pyramid Trapezoid	7 weeks 3 days/week 5 h daily	CSTR, 15 plants per chamber	Least injury: profiles 2 and 4 Greatest injury: 3 > 1 but less than 2 and 4	Musselman et al. (1994)
Alfalfa, *Medicago sativa* L.	Daily 7-h mean: 0.063, 0.064, 0.083, 0.084, peaks ≈ 0.2 7-h mean 0.074, 0.094, 0.099, peaks ≈ 0.10-0.15	Daily for 30 days: low episodic, high episodic, peaks at 1400-1500 h 30 days: low daily peak, high daily peak, peaks at 1400 h	0900-1600 h; 30 days × 5	8 OTC, in pots; alfalfa cut 3× during exposure period	Growth reduced more for alfalfa under episodic exposures Growth reduced less than with episodic exposures	Hogsett et al. (1985b)

Table 5-18 (cont'd). A Summary of Studies Reporting Effects of Peaks or Mid-Range Concentrations[a]

Species	Concentration (ppm)	Exposure Pattern	Exposure Duration	Methodology	Response	Reference
Tobacco cv. Bel W3 *Nicotiana tobacum* L.	Yearly mean range 1979-88: 0.025-0045	Ambient, daily not given	1 week	4 pots in soil in field: 17 locations	Foliar injury	Tonneijck and Bugter (1991)
	Weekly mean range 1988: 0.01-0.055	Ambient, daily not given	1 week	4 pots in soil in field: 17 locations	Foliar injury	
Tobacco cv. Bel W3, *Nicotiana tobacum* L.	Years, 1979-1983 0.005-0.15, combined in classes of 10 $\mu g/m^3$	Ambient, daily not given	1 week	4 pots in soil in field: 40 locations	Foliar injury	Tonneijck (1994)
Bean, *Phaseolus vulgaris* L. cv. Stratego cv. Groffy	Years 1982-1983 0.015-0.075, combined in classes of $\mu g/m^3$	Ambient, daily not given	1 week	4 pots in soil in field: 10 locations	Foliar injury on Stratego	
Tobacco cv. Bel W3 Bel B *Nicotiana tobacum* L.	0.06-0.100	Montague weekly max	1 week	OTC (CF); OTC (NF); ambient	Foliar injury on bottommost expanded leaf	Krupa et al. (1993)
	0.06-0.103	Mt. Equinox weekly max	1 week	6 plants in pots in peat and Perlite		
				6 plants in pots in peat and Perlite		

[a]See Appendix A for abbreviations and acronyms.
[b]UH = Uniform high.
[c]UL = Uniform low.

and that caution should be used in applying their results on a broader scale. Had this advice been adhered to, then many apparent discrepancies in conclusions across the papers would likely not have arisen.

Musselman et al. (1983) exposed bean plants (*Phaseolus vulgaris* cv. California Red Kidney) grown in pots in soil in CSTR chambers in a greenhouse with CF air to simulated ambient O_3 concentration distributions specific for their region (Riverside, CA), as well as to two uniform concentration levels (Table 5-18). Plants were exposed to a 6-h O_3 fumigation from 0915 to 1515 Pacific Standard Time (PST) at 6, 7, and 8 weeks of age. The four exposure regimes were (1) uniform high, 0.28 ppm; (2) uniform low, 0.2 ppm; (3) variable low concentrations ranging from 0.1 to 0.5 ppm that simulated ambient exposures distributions (i.e., O_3 concentrations increased during the morning, peaked in the afternoon, and then decreased in the evening); and (4) variable high exposures ranging from 0.14 to 0.71 ppm that also simulated ambient concentration distributions (Table 5-18; Figure 5-22). Six days after each of the three fumigations, one-third of the plants were measured for leaflet oxidant stipple and destructively analyzed for leaf area and dry weight of plant parts. Therefore, one-third of the plants received one fumigation, the second third received two fumigations, and the remaining third received three fumigations at 6, 7, and 8 weeks of age. Simulated ambient O_3 distribution treatment produced significantly greater leaf injury and reduced growth and yield response than the uniform low or high exposure patterns. In addition, the simulated Riverside ambient O_3 concentration distribution reduced the total dry weight at both the 6- and 7-week fumigations; both pod and seed weights were reduced. The reduction in dry weights of pods resulted after the first fumigation at 6 weeks and did not change with subsequent fumigations. At 8 weeks, plants had begun to senesce. In this experiment, levels of concentration ranged from the lowest, 0.1 ppm, to the highest, 0.5 ppm. No exposure concentration, therefore, was below the "peak" level. Musselman et al. (1983) pointed out that the simulated ambient pollutant distribution used in their studies was specific for their geographic region. They also suggested that other studies determining the responses of additional species at different developmental stages to ambient O_3 distributions typical of other regions of the country were needed to put their findings in perspective.

Exposures in the Musselman et al. (1986b) study were designed to compare plant response to simulated ambient and uniform O_3 concentration distributions at two equivalent dose levels under controlled conditions (Table 5-18; Figure 5-23). Plants were fumigated in eight negative pressure chambers located within the greenhouse and received either one ambient or one uniform O_3 treatment during Week 6, during Weeks 6 and 7, or during Weeks 6, 7, and 8. Therefore, as in the previous study, one-third received one fumigation, the second third received two fumigations, and the other third received three fumigations. Plants were harvested 6 days after their last fumigation (Musselman et al., 1986b).

The uniform distribution in the above study was selected so that the constant concentration matched the total dose and peak concentration of the ambient distribution. Matching the peak concentration and the total dose required that plants exposed to the uniform distribution be exposed to the peak concentration (either 0.3 or 0.4 ppm) during the entire fumigation period, whereas plants in the ambient distribution were exposed to the same peak for only half an hour. The O_3 concentrations during the ambient exposure distribution had a fluctuating rising and falling pattern and were of longer duration overall, and the time of the peak exposure was shorter when compared with the uniform O_3 concentration

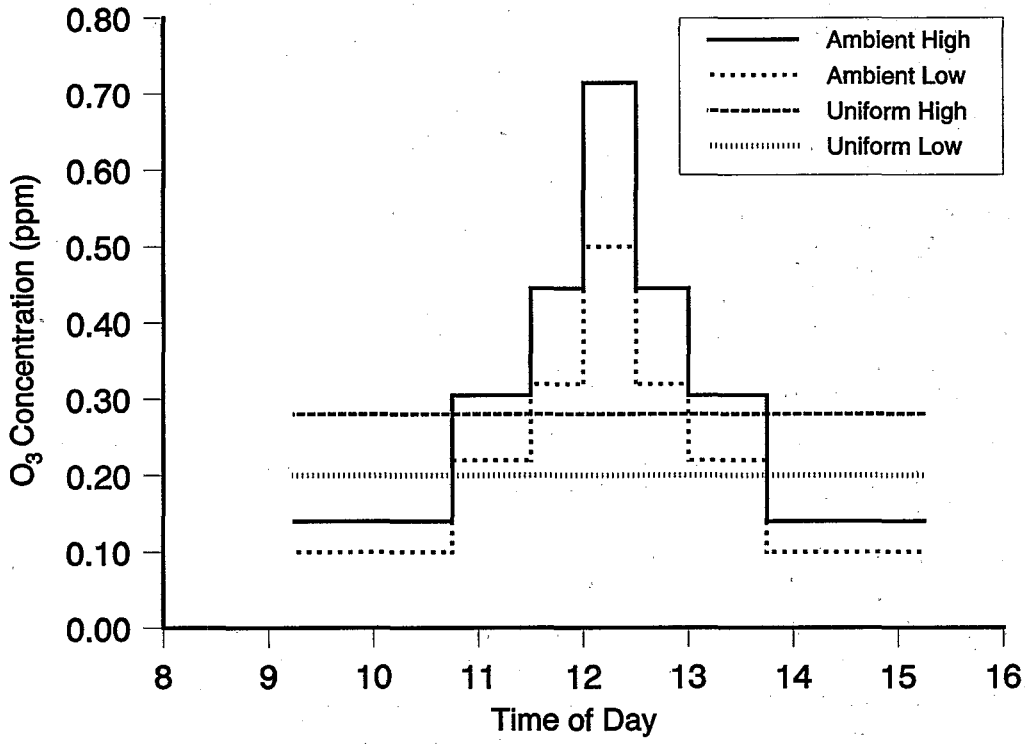

Figure 5-22. Fumigation schedule of uniform and simulated ambient ozone (O_3) concentration distributions at two equivalent dose levels.

Source: Musselman et al. (1983).

treatment. Total exposure time for the uniform distribution was 2 h and 18 min, and, for the ambient distribution, it was 6 h (Figure 5-23). Simulated ambient O_3 concentrations for the low dose ranged from 0.058 to 0.30 ppm, and for high dose, from 0.077 to 0.40 ppm.

The authors point out that ambient air quality data are generally reported as hourly average concentrations, and the dynamics of changes in O_3 concentrations during the hour are not considered in the summaries of air quality data, although these have been considered important in plant response. They also state that the results of this experiment demonstrate that, when peak O_3 concentrations and total dose are equivalent, the shape of the O_3 distribution (normal versus square wave) had no effect on the magnitude of response. Beans responded similarly to both an ambient and a uniform O_3 concentration distribution. No significant difference in injury, growth, or yield was observed. The authors conclude with the statement that "Further research is needed to examine whether peak concentration is the most important component of the concentration distribution causing plant response" (Musselman et al., 1986b).

In a further attempt to determine the response of plants to different exposure profiles but equal total exposures (C $\times$ T), Musselman et al. (1994) exposed the same bean cultivar, California Red Kidney, grown as in the previous studies, in CSTRs in a CF greenhouse to four different profiles having the same total cumulative exposure and the same

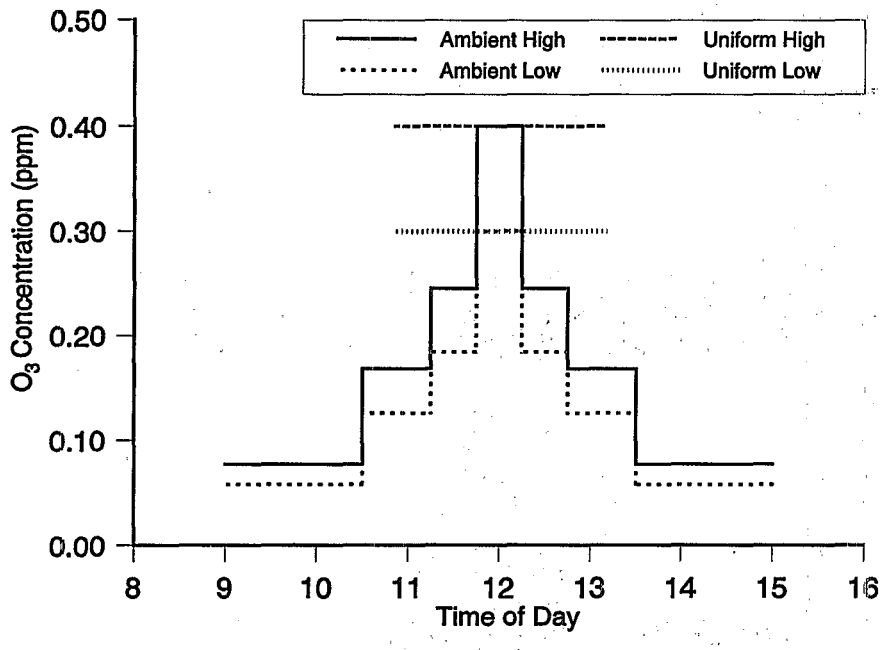

Figure 5-23. Fumigation schedule of uniform and simulated ambient ozone (O_3) concentration distributions at two dose levels.

Source: Musselman et al. (1986b).

7-, 12-, and 24-h seasonal means (Table 5-18; Figure 5-24). Ozone exposures began 21 days after germination. Plants were exposed for approximately 5 h, three times a week over the seven-week growing season. The first profile used was a "square-wave" concentration of 0.12 ppm; the second exposure resembled a narrow-based triangle, during which the O_3 concentrations rose rapidly to a peak of 0.36 ppm with a maximum 1-h average of 0.28 ppm and then dropped off rapidly; the third profile was in the shape of a broad-based pyramid, during which the O_3 concentration rose slowly to a peak of 0.24 ppm and then slowly dropped off; the fourth profile rose rapidly to a plateau with a peak of 0.24 ppm that lasted for 1 h and then dropped off slowly. The maximum 1-h average concentrations of 0.22 ppm for Profiles 3 and 4 simulated the more typical summer patterns for Southern California, where hourly peaks of >0.2 ppm occurred with regularity. Each of the last three profiles had the same total O_3 exposure, but at least 1 h of each daily exposure had at an average peak concentration that exceeded 0.12 ppm.

Significant differences were found for all measured variables. Plants exposed using the 0.12-ppm square-wave exposure (Profile 1) exhibited the least injury. Profile 3, with the mean hourly pyramidal peak of 0.22-ppm exposure, exhibited significantly less necrosis than did Profiles 2 and 4, which also had peak exposures. Plants responded similarly to Profiles 2 and 4. There were no significant differences in plant responses for any of the measured response variables, even though the mean 1-h peak for Profile 2 (0.28 ppm) was higher than the 1-h peak mean (0.22) for Profile 4. Both of these profiles had higher peaks or a longer duration of high concentrations, those above 0.16 ppm, than did

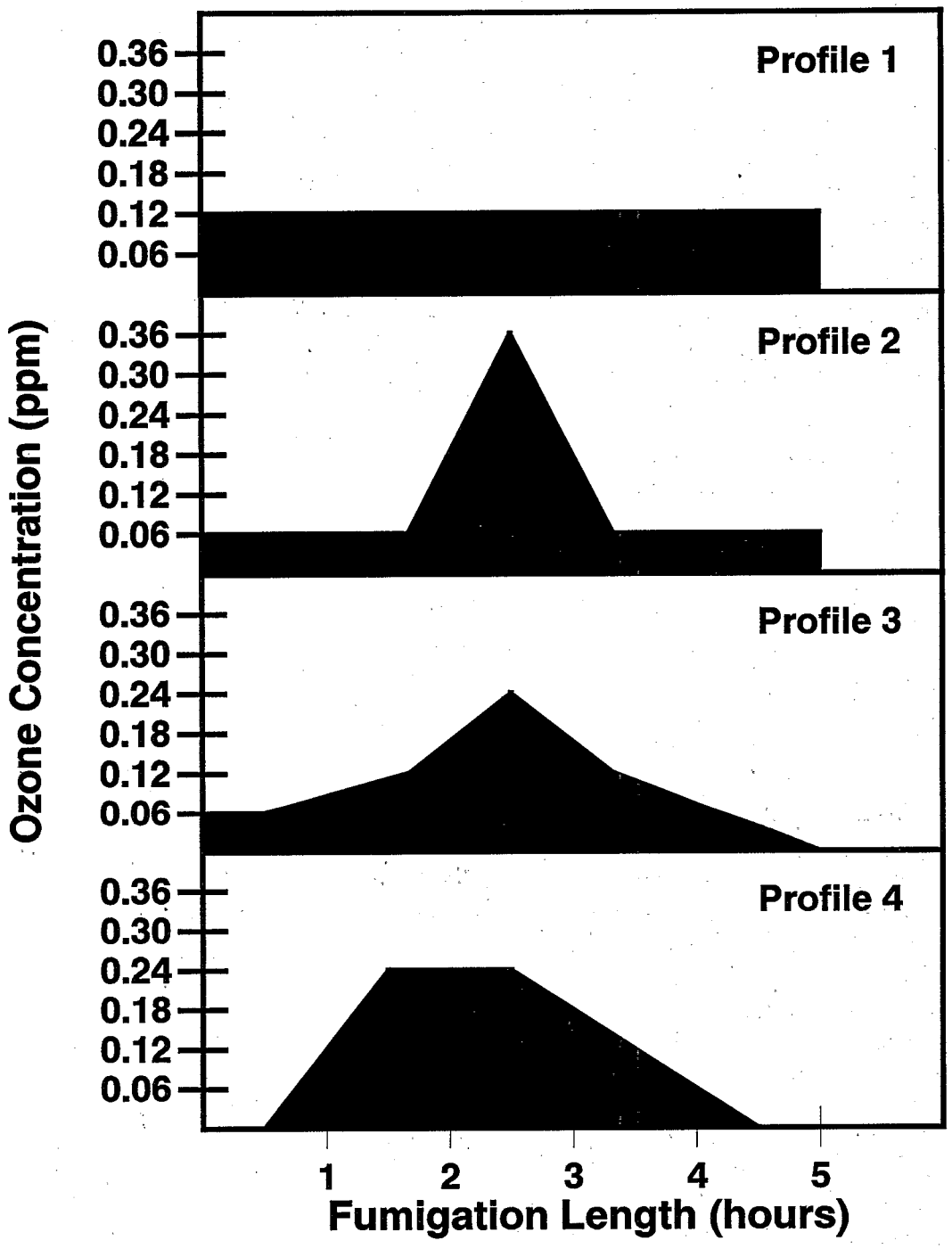

Figure 5-24. Experimental ozone exposure profiles.

Source: Musselman et al. (1994).

Profile 3. The three exposure profiles that incorporated peaks impacted plant response more severely than the steady-state profile, thus providing evidence of the importance of peak concentrations in defining an exposure index (Musselman et al., 1994). Total exposure, however, could not relate O_3 impact to plant response unless the exposure shape was held constant. The authors caution against the application of summary exposure statistics that do not give increased weight to higher concentrations for comparison of plant response in areas with differing exposure regimes. In addition, the authors state that, for Southern California, which experiences high peak O_3 levels, a descriptor of exposure that gives greater weight to peak concentrations is more useful when relating plant response to O_3 exposure. They also suggest that environmental conditions may influence stomatal conductance and O_3 uptake. Therefore, summary statistics might necessitate the inclusion of other parameters that relate to environmental factors. Finally, it is suggested that flattening out concentrations so that peaks remain lower than 0.10 ppm might be expected to benefit the vegetation of Southern California. Again, it should be noted that in all of the studies by Musselman et al. (1983, 1986b, 1994) peaks greatly exceed those in any of the other exposure studies.

The experiments of Hogsett et al. (1985a) were the initial studies using a newly designed modified OTC, with an automated control system in which plants were exposed to simulated ambient concentrations typical of the midwest. In the study, alfalfa and tall fescue growing in pots were exposed to generator-produced O_3 in OTCs using two different types of exposure profiles (Table 5-18). Concentrations used were based on a 1978 Storage and Retrieval of Aerometric Data (SAROAD) database for a selected midwestern site where a substantial acreage of hay was grown. This study used the longest exposures of any of the papers reviewed. The first exposure was a 30-day episodic profile of varying peak frequency, concentration, and duration; a profile that was repeated every 30 days throughout the growing season (Table 5-18; Figure 5-25). The second exposure was a daily peak profile of equivalent peak concentration and duration each day. Daily 7-h exposures of alfalfa were from 0900 to 1600 hours (9 a.m. to 4 p.m.) for the 133-day growing season. Episodic 7-h mean concentrations ranged from 0.064 to 0.084 ppm, with peaks of nearly 0.2 ppm occurring at 1400 to 1500 hours, whereas the profile for the mean daily peak concentrations varied from 0.074 to 0.099 ppm, with peaks ranging between 0.10 to 0.15 ppm occurring at 1400 hours. Reduction in alfalfa growth was reported under both exposure profiles; however, response to the episodic exposures was greater. Actual response data is not given in the paper. The response of tall fescue was reduced only slightly over a period of 90 days when exposed to either regime. Both alfalfa and fescue were cut three times during the exposure period. This is the only study exposing a perennial plant, alfalfa, and a grass. The growth habit of grasses differs from that of dicotyledonous plants because the growth of each leaf blade results from a meristem at the base of the leaf, not from the apical meristem. Therefore, cutting or injury to the leaf blade does not prevent its continued growth. Of the papers cited, this OTC experiment is the only long-term study in which plants were exposed to both mid-range and peak concentrations. The fluctuating episodic O_3 pattern in the Hogsett et al. (1985b) and the single 6-h/week exposure of the Musselman et al. (1983, 1986b) studies permit plants a brief recovery period between exposures to peak concentrations. Also, in the above studies, plant response to O_3 exposure resulted in a reduction in growth, whereas, in the studies discussed below, foliar injury is the plant response observed.

Tonneijck and Bugter (1991), Tonneijck (1994), and Krupa et al. (1993) were reviewed by Krupa et al. (1995) who cited these Bel W3 studies in support of the concept

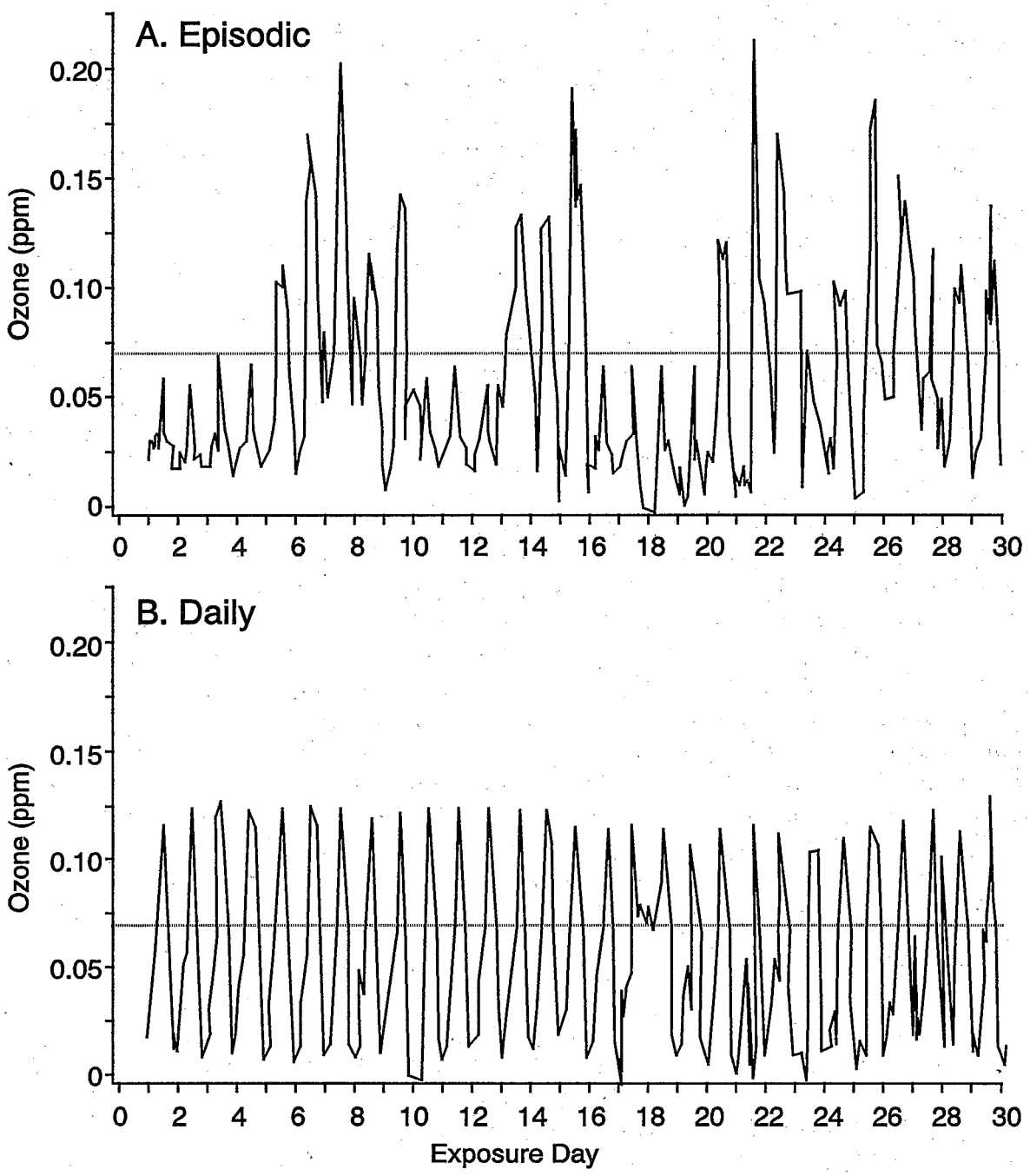

Figure 5-25. Ozone exposure profiles for the 1983 season.

Source: Hogsett et al. (1985b).

that "mid-range" concentrations (0.05 to 0.09 ppm) play a greater role than peak concentrations in causing plant response (Figures 5-5, A, B, and D, and 5-6). Bel W-3, is a variety of tobacco noted for its sensitivity to O_3 and has been used as a sensitive monitor for photochemical ambient air pollution for many years. Visible foliar injury is a clear and unequivocal indication of O_3 exposure. Heggestad and Middleton (1959) discovered Bel W3 and first reported on its sensitivity to O_3. Heggestad and Menser (1962), Heck et al. (1969) and Heck and Heagle (1970) all reported its value as a sensitive monitor of photochemical ambient air pollution. Both Heck et al. (1969) and Heck and Heagle (1970) reported, however, that there was no consistent relationship between oxidant values (O_3 concentrations measured as total oxidants) and foliar injury. They state, however, that a monitoring system such as they describe can provide a community with estimates of the frequency of phytotoxic levels of oxidants, of the relative severity of each episode, and of regional distribution of phytotoxic air pollution (Heck and Heagle, 1970).

The papers of Tonneijck and Bugter (1991) report on observations made in the Netherlands from 1984 to 1988, during which Bel W3 was used as a part of an extensive network for monitoring the effects of ambient air pollution along with the O_3-sensitive indicator plant subterranean clover cv. Geraldton (*Trifolium subterraneum*).

Indicator plants grown in the greenhouse in pots were taken to 17 field locations at weekly intervals and were exposed to ambient air for 1 week for Bel W3 tobacco and 2 weeks for clover. Foliar injury on the tobacco Bel W3 cultivar used in 1988 was greater than that on the variety used during the years 1984 through 1987 (Figure 5-5A), although mean O_3 concentrations to which the varieties were exposed were similar (Figure 5-26, B). The increased injury appeared to be associated with the new line of "relatively sensitive" tobacco used in 1988 when compared with the "rather tolerant" strains used from 1984 to 1987. Exposures were reported as mean weekly O_3 concentrations, 24-h means, daytime average concentrations, number of hours >80 μg m^3 (≈ 0.04 ppm), and cumulative dose of hourly values >120 μg m^3 (≈ 0.06 ppm). No peak concentrations were listed. The highest effect intensity, a mean O_3 concentration of 100 $\mu g/m^3$ (≈ 0.05 to 0.06 ppm), was observed during Week 22 of the exposures at the field site in 1988 (Figure 5-26, B). The mean O_3 concentration was the highest in Week 32.

The authors state that "foliar injury on tobacco Bel W3 was poorly related to the ambient ozone in the Netherlands" (Figure 5-26, A, B, and C), whereas foliar injury on subterranean clover correlated well with O_3 exposure concentrations (Figure 5-26, D). Ozone exposure indices emphasizing the importance of peak values did not correlate better with injury than those based on mean values (Figure 5-26, E). Even though no peaks, as previously defined above, were listed in their paper, foliar injury of tobacco was observed. Tobacco plants appeared to be "relatively" more sensitive to O_3 than did clover at the end of the season. The main reason for using Bel W3 was to demonstrate the occurrence of symptoms induced by O_3 and "not to examine the relationship between the level of ambient ozone and foliar injury intensity," as stated by Tonneijck and Bugter (1991). These authors further noted that care should be taken when comparing the responses of both species because of the difference in length of exposure and effect parameter. Even when both species of plants were exposed to ambient air at the same location for the same length of time (7 days), foliar injury on tobacco was not related to foliar injury on primary leaves of bean plants. Finally, the authors state, "From these results, it can be concluded that ozone injury on tobacco Bel W3 does not adequately indicate the concentration of ambient ozone nor is it a good indication of the risk of ozone to other plant species or to vegetation as a

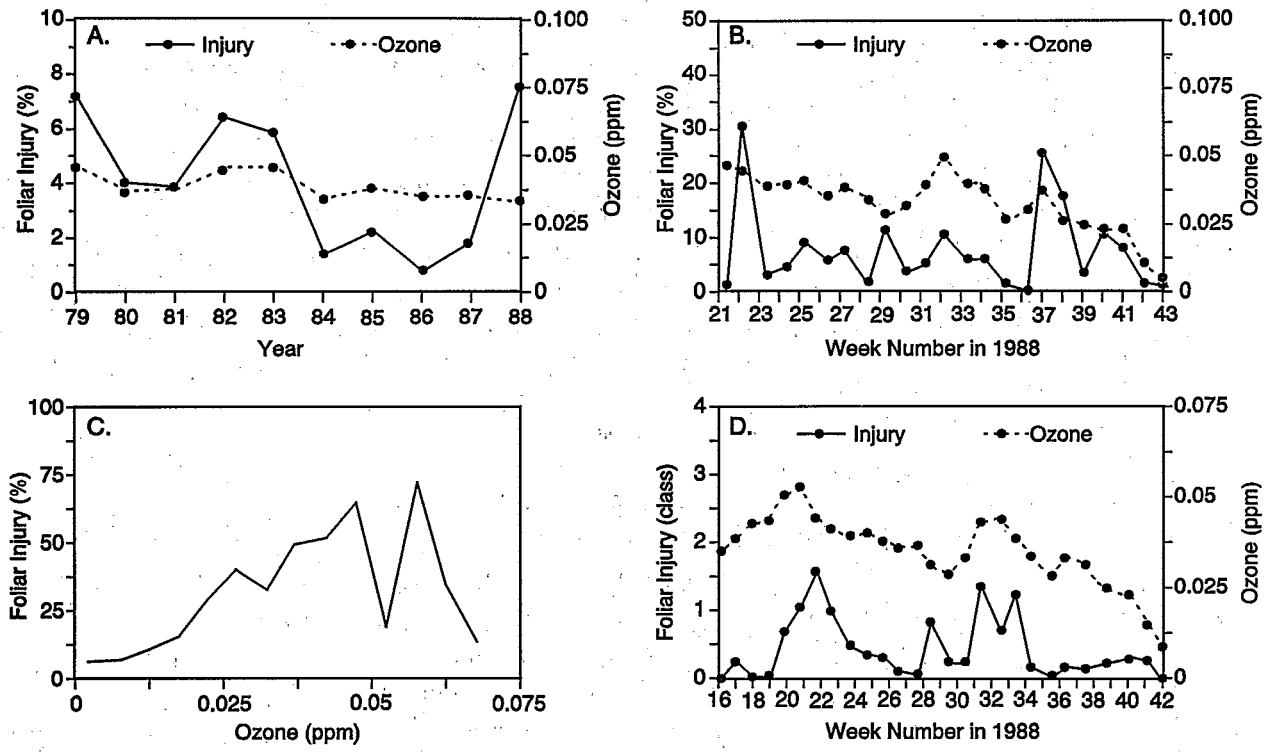

Figure 5-26. (A) Mean foliar injury on tobacco Bel W3 and mean ozone (O_3) concentrations for the years 1979 to 1988, (B) mean foliar injury on tobacco Bel W3 and O_3 concentrations for weekly exposures during the 1988 growing season, (C) maximal foliar injury on tobacco Bel W3 in relation to O_3 concentrations for 1988, and (D) mean foliar injury on subterranean clover cv. Geraldton and mean O_3 concentrations for two weekly exposures during the 1988 growing season.

Source: Tonneijck and Bugter (1991).

whole" (Tonneijck and Bugter, 1991). In other words, Tonneijck and Bugter (1991) concur with the reports of Heck et al. (1969) and Heck and Heagle (1970), who much earlier had reported similar views based on the results of their studies. Also, in their studies they observed that ratios of weekly tobacco injury indices to oxidant indices at an oxidant-monitoring site revealed no consistent relationship between weekly oxidant concentrations and weekly plant injury. In addition, they observed that, although considerable new injury was recorded each week of the season, the relationship between oxidant values and plant injury was not consistent. In other words, data from Bel W3 exposures is not a good basis from which to make extrapolations.

Tonneijck (1994) used data from the Dutch monitoring network for the years 1979 to 1983 (Figure 5-27, A) for Bel W3 and from 1982 to 1983 (Figure 5-27, B) for two bean cultivars, the O_3-sensitive "Stratego" and the O_3 tolerant "Groffy", to evaluate injury-response relationships among certain indicator plants. Various O_3 exposure indices were

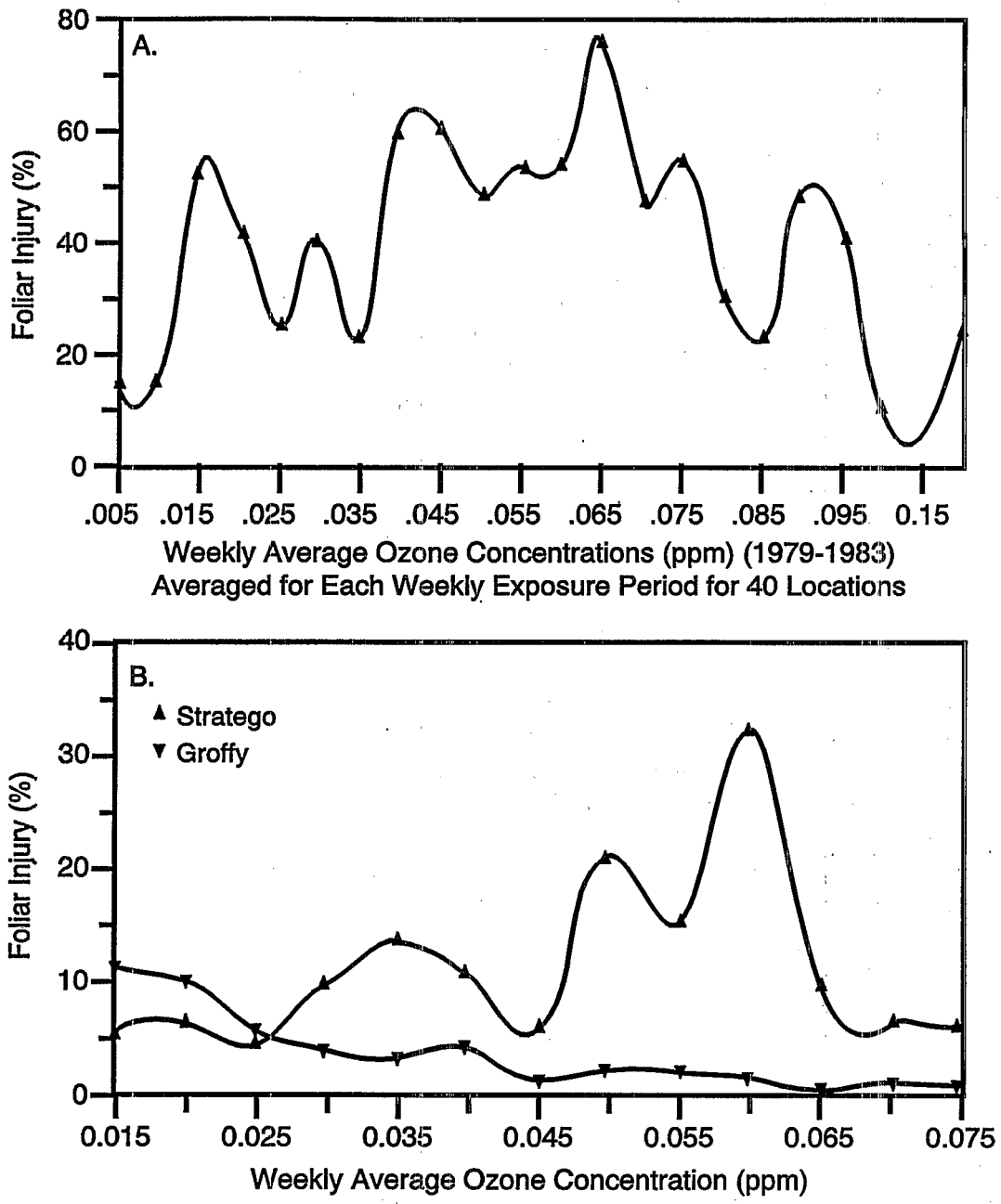

Figure 5-27. ***(A) Maximum foliar injury (percent of leaf area affected) on tobacco Bel W3 in relation to ozone (O_3) concentrations expressed in classes of 10 μg/m^3 for 1979 to 1983, and (B) maximum foliar injury (percent of leaf area affected) on two bean cultivars in relation to O_3 concentrations for 1982 to 1983.***

Source: Tonneijck (1994).

calculated from hourly O_3 concentrations for all exposure periods. Data of foliar injury to Bel W3 tobacco based on 20 to 22 weekly observations for 5 years (1979 to 1983) at 40 locations (Figure 5-27, A) were regressed against several exposure indices. Results of correlation analysis indicated that the weekly sum of all hourly concentrations >40 $\mu g/m^3$ (0.02 ppm) has a negligibly better linear association with maximum weekly foliar injury response than does the 24-h mean. Tonneijck (1994) does not present strong evidence in favor or against the importance of mid-range concentrations in causing foliar injury response due to low correlations (<0.28). The role of mid-range concentrations is difficult to substantiate using correlation analyses because the effects of O_3 on maximum foliar injury response are not linear (Figures 5-27, B and C) and are confounded with environmental factors. Tonneijck stated that the results of the Dutch monitoring network generally do not support the conclusion that hourly concentrations of ambient O_3 above 80 to 120 $\mu g/m^3$ (0.05 to 0.06 ppm) may be relatively more important in causing tobacco injury. Problems with weak associations between weekly pollutant concentrations and visible foliar injury that make the ability to discriminate among exposure indices difficult, which were reported by Tonneijck (1994), also were experienced by Tonneijck and Bugter (1991) and Heck et al. (1969), and Heck and Heagle (1970).

Based on his study, Tonneijck (1994) concluded that "the greatest injury to the ozone-sensitive indicators, tobacco Bel-W3 and bean cv. Stratego, seems to occur at moderate levels of ambient ozone." At relatively high O_3 concentrations (>115 to 135 $\mu g/m^3$; ≈ 0.055 to 0.065 ppm), less injury was observed than at "moderately enhanced concentrations". Results of the above study do not support the "concept that higher O_3 concentrations should be given more weight in terms of plant response than lower ones, since higher concentrations do not necessarily cause greater effects." In Figure 5-27, A, it can be noted that foliar injury on Bel W3 tobacco did not increase even when O_3 concentrations neared 0.15 ppm. However, the manner in which the data in the above study is presented makes it difficult to determine the actual concentrations to which the plants were exposed.

In neither the Tonneijck and Bugter (1991) nor Tonneijck (1994) papers are the actual O_3 concentrations to which the plants were exposed stated, except as mean values. Also, the terms "peak", "moderate", "moderately enhanced", and "circa" are used, but never defined. The problems associated with attempting to make extrapolations from Bel W3 have already been mentioned. In addition, Posthumus (1984) points out, in a paper describing the Dutch monitoring program, that plants grown in the greenhouse may be "more vulnerable" to ambient air pollutants than are crops grown in the field because those grown in a greenhouse have been grown under ideal circumstances.

Krupa et al. (1993) used two tobacco cv. (the sensitive Bel W3 and the tolerant Bel B) as differential indicators of ambient O_3 pollution. When reviewing previous studies in the introduction to their paper, Krupa et al. (1993) mention that the tobacco cultivars Bel W3 and Bel B have been used for over 25 years and indicate that other studies using Bel W3 have produced conflicting results. The aim of their present study was to further examine this subject. Seedlings of the two cultivars grown in pots containing Fafard Mix No. 2 (screened peat + Perlite) in CF air and fertilized every 7 days with liquid fertilizer until the day prior to exposure were transferred to the two field sites when each set of plants reached its "true four-leaf stage" after removing the two juvenile leaves. Exposures to ambient O_3 concentrations were made at two different sites (near Amherst, MA, and in the Green Mountains of southern Vermont) from mid-June to August during the 9 weeks of the study

(Figure 5-28, A and B). Ambient O_3 concentrations were measured continuously. Exposures occurred in an OTC with CF air, an OTC with NF air, and a chamberless ambient-air field plot (Table 5-18). There were two replicates per treatment, with six plants of each cultivar in each replicate. Visual estimates of leaf area showing O_3 injury were made, beginning with the bottommost fully expanded leaf (leaf no. 1) at the end of each weekly exposure. Ratings were given a value from 1 to 10. A new set of plants was exposed each week. Maximum hourly average concentrations for the 9-week period ranged from 0.06 to 0.1 ppm, with the highest concentrations occurring during week seven.

Observations, based on foliar injury scores, indicated that injury to leaves no. 1 and 2 on Bel W3 was much greater than corresponding leaves on Bel B. Foliar injury on Bel W3 was much higher in the NF OTCs and chamberless ambient-air exposures than in the filtered-air OTC exposures. Injury scores indicated that leaf no. 1 on Bel W3 was more sensitive than leaf no. 2. Also, injury scores on leaf no. 1 were very similar in the NF OTC and the chamberless ambient field plot. Study results indicated that, in all cases, of the several O_3 descriptors tested, the number of hours with O_3 concentrations >40 ppb (N40) and >60 ppb (N60) or the number of hours with O_3 concentrations >40 ppb (SUM40) and >60 ppb (SUM60) were best predictors of O_3 injury. Neither the N40 or N60 nor the SUM40 or SUM60 performed well independently of the corresponding variable in the best regression.

The authors state that the results of the present study support the conclusions of Menser et al. (1963), who pointed out that mature leaves were more sensitive than over-mature and rapidly expanding younger leaves. Consequently, all subsequent analyses were based on the responses of leaf no. 1. The authors also point out that their analysis had two limitations: (1) the number of foliar injury observations was low (nine) on a per-site basis, and, hence, results had to be pooled; and (2) foliar injury observations each week involved new groups of plants, and the results on consecutive weeks were thus independent of each other. This is the only study, of those being discussed, in which plants were grown in an artificial medium.

Krupa et al. (1994) suggested that mid-level hourly average concentrations of O_3 (0.05 to 0.087 ppm) are more important than higher hourly average concentrations in affecting vegetation. The key result of Krupa et al. (1994) is questioned because the CF-NF and AA-NF (i.e., comparisons between CF and NF OTC plots and between ambient air nonchambered and NF chambered plots) differences, as reported by the authors, were inconsistent with earlier publications of the same NCLAN studies, which found few cases with significant CF-NF differences (e.g., Heagle et al., 1988a; Rawlings et al., 1988a; Kress et al., 1985; Kohut and Laurence, 1983). For three of the eight harvests, which Krupa et al. (1994) reported as having significant CF-NF difference, Kohut and Laurence (1983) reported a 2% yield reduction at NF for kidney bean plants at the Ithaca site in 1980; Heagle et al. (1987a) reported 0 and 34% yield reductions at NF for well-watered and water-stressed soybean plants, respectively, at the Raleigh, NC, site in 1983; and Kohut et al. (1987) reported an 11% yield reduction at NF for wheat plants at the Ithaca site in 1983, which was not significant at the 5% level. Another two harvests of clover in the 1985 Raleigh experiment should not have been used by Krupa et al. (1994) because Heagle et al. (1989b) reported significant chamber effects on total biomass, based on a 33% yield reduction at NF relative to AA. Two other inconsistencies were found in Krupa et al. (1994). First, the two clover studies conducted at Raleigh in 1984 and 1985 had six and seven harvests during each year of the studies (Heagle et al., 1989b), not 12 and 14 as reported by Krupa et al. (1994).

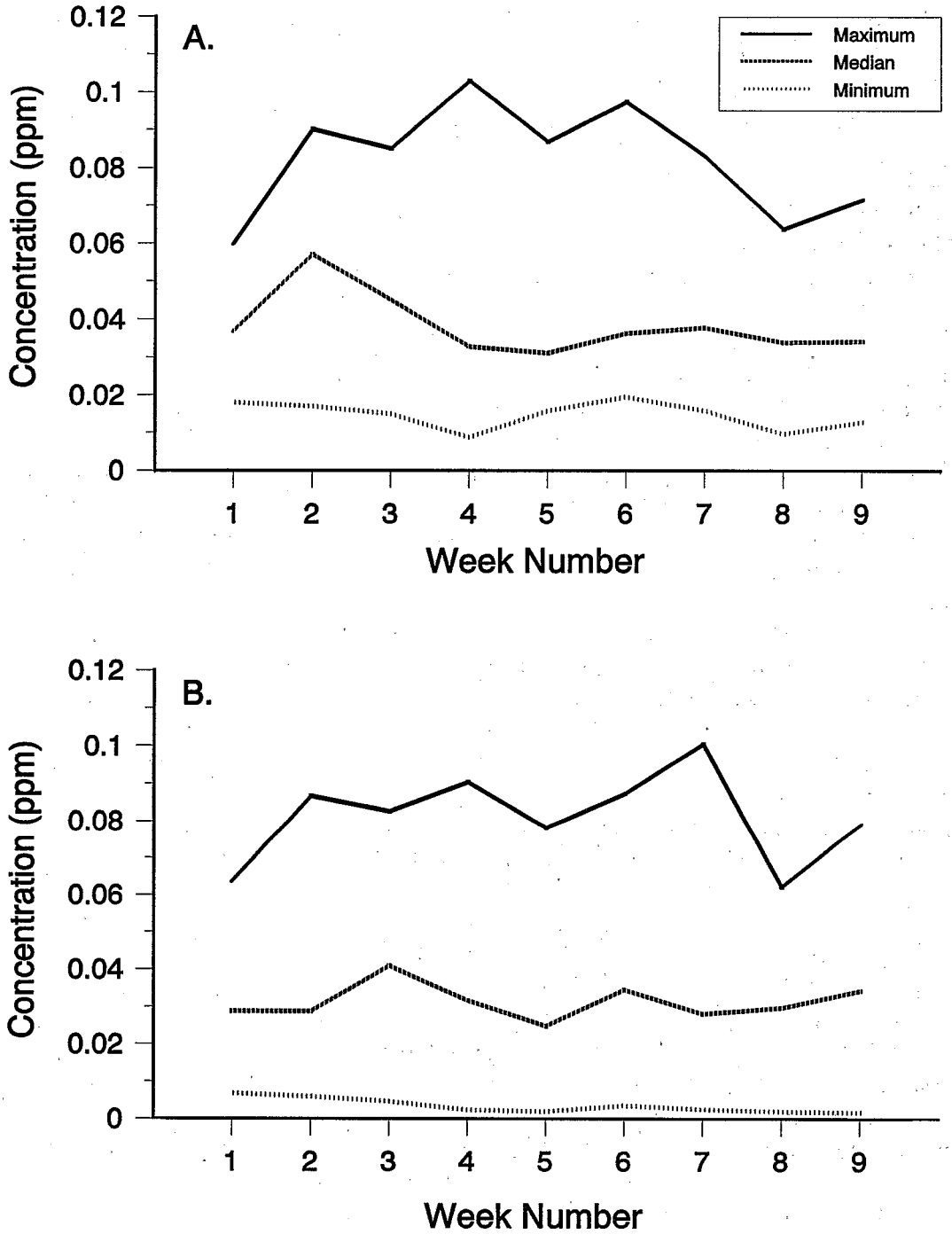

Figure 5-28. (A) Summary hourly ambient ozone (O_3) concentrations during 9 weeks of experimentation (1990) at Montague-Amherst, MA, and (B) summary hourly ambient O_3 concentrations during 9 weeks of experimentation (1990) at Mount Equinox.

Source: Krupa et al. (1993).

Second, the two clover studies conducted at Ithaca in 1984 and 1985 had three harvests during each year of the studies (Kohut et al., 1988a), not six as reported by the authors.

Krupa et al. (1995) attempted in another paper to present "a cohesive view of the dynamics of ambient O_3 exposure and adverse crop response relationships, coupling the properties of photochemical O_3 production, flux of O_3 from the atmosphere into crop canopies and the crop response per se." The results from two independent approaches, (1) statistical and (2) micrometeorological, were analyzed for understanding cause and effect relationships of foliar injury responses of tobacco Bel W3 to the exposure dynamics of ambient O_3 concentrations. Additionally, other results from two independent approaches were analyzed to (1) establish a micrometeorological relationship between hourly ambient O_3 concentrations and their vertical flux from the atmosphere into a grassland canopy and (2) establish a statistical approach relationship between hourly O_3 concentrations in long-term, chronic exposures and crop yield reductions. Based on the above approaches, Krupa et al. (1995) noted that atmospheric conditions appeared to be most conducive and crop response appeared to be explained best statistically by the cumulative frequency of hourly ambient O_3 concentrations between 0.05 and 0.09 ppm. The diurnal occurrence of this concentration range, frequently between the hours 9:00 a.m. and 4:00 p.m. in a polluted agricultural environment, coincided with the optimal CO_2 flux from the atmosphere into the crop canopy, thus facilitating high uptake. The frequency of hourly concentration >0.90 ppm appeared to be of little importance. The higher concentrations, generally appeared to occur when atmospheric conditions did not facilitate optimal vertical flux into the crop canopy, therefore uptake was low.

Krupa et al. (1995) concluded, based on their overall results, that, if the cumulative frequency of hourly ambient O_3 concentrations between 0.05 and 0.062 ppm (100 and 124 $\mu g\ m^3$) occurred during 53% of the growing season, and the corresponding cumulative frequency of hourly concentrations between 0.05 and 0.074 ppm occurred during 71% of the growing season, a potential yield reduction in sensitive crops could be expected, if other factors supporting growth, such as adequate soil moisture, are not limiting. In summary, they concluded that these results need further verification.

High correlations can be obtained from chamber experiments because exchange properties inside chambers are more or less constant in time (Grünhage and Jäger, 1994b). Under ambient conditions, however, exposure indices obtained from the chamber studies frequently yield unsatisfactory results (Grünhage and Jäger, 1994a). Grünhage and Jäger (1994a,b) support this view by presenting the results of O_3 flux density measurements above a permanent grassland in Germany. Two years of observations demonstrate the influence of atmospheric conditions on O_3 exposure potential (i.e., how vertical flux and stomatal conductance change during the day). Diurnal flux densities of O_3 varied during the growing seasons of 1990 and 1991 (Grünhage et al., 1994). Vertical flux densities have to be calculated using micrometeorological approaches. Though similar in pattern, the higher flux densities in 1991 coincided with lower O_3 concentrations. Therefore, under ambient conditions, exposures cannot be expressed as a simple function of the concentration in the air. Flux densities and deposition velocities of O_3, as well as the biological activity of the canopy, need to be considered when determining the effects of ambient air exposures on vegetation. Grünhage and Jäger (1994a,b) and Grünhage et al. (1994), using the information obtained from the micrometeorological measurements of vertical flux densities of CO_2 and O_3 above the native grassland, developed a mathematical model. Grünhage and Jäger (1994b) fit this mathematical model to Bel W3 tobacco data to describe a dose-response

relationship for leaf injury. They concluded that it is possible with this model to attribute the DLA on Bel W3 tobacco to O_4 flux densities. Correlations between O_3 fluxes and leaf injury to tobacco are significantly higher than those using exposure indices based on chamber studies. Grünhage and Jäger (1994b) emphasize the need for taking ambient conditions into account when developing exposure indices to determine critical levels that will prevent injury to vegetation.

Finally, it is not possible at this time, based on a comparison of data from the above mixed studies, to conclude whether the cumulative effects of mid-range concentrations are of greater importance than those of peak hourly average concentrations in determining plant response. The data are not comparable; exposure methods, concentrations and durations used, age of plants at exposure, length of exposure, the plants exposed, and the media in which they were grown all differ across experiments. Some exposures were in chambers in the greenhouse, others in OTCs and others in the ambient air. Many of the exposures in the studies supporting the importance of mid-level O_3 concentrations were only 1 week in duration. It is doubtful that an exposure duration of only 1 week and foliar response data from a sensitive plant species like Bel W3 or from any other plant species are sufficient to ascertain whether cumulative peaks or mid-range concentrations play a greater role in plant growth response. It should be noted, however, that plants are not exposed just to peak O_3 concentrations, therefore, response to O_3 involves the cumulative effect of all concentrations that enter the plants. The short-term exposures indicate that foliar injury can occur even in the absence of peaks. The timing is the key to plant response. Peak and mid-range concentrations do not occur at the same time. A plant effect is determined by which concentrations occur when stomatal conductance is highest. Peaks are important in plant response only where and when plants are exposed to them.

Most important of all is that the response parameters measured in the studies of Musselman et al. (1983, 1986b, 1994) and Hogsett et al. (1985b) differ from those of Tonneijck and Bugter (1991), Tonneijck (1994), and Krupa et al. (1993, 1994). The former measured both foliar injury and growth reductions; all but one of the latter based their conclusions on foliar injury alone. Although foliar injury in tobacco can result in important economic loss to the grower, for the majority of crops, reduction in growth and yield is the measure of importance. As stated in the previous criteria document (U.S. Environmental Protection Agency, 1986), foliar injury in crops does not necessarily signify growth or yield loss. Many studies can be cited to illustrate the inconsistency of relationship between foliar injury and yield loss when foliage is not the yield component.

The studies of Musselman et al. (1983, 1986b) and Hogsett et al. (1985b) have been cited previously (U.S. Environmental Protection Agency, 1986, 1992) as a basis for emphasizing the importance of episodic peak exposures. In addition, the conclusions discussed in previous sections that favored the concept that cumulative effects of hourly O_3 (>0.10 ppm) concentrations are of greater importance than seasonal mean exposures in causing vegetation injury are based on subsequent reanalyses of the NCLAN data. The information presented above in Section 5.5.2.5 does not alter the conclusions reached in the retrospective statistical analyses of NCLAN (Lee et al., 1987, 1991; Tingy et al., 1989; Lefohn and Foley, 1992) that episodic peaks are of importance in causing growth effects, nor does it rule out the possibility that mid-range exposures also could have had an effect.

5.5.3 Summary

The effects of O_3 on individual plants and the factors that modify plant response to O_3 are complex and vary with species and environmental soil and nutrient conditions. Because the effects of O_3 and its interactions with physical and genetic factors that influence response are complex, it is difficult to develop a measure of exposure that relates well with plant response based on experimental data. At best, experimental evidence of the impact of O_3 on biomass production can suggest the important factors of O_3 exposure that modify plant response, which should be considered when developing an exposure index.

Considerable evidence of the primary mode of action of O_3 on plants (injury to proteins and membranes, reduction in photosynthesis, changes in allocation of carbohydrate, and early senescence), which ultimately lead to reductions in biomass production, identifies O_3 uptake as an important factor (see Section 5.2). Ozone uptake is controlled by canopy conductance, stomatal conductance, O_3 concentration outside the leaf and gases emitted from the leaf (see Figure 5-2). Any factor that will affect stomatal conductance (e.g., light, temperature, humidity, soil and atmospheric chemistry and nutrients, time of day, phenology, biological agents) will affect O_3 uptake and, consequently, plant response.

The factors such as respite time, temporal variation, phenology, canopy structure, physiological processes, environmental conditions, and soil and nutrient conditions are important in determining the impact of O_3 on crops and trees but are not well understood and interact with concentration and duration in different fashions depending on species. Ozone uptake integrates these factors with atmospheric conditions and relates well with plant response, but is difficult to measure. Empirical functions to predict stomatal conductance have been developed for particular species (e.g., Lösch and Tenhunen, 1981) but have not been used to estimate O_3 uptake or used in development of exposure indices. Based on atmospheric measurement of deposition and diurnal patterns of O_3 and gas exchange in a natural grassland ecosystem, Grünhage and Jäger (1994a,b) and Grünhage et al. (1993a) proposed an ambient O_3 exposure potential for characterizing O_3 uptake and related it to the DLA of Bel W3 tobacco. Grünhage and Jäger (1994a,b) proposed a weighting scheme that preferentially weights the hourly O_3 concentrations occurring during periods of optimal vertical flux into the canopy. For the diurnal pattern of distribution at the natural grassland site in Germany, there was a greater frequency of concentrations in the 0.05- to 0.09-ppm range during the 0900 to 1559 period that matched the DLA of Bel W3 when atmospheric and canopy resistance was minimal.

Further, the biochemical mechanisms, discussed in Section 5.2, describe the mode of action of O_3 on plants as the culmination of a series of physical, biochemical, and physiological events leading to alterations in plant metabolism. Ozone-induced injury is cumulative, resulting in net reductions in photosynthesis, changes in allocation of carbohydrate, and early senescence, which lead to reductions in biomass production (Section 5.2). Increasing O_3 uptake will result in increasing reductions in biomass production.

The optimum exposure index that relates well with plant response should incorporate the factors (directly or indirectly) described above; unfortunately, such an index has not yet been identified. At this time, exposure indices that weight the hourly O_3 concentrations differentially appear to be the best candidates for relating exposure with predicted plant response. Peak concentrations in ambient air occur primarily during daylight, thus, these indices, by providing preferential weight to the peak concentrations, give greater

weight to the daylight concentrations than to the nighttime concentrations (when stomatal conductance is minimal). The timing of peak concentrations and maximum plant uptake is critical in determining their impact on plants.

Some studies reported in the literature show that, when O_3 is the primary source of variation in response, year-to-year variations in plant response are minimized by the peak-weighted, cumulative exposure indices. However, the study of Fuhrer et al. (1992) illustrates some of the limitations in applying exposure indices. The study is significant for its use of the mean O_3 flux in minimizing the year-to-year variation in response when combining replicate studies, indicating the importance of environmental conditions in quantifying the relationship between O_3 exposure and plant response.

5.6 Exposure-Response of Plant Species

5.6.1 Introduction

Determining the response of plants to O_3 exposures continues to be a major challenge. The effects of exposure usually are evaluated by exposing various plant species under controlled experimental conditions, such as those discussed in Section 5.2, to known concentrations and exposure periods. Plant responses are influenced not only by the biochemical and physiological changes that may occur within the plant after O_3 entry (Section 5.3, Mode of Action, see also Figure 5-5) but also by the many factors (both internal and external) that modify plant response (Section 5.4). Of the internal factors discussed in Section 5.4, those that are most likely to apply under controlled experimental conditions are the genetic makeup and age of the plant at the time of exposure. Compensatory responses (Section 5.3.4.2) also will influence plant response. This section analyzes, summarizes, and evaluates what is known about the response of various plant species or cultivars, either as individuals or in populations, to O_3 exposure. Species as populations will be considered only in the case of pasture grasses, or forage mixes, which commonly occur as mixed stands. The response of forest and trees in their natural habitats is discussed in the next section. Emphasis will be placed on those studies conducted since the publication of the previous criteria document 1986 (U.S. Environmental Protection Agency, 1986). Much of the discussion of vegetation response to O_3 exposure in the current document is based on the conclusions of both the 1978 and 1986 criteria documents (U.S. Environmental Protection Agency, 1978, 1986); therefore, to provide a basis for understanding the effects presented below, the conclusions of the two documents are summarized.

Finally, the results of O_3 exposure-response presented in this section must be related to one or more assessment endpoints. Historically, the dollar value of lost production was the endpoint of interest; however, other endpoints (e.g., biodiversity, habitat, aesthetics, recreation) must be considered now, particularly as the impacts of O_3 on long-lived species of ecological importance are evaluated (Tingey et al., 1990).

5.6.2 Summary of Conclusions from the Previous Criteria Documents

The experimental data presented in the 1978 and 1986 criteria documents dealt with the effects of O_3 primarily on agricultural crops species (U.S. Environmental Protection Agency, 1978, 1986). The chapter on vegetation effects in the 1978 criteria document (U.S. Environmental Protection Agency, 1978) emphasized visible injury and growth effects;

however, the growth effects were not those that affected yield. This emphasis was dictated by the kind of data available at the time. The document also presented data dealing with the response of the San Bernardino forest ecosystem to O_3. This information also was discussed in the 1986 document (U.S. Environmental Protection Agency, 1986). It remains the best and most comprehensive study of forest ecosystem responses to O_3 stresses (see Section 5.7).

The 1986 document emphasized the fact that although foliar injury on vegetation is one of the earliest and most obvious manifestations of O_3 exposure, the effects of exposure are not limited to visible injury. Foliage is the primary site of plant response to O_3 exposures. Significant secondary effects include reduced growth, both in foliage and roots. Impacts range from reduced plant growth and decreased yield to changes in crop quality and alterations in plant susceptibility to biotic and abiotic stresses. Also, the 1986 document noted that O_3 exerts a phytotoxic effect only if a sufficient amount reaches sensitive sites within the leaf (see Section 5.3). Ozone injury will not occur if the rate of uptake is low enough that the plant can detoxify or metabolize O_3 or its metabolites or if the plant is able to repair or compensate for the effects (Tingey and Taylor, 1982; U.S. Environmental Protection Agency, 1986). Cellular disturbances that are not repaired or compensated are ultimately expressed as visible injury to the leaf or as secondary effects that can be expressed as reduced root growth or as reduced yield of fruits or seeds, or both. Ozone would be expected to reduce plant growth or yield if it directly impacts the plant process (e.g., photosynthesis) that limits plant growth or if it impacts another step to the extent that it becomes the step limiting plant growth (U.S. Environmental Protection Agency, 1986; Tingey, 1977). Conversely, if the process impacted is not or does not become rate-limiting, O_3 will not limit plant growth. These conditions also suggest that there are combinations of O_3 concentration and exposure duration that a plant can experience that will not result in visible injury or reduced plant growth and yield. Indeed, numerous studies have demonstrated this fact. This information is still pertinent today (Section 5.3)

Ozone can induce a diverse range of effects beginning with individual plants and then proceeding to plant populations and, ultimately, communities. The effects may be classified as either injury or damage. Injury encompasses all plant reactions, such as reversible changes in plant metabolism (e.g., altered photosynthesis), leaf necrosis, altered plant quality, or reduced growth that does not impair yield or the intended use or value of the plant (Guderian, 1977). In contrast, damage or yield loss includes all effects that reduce or impair the intended use or value of the plant. Thus, for example, visible foliar injury to ornamental plants, detrimental responses in native species, and reductions in fruit and grain production by agricultural species all are considered damage or yield loss. Although foliar injury can not always be classified as damage, its occurrence indicates that phytotoxic concentrations of O_3 are present, and, therefore, studies should be conducted to assess the risk to vegetation.

The concept of limiting values used to summarize visible foliar injury in the 1978 document also was considered valid in the 1986 document (U.S. Environmental Protection Agency, 1978, 1986). Jacobson (1977) developed limiting values by reviewing the scientific literature and identifying the lowest concentration and exposure duration reported to cause visible injury to a variety of plant species. Expressed in another way, limiting values were concentrations and durations of exposure below which visible injury did not occur. A graphical analysis presented in both of the previous documents indicated the limit for reduced plant performance was an exposure to 0.05 ppm for several hours per day for more than 16 days. Decreasing the exposure period to 10 days increased the concentration

required to cause injury to 0.1 ppm, and a short, 6-day exposure further increased the concentration to cause injury to 0.3 ppm.

By 1986, a great deal of new information concerning the effects of O_3 on the yield of crops plants had become available, both through EPA's NCLAN and the results of research funded by other agencies. The NCLAN project was initiated by EPA in 1980, primarily to improve estimates of yield loss in the field and of the magnitude of crop losses caused by O_3 (Heck et al., 1982, 1991). The primary objectives were:

(1) to define the relationships between yields of major agricultural crops and O_3 exposure as required to provide data necessary for economic assessments and the development of National Ambient Air Quality Standards;

(2) to assess the national economic consequences resulting from the exposure of major agricultural crops to O_3; and

(3) to advance understanding of the cause and effect relationships that determine crop responses to pollutant exposures.

The cultural conditions used in the NCLAN studies approximated typical agronomic practices. The methodology used in these studies is described in Section 5.2.

Yield loss in the 1986 document was defined as "damage", an impairment in the intended use of the plant. This concept included reductions in aesthetic values, the occurrence of foliar injury (changes in plant appearance), and losses in terms of weight, number, or size of the plant part that is harvested. Yield loss also may include changes in physical appearance, chemical composition, or the ability to withstand quality storage (collectively termed crop quality). Losses in aesthetic values are difficult to quantify. Foliar injury symptoms can substantially reduce the marketability of ornamental plants or crops in which the foliage is the plant part (e.g., spinach, lettuce, cabbage) and constitute yield loss with or without concomitant growth reductions. At that time (1986), most studies of the relationship between yield loss and O_3 concentration focused on yields as measured by weight of the marketable organ of the plant.

The OTC studies conducted to estimate the impact of O_3 on the yield of various crop species (e.g., the NCLAN program) were grouped into two types, depending on the experimental design and statistical methods used to a analyze the data: (1) studies that developed predictive equations relating O_3 exposure to plant response and (2) studies that compared discrete treatment level to a control. The advantage of the regression approach is that exposure-response models can be used to interpolate results between treatment levels (see Section 5.2.2).

Using NCLAN data as an example of plant response, the O_3 concentrations that could be predicted to cause 10 or 30% yield loss were estimated using the Weibull function (Table 5-19). The data in Table 5-19 are based on yield-response functions for 38 species or cultivars developed from studies using OTCs. Review of that data indicated that 10% yield reductions could be predicted for 58% of the species or cultivars, when 7-h seasonal mean concentrations were below 0.05 ppm, and for 34%, when seasonal mean concentrations were between 0.04 and 0.05 ppm, but only 18% required 7-h seasonal mean concentrations in excess of 0.08 ppm to suffer a 10% loss in yield. Furthermore, approximately 11% of the 38 species or cultivars would be expected to have a yield reduction of 10% loss at 7-h seasonal mean concentrations below 0.035 ppm, suggesting that these plants are very sensitive to O_3.

Grain crops were apparently less sensitive than the other crops. The data also demonstrate that the sensitivity within species may be as great as differences between

Table 5-19. Estimates of the Parameters for Fitting the Weibull Model Using the 7-Hour Seasonal Mean Ozone Concentrations[a,b]

Crop	Parameters for Weibull Model				Concentration for Predicted Yield Losses of:	
	$\hat{\alpha}$	$\hat{\sigma}$	$\hat{c}$	CF[c]	10%[d]	30%[d]
LEGUME CROPS						
Soybean, Corsoy	2,785.00	0.133	1.952	0.022	0.048	0.082
Soybean, Davis (81)	5,593.00	0.128	0.872	0.025	0.038	0.071
Soybean, Davis (CA-82)[e]	4,931.00	0.12/	2.144	0.019	0.048	0.081
Soybean, Davis (PA-82)$_e$	4,805.00	0.103	4.077	0.019	0.059	0.081
Soybean, Essex (81)	4,562.00	0.187	1.543	0.014	0.048	0.099
Soybean, Forrest (82-I)	4,333.00	0.171	2.752	0.017	0.076	0.118
Soybean, Williams (81)	4,992.00	0.211	1.100	0.014	0.039	0.093
Soybean, Williams (82-I)	5,884.00	0.162	1.577	0.017	0.045	0.088
Soybean, Hodgson	2,590.00	0.138	1.000	0.017	0.032	0.066
Bean, Kidney (FP)[f]	2,878.00	0.120	1.171	0.019	0.033	0.063
Peanut, NC-6	7,485.00	0.111	2.249	0.025	0.046	0.073
GRAIN CROPS						
Wheat, Abe (82)	5,363.00	0.143	2.423	0.023	0.059	0.095
Wheat, Arthur 71 (82)	4,684.00	0.148	2.154	0.023	0.056	0.094
Wheat, Roland	5,479.00	0.113	1.633	0.023	0.039	0.067
Wheat, Vona	7,857.00	0.053	1.000	0.022	0.028	0.041
Wheat, Blueboy II (T)	5.88	0.175	3.220	0.030	0.088	0.127
Wheat, Coker 47-27 (T)	5.19	0.171	2.060	0.030	0.064	0.107
Wheat, Holly (T)	4.95	0.156	4.950	0.030	0.099	0.127
Wheat, Oasis (T)	4.48	0.186	3.200	0.030	0.093	0.135
Corn, PAG 397	13,968.00	0.160	4.280	0.015	0.095	0.126
Corn, Pioneer 3780	12,533.00	0.155	3.091	0.015	0.075	0.111
Corn, Coker 16 (T)	240.00	0.221	4.460	0.020	0.133	0.175
Sorghum, DeKalb-28	8,137.00	0.296	2.217	0.016	0.108	0.186
Barley, Poco	1.99	0.205	4.278	0.020	0.121	0.161
FIBER CROPS						
Cotton, Acala SJ-2 (81-I)	5,546.00	0.199	1.228	0.018	0.044	0.096
Cotton, Acala SJ-2 (82-I)	5,872.00	0.088	2.100	0.012	0.032	0.055
Cotton, Stoneville	3,686.00	0.112	2.577	0.026	0.047	0.075
HORTICULTURAL CROPS						
Tomato, Murrieta (81)	32.90	0.142	3.807	0.012	0.079	0.108
Tomato, Murrieta (82)	32.30	0.082	3.050	0.012	0.040	0.059
Lettuce, Empire (T)	1,245.00	0.098	1.220	0.043	0.053	0.075
Spinach, America (T)	21.20	0.142	1.650	0.024	0.046	0.082
Spinach, Hybrid (T)	36.60	0.139	2.680	0.024	0.043	0.082
Spinach, Viroflay (T)	41.10	0.129	1.990	0.024	0.048	0.080
Spinach, Winter Bloom (T)	20.80	0.127	2.070	0.024	0.049	0.080

Table 5-19 (cont'd). Estimates of the Parameters for Fitting the Weibull Model Using the 7-Hour Seasonal Mean Ozone Concentrations[a,b]

Crop	Parameters for Weibull Model				Concentration for Predicted Yield Losses of:	
	$\hat{\alpha}$	$\hat{\sigma}$	$\hat{c}$	CF[c]	10%[d]	30%[d]
HORTICULTURAL CROPS (cont'd)						
Turnip, Just Right (T)	10.89	0.090	3.050	0.014	0.043	0.064
Turnip, Pur Top W.G. (T)	6.22	0.095	2.510	0.014	0.040	0.064
Turnip, Shogoin (T)	4.68	0.096	2.120	0.014	0.036	0.060
Turnip, Tokyo Cross (T)	15.25	0.094	3.940	0.014	0.053	0.072

[a]Data are from Heck et al. (1984) and are based on individual plot means unless the crop name is followed by "(T)". The "T" indicates that the parameters were based on treatment means and the data are from Heck et al. (1983). The parameters given in Heck et al. (1983, 1984) also contain the standard errors of the parameters.
[b]All estimates of $\hat{\sigma}$ are in ppm. The yield is expressed as kilograms per hectare for all crops except barley—see weight (grams per head); tomato (both years)—fresh weight (kilograms per plot); cotton—lint + seed weight (kilograms per hectare); peanut—pod weight (kilograms per hectare). In cases where the estimated $\hat{c}$ parameter is exactly 1.0, it has been bounded from below to obtain convergence in the nonlinear model fitting routine. Parameters were estimated from data not showing the expected Weibull form. Caution should be used in interpreting these Weibull models. Other models might better describe the behavior observed in these experiments. For those crops whose name is followed by "(T)", the yield is expressed as grams per plant.
[c]The ozone (O_3) concentration in the charcoal-filtered (CF) chambers expressed as a 7-h seasonal mean concentration.
[d]The 7-h seasonal mean O_3 concentration (parts per million) that was predicted to cause a 10 or 30% yield loss (compared to CF air).
[e]CA and PA refer to constant and proportional O_3 addition.
[f]Only the bean data from the full plots are shown. The partial plot data are given Heck et al. (1984).

Source: U.S. Environmental Protection Agency (1986).

species. For example, at 0.04 ppm O_3, estimated yield losses ranged from 2 to 15% in soybean and from 0 to 28% in wheat. Year-to-year variations in plant response also were observed during the studies.

Discrete treatments were used to determine yield loss in some studies. These experiments were designed to test whether specific O_3 treatments were different from the control rather than to develop exposure-response equations, and the data were analyzed using analyses of variance. When summarizing these studies using discrete treatment levels, as opposed to the variable concentrations used in NCLAN, the lowest O_3 concentration that significantly reduced yield was determined from analyses done by the authors. Frequently, the lowest concentration used in the study was the lowest concentration reported to reduce yield; hence, it was not always possible to estimate a no-effect exposure concentration. In general, the data indicated that O_3 concentrations of 0.10 ppm (frequently the lowest concentration used in the studies) for a few hours per day for several days to several weeks generally caused significant yield reductions. The concentrations derived from the regression

studies were based on a 10% yield loss, whereas, in the studies using the analysis of variance, the 0.10-ppm concentration frequently induced mean yield losses of 10 to 50%.

A chemical protectant, EDU was used to provide estimates of yield loss. The impact of O_3 on yield was determined by comparing the yield data from plots treated with EDU with those that were not. Studies indicated that yields were reduced by 18 to 41% when ambient O_3 concentrations exceeded 0.08 ppm during the day for 5 to 18 days over the growing season.

In summary, the 1986 criteria document (U.S. Environmental Protection Agency, 1986) states that several general conclusions can be drawn from the various approaches used to estimate crop loss yield.

(1) Based on the comparison of crop yield in CF and unfiltered (ambient) exposures, data clearly indicate that O_3 at ambient levels is elevated sufficiently in several parts of the country to impair the growth and yield of plants. Data from the chemical protectant studies support the conclusion and extend it to other plant species.

(2) Both of the above-mentioned approaches indicate that effects occur with only a few O_3 occurrences above 0.08 ppm.

(3) The growth and yield data cited in the 1978 criteria document (U.S. Environmental Protection Agency, 1978) indicate that several plant species exhibited growth and yield effects when the mean O_3 concentration exceeded 0.05 ppm for 4 to 6 h/day for at least 2 weeks.

(4) The data obtained from regression studies conducted to develop exposure-response functions for estimating yield loss indicated that at least 50% of the species and cultivars tested were predicted to exhibit a 10% yield loss at 7-h season mean O_3 concentrations of 0.05 ppm or less.

Though most of the data from the discrete treatment studies (non-NCLAN studies) did not use concentrations low enough to support the values cited above, the magnitude of yield losses reported at 0.10 ppm under a variety of exposure regimes indicate that, to prevent O_3 effects, a substantially lower concentration is required (U.S. Environmental Protection Agency, 1986).

The limiting values established in 1978 were still deemed appropriate in the 1986 criteria document for ornamentals and certain vegetable crops where visible injury was still considered the response of interest because appearance is of importance (e.g., spinach, lettuce, cabbage) (U.S. Environmental Protection Agency, 1986). This remains the case today.

5.6.3 Information in the Published Literature Since 1986

The major question to be addressed in this section is whether the conclusions of the 1986 criteria document summarized in the previous section, remain valid, given the results of research published since 1988. In particular, whether the response of plants to experimental treatments at or near concentrations of 0.05 ppm (7-h seasonal mean), which are characteristic of ambient concentrations in many areas, can be compared to a control or to reduced O_3 treatment to establish a potential adverse effect.

The 1986 criteria document (U.S. Environmental Protection Agency, 1986) made the following statement: "The characterization and representation of plant exposures to O_3 has been and continues to be a major problem because research has not yet clearly

identified which components of the pollutant exposure cause plant response." This is still true today, although some insight into the importance of peak concentrations versus long-term means has been gained (See Section 5.5). The importance of the timing of exposure during the growing season, the duration of peaks, the rate of increase of concentration, and the respite periods is unresolved.

The aim of most air pollution research experiments have been designed to quantify the relationship between pollutant exposure and agricultural crop yield. The problem is the incorporation of the concentration, duration, frequency, age, genetic composition, and respite time into an exposure statistic or index that may be used to predict yield loss. The correct exposure representation is the amount of pollutant entering the plant, not the ambient concentration to which the plant is exposed (Taylor et al., 1982; Tingey and Taylor, 1982). Unfortunately, it is rarely possible to know the amount of pollutant taken up by the plant, so therefore, an appropriate index of exposure must be chosen. Most indices were not developed from a biological basis, nor were they developed using an experimental approach specifically designed to address all key factors (Lee et al., 1991). A number of exposure indices have been developed in an attempt for depicting plant response to O_3 exposure (see Section 5.5). Much of the data in this section is evaluated using these indices. For this reason, several different exposure statistics are used to determine the effect of an exposure on plant response. It should be remembered that the SUM06, which is used more than any of the other indices, is the seasonal sum of hourly concentrations at or above 0.06 ppm (see Section 5.5).

Exposure indices calculated for each of 10 years (1982 to 1991) and two exposure periods, June through August (3 mo) and May through September (5 mo), are presented in Table 5-20 (modified from Tingey et al., 1991). The monitoring data, collected at nonurban sites, show that ambient O_3 is frequently at, or near, the 7-h seasonal mean that would be expected to cause a yield loss in crops, based on the conclusions of the 1986 criteria document. This table may be used for comparison of ambient-O_3 concentrations to those used in experiments. Although the examples here are based on 10% loss figures, losses below that level may occur and be important. Thirty-four percent of the 38 species or cultivars under consideration would be predicted to have a 10% yield loss at a 7-h mean concentration of between 0.04 and 0.05 ppm, but only 19% required a 7-h mean concentration of greater than 0.08 ppm to suffer a predicted 10% loss in yield. Furthermore, 11% of the 38 species or cultivars would be expected to have a yield reduction of 10% at a 7-h mean, or less than 0.028 to 0.035 ppm (Tables 6-17 and 6-19; U.S. Environmental Protection Agency, 1986). It also was concluded that grain crops (with the exception of a few very sensitive cultivars) were generally less sensitive than others, but that within-species variability in sensitivity may be as great or greater than between species. The preceeding results are similar to those previously obtained from Table 6-19 in the 1986 document. Lee et al. (1994a,b) have revised Table 6-19 in U.S. Environmental Protection Agency (1986) (see Table 5-19) using recalculated peak-weighted exposure indices (shown to be more appropriate than long-term means for relating effects to ambient concentrations) for the 54 studies (listed in Tables 5-21 and 5-22).

In 1992, the Supplement to the Air Quality Criteria Document for Ozone and Other Photochemical Oxidants (1986) reviewed effects of oxidant exposure on vegetation. Considerable emphasis was placed on the appropriate exposure index for relating biological effects of O_3 on plants (U.S. Environmental Protection Agency, 1992). An analysis of the data at that time indicated that a seasonal mean concentration (e.g., 7 or 24 h) might not be

the best expression of the exposure because it did not weight high concentrations differently from low concentrations, and it did not account for the variable length of growing seasons or exposure durations. Unfortunately, it is often impossible to calculate the different possible exposure indices (means, cumulative peak- or threshold-weighted, or continuously weighted [sigmoid] cumulative) from information given in published papers. Thus, difficulties remain when comparing exposure-response studies that utilize different exposure indices. However, reported responses and concentrations of O_3 can be compared to those that occur at ambient concentrations and then to other exposure indices (Table 5-20).

5.6.3.1 Effects of Ozone on Short-Lived (Less Than One Year) Species

Plant species can be characterized by their life span. They are either short-lived annual species or longer lived perennials and trees. Physiological processes may be related to life span (for instance, leaf gas exchange tends to be lower in longer-lived trees than in crop species), so the response to O_3 may be different (Reich, 1987). In addition, multiple-year exposures and carry-over effects may be of importance in long-lived species, but of no concern in annuals. Accordingly, annuals and perennials will be discussed separately. The response of plants to O_3 also is affected by interactions with other physical, chemical, and biological factors. Those interactions are discussed elsewhere in this document (Section 5.3). In most cases, the research analyzed here was conducted under near-optimal conditions of water and nutrient availability. Although deviations from these conditions may affect the magnitude of response, it is important to understand the potential of O_3 exposure and its consequences.

Several papers (Lee et al., 1988, 1991, 1994a,b; Lefohn et al., 1988a; Lesser et al., 1990; Tingey et al., 1991) present a reanalysis of NCLAN data and data from field studies conducted on potato that were not part of the NCLAN project. Lee et al. (1988, 1991) examined a number of measures of O_3 exposure in relation to response data collected in the experiments. The investigators were particularly interested in examining the ability of a seasonal mean, a cumulative exposure index, and the second-highest daily maximum concentration (2HDM) to predict the biological response of the plant. They found that no particular index of O_3 concentration dominated as best in all studies, but that cumulative indices that weighted high concentrations at the "grain-filling" stage of the life cycle were better than a seasonal mean. Seasonal means did work well within a given experiment where treatments were highly correlated. The 2HDM was consistently a poor predictor of plant response.

In a reanalysis of NCLAN data, Lesser et al. (1990) presented composite exposure-response functions for a number of crop species, or groups of species. Predicted yield losses (compared to yield at an assumed background concentration of 0.025 ppm) of up to 20% occurred at a 12-h seasonal mean of 0.06 ppm, with a loss of 10% at a 12-h mean concentration of about 0.045 ppm.

Tingey et al. (1991) and Lee et al. (1991) went on to reanalyze the crop response data using three measures of exposure: (1) the SUM06, (2) the 7-h seasonal mean, and (3) the 2HDM. Their analysis included crops that account for 70% of all crop land in the United States and 73% of the agricultural receipts. The analysis included 31 field experiments with 12 crop species, conducted in OTCs and resulted in composite exposure-response functions. The results of their studies and additional reanalyses done since then are summarized in Tables 5-23 and 5-24. They concluded that to limit yield loss to 10% or less in 50% of the cases (all experiments and crops), a SUM06 of 24.4 ppm·h (or 26.4 ppm·h,

Table 5-20. Summary of Ozone Exposure Indices Calculated for 3- or 5-Month Growing Seasons from 1982 to 1991[a]

3 mo (June-August)

Year	No. of Sites[b]	HDM2[c] ppm Mean	HDM2[c] ppm CV[d]	M7 ppm Mean	M7 ppm CV	SUM00 ppm·h Mean	SUM00 ppm·h CV	SUM06 ppm·h Mean	SUM06 ppm·h CV	SIGMOID ppm·h Mean	SIGMOID ppm·h CV
1982	99	0.114	23.7%	0.052	18.7%	82.9	19.1%	26.8	68.8%	26.3	56.7%
1983	102	0.125	24.9%	0.056	21.9%	86.1	22.1%	34.5	58.1%	33.0	52.3%
1984	104	0.117	24.6%	0.052	18.2%	84.1	19.9%	27.7	58.4%	27.4	47.9%
1985	117	0.117	24.6%	0.052	17.1%	84.6	18.0%	27.4	59.6%	27.4	47.6%
1986	123	0.115	21.8%	0.052	19.1%	85.3	18.0%	27.7	65.0%	27.7	51.8%
1987	121	0.119	22.9%	0.055	17.6%	86.9	17.3%	31.2	56.4%	30.4	46.8%
1988	139	0.129	21.3%	0.060	17.8%	97.6	19.6%	45.2	46.8%	42.9	42.4%
1989	171	0.105	23.1%	0.051	17.5%	86.4	19.9%	24.8	78.7%	25.8	59.4%
1990	188	0.105	21.6%	0.053	18.3%	85.7	21.0%	25.8	76.2%	26.6	59.2%
1991	199	0.106	22.0%	0.054	18.4%	87.7	21.3%	28.3	74.2%	28.9	59.5%
Among Years		0.113	11.1%	0.054	10.0%	87.0	9.9%	29.5	42.1%	29.4	31.0%

5 mo (May-September)

Year	No. of Sites	M7 ppm Mean	M7 ppm CV	SUM00 ppm·h Mean	SUM00 ppm·h CV	SUM06 ppm·h Mean	SUM06 ppm·h CV	SIGMOID ppm·h Mean	SIGMOID ppm·h CV
1982	88	0.048	20.6%	122.9	22.3%	37.3	70.9%	37.1	57.8%
1983	87	0.051	22.1%	129.6	24.4%	44.4	61.9%	43.8	52.7%
1984	95	0.048	18.0%	126.2	19.1%	36.7	60.8%	37.6	46.9%
1985	114	0.048	18.4%	124.5	19.4%	36.2	63.8%	37.0	50.3%
1986	118	0.048	20.3%	123.3	21.4%	34.9	70.7%	35.6	55.7%
1987	116	0.050	20.3%	128.7	20.4%	42.2	62.0%	41.8	50.3%
1988	134	0.054	18.7%	141.7	22.0%	58.0	50.5%	55.6	45.0%
1989	158	0.047	18.6%	127.8	22.5%	32.7	87.8%	35.2	64.1%
1990	172	0.049	19.8%	129.4	22.7%	34.6	82.7%	37.0	62.1%
1991	190	0.050	19.8%	130.6	23.6%	36.8	80.7%	38.8	62.9%
Among Years		0.049	9.8%	129.0	9.9%	38.7	42.5%	39.6	29.8%

[a]Updated and additional years from data given in Table III of Tingey et al. (1991), where the spatial and temporal variation in ambient O_3 exposures is expressed in terms of several exposure indices.
[b]Indicates the number of separate monitoring sites included in the analysis; fewer sites had 5 mo of available data than had 3 mo of available data.
[c]The 2HDM index is calculated for sites with at least 3 mo of available data. SUM00, SUM06, M7, SIGMOID, and 2HDM are the cumulative sum above 0.0 ppm, the cumulative sum above 0.06 ppm, the 7-h seasonal mean, the sigmoid weighted summed concentration, and the second highest daily maximum 1-h concentration, respectively.
[d]CV = coefficient of variation.

Source: Tingey et al. (1991).

Table 5-21. Comparison of Exposure-Response Curves Calculated Using the 3-Month, 24-Hour SUM06 Values for 54 National Crop Loss Assessment Network Cases[a]

Species	Cultivar	Moisture[b]	Wiebull/Linwear Model Parameters[c] A	B	C	RMSE[d]	R^2[e]	3 mo 24-h SUM06[f] Values for Yield Losses of 10%	30%
Barley (Linear)	CM-72	Dry	7,741.1	−4.412		1,215	0.12	175.5	526.4
Barley (Linear)	CM-72	Wet	8,776.6	15.485		1,175	NA	250.0	250.0
Corn (L)	Pio		9,627.4	92.61	2.823	680	0.93	41.7	64.3
Corn (L)	Pag		10,730.1	94.36	4.316	1,248	0.80	56.0	74.3
Cotton (L)	Acala	Dry	6,465.0	92.59	2.361	1,097	0.45	35.7	59.8
Cotton (L)	Acala	Wet	9,808.0	71.17	1.997	521	0.96	23.1	42.5
Cotton (L)	Acala	Dry	7,009.8	83.78	1.849	949	0.80	24.8	48.0
Cotton (L)	Acala	Wet	7,858.8	78.01	1.311	937	0.85	14.0	35.5
Cotton (L, Linear)	Acala	Dry	5.693	−0.0011		104	0.06	94.9	321.3
Cotton (L, Linear)	Acala	Wet	5.,883	−0.0017		90	0.20	60.3	204.0
Cotton	Stoneville		3,576.1	94.6	2.012	226	0.91	30.9	56.7
Cotton	McNair	Dry	3,698.8	165.81	2.778	342	0.46	73.8	114.4
Cotton	McNair	Wet	4,811.0	117.02	1.534	366	0.89	27.0	59.7
Kidney Bean	California Light Red		2,488.2	27.41	3.885	333	0.72	15.4	21.0
Kidney Bean (L)	California Light Red		2,484.3	44.24	2.691	397	0.71	19.2	30.2
Lettuce (T)	Empire		7,196.6	54.87	5.512	613	0.74	36.5	45.5
Peanut (L)	NC-6		6,402.5	100.12	2.226	351	0.97	36.4	63.0
Potato	Norchip		5,900.7	93.84	1.000	742	0.63	9.9	33.5
Potato	Norchip		5,755.6	79.26	1.654	675	0.49	20.3	42.5
Sorghum	Dekalb		8,046.2	178.05	2.338	441	0.48	68.0	114.6
Soybean	Corsoy		2,652.6	57.1	1.726	166	0.91	15.5	31.4
Soybean	Corsoy		1,891.7	65.21	5.160	282	0.63	42.2	53.4
Soybean	Amsoy		1,907.2	75.91	2.739	390	0.41	33.4	52.1
Soybean	Pella		2,619.9	174.13	1.000	311	0.51	18.3	62.1

Table 5-21 (cont'd). Comparison of Exposure-Response Curves Calculated Using the 3-Month, 24-Hour SUM06 Values for 54 National Crop Loss Assessment Network Cases[a]

			Wiebull/Linwear Model Parameters[c]					3 mo 24-h SUM06[f] Values for Yield Losses of	
Species	Cultivar	Moisture[b]	A	B	C	RMSE[d]	R^2[e]	10%	30%
Soybean	Williams		2,368.4	146.37	1.000	527	0.27	15.4	52.2
Soybean	Corsoy	Dry	2,229.8	92.0	9.593	193	0.16	72.8	82.6
Soybean	Corsoy	Wet	2,913.8	311.04	1.527	330	0.38	71.3	158.4
Soybean	Corsoy	Dry	3,528.1	103.83	15.709	400	0.55	90.0	97.2
Soybean	Corsoy	Wet	4,905.0	117.98	3.590	401	0.80	63.0	88.5
Soybean	Corsoy	Dry	5,676.1	97.46	1.000	508	0.81	10.3	34.8
Soybean	Corsoy	Wet	5,873.9	65.73	1.319	512	0.89	11.9	30.1
Soybean	Williams	Dry	6,305.2	99.18	1.456	389	0.87	21.1	48.8
Soybean	Williams	Wet	7,338.4	78.71	1.344	377	0.94	14.8	36.5
Soybean	Hodgson		2,052.4	79.97	1.000	361	0.78	8.4	28.5
Soybean	Davis		3,929.7	131.57	1.000	524	0.64	13.9	46.9
Soybean	Davis		4,815.5	85.71	1.734	346	0.87	23.4	47.3
Soybean	Davis	Dry	2,007.1	542.36	1.000	556	0.04	57.1	193.4
Soybean	Davis	Wet	4,568.0	158.57	1.539	495	0.61	36.8	81.2
Soybean	Davis	Dry	5,775.6	90.18	3.348	920	0.55	46.0	66.3
Soybean	Davis	Wet	8,082.7	113.89	1.442	927	0.71	23.9	55.7
Soybean	Young	Dry	5,978.8	183.63	1.448	244	0.93	38.8	90.1
Soybean	Young	Wet	7,045.0	145.63	1.277	424	0.93	25.0	65.0
Tobacco (L)	McNair		5,177.4	172.55	1.186	306	0.81	25.9	72.3
Turnip (T)	Just Right		12.7	25.68	1.806	0.810	0.96	7.4	14.5
Turnip (T)	Purple Top		5.7	29.26	1.437	0.590	0.92	6.1	14.3
Turnip (T)	Shogon		4.4	29.18	1.548	0.660	0.81	6.8	15.0
Turnip (T)	Tokyo Cross		11.7	27.83	2.142	3.250	0.78	9.7	17.2
Wheat	Abe		5,149.8	52.89	3.077	399	0.90	25.5	37.8
Wheat	Arthur		4,455.8	60.87	2.176	264	0.92	21.6	37.9

Table 5-21 (cont'd). Comparison of Exposure-Response Curves Calculated Using the 3-Month, 24-Hour SUM06 Values for 54 National Crop Loss Assessment Network Cases[a]

			Wiebull/Linwear Model Parameters[c]					3 mo 24-h SUM06[f] Values for Yield Losses of	
Species	Cultivar	Moisture[b]	A	B	C	RMSE[d]	R^2[e]	10%	30%
Wheat	Roland		5,028.9	52.32	1.173	405	0.91	7.7	21.7
Wheat	Abe		6,043.1	47.39	7.711	226	0.74	35.4	41.5
Wheat	Arthur		5,446.9	72.34	2.462	349	0.57	29.0	47.6
Wheat	Vona		5,384.0	27.74	1.000	608	0.88	2.9	9.9
Wheat	Vona		4,451.0	33.5	1.818	654	0.64	9.7	19.0

[a]See Appendix A for abbreviations and acronyms.
[b]Wet refers to experiments conducted under well-watered conditions, whereas dry refers to experiment conducted under some controlled level of drought stress.
[c]For those studies whose species name is followed by "Linear", a linear model was fit. A Weibull model was fit to all other studies, and estimates of "B" parameter are in parts per million per hour. The yield is expressed in kilograms per hectare for all crops except turnip (grams per meter per plant) and lettuce (grams per meter). In cases where the estimated "C" parameter is exactly 1.0, the shape parameter has been bounded from below to obtain convergence in the nonlinear-model-fitting routine. For those studies whose species name is followed by "L", a log transformation was used to stabilize the variance. For those crops whose name is followed by "T", the yield is expressed as either grams per plant or grams per meter.
[d]The root mean square error, based on individual plot means.
[e]Multiple correlation coefficient (R^2) measures the proportion of total variation about the mean response explained by the regression on individual plot means.
[f]The 24-h SUM06 value (ppm-h) that was predicted to cause a 10 or 30% yield loss (compared to zero SUM06).

Source: Based on analyses by Lee et al. (1991, 1994a,b).

based on 24 h), a 7-h seasonal mean of 0.049 ppm, or a 2HDM of 0.094 ppm would be required. A SUM06 of about 37 ppm·h should limit yield losses to 20% in 50% of the cases. If one standard error were added to or subtracted to account for the variability, the metrics would be reduced to 21 ppm·h, 0.046 ppm, and 0.088 ppm or increased to 27.8 ppm·h, 0.049 ppm, and 0.10 ppm, respectively. To limit the loss to 10% or less in 75% of the cases would require 14.2 ppm·h, 0.040 ppm, and 0.051 ppm, respectively (Table 5-23). These values are based on studies of both well-watered and drought stressed plants.

Further analyses by Lee et al. (1991, 1994a,b) provides composite exposure-response functions for all NCLAN studies, as well as for soybean and wheat experiments (Table 5-22). In the analysis, they calculated the SUM06 based on 24-h/day O_3 concentrations, and the resulting exposure to prevent crops from yield loss is slightly higher than they previously calculated (26.4 ppm·h versus 24.4 ppm·h; Table 5-23).

Table 5-22. Comparison of Exposure-Response Curves Calculated Using the 24-Hour W126 Values for 54 National Crop Loss Assessment Network Cases[a]

Species	Cultivar	Moisture[b]	Weibull[c] A	B	C	RMSE[d]	R^2[e]	24-h W126[f] Values for Yield Losses of 10%	30%
Barley	CM-72	Dry	8,133.2	1,109.6	1.000	1,214	0.13	116.9	395.8
Barley	CM-72	Wet	8,927.2	57,439.6	1.000	1,175	NA	6,051.9	20,487.3
Corn (L)	Pio		9,605.0	92.9	2.594	650	0.93	39.0	62.4
Corn (L)	Pag		10,686.7	94.5	4.190	1,253	0.80	55.2	73.9
Cotton (L)	Acala	Dry	6,482.8	89.9	1.949	1,075	0.47	28.3	53.0
Cotton (L)	Acala	Wet	9,817.3	66.6	1.603	514	0.96	16.4	35.0
Cotton (L)	Acala	Dry	7,022.7	81.3	1.540	948	0.80	18.8	41.6
Cotton (L)	Acala	Wet	7,927.1	74.7	1.070	943	0.85	9.1	28.5
Cotton (L)	Acala	Dry	310.1	174.1	2.189	104	0.06	62.3	108.7
Cotton (L)	Acala	Wet	393.2	582.6	1.000	90	0.20	61.4	207.8
Cotton	Stoneville		3,592.1	94.1	1.582	223	0.91	22.7	49.1
Cotton	McNair	Dry	3,700.9	174.1	2.430	344	0.45	68.9	113.9
Cotton	McNair	Wet	4,817.6	113.5	1.410	360	0.89	23.0	54.6
Kidney bean	California Light Red		2,484.7	28.0	3.706	332	0.72	15.3	21.2
Kidney bean (L)	California Light Red		2,475.2	44.2	2.353	401	0.70	17.0	28.5
Lettuce (T)	Empire		7,197.4	54.6	4.921	614	0.74	34.6	44.3
Peanut (L)	NC-6		6,386.0	97.4	1.905	370	0.96	29.9	56.7
Potato	Norchip		5,867.2	96.3	1.000	754	0.62	10.1	34.3
Potato	Norchip		5,777.9	113.9	1.299	675	0.48	20.1	51.5
Sorghum	Dekalb		8,049.7	205.9	1.963	439	0.48	65.4	121.8
Soybean	Corsoy		2,660.3	58.8	1.455	169	0.91	12.5	28.9
Soybean	Corsoy		1,895.6	63.3	4.032	280	0.63	36.2	49.0
Soybean	Amsoy		1,926.1	79.0	1.977	390	0.41	25.3	46.9
Soybean	Pella		2,602.4	161.5	1.000	314	0.50	17.0	57.6
Soybean	Williams		2,341.8	138.6	1.000	533	0.25	14.6	49.4
Soybean	Corsoy	Dry	2,229.3	88.2	8.632	192	0.16	67.9	78.2

Table 5-22 (cont'd). Comparison of Exposure-Response Curves Calculated Using the 24-Hour W126 Values for 54 National Crop Loss Assessment Network Cases[a]

Species	Cultivar	Moisture[b]	Weibull[c] A	B	C	RMSE[d]	R^2[e]	24-h W126[f] Values for Yield Losses of 10%	30%
Soybean	Corsoy	Wet	2,929.7	470.2	1.128	329	0.39	64.0	188.6
Soybean	Corsoy	Dry	3,533.5	113.2	11.095	403	0.54	92.4	103.1
Soybean	Corsoy	Wet	4,909.5	126.5	2.803	405	0.80	56.7	87.6
Soybean	Corsoy	Dry	5,597.1	95.7	1.000	526	0.80	10.1	34.1
Soybean	Corsoy	Wet	5,884.8	65.6	1.139	515	0.88	9.1	26.6
Soybean	Williams	Dry	6,314.1	106.3	1.243	391	0.87	17.4	46.4
Soybean	Williams	Wet	7,352.3	80.7	1.162	368	0.95	11.6	33.2
Soybean	Hodgson		2,044.6	76.2	1.000	361	0.78	8.0	27.2
Soybean	Davis		3,837.6	130.3	1.000	530	0.63	13.7	46.5
Soybean	Davis		4,810.8	87.5	1.494	352	0.86	19.4	43.9
Soybean	Davis	Dry	1,992.3	537.6	1.000	558	0.03	56.6	191.7
Soybean	Davis	Wet	4,595.4	170.9	1.253	496	0.61	28.4	75.1
Soybean	Davis	Dry	5,770.1	90.6	2.796	928	0.54	40.5	62.7
Soybean	Davis	Wet	8,101.3	118.2	1.220	939	0.70	18.7	50.8
Soybean	Young	Dry	5,994.2	199.8	1.251	244	0.93	33.1	87.7
Soybean	Young	Wet	7,075.0	149.7	1.133	418	0.93	20.5	60.2
Tobacco (L)	McNair		5,223.9	179.8	1.018	291	0.83	19.7	65.3
Turnip (T)	Just Right		12.7	24.1	1.473	1.0	0.96	5.2	12.0
Turnip (T)	Purple Top		5.8	28.2	1.155	1	0.92	4.0	11.6
Turnip (T)	Shogon		4.4	28.2	1.174	1	0.82	4.1	11.7
Turnip (T)	Tokyo Cross		11.7	26.8	1.710	3	0.78	7.2	14.7
Wheat	Abe		5,138.1	53.3	2.602	407	0.89	22.4	35.8
Wheat	Arthur		4,467.4	63.8	1.747	264	0.92	17.6	35.4
Wheat	Rol		5,074.4	51.2	1.000	397	0.91	5.4	18.3
Wheat	Abe		6,042.8	48.5	5.843	225	0.75	33.0	40.6

Table 5-22 (cont'd). Comparison of Exposure-Response Curves Calculated Using the 24-Hour W126 Values for 54 National Crop Loss Assessment Network Cases[a]

			Weibull[c]					24-h W126[f] Values for Yield Losses of	
Species	Cultivar	Moisture[b]	A	B	C	RMSE[d]	R^2[e]	10%	30%
Wheat	Arthur		5,440.0	76.1	2.100	349	0.57	26.1	46.6
Wheat	Vona		5,300.8	25.0	1.000	679	0.85	2.6	8.9
Wheat	Vona		4,462.7	32.3	1.517	665	0.63	7.3	16.4

[a]See Appendix A for abbreviations and acronyms.
[b]Wet refers to experiments conducted under well-watered conditions, whereas dry refers to experiments conducted under some controlled level of drought.
[c]All estimates of "B" parameter are in parts per million per hour. The yield is expressed in kilograms per hectare for all crops except turnip (grams per plant) and lettuce (grams per meter). In cases where the estimated "C" parameter is exactly 1.0, the shape parameter has been bounded from below to obtain convergence in the nonlinear-model-fitting routine. For those studies whose species name is followed by "L", a log transformation was used to stabilize the variance. For those crops whose name is followed by "T", the yield is expressed as either grams per plant or grams per meter.
[d]The root mean square error, based on individual plot means.
[e]Multiple correlation coefficient (R^2) measures the proportion of total variation about the mean response explained by the regression on individual plot means.
[f]The 24-h W126 value (parts per million per hour) that was predicted to cause a 10 or 30% yield loss (compared to zero W126).

Source: Based on analyses by Lee et al. (1991, 1994a,b).

Research since 1986 has focused largely on understanding the response of trees and other perennials to O_3 (covered in the next section) and of five crop species: (1) cotton, (2) wheat, (3) spring rape, (4) bean, and (5) soybean. A number of the studies were conducted as part of NCLAN, but many also were the result of research activity in Europe. Results of these studies, as well as those species studied less intensively, are summarized in Table 5-25. A composite exposure-response function is illustrated in Figure 5-29.

Yield losses in cotton of 13 to 19% have been reported at 12-h mean concentrations of 0.050 or 0.044 ppm by Heagle et al. (1988a) and Temple et al. (1988b) (Table 5-25). These are typical ambient concentrations, as listed under M7 (Table 5-20). The same experiments showed that drought stress reduced the predicted yield loss due to O_3, but did not eliminate it.

Wheat yields have been reduced by 0 to 29%, depending on the cultivar and exposure conditions (Adaros et al., 1991a; Fuhrer et al., 1989; Grandjean and Fuhrer, 1989; Kohut et al., 1987; Pleijel et al., 1991) (Table 5-25). In no case was a 7-h average of greater than 0.062 ppm required to cause the reported loss, but Slaughter et al. (1989) suggest that hourly concentrations above 0.06 ppm during the period following anthesis may be particularly effective in reducing yield.

Table 5-23. The Exposure Levels (Using Various Indices) Estimated To Cause at Least 10% Crop Loss in 50 and 75% of Experimental Cases[a]

50th PERCENTILE[b]	SUM06	SE[c]	SIGMOID	SE	M7	SE	2HDM	SE
NCLAN Data (N = 49; wet and dry)[d]	24.4	3.4	21.5	2.0	0.049	0.003	0.094	0.006
NCLAN Data (N = 39; wet only)	22.3	1.0	19.4	2.3	0.046	0.003	0.090	0.010
NCLAN Data (N = 54; wet and dry)[e]	26.4	3.2	23.5	2.4	NA	NA	0.099	0.011
NCLAN Data (N = 42; wet only)[e]	23.4	3.1	22.9	4.7	NA	NA	0.089	0.008
NCLAN Data (N = 10; wet)	25.9	4.5	23.4	3.2	0.041	0.001	0.110	0.042
NCLAN Data (N = 10; dry)	45.7	23.3	40.6	0.1	0.059	0.014	0.119	0.017
Cotton Data (N = 5)	23.6	2.3	19.3	2.3	0.041	0.001	0.066	0.032
Soybean Data (N = 13)	26.2	5.4	22.6	3.6	0.044	0.005	0.085	0.013
Wheat Data (N = 6)	21.3	15.2	19.3	12.7	0.061	0.018	0.098	0.059
Cotton Data (N = 5)[e]	30.0	12.7	27.2	12.8	NA	NA	0.075	0.012
Soybean Data (N = 15)[e]	23.9	6.5	22.0	8.0	NA	NA	0.088	0.008
Wheat Data (N = 7)[e]	25.9	10.5	21.4	9.4	NA	NA	0.097	0.028
75th PERCENTILE[b]								
NCLAN Data (N = 49; wet and dry)	14.2	4.2	11.9	5.6	0.040	0.007	0.051	0.010
NCLAN Data (N = 39; wet only)	14.3	2.7	12.6	2.3	0.039	0.005	0.056	0.006
NCLAN Data (N = 54; wet and dry)[e]	16.5	4.3	14.5	3.2	NA	NA	0.073	0.006
NCLAN Data (N = 42; wet only)[e]	17.2	3.0	14.7	2.4	NA	NA	0.070	0.006
NCLAN Data (N = 10; wet)	16.4	3.7	13.7	3.2	0.040	0.001	0.080	0.032
NCLAN Data (N = 10; dry)	24.0	0.8	22.3	0.1	0.053	0.022	0.093	0.003
Cotton Data (N = 5)	21.8	5.0	17.5	2.8	0.041	0.001	0.065	0.014
Soybean Data (N = 13)	14.2	0.1	12.4	0.1	0.041	0.006	0.069	0.004
Wheat Data (N = 6)	11.7	2.5	10.9	2.4	0.054	0.032	0.062	0.035
Cotton Data (N = 5)[e]	21.1	6.0	16.7	5.7	NA	NA	0.070	0.034
Soybean Data (N = 15)[e]	15.3	4.1	13.4	4.1	NA	NA	0.078	0.007
Wheat Data (N = 7)[e]	5.1	2.6	8.5	3.4	NA	NA	0.054	0.027

[a]See Appendix A for abbreviations and acronyms.
[b]The numbers in parentheses are the number of cases used in deriving the various exposure levels.
[c]Standard error (SE).
[d]NCLAN data refers to studies conducted as part of the NCLAN project. Wet and dry refer to watery regimes used in the studies, wet being well-watered, and dry meaning some level of drought stress was imposed.
[e]24-h exposure statistics reported in Lee et al. (1994b). Relative yield loss for 2HDM is relative to yield at 0.04 ppm rather than 0.00 ppm as was used in Tingey et al. (1991).

Source: Modified from Tingey et al. (1991).

Studies with spring rape in Europe have documented yield losses of 9.5 to 26.9% at 8-h growing season average concentrations ranging from 0.03 to 0.06 ppm (Adaros et al., 1991b,c) (Table 5-26).

The yield of beans (fresh pods) was reduced by 17% at a 7-h average of 0.045 ppm (Schenone et al., 1992) or 20% at an 8-h growing season average of 0.080 ppm (Bender et al., 1990). In a similar study, Heck et al. (1988) found that the predicted yield of sensitive cultivars was reduced an average of 17.3% by exposure to a 7-h growing season

Table 5-24. SUM06 Levels Associated with 10 and 20% Yield Loss for 50 and 75% of the National Crop Loss Assessment Network (NCLAN) Crop Studies[a]

Weibull Equations (all 54 NCLAN studies):
50th Percentile[b] PRYL = 1 − exp(−[SUM06/89.497]**1.84461)
75th Percentile PRYL = 1 − exp(−[SUM06/60.901]**1.72020)

Weibull Equations (all 22 NCLAN soybean studies; 15 well-watered, 7 water-stress):
50th Percentile PRYL = 1 − exp(−[SUM06/117.68]**1.46509)
75th Percentile PRYL = 1 − exp(−[SUM06/88.99]**1.47115)

Weibull Equations (15 NCLAN well-watered soybean studies):
50th Percentile PRYL = 1 − exp(−[SUM06/112.75]**1.46150)
75th Percentile PRYL = 1 − exp(−[SUM06/79.62]**1.36037)

Weibull Equations (7 NCLAN wheat studies):
50th Percentile PRYL = 1 − exp(−[SUM06/49.02]**3.52788)
75th Percentile PRYL = 1 − exp(−[SUM06/29.56]**1.29923)

SUM06 Levels Associated with 10 and 20% Yield Loss for 50 and 75% of the Crops:

All 54 NCLAN Cases

		Percent of Crops	
		50%	75%
Relative	10%	26.4	16.5
Yield Loss	20%	39.7	25.5

All 22 NCLAN Soybean Cases

		Percent of Crops	
		50%	75%
Relative	10%	25.3	19.3
Yield Loss	20%	42.3	32.1

15 Well-Watered Soybean Cases

		Percent of Crops	
		50%	75%
Relative	10%	24.2	15.2
Yield Loss	20%	40.4	26.4

All Seven NCLAN Wheat Cases

		Percent of Crops	
		50%	75%
Relative	10%	25.9	5.2
Yield Loss	20%	32.0	9.3

[a]See Appendix A for abbreviations and acronyms.
[b]50th and 75th percentiles refer to the percentage of studies analyzed in which loss of the stated magnitude would have been prevented.

Source: Based on analyses by Lee et al. (1994b).

Table 5-25. A Summary of Studies Reporting the Effects of Ozone on the Growth, Productivity, or Yield of Annual Plants Published Since U.S. Environmental Protection Agency (1986)[a]

Species	Concentration[b]	Duration	Facility[c]	Variable[d]	Effect[e]	Reference
Soybean	18 or 24 ppb vs. 59 or 72 ppb 9-h mean	13 weeks, two growing seasons	OTC	Seed yield	12.5% reduction over filtered air averaged over cultivars. Between-cultivar differences as great as ozone effect.	Mulchi et al. (1988)
Soybean	23, 40, and 66 ppb 7-h mean	84 days	OTC	Seed yield	15.8 and 29% reduction over 23 ppb.	Mulchi et al. (1992)
Soybean	97 ppb vs. 38, 23, 16, and 23 ppb 7-h mean	Four 31-day periods, one growing season	OTC in pots	Seed yield	30 to 56% reduction over control, most loss in mid- to late-growth stage.	Heagle et al. (1991b)
Soybean	17 to 122 ppb 7-h mean	69 days	OTC	Seed yield	8% at 35 ppb to 41% at 122 ppb.	Kohut et al. (1986)
Soybean	25 and 50 ppb 7-h mean	About 90 days	OTC	Seed yield	Predicted loss of 10%.	Heagle et al. (1986b)
Soybean	20 and 50 ppb 12-h mean	107 days	OTC	Seed yield	Predicted loss of 13%.	Miller et al. (1989b)
Soybean	25 and 55 ppb 7-h mean	64, 70, and 62 days, three growing seasons	OTC	Seed yield	Predicted loss of 15%.	Heggestad and Lesser (1990)
Soybean	27 and 54 ppb 7-h means	About 109 and 103 days, two growing seasons	OTC	Seed yield	Predicted loss of 12 and 14%.	Heagle et al. (1987a)
Soybean	Filtered and nonfiltered air-concentration not reported	About 125 days, two growing seasons	OTC	Seed yield	No difference.	Johnston and Shriner (1986)
Soybean	10 to 130 ppb	8 weeks, 6.8 h/day	GC	Biomass	Predicted reduction of 16 or 33% at 60 and 100 ppb vs. 25 ppb.	Amundson et al. (1986)
Soybean	200 ppb	12 h, up to four times	GC	Shoot and root weight	No effect at maturity.	Smith et al. (1990)

Table 5-25 (cont'd). A Summary of Studies Reporting the Effects of Ozone on the Growth, Productivity, or Yield of Annual Plants Published Since U.S. Environmental Protection Agency (1986)[a]

Species	Concentration[b]	Duration	Facility[c]	Variable[d]	Effect[e]	Reference
Cotton	15 to 111 ppb 12-h mean	123 days	OTC	Leaf, stem, and root weight	Up to 42% reduction in leaf and stem and 61% reduction in root weights.	Temple et al. (1988c)
Cotton	10 to 90 ppb 12-h mean	102 days	OTC	Lint weight	40 to 71% reduction at highest concentration determinant cultivars more susceptible.	Temple (1990b)
Cotton	25 to 74 ppb 12-h mean	123 days	OTC	Lint weight	Predicted loss of 26.2% at 74 ppb.	Temple et al. (1988b)
Cotton	22 to 44 ppb 12-h mean	124 days	OTC	Lint weight	Predicted loss of 19% at 44 ppb.	Heagle et al. (1988a)
Cotton	26 to 104 ppb 7-h mean	119 days	OTC	Lint weight	Predicted loss of 11% at 53 ppb.	Heagle et al. (1986a)
Bean, fresh	35 to 132 ppb 7-h mean	42 days	OTC in pots	Green pod weight	Significant yield reductions of >10% in eight lines at 63 ppb 7-h mean.	Eason and Reinert (1991)
Bean, fresh	11 to 40 ppb 12-h mean, 7 to 42 ppm·h	69 days	OTC	Pod weight	15.5% reduction at 45 ppb (39 ppm·h).	Schenone et al. (1992)
Bean, fresh	26 to 126 ppb 7-h mean	26 days and 44 days, early and late in season	OTC in pots	Pod weight	3.5 to 26% reduction in resistant and sensitive cultivars at 55 to 60 ppb.	Heck et al. (1988)
Bean, fresh	24 to 109 ppb 8-h mean	43 days 34 days, two growing seasons	OTC	Pod weight	20% reduction at 80 ppb.	Bender et al. (1990)
Bean, dry	15 to 116 ppb 12-h mean, 339 ppb highest hour	54 days	OTC	Seed yield	55 to 75% reduction at 72 ppb 12-h mean, 198 highest hour.	Temple (1991)

Table 5-25 (cont'd). A Summary of Studies Reporting the Effects of Ozone on the Growth, Productivity, or Yield of Annual Plants Published Since U.S. Environmental Protection Agency (1986)[a]

Species	Concentration[b]	Duration	Facility[c]	Variable[d]	Effect[e]	Reference
Bean, dry	10 to 50 ppb 7-h mean	86 days	OTC	Seed weight	26 to 42% reduction at 38 to 50 ppb.	Sanders et al. (1992)
Bean, dry	300 ppb	3 h, two exposures	GC	Dry weight	Growth response detected if exposure separated by 3 to 5 days.	McCool et al. (1988)
Wheat, spring	14 to 46 ppb 24-h mean	79, 92, and 79 days in three growing seasons	OTC	Seed weight	13% reduction at 40 ppb.	Fuhrer et al. (1989)
Wheat, spring	21.6 to 80 and 24.6 to 93.5 ppm·h	82 and 88 days in two growing seasons	OTC	Seed weight	48 to 54% reduction at 80 and 93.5 ppm·h.	Grandjean and Fuhrer (1989)
Wheat, spring	3 to 56 ppb 7-h mean	61 and 55 days in two growing seasons	OTC	Seed weight	7% reduction at 15 and 22 ppb.	Pleijel et al. (1991)
Wheat, spring	8 to 101 and 20 to 221 ppb 8-h mean	118 and 98 days in two growing seasons	OTC	Seed weight	10% reduction at 17 to 23 ppb.	Adaros et al. (1991a)
Wheat, spring	0 to 38 ppb 8-h mean	Entire growing season	OTC	Seed weight	5% reduction at 38 ppb.	De Temmerman et al. (1992)
Wheat, spring	17 to 77 ppb 7-h mean	90 and 87 days in two growing seasons	OTC	Seed weight	9.5 to 11.6 reduction at 37 and 45 ppb.	Fuhrer et al. (1992)
Wheat, spring	25 to 75 ppb 8-h mean	40 days	OTC	Total weight	Reductions at 75 ppb.	Johnsen et al. (1988)
Wheat, spring	6 to 10 ppb, 6 h/day	21 days	GC	Shoot dry weight	Decreased 35 to 60% at 101 ppb in low and high light.	Mortensen (1990b)
Wheat, spring	10 to 125 ppb, 6 h/day	21 and 17 days	GC	Top dry weight	Reduced by up to 35%.	Mortensen (1990c)

Table 5-25 (cont'd). A Summary of Studies Reporting the Effects of Ozone on the Growth, Productivity, or Yield of Annual Plants Published Since U.S. Environmental Protection Agency (1986)[a]

Species	Concentration[b]	Duration	Facility[c]	Variable[d]	Effect[e]	Reference
Wheat, winter	11 to 42 ppb 14-week mean	109 days	OTC	Seed weight	No effect.	Olszyk et al. (1986b)
Wheat, winter	30 to 93 ppb 4-h mean	39 and 40 days in two growing seasons 5 days/week 4 h/day	OTC	Seed weight	Exposures >60 ppb during anthesis reduce yield.	Slaughter et al. (1989)
Wheat, winter	27 to 96 ppb 7-h mean	36 days	OTC	Seed weight/ head	50% reduction at 96 ppb.	Amundson et al. (1987)
Wheat, winter	22 to 96 ppb 7-h mean	65 days and 36 days in two growing seasons	OTC	Seed weight	33 and 22% reductions at 42 and 54 ppb, respectively.	Kohut et al. (1987)
Wheat, winter	23 to 123 ppb 4 h/day	5 days at anthesis	OTC	Seed weight	Up to 28% reduction.	Mulchi et al. (1986)
Barley, spring	6 to 45 ppb 7-h mean	96 days	OTC	Seed weight	No effect.	Pleijel et al. (1992)
Barley, spring	0.6 to 27 ppb monthly mean	Growing season	OTC	Seed weight	No effect.	Weigel et al. (1987)
Barley, spring	0.8 to 83 ppb 8-h mean	97, 108, and 98 days in three growing seasons	OTC in pots	Seed weight	0 to 13% reduction at highest.	Adaros et al. (1991b)
Rape, spring	25 to 75 ppb 8-h mean	31 days	OTC	Premature senescence	Increased at 75 ppb.	Johnsen et al. (1988)
Rape, spring	0.8 to 83 ppb 8-h mean	89, 113, and 84 days in three growing seasons	OTC in pots	Seed weight	9.4 to 16% reduction at 30 or 51 ppb.	Adaros et al. (1991b)

Table 5-25 (cont'd). A Summary of Studies Reporting the Effects of Ozone on the Growth, Productivity, or Yield of Annual Plants Published Since U.S. Environmental Protection Agency (1986)[a]

Species	Concentration[b]	Duration	Facility[c]	Variable[d]	Effect[e]	Reference
Rape, spring	43 to 60 ppb 8-h mean	89, 113, and 84 days in three growing seasons	OTC in pots	Seed weight	12 to 27% reduction.	Adaros et al. (1991c)
Tomato	13 to 0.109 ppm 12-h mean, 79.5 ppm-h	75 days	OTC	Fresh weight	17 to 54% reduction at 0.109 ppm; no reduction at ambient.	Temple (1990a)
Tomato	10 to 85 ppb, 6 h/day	12 to 21 days	GC	Shoot dry weight	35 to 62% reduction.	Mortensen (1992b)
Tomato	18 to 66 ppb 12-h mean	11 weeks	OTC	Fresh fruit weight	No effect.	Takemoto et al. (1988c)
Moss campion	5 to 80 ppb, 8 h/day	Up to 90 days	GC	Dry weight	25% reduction at 80 ppb.	Mortensen and Nilsen (1992)
Buckhorn	5 to 80 ppb, 8 h/day	Up to 90 days	GC	Dry weight	14% reduction at 50 ppb.	Mortensen and Nilsen (1992)
16 Other species	5 to 80 ppb, 8 h/day	Up to 90 days	GC	Dry weight	No effect.	Mortensen and Nilsen (1992)
Radish	20 or 70 ppb 24-h mean	27 days	GC	Shoot and root growth	36 and 45% reduction at 70 ppb.	Barnes and Pfirrman (1992)
Lettuce	21 to 128 ppb 7-h mean	52 days	OTC	Head weight	Significant reduction at 83 ppb, 35% at 128 ppb.	Temple et al. (1986)
Lettuce	10 to 34 ppb 7-week mean	64 days	OTC	Fresh weight	No effect.	Olszyk et al. (1986b)
Faba bean	6 or 15 ppb 24-h mean	134 days	OTC	Seed weight	No effect.	Sanders et al. (1990)
Fenugreek	120 ppb, 7 h/day	4 weeks	CC	Dry weight	No significant effect.	Kasana (1991)

Table 5-25 (cont'd). A Summary of Studies Reporting the Effects of Ozone on the Growth, Productivity, or Yield of Annual Plants Published Since U.S. Environmental Protection Agency (1986)[a]

Species	Concentration[b]	Duration	Facility[c]	Variable[d]	Effect[e]	Reference
Chickpea	120 ppb, 7 h/day	4 weeks	CC	Dry weight	No significant effect.	Kasana (1991)
Gram, black	120 ppb, 7 h/day	4 weeks	CC	Dry weight	No significant effect.	Kasana (1991)
Rice	0 to 200 ppb, 5 h/day	5 days/week 15 weeks	OTC	Seed weight	12 to 21% reduction at 200 ppb.	Kats et al. (1985)
Rice	50 ppb 24-h mean	8 weeks	GC	Dry weight	No effect at 50 ppb.	Nouchi et al. (1991)
Watermelon	15 to 27 ppb 7-h mean	81 days	OTC	Fresh weight and number (marketable)	20.8 and 21.5% reduction at 27 ppb.	Snyder et al. (1991)
Pea	10 to 35 ppb 12-h mean	58 and 52 days in two growing seasons	OF	Fresh weight	Linear decrease in yield with increasing O_3.	Runeckles et al. (1990)
Green pepper	19 to 66 ppb 12-h mean	77 days	OTC	Fresh fruit weight	12% reduction at 66 ppb.	Takemoto et al. (1988c)
Green pepper	18 to 66 ppb 12-h mean	11 weeks	OTC	Fresh fruit weight	13% reduction in fruit weight at 66 ppb.	Takemoto et al. (1988c)
Celery	18 to 66 ppb 12-h mean	11 weeks	OTC	Shoot dy weight	12% reduction at 66 ppb.	Takemoto et al. (1988c)

[a]See Appendix A for abbreviations and acronyms.
[b]Means are seasonal means unless specified. Maximums are 1-h seasonal maxima unless otherwise specified. Cumulative exposures are SUM00 unless otherwise specified; accumulation based on 24 h/day unless otherwise noted.
[c]OTC = open-top chamber with plants in ground unless specified in pots; CC = closed chamber, outside; GC = controlled environment growth chamber or CSTR; OF = open-field fumigation.
[d]The effect reported in the study that is a measure of growth, yield, or productivity.
[e]Effect measured at specified ozone concentration, over the range specified under concentration, or predicted (if specified) to occur based on relationships developed in the experiment.

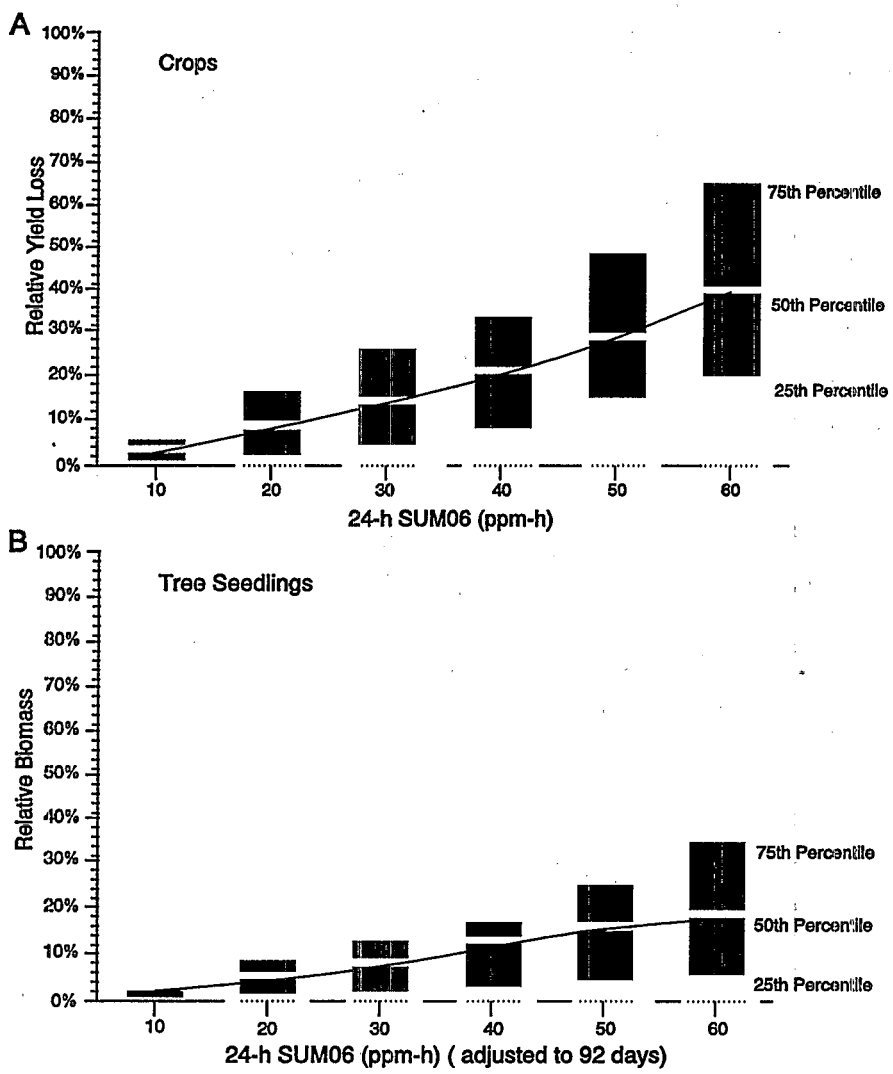

Figure 5-29. ***Box-plot distribution of biomass loss predictions from Weibull and linear exposure-response models that relate biomass and ozone exposure as characterized by the 24-h SUM06 statistic using data from (A) 31 crop studies from National Crop Loss Assessment Network (NCLAN) and (B) 26 tree seedling studies conducted at U.S. Environmental Protection Agency's Environmental Research Laboratory in Corvallis, OR; Smoky Mountains National Park, TN; Michigan; Ohio; and Alabama. Separate regressions were calculated for studies with multiple harvests or cultivars, resulting in a total of 54 individual equations from the 31 NCLAN studies and 56 equations from the 26 seedling studies. Each equation was used to calculate the predicted relative yield or biomass loss at 10, 20, 30, 40, 50, and 60 ppm·h, and the distributions of the resulting loss were plotted. The solid line is the calculated Weibull fit at the 50th percentile (from Hogsett et al., 1995).***

Table 5-26. A Summary of Studies Reporting the Effects of Ozone on the Growth, Productivity, or Yield of Perennial Crop Plants Published Since U.S. Environmental Protection Agency (1986)[a]

Species	Concentration[b]	Duration	Facility[c]	Variable[d]	Effect[e]	Reference
Strawberry	18 to 66 ppb 12-h mean	11 weeks	OTC	Fresh fruit weight	20% increase in fruit weight at 66 ppb.	Takemoto et al. (1988c)
Timothy	10 to 55 ppb 7-h mean	5 weeks	GC	Shoot dry weight	45% reduction at 55 ppb.	Mortensen (1992a)
Orchard grass	10 to 55 ppb 7-h mean	5 weeks	GC	Shoot dry weight	28% reduction at 55 ppb.	Mortensen (1992a)
Kentucky blue grass	10 to 55 ppb 7-h mean	5 weeks	GC	Shoot dry weight	28% reduction at 55 ppb.	Mortensen (1992a)
Red grass	10 to 55 ppb 7-h mean	5 weeks	GC	Shoot dry weight	23% reduction at 55 ppb.	Mortensen (1992a)
Tall fescue	10 to 55 ppb 7-h mean	5 weeks	GC	Shoot dry weight	16% reduction at 55 ppb.	Mortensen (1992a)
Colonial bent grass	10 to 55 ppb 7-h mean	5 weeks	GC	Shoot dry weight	No effect.	Mortensen (1992a)
Rye grass	62 ppb 7-h mean	5 weeks	GC	Shoot dry weight	No effect.	Mortensen (1992a)
Red clover	6 to 59 ppb 7-h mean	5 weeks	GC	Shoot dry weight	30% reduction at 59 ppb.	Mortensen (1992a)
Common plantain	70 ppb 7-h mean	8 weeks	GC	Total dry weight	Reduced up to 36% depending on growth stage.	Reiling and Davison (1992c)
Red clover	19 to 62 ppb 12-h mean	83 and 91 days in two growing seasons	OTC	Dry weight	11% reduction at 62 ppb.	Kohut et al. (1988a)
Timothy	19 to 62 ppb 12-h mean	83 and 91 days in two growing seasons	OTC	Dry weight	No effect.	Kohut et al. (1988a)

Table 5-26 (cont'd). A Summary of Studies Reporting the Effects of Ozone on the Growth, Poductivity, or Yield of Perennial Crop Plants Published Since U.S. Environmental Protection Agency (1986)[a]

Species	Concentration[b]	Duration	Facility[c]	Variable[d]	Effect[e]	Reference
Ladino clover-tall fescue pasture	22 to 114 ppb 12-h mean	Five 3- to 4-week exposure periods. Six 3- to 4-week exposures in 2 years	OTC	Shoot dry weight, root dry weight	18 to 50% reduction in shoot dry weight (SDW) at 40 to 47 ppb clover; 25% reduction root dry weight at 40 to 47 ppb. SDW increased by up to 50% in fescue.	Rebbeck et al. (1988)
Ladino clover	28 to 46 ppb 12-h mean	180 and 191 days in two growing seasons	OTC	Dry weight	Predicted yield of mix reduced 10%, with 19% decrease in clover and 19% increase in fescue at 46 ppb.	Heagle et al. (1989b)
Alfalfa	14 to 98 ppb 12-h mean	32 days	OTC	Dry weight	2.4% reduction at 40 ppb, 18.3% reduction at 66 ppb.	Temple et al. (1987)
Alfalfa	20 to 53 ppb 12-h mean	11 weeks	OTC	Dry weight	22% reduction at 53 ppb.	Takemoto et al. (1988a)
Alfalfa	18 to 66 ppb 12-h mean	11 weeks	OTC	Shoot dry weight	22% reduction at 36 ppb.	Takemoto et al. (1988c)
Alfalfa	10 to 109 ppb 12-h mean	208 and 200 days in two growing seasons	OTC	Dry weight	0 to 25% reduction at levels of 38 ppb and above.	Temple et al. (1988a)
Alfalfa	60 to 80 ppb 6-h day	5 days/week for 8 weeks	GH	Relative growth rate	Reduced up to 40% in Saranac.	Cooley and Manning (1988)
Grape	Not reported	Two growing seasons	OTC	Yield	No effects of ambient air vs. filtration.	Musselman et al. (1985)

[a]See Appendix A for abbreviations and acronyms.
[b]Means are seasonal means unless specified. Maximums are 1-h seasonal maxima unless otherwise specified. Cumulative exposures are SUM00 unless otherwise specified; accumulation based on 24 h/day unless otherwise noted.
[c]OTC = open-top chamber with plants in ground unless specified in pots; GC = controlled environment growth chamber or CSTR; GH = greenhouse.
[d]The effect reported in the study that is a measure of growth, yield, or productivity.
[e]Effect measured at specified ozone concentration, over the range specified under concentration, or predicted (if specified) to occur based on relationships developed in the experiment.

mean of 0.05 ppm, but resistant cultivars suffered only a 1.6% loss. Temple (1991) reported reductions in dry bean yield of 44 to 73% in three cultivars grown in California and exposed to a 12-h seasonal mean of 0.072 ppm. One other cultivar increased in yield in NF chambers but was severely affected in higher concentration O_3 treatments. Sanders et al. (1992) also observed yield stimulation at a 7-h growing season mean of 0.025 ppm; however, significant yield reductions were measured as O_3 concentrations increased to 50 ppb (7-h seasonal mean).

Several studies have shown soybean yields to be reduced by 10 to 15% at 7- or 12-h seasonal mean concentrations of 0.05 to 0.055 ppm (Table 5-26; Heagle et al., 1986b, 1987a; Heggestad and Lesser, 1990; Miller et al., 1989b).

A number of the studies cited above and some of those in Table 5-26 were conducted as part of NCLAN and are considered in the discussions of Tingey et al. (1991), Lee et al. (1993), and Lesser et al. (1990), but many of the experiments (primarily those not part of NCLAN) were not included in their analyses. Although the range of variability in species response to O_3 is apparent, these studies support, for the most part, the conclusions of U.S. Environmental Protection Agency (1986), Tingey et al. (1991), and Lesser et al. (1990). Table 5-24 summarizes the studies reporting the response of annual plants, particularly crops, as growth, dry weight, or yield to O_3 exposures (C $\times$ T) under experimental conditions since the previous criteria document (U.S. Environmental Protection Agency, 1986). Based on the results of the studies reviewed in this section, including the reanalysis of NCLAN, exposures for a 3-mo period to O_3 concentrations currently occurring in the ambient air (0.048 to 0.06 ppm, 7-h seasonal mean; see M7, Table 5-20) have been shown to cause losses of 10% or more in the yield of the majority of major crop plants grown in the country. A number of crop species are more sensitive, and greater losses could be expected (Tables 5-21 through 5-25). It should be noted that a variety of methodologies has been used to generate these data. Generally speaking, data obtained through growth chamber experiments and experiments conducted using potted plants, in fact, are more scientifically reliable but less relevant to ambient conditions when assessing the effects of O_3 than are results from field growth plants.

5.6.4 Effects of Ozone on Long-Lived Plants

Quantifying exposure-response in the case of perennial plants (agricultural crops such as pastures, alfalfa, and shrubs and trees) is complicated by they fact that they can receive multi-year exposures and because the results of exposures in a previous year, or over a number of years, may be cumulative. Reduction in growth and productivity, a result of altered carbon allocation, may appear only after a number of years or when carbohydrate reserves are depleted (U.S. Environmental Protection Agency, 1986; Laurence et al., 1993; Garner, 1991; Garner et al., 1989). A further complication is that, in the case of evergreen plants, the life span of a leaf exceeds 1 year and usually persists for several years. In such cases, loss of a leaf or a reduction in photosynthetic performance may have a large effect on a plant's ability to survive and grow. Physiological differences among species (rates of gas exchange, for instance) may have a tendency to equalize exposure over a number of years, however, as shown in Reich's (1987) analysis of crops, hardwoods, and conifers and in Pye's analysis of tree species (1988). Unfortunately, there is little experimental data regarding the effects of long-term O_3 exposure on perennial plants, because only a few experimental studies have extended exposures beyond a single growing season. Most of what

is known regarding the effects of O_3 on mature trees is from field observations. There have been some studies that have extended observation of growth alterations into the season following exposures and, thus, observed "carry-over effects" in several species. Hogsett et al. (1989) reported altered bud elongation in ponderosa pine, lodgepole pine (*Pinus contorta*), and western hemlock (*Tsuga heterphylla*), following a season of O_3 exposure. Altered root regrowth in ponderosa pine in the season following exposure that was correlated with root storage carbohydrate was observed by Andersen et al. (1991). Most studies have used seedlings because of the difficulty of exposing large trees. The extrapolation from seedlings to large trees and to forest stands is not straight-forward and, most likely, will depend on the use of models (Hogsett et al., 1995; Laurence et al., 1993; Taylor and Hanson, 1992). Correlative studies, such as those conducted in the San Bernardino Mountains of California, indicate potentially large impacts on ecosystems (U.S. Environmental Protection Agency, 1986). Cregg et al. (1989), however, point out that notable differences between trees and seedlings are their carbon allocation and use patterns. There is a significantly higher ratio of respiring to photosynthetic tissue in mature trees. This section will address three distinct types of long-lived plants: (1) multiple-year agricultural crops, (2) deciduous shrubs and trees, and (3) evergreen coniferous trees.

5.6.4.1 Perennial Agricultural Crops

Cooley and Manning (1988) conducted a greenhouse study of the response of alfalfa to O_3 applied at 0.06 to 0.08 ppm for 6 h/day, 5 days/week for 8 weeks during 2 different years (to different plants). Ozone treatment reduced the growth and relative growth rate (by about 15 to 20% for tops and 20 to 40% for roots) of plants before cutting, when compared to a filtered-air control. The growth of roots was affected more than the growth of tops, with a shift in the allocation pattern. In the second year of the study, O_3 exposure was continued after the plants were harvested and the impact of exposure on regrowth was determined. In this case, they found that the relative growth rate in O_3 exposed plants was higher, perhaps because of an increased demand for carbon by the root systems of the O_3-stressed plants. It is unclear whether these plants would sustain their increased growth, and, in fact, the authors speculate that the increased growth, in lieu of partitioning carbon to other compounds, might alter the cold hardiness of the plants.

Ozone has been demonstrated to affect the growth of field grown alfalfa. Temple et al. (1988a) reported a 2-year study of alfalfa in which O_3 at ambient concentrations (0.049 in 1984 and 0.042 ppm in 1985 for the seasonal 12-h means, April to October) did not affect the growth and yield of the plants, but at 12-h seasonal means of 0.063 and 0.078 ppm, yield was reduced by about 15 and 19%, respectively. The exposure-response functions for the 2 years were homogeneous; there was no indication of cumulative effect of O_3 exposure; however, crown weight (an indicator of health and vigor) of exposed plants was reduced significantly.

In a different field experiment conducted to determine the interactive effects of O_3 and simulated acid fog on stomatal conductance, photosynthesis, foliar injury, and yield of an established stand of alfalfa, plants were exposed 12 h daily for 4 weeks (Temple et al., 1987). Ozone was added in proportion to its concentration in the ambient air. Ambient O_3 concentrations during the experiment were 0.043 ppm. Ozone injury symptoms appeared on the alfalfa exposed to 0.098 ppm (NF $\times$ 2.0), 1 week after the start of the regrowth period. When exposures were at 0.081 and 0.066 ppm (NF $\times$ 1.7 and NF $\times$ 1.3), more than a week was required for injury to appear. A 1-mo exposure of the plants at the end of

the growing season resulted in a reduction of about 2.5% in aboveground yield at a 12-h seasonal mean concentration of 0.04 ppm. At a concentration of 0.066 ppm, the exposure resulted in a reduction in yield of approximately 18%. It should be noted that the whole plant was exposed to ambient O_3 for the growing season, only new leaves that had developed after harvest received the 1-mo exposure. Ozone exposures could shorten the productive life of alfalfa stands, in addition to its affecting yield.

Kohut et al. (1988a) and Heagle et al. (1989b) experimented with forage mixtures characteristic of the northeast and southeast, respectively. In both cases, exposure to O_3 resulted in a reduction in total forage yield of about 10 to 20% at 12-h seasonal mean O_3 concentrations of 0.045 to 0.05 ppm. In both cases, the clover component of the mix was more sensitive than the grass and was reduced in prevalence in the stand. The relevance of these studies to competition and species composition is discussed in the section on ecosystem response (Section 5.7).

Results of studies on perennial plants conducted since 1986 are summarized in Table 5-26. As with single-season agricultural crops, yields of multiple-year forage crops are reduced at concentrations at or near ambient (0.05 to 0.06 ppm for 5 weeks) in many parts of the country.

5.6.4.2 Effects of Ozone on Deciduous Shrubs and Trees

Most of the information concerning the response of deciduous shrubs and trees to episodes or season-long or multiple-year exposures to O_3 is based on field observations. The longevity of perennial plants and their size, in the case of trees, makes their study under experimental conditions difficult. For this reason, there is little experimental data concerning the response of deciduous shrubs and trees.

Trees, because of their size, are difficult to study under controlled conditions, therefore, most experiments have used seedlings in pots or in OTCs. Most of the hardwood experiments included in Reich's analysis (1987), for example, were exposed under laboratory or greenhouse conditions to relatively high concentrations for short periods of time. Although exposure durations of weeks were used, square-wave exposure regimes that do not capture important characteristics of ambient exposure were used. In addition, in Pye (1988), the majority of the studies were conducted in a laboratory or greenhouse. The results of a few OTC studies are cited; however, the majority of these studies used O_3 concentrations of 0.10 ppm or higher, a condition found only during peak exposures in the ambient air. Although the studies reported in the previous criteria document (U.S. Environmental Protection Agency, 1986) (see Section 5.6.2) support the sensitivity of the seedlings of some species grown in chambers, little information of value with regard to tree growth or biomass production in the long-term can be extrapolated from the experiments. Since 1986, a number of studies have been conducted documenting the sensitivity of hardwoods to O_3 (Table 5-27). Some species, such as black cherry, are very sensitive, although great variability in foliar injury was observed among individual trees, indicating that sensitivity varies greatly within species (Davis and Skelly, 1992a,b; Simini et al., 1992). No significant reductions in basal diameter and height growth were observed during the 3 years of the study, although growth was reduced during 1988 at two sites where O_3 concentrations exceeded 0.12 ppm (Simini et al., 1992), with SUM06 exposures as low as 12.9 ppm·h over 92 days (concentrations not given) predicted to cause a 10% yield loss (Hogsett et al., 1995; Table 5-28).

Based on studies previously reviewed, the growth of some hardwood species, particularly those of the genus *Populus*, may be affected by ambient concentrations of

Table 5-27. A Summary of Studies Reporting the Effects of Ozone on the Growth or Productivity of Deciduous Shrubs and Trees Published Since U.S. Environmental Protection Agency (1986)[a]

Species	Concentration[b]	Duration	Facility[c]	Variable[d]	Effect[e]	Reference
Almond	38 to 112 ppb 12-h mean	153 days	OTC	Total dry weight	Linear reduction in two cultivars, no effect in three.	Retzlaff et al. (1992a)
Almond	30 to 117 ppb 12-h mean	3.5 mo	OTC	Cross-sectional area	6% reduction at 51 ppb.	Retzlaff et al. (1991)
Almond	250 ppb, 4 h/week	16 weeks in each of two growing seasons	CC	Net growth	28 and 36% reduction in years 1 and 2.	McCool and Musselman (1990)
Plum	44 to 111 ppb 12-h mean	191 and 213 days	OTC	Number of fruit per tree	29% fewer fruit at ambient and above.	Retzlaff et al. (1992b)
Plum	30 to 117 ppb 12-h mean	3.5 mo	OTC	Cross-sectional area	19% reduction at 51 ppb.	Retzlaff et al. (1991)
Pear	30 to 117 ppb 12-h mean	3.5 mo	OTC	Cross-sectional area	8% reduction at 51 ppb.	Retzlaff et al. (1991)
Apricot	30 to 117 ppb 12-h mean	3.5 mo	OTC	Cross-sectional area	53% reduction at 117 ppb.	Retzlaff et al. (1991)
Skunk bush	10 to 75 ppb 12-h mean	3 mo	OTC in pots	Growth	Increase in leaf weight in ambient air; no other effect.	Temple (1989)
Black cherry	16 to 67 ppb 12-h mean	Three growing seasons	OTC	Growth and leaf dynamics	Leaf abscission increased with increasing ozone.	Simini et al. (1992)
Black cherry	40 or 80 ppb, 7 h/day, 5 days/week	8 or 12 weeks	GC	Growth	Reduced leaf, stem, and root dry weight, and height at 80 ppb.	Davis and Skelly (1992b)
Red oak	18 to 87 ppm·h 15 to 69 ppb 7-h mean	177 days	OTC	Tree canopy	Reduced 41% at 82 ppm·h or 69 ppb 7-h mean.	Samuelson and Edwards (1993)
Red oak	16 to 67 ppb 12-h mean	Three growing seasons	OTC	Growth and leaf dynamics	No effect.	Simini et al. (1992)

Table 5-27 (cont'd). A Summary of Studies Reporting the Effects of Ozone on the Growth or Productivity of Deciduous Shrubs and Trees Published Since U.S. Environmental Protection Agency (1986)[a]

Species	Concentration[b]	Duration	Facility[c]	Variable[d]	Effect[e]	Reference
Red oak	40 or 80 ppb, 7 h/day, 5 days/week	8 or 12 weeks	GC	Growth	Reduced root dry weight at 80 ppb.	Davis and Skelly (1992b)
Red maple	16 to 67 ppb 12-h mean	Three growing seasons	OTC	Growth and leaf dynamics	No effect.	Simini et al. (1992)
Red maple	40 or 80 ppb, 7 h/day, 5 days/week	8 or 12 weeks	GC	Growth	Reduced stem diameter and dry weight at 80 ppb.	Davis and Skelly (1992b)
Tulip poplar	16 to 67 ppb 12-h mean	Three growing seasons	OTC	Growth and leaf dynamics	Leaf abscission increased with increasing ozone.	Simini et al. (1992)
Yellow poplar	40 or 80 ppb, 7 h/day, 5 days/week	8 or 12 weeks	GC	Growth	Reduced leaf dry weight and stem diameter at 80 ppb.	Davis and Skelly (1992b)
European beech	10 to 90 ppb weekly mean	5 years	OTC	Growth	Reduced shoot growth and leaf area.	Billen et al. (1990)
Aspen	80 ppb, 6 h/day, 3 days/week	70 and 92 days in two growing seasons	OTC	Stem weight	No effect on tolerant clones; 46% reduction for sensitive clones in 1 year 5% (tolerant), and 74% (sensitive) reductions in the second year.	Karnosky et al. (1992b)
Aspen	Filtered air or 80 ppb, 6 h/day, 3 days/week	93 days at two sites in Michigan	OTC	Growth	18 to 26% reduction in diameter growth.	Karnosky et al. (1992a)
Aspen	Ambient + 27, 51, or 102-ppb exposure period mean	105 days	CC	Dry weight	40% reduction; 44% reduction in early growth the following year.	Keller (1988)

Table 5-27 (cont'd). A Summary of Studies Reporting the Effects of Ozone on the Growth or Productivity of Deciduous Shrubs and Trees Published Since U.S. Environmental Protection Agency (1986)[a]

Species	Concentration[b]	Duration	Facility[c]	Variable[d]	Effect[e]	Reference
Yellow poplar	0 to 200 ppb, 8 h/day, 3 days/week	4.5 mo	GC	Growth	Up to a 24% reduction at 200 ppb but moderated by pH treatment.	Jensen and Patton (1990)
Paper birch	60 to 80 ppb, 7 h/day, 5 days/week	12 weeks	GH	Dry weight	Decreased shoot and root weight and leaf area.	Keane and Manning (1988)
Downy birch	25 to 82 ppb, 7 h/day	50 days	GC	Dry weight	Shoot and root dry weight decreased linearly with ozone.	Mortensen and Skre (1990)
Downy birch	25 to 82 ppb, 7 h/day	50 days	GC	Dry weight	Shoot and root dry weight decreased linearly with ozone.	Mortensen and Skre (1990)
Red alder	25 to 82 ppb, 7 h/day	50 days	GC	Dry weight	Shoot and root dry weight decreased linearly with ozone.	Mortensen and Skre (1990)

[a]See Appendix A for abbreviations and acronyms.
[b]Means are seasonal means unless specified. Maximums are 1-h seasonal maxima unless otherwise specified. Cumulative exposures are SUM00 unless otherwise specified, accumulation based on 24 h/day unless otherwise noted.
[c]OTC = open-top chamber with plants in ground unless specified in pots; CC = closed chamber, outside; GC = controlled environment growth chamber or CSTR; GH = greenhouse.
[d]The effect reported in the study that is a measure of growth, yield, or productivity.
[e]Effect measured at specified ozone concentration, over the range specified under concentration, or predicted (if specified) to occur based on relationships developed in the experiment.

Table 5-28. Exposure-Response Equations That Relate Total Biomass (Foliage, Stem, and Root) to 24-Hour SUM06 Exposures (C) Adjusted to 92 Days (ppm-h/year)[a]

Rate of Growth	Habit	Study	Species	Location (State)	Exposure[b] Days	Year	Harvests[c]	Weibull Parameters A	B	C	SUM06 for Loss of[d] 10%	30%
Fast	D	1	Aspen, wild	OR	84	1989	1	9.9	96.3	1.316	19.09	48.21
Fast	D	1	Aspen, wild	OR	84	1989	2	17.7	165.2	1.000	19.06	64.54
Fast	D	2	Aspen, wild	OR	118	1991	1	31.0	130.0	3.062	48.62	72.41
Fast	D	2	Aspen, wild	OR	118	1991	2	75.6	124.9	5.529	64.80	80.79
Fast	D	3	Aspen, wild	OR	112	1990	1	67.8	111.0	6.532	64.60	77.86
Fast	D	3	Aspen, wild	OR	112	1990	2	96.9	142.1	1.257	19.48	51.40
Fast	D	4	Aspen 216	MI	82	1990	1	54.5	121.1	1.609	33.56	71.60
Fast	D	4	Aspen 253	MI	82	1990	1	73.1	265.5	1.000	31.38	106.23
Fast	D	4	Aspen 259	MI	82	1990	1	79.1	92.7	1.000	10.96	37.10
Fast	D	4	Aspen 271	MI	82	1990	1	91.3	44.9	8.964	39.20	44.91
Fast	D	5	Aspen 216	MI	98	1991	1	37.4	128.6	1.000	12.72	43.06
Fast	D	5	Aspen 259	MI	98	1991	1	35.2	95.9	1.000	9.49	32.11
Fast	D	5	Aspen 271	MI	98	1991	1	35.7	73.1	4.012	39.16	53.07
Fast	D	6	Aspen, wild	MI	98	1991	1	19.0	263.1	1.000	26.02	88.08
Slow	E	7	Douglas fir	OR	113	1989-90	1	16.8	462.7	1.844	111.17	215.37
Slow	E	7	Douglas fir	OR	113	1989-90	2	27.9	3.8E+17	1.000	250.00	250.00
Slow	E	7	Douglas fir	OR	234	1989-90	3	33.3	438.9	5.383	113.61	142.49
Slow	E	7	Douglas fir	OR	234	1989-90	4	83.5	2,887.0	1.000	119.61	404.91
Slow	E	8	Douglas fir	OR	118	1991-92	1	26.7	109.5	57.655	82.13	83.88
Slow	E	8	Douglas fir	OR	118	1991-92	2	85.9	-0.0058	(lin)	250.00	250.00
Slow	E	8	Douglas fir	OR	230	1991-92	3	119.1	218.7	12.254	72.80	80.42
Slow	E	9	Ponderosa pine	OR	111	1989	1	12.8	246.9	1.000	21.56	73.00
Slow	E	9	Ponderosa pine	OR	111	1989	2	25.8	365.2	1.000	31.89	107.95
Slow	E	10	Ponderosa pine	OR	113	1989-90	1	12.9	233.7	1.000	20.05	67.87
Slow	E	10	Ponderosa pine	OR	113	1989-90	2	25.7	358.8	1.000	30.77	104.18
Slow	E	10	Ponderosa pine	OR	234	1989-90	3	32.1	327.8	1.000	13.58	45.97
Slow	E	10	Ponderosa pine	OR	234	1989-90	4	90.1	634.3	1.000	26.27	88.94
Slow	E	11	Ponderosa pine	OR	118	1991-92	1	20.2	266.4	1.000	21.88	74.09
Slow	E	11	Ponderosa pine	OR	118	1991-92	2	47.1	206.5	1.000	16.96	57.42
Slow	E	11	Ponderosa pine	OR	230	1991-92	3	44.5	458.5	1.257	30.61	80.77
Slow	E	12	Ponderosa pine	OR	140	1992	1	134.6	235.8	2.570	64.56	103.76
Slow	E	13	Ponderosa pine	OR	84	1991	1	136.0	442.8	1.000	51.10	172.98
Fast	D	14	Red alder	OR	121	1990	1	42.4	217.0	1.427	34.08	80.10
Fast	D	15	Red alder	OR	113	1989	1	84.4	253.0	1.000	21.70	73.46
Fast	D	15	Red alder	OR	113	1989	2	206.8	179.9	5.294	95.76	120.57
Fast	D	16	Red alder	OR	118	1991	1	63.5	501.7	1.000	41.21	139.51
Fast	D	16	Red alder	OR	118	1991	2	248.8	2.0E+13	1.000	250.00	250.00
Fast	D	17	Red alder	OR	112	1992	1	54.1	274.4	1.107	29.50	88.79
Fast	D	18	Black cherry	TN	76	1989	1	53.7	79.1	1.123	12.91	38.23
Fast	D	19	Black cherry	TN	140	1992	1	37.1	176.6	1.168	16.90	48.00
Slow	D	20	Red maple	TN	55	1988	1	28.5	387.1	1.537	149.75	331.07
Fast	D	21	Tulip poplar	TN	75	1990-91	1	45.8	46.4	4.518	34.56	45.27
Fast	D	21	Tulip poplar	TN	184	1990-91	3	334.1	623.5	1.000	32.85	111.19
Fast	D	22	Tulip poplar	TN	81	1992	1	150.1	50.8	1.852	17.12	33.07

Table 5-28 (cont'd). Exposure-Response Equations That Relate Total Biomass (Foliage, Stem, and Root) to 24-Hour SUM06 exposures (C) Adjusted to 92 days (ppm-h/year)[a]

Rate of Growth	Habit	Study	Species	Location (State)	Exposure[b] Days	Year	Harvests[c]	Weibull Parameters A	B	C	SUM06 for Loss of[d] 10%	30%
Fast	E	23	Loblolly GAKR 15-91	AL	555	1988-89	3	22.7	4,402.5	1.000	76.89	260.30
Fast	E	23	Loblolly GAKR 15-23	AL	555	1988-89	3	20.4	13,125.4	1.000	229.24	250.00
Slow	D	24	Sugar maple	MI	83	1990-91	1	4.12	100.0	40.069	104.79	108.03
Slow	D	24	Sugar maple	MI	180	1990-91	3	24.63	110.2	5.987	38.68	47.42
Slow	E	25	Eastern white pine	MI	83	1990-91	1	0.35	63.1	4.191	40.90	54.72
Slow	E	25	Eastern white pine	MI	180	1990-91	3	1.21	719.5	1.000	38.74	131.16
Slow	E	26	Virginia pine	MI	98	1992	1	78.3	3,045.1	1.000	250.00	250.00

[a]See Appendix A for abbreviations and acronyms.
[b]Duration corresponds to the length in days of the first year of exposure for Harvests 1 and 2 and to the total length of the first and second years of exposure for Harvests 3 and 4.
[c]Harvest 1 occurs immediately following the end of the first year of exposure. Harvest 2 occurs in the spring following the first year of exposure. Harvest 3 occurs immediately following the end of the second year of exposure. Harvest 4 occurs in the spring following the second year of exposure.
[d]To compare the results from seedling studies of varying exposure duration, the SUM06 value is calculated for an exposure of fixed period of 92 days per year. For example, Study 1 Harvest 1 has an exposure duration of 84 days and a SUM06 value of 19.09 ppm-h over 92, days which corresponds to a SUM06 value of 19.09*84/92 = 17.43 ppm-h over 84 days, at which biomass loss is 10%. The calculation assumes that exposures can be scaled up or down in uniform fashion.
[e]Based on GIS, TREGRO, and ZELIG models projections. No data given in paper.

Source: Hogsett et al. (1995).

O_3 (U.S. Environmental Protection Agency, 1978, 1986). In studies of the response of aspen clones to O_3 at two field sites in Michigan, Karnosky et al. (1992a,b) documented reductions in stem weight of up to 46% in sensitive aspen clones after 70 days of exposure in OTCs to 0.08 ppm for 6 h/day, 3 days/week.

Tjoelker and Luxmoore (1991) found leaf abscission on tulip poplar (*Liriodendron tulipifera*) seedlings to be increased by exposure to a 7-h seasonal mean concentration of 0.108 ppm, resulting in a doubling of the leaf turnover rate, but this was not translated into an effect on growth, perhaps due to the indeterminate growth habit of the plant. In such plants, leaf production continues throughout the growing season, which may permit the tree to maintain an optimal leaf area; however, continued leaf growth could deplete carbon or nitrogen reserves.

Samuelson and Edwards (1993), in a study to determine if seedlings and trees responded similarly to O_3, found canopy weight of 30-year-old northern red oak, exposed in large OTCs, to be reduced by 41% after exposure for 177 days at a 7-h seasonal mean of 0.069 ppm (87 ppm·h SUM08), compared to a subambient treatment at a 7-h seasonal mean of 0.015 ppm (18 ppm·h SUM00). Two-year-old seedlings were not affected by similar exposures. Trees produced only one flush of leaves, seedlings produced as many as three.

Hogsett et al. (1995) developed exposure-response functions for aspen, red alder (*Alnus rubra*), black cherry (*Prunus serotina*), red maple (*Acer rubrum*), and tulip poplar (Table 5-28), as well as composite functions for deciduous tree seedlings (Table 5-29). Their results suggest that, for 28 deciduous seedling cases, a SUM06 exposure of 31.5 ppm·h over 92 days with a mean concentration of approximately 0.055 ppm could result in less than a

Table 5-29. SUM06 Levels Associated with 10 and 20% Total Biomass Loss for 50 and 75% of the Seedling Studies (The SUM06 value is adjusted to an exposure length of 92 days per year.)[a]

Weibull Equations (all 51 seedling studies):
50th Percentile PRYL = 1 − exp(−[SUM06/176.342]**1.34962)
75th Percentile PRYL = 1 − exp(−[SUM06/104.281]**1.46719)

Weibull Equations (27 fast-growing seedling studies):
50th Percentile PRYL = 1 − exp(−[SUM06/150.636]**1.43220)
75th Percentile PRYL = 1 − exp(−[SUM06/89.983]**1.49261)

Weibull Equations (24 slow to moderate growing seedling studies):
50th Percentile PRYL = 1 − exp(−[SUM06/190.900]**1.49986)
75th Percentile PRYL = 1 − exp(−[SUM06/172.443]**1.14634)

Weibull Equations (28 deciduous seedling studies):
50th Percentile PRYL = 1 − exp(−[SUM06/142.709]**1.48845)
75th Percentile PRYL = 1 − exp(−[SUM06/87.724]**1.53324)

Weibull Equations (23 evergreen seedling studies):
50th Percentile PRYL = 1 − exp(−[SUM06/262.911]**1.23673)
75th Percentile PRYL = 1 − exp(−[SUM06/201.372]**1.01470)

Levels Associated with Prevention of a 10 and 20% Total Biomass Loss for 50 and 75% of the Seedlings:

All 51 Seedling Cases

		Percent of Seedlings	
		50%	75%
Relative	10%	33.3	22.5
Biomass Loss	20%	58.0	37.5

27 Fast-Growing Seedling Cases

		Percent of Seedlings	
		50%	75%
Relative	10%	31.3	19.4
Biomass Loss	20%	52.9	32.4

24 Slow-to-Moderate-Growth Seedling Cases

		Percent of Seedlings	
		50%	75%
Relative	10%	42.6	24.2
Biomass Loss	20%	70.2	46.6

28 Deciduous Seedling Cases

		Percent of Seedlings	
		50%	75%
Relative	10%	31.5	20.2
Biomass Loss	20%	52.1	33.0

Table 5-29 (cont'd). SUM06 Levels Associated with 10 and 20% Total Biomass Loss for 50 and 75% of the Seedling Studies (The SUM06 value is adjusted to an exposure length of 92 days per year.)[a]

23 Evergreen Seedling Cases

		Percent of Seedlings	
		50%	75%
Relative	10%	42.6	21.9
Biomass Loss	20%	78.2	45.9

[a]See Appendix A for abbreviations and acronyms.

Hogsett et al. (1995).

10% growth (biomass) reduction in 50% of the cases. A 20% reduction in growth should result from a SUM06 exposure of greater than 52.1 ppm·h. Comparison with Table 5-20 shows a SUM06 for 3 mo of 29.5 ppm·h at ambient concentrations, a value near that (33.3 ppm·h) expected to prevent a 10% growth reduction in 50% of the cases (Table 5-27). An individual year, such as 1988, might be significantly above the no-injury exposure value (Table 5-20). By further grouping the seedlings by rate of growth (fast or slow), the investigators were able to refine estimates of the SUM06 exposure that would protect seedlings, based on growth strategy. Deciduous seedlings, and fast-growing species are more sensitive than evergreen and slow-growing seedlings (Table 5-27). Seedlings utilize more of the carbon compounds formed during photosynthesis for growth, whereas mature trees use more for maintenance; therefore, extrapolation of exposure response from seedlings to mature trees may lead to inaccurate assumptions.

The response of a number of fruit and nut trees to O_3 has been reported (McCool and Musselman, 1990; Retzlaff et al., 1991, 1992a,b). Almond (*Prunus amygdalis* Batsch) has been identified as the most sensitive, but peach (*Prunus persica*), apricot, pear, and plum (*Prunus domestica*) also have been affected. Net growth of almond, the stem diameter of peach, and the stem diameter and number of shoots produced on apricot were reduced by 4 mo (the exposure duration specified by the authors) of once-weekly exposure to 0.25 ppm for 4 h (an exposure found only in California), a relatively small exposure cumulatively (16 ppm·h as a SUM00 or as a SUM06) (McCool and Musselman, 1990), but one with a high peak value. Cross-sectional area of almond, plum, apricot, and pear stems decreased linearly with increasing O_3, with a significant reduction at a 12-h seasonal mean of 0.051; dry weight of roots, trunk, and foliage also was reduced in one variety of almond (Retzlaff et al., 1992a).

Finally, two studies report the response of citrus and avocado to O_3 (Eissenstat et al., 1991a; Olszyk et al., 1990b). These species retain their leaves for more than 1 year, but fit best in the deciduous category because, although evergreen, leaves are replaced more frequently than in most evergreen species. Valencia orange trees (*Citrus sinensis*), exposed during a production year to a seasonal 12-h mean of 0.04 or 0.075 ppm, had 11 and 31% lower yields than trees grown in filtered air at 0.012 ppm and atypical concentration. During an off-production year, yield was not affected. Growth of Ruby Red grapefruit (*Citrus paradisi*) was not affected by concentrations of three times that of the ambient concentration

(Eissenstat et al., 1991b). Avocado growth was reduced by 20 or 61% by exposure during two growing seasons at 12-h seasonal mean concentrations of 0.068 and 0.096 ppm.

In summary, deciduous trees appear to be less sensitive to O_3 than are most crop plants, but there are species that are as sensitive or more so because of their genetic composition than are crops (e.g., *Populus* species and perhaps black cherry; see discussion in Section 5.4.2). Analysis of the shrub and tree data presented in Table 5-25 and discussed above suggests that a 7-h seasonal mean exposure of approximately 0.055 ppm over a 3-mo period would not result in injury to tree seedlings. However, the absence of multiple-year studies, or studies using older, more mature trees, leaves unanswered the question of long-term and cumulative effects.

5.6.4.3 Effects of Ozone on Evergreen Trees

As with hardwoods, little long-term data from controlled studies of evergreen trees were available at the time the literature was reviewed for the previous criteria document (U.S. Environmental Protection Agency, 1986). The 1986 document did point out, however, that studies conducted on eastern white pine on the Cumberland Plateau in Tennessee indicated that ambient O_3 may have reduced the radial growth of sensitive individuals by as much as 30 to 50% annually over a period of 15 to 20 years (Mann et al., 1980). Also, field studies in the San Bernardino National Forest indicated that, over a period of 30 years, O_3 may have reduced the growth in height of ponderosa pine by as much as 25%, radial growth by 37%, and total volume of wood produced by 84% (Miller et al., 1982). Calculations of biomass in these studies were based on apparent reductions in radial growth without standardization of the radial growth data with respect to tree age. Since 1986, studies on the effects of O_3 on evergreen trees have focused primarily on three species or groups: (1) red spruce in the eastern United States, (2) southern pines (loblolly and slash), and (3) western conifers (primarily ponderosa pine). For the most part, the research has been conducted with tree seedlings or saplings and has involved exposures lasting one to four growing seasons. In many cases, the research has concentrated on defining the mode of action of O_3 in conifers and is discussed elsewhere in this document (Section 5.3). Results of studies with evergreen trees are summarized in Table 5-30.

Studies of the response of red spruce to O_3 exposures, regardless of whether they have been conducted in growth chambers (Lee et al., 1990a,b; Patton et al., 1991; Taylor et al., 1986) or in the field (Kohut et al., 1990; Laurence et al., 1993; Thornton et al., 1992) have failed to detect effects on growth of seedlings or saplings, even after exposure to 12-h seasonal means of up to approximately 0.09 ppm (concentrations that are considerably greater than those expected in ambient air) each year for up to 4 years. There was an indication that total nonstructural carbohydrate content was reduced by O_3, which might be an indicator of cumulative stress (Woodbury et al., 1992). However, results of these studies indicate red spruce is tolerant of O_3, at least for exposures of a few years.

Growth of seedlings of loblolly pine (a much faster growing species than red spruce) has been reduced by O_3 under some conditions. In growth chamber experiments, height growth was reduced after exposure to 0.10 ppm for 4 h/day, 3 days/week for 10 weeks, but only in combination with a "control" rain treatment. The effect was not observed in trees that received significant inputs of potential nutrients in simulated rain. Conversely, Tjoelker and Luxmoore (1991) reported a significant reduction in the weight of current year needles following an OTC exposure to O_3 at a 7-h seasonal mean of 0.056 or 0.108 ppm, only in a high-nitrogen treatment.

Table 5-30. A Summary of Studies Reporting the Effects of Ozone on the Growth or Productivity of Evergreen Trees Published Since U.S. Environmental Protection Agency (1986)[a]

Species	Concentration[b]	Duration	Facility[c]	Variable[d]	Effect[e]	Reference
Avocado	0.010 to 0.108 ppm 12-h mean	4 and 8 mo in two growing seasons	OTC in pots	Leaf mass	20 and 61% reduction in leaf mass at 86 and 108 ppb.	Eissenstat et al. (1991a)
Orange	0.010 to 0.108 ppm 12-h mean	4 and 8 mo in two growing seasons	OTC in pots	Leaf mass	No effect.	Eissenstat et al. (1991a)
Orange	0.012 to 0.075 ppm 12-h mean	7 mo/season for 5 years	OTC	Fruit weight	"On" production year: 11 and 31% reduction at 40 and 75 ppb; "off" year: no effect.	Olszyk et al. (1990b)
Ponderosa pine	0.036 to 0.051 ppm 24-h mean	June to August	F	Radial growth rate	No change in growth rate on symptomatic trees.	Peterson and Arbaugh (1988)
Ponderosa pine	0.013 to 0.095 ppm 12-h mean, 0.047 to 0.0350 ppm·h over 3 years	Three growing seasons	OTC	Growth	19.5% reduction at 95 ppb.	Beyers et al. (1992)
Ponderosa pine	0.011 to 0.087 ppm 12-h mean	Three growing seasons	OTC	Leaf weight	70 and 48% loss of 1- and 2-year-old needles at 87 ppb.	Temple et al. (1993)
Ponderosa pine	5, 122, or 169 ppm·h	112 days	OTC in pots	Root growth	43% reduction in coarse and fine nongrowing roots; 50, 65, and 62% reduction in coarse, fine, and new growing roots, respectively.	Andersen et al. (1991)
Ponderosa pine	0.067 to 0.071 ppm 7-h mean	134 days	OTC in pots	Leaf, stem, and root dry weight	20 to 33% reduction from filtered air at 67 ppb.	Hogsett et al. (1989)
Lodgepole pine	0.067 to 0.071 ppm 7-h mean	134 days	OTC in pots	Leaf, stem, and root dry weight	No effect.	Hogsett et al. (1989)
Jeffrey pine	0 to 0.0200 ppm, 4 h/day, 3 days/week	44 and 58 days in two growing seasons	GC	Root, stem, and needles dry weight	Reduced 10 to 20% ppb in 1 year.	Temple (1988)

Table 5-30 (cont'd). A Summary of Studies Reporting the Effects of Ozone on the Growth or Productivity of Evergreen Trees Published Since U.S. Environmental Protection Agency (1986)[a]

Species	Concentration[b]	Duration	Facility[c]	Variable[d]	Effect[e]	Reference
Jeffrey pine	>0.10 ppm	On 34 days of 1985	F	Radial growth	11% reduction in symptomatic trees.	Peterson et al. (1987)
Western hemlock	0.067 to 0.071 ppm 7-h mean	134 days	OTC in pots	Leaf, stem, and root dry weight	11 to 30% reduction at 71 ppb.	Hogsett et al. (1989)
Western red cedar	0.067 to 0.071 ppm 7-h mean	134 days	OTC in pots	Leaf, stem, and root dry weight	No effect.	Hogsett et al. (1989)
Douglas fir	0.067 to 0.071 ppm 7-h mean	134 days	OTC in pots	Leaf, stem, and root dry weight	No effect.	Hogsett et al. (1988)
Giant sequoia	0-0.0200 ppm, 4 h/day, 3 days/week	44 and 58 days in two growing seasons	GC	Root, stem, and needles dry weight	No effect.	Temple (1988)
Red spruce	0.08 to 0.0166 ppm 8-h mean, 8 to 156 ppm·h	135 days	OTC	Scion growth	No effect on juvenile or mature scion growth.	Rebbeck et al. (1992)
Red spruce	0.023 to 0.087 ppm 12-h mean	Two growing seasons	OTC in pots	Dry weight	No effect.	Kohut et al. (1990)
Red spruce	0.120 ppm, 4 h/day, twice per week	4 mo	GC	Growth	No effect.	Taylor et al. (1986)
Red spruce	0, 0.150 ppm, 6 h/day or 150 ppb, 6 h, plus 70 ppb 18 h/day	195 days	GC	Dry weight	No effect.	Patton et al. (1991)
Red spruce	0.025 or 0.100 ppm, 4 h/day, 3 day/week	10 weeks	GC	Growth	No effect.	Lee et al. (1990b)

Table 5-30 (cont'd). A Summary of Studies Reporting the Effects of Ozone on the Growth or Productivity of Evergreen Trees Published Since U.S. Environmental Protection Agency (1986)[a]

Species	Concentration[b]	Duration	Facility[c]	Variable[d]	Effect[e]	Reference
Red spruce	0.027 to 0.054 ppm 12-h mean	Three growing seasons	OTC in pots	Dry weight, diameter, and height	No effect.	Thornton et al. (1992)
Norway spruce	0.080 to 0.100 ppm, 7 to 8 h/day	100 days	GC	Dry weight	0 to 14% reduction vs. filtered air, five provenances.	Mortensen (1990a)
Norway spruce	0.014 to 0.070 ppm 8-h mean	5 to 6 mo in two growing seasons	OTC in pots	Growth	No effect.	Nast et al. (1993)
Norway spruce	0.010- to 0.090-ppm weekly mean	5 years	OTC	Growth	Reduced lateral shoot growth in last year.	Billen et al. (1990)
Sitka spruce	0.05 to 0.170 ppm, 7 h/day, 5 days/week	65 days	GH	Growth and winter hardiness	No effect on growth, reduced winter hardiness.	Lucas et al. (1988)
Silver fir	0.010- to 0.090-ppm weekly mean	5 years	OTC	Growth	Increased dry matter production.	Billen et al. (1990)
Fraser fir	0.020 to 0.100 ppm, 4 h/day, 3/week	10 weeks	GC	Biomass	No effect.	Tseng et al. (1988)
White pine	0.020 to 0.140 ppm, 7 h/day, 3 day/week	3.5 mo	GC	Dry weight	No effect.	Reich et al. (1987)
Loblolly pine	0.021 to 0.086 ppm 7-h mean	96 days	OTC in pots	Dry weight	18% reduction at 86 ppb; 20% reduction in foliage at 40 or 86 ppb.	Adams et al. (1988)
Loblolly pine	0.021 to 0.117 ppm 7-h mean	Three growing seasons	OTC in pots	Growth	No effect on five families.	Adams et al. (1990b)
Loblolly pine	0.022 to 0.094 ppm 7-h mean	Three growing seasons	OTC in pots	Dry weight	4% reduction at 30 to 38 ppm; 8% reduction at 51 to 65 ppm.	Edwards et al. (1992a)
Loblolly pine	0.032 to 0.108 ppm 7-h mean	18 weeks	OTC in pots	Dry weight	20% reduction in needles at 108 ppm.	Tjoelker and Luxmoore (1991)
Loblolly pine	0.023 to 0.090 ppm 12-h mean, 46 to 0.209 max 12-h	150 days	OTC in pots	Growth	10% reduction at 46 ppm.	Shafer et al. (1987)

Table 5-30 (cont'd). A Summary of Studies Reporting the Effects of Ozone on the Growth or Productivity of Evergreen Trees Published Since U.S. Environmental Protection Agency (1986)[a]

Species	Concentration[b]	Duration	Facility[c]	Variable[d]	Effect[e]	Reference
Loblolly pine	0.022 to 0.092 ppm 12-h mean, 37 to 0.143 ppm 1-h max	Three growing seasons	OTC in pots	Dry weight	0 to 13% reduction after 3 years at about 45 to 50 ppm 12-h seasonal mean, depending on family.	Shafer and Heagle (1989)
Loblolly pine	0.007 to 0.166 ppm 12-h mean, 12-h max 248 ppm	245 days	OTC in pots	Foliar weight	35% reduction at 166 ppm.	Qiu et al. (1992)
Loblolly pine	0.007 to 0.132 ppm 12-h mean 17 to 382 ppm·h	Three growing seasons	OTC	Foliage abscission	Initiated above 130 to 220 ppm·h in trees exposed to ambient or above.	Stow et al. (1992)
Loblolly pine	0.021 to 0.137 ppm 12-h mean 60 to 397 ppm·h	241 days	OTC	Shoot growth	Shoot length reduced 30% at 137 ppm.	Mudano et al. (1992)
Loblolly pine	0.020 to 0.137 ppm 12-h mean 0.050 to 0.286 ppm max 12-h mean	Two growing seasons	OTC	Needle retention and fascicle length	Needle retention decreased in elevated ozone—fascicle length reduced by ozone in early flushes, increased in later flushes.	Kress et al. (1992)
Loblolly pine	0 to 0.150 ppm, 5 h/day, 5 days/week	6 to 12 weeks	GC	Dry weight	8% reduction at 150 ppm.	Meier et al. (1990)
Loblolly pine	0 to 0.320 ppm, 6 h/day, 4 days/week	8 weeks	GC	Height and diameter growth	20% reduction in height growth; 36% reduction in diameter growth in three open-pollinated families.	Horton et al. (1990)
Loblolly pine	0 to 0.120 ppm, 7 h/day, 5 days/week	12 weeks	GC	Dry weight	Top dry weight increased up to 60%; root dry weight reduced 6%.	Spence et al. (1990)
Loblolly pine	0 to 0.320 ppm, 8 h/day, 4 days/week	9 weeks	GC	Relative growth rate (RGR)	36% reduction in height RGR; 10% reduction in diameter RGR.	Wiselogel et al. (1991)

Table 5-30 (cont'd). A Summary of Studies Reporting the Effects of Ozone on the Growth or Productivity of Evergreen Trees Published Since U.S. Environmental Protection Agency (1986)[a]

Species	Concentration[b]	Duration	Facility[c]	Variable[d]	Effect[e]	Reference
Loblolly pine	0.020 to 0.100 ppm, 4 h/day, 3 days/week	10 weeks	GC	Dry weight	No effect.	Lee et al. (1990a)
Slash pine	0.076 to 0.104 ppm 7-h mean 0.126 ppm 1-h max 122 and 155 ppm·h	112 days	GC	Top and root dry weight	18% reduction in top dry weight and 39% reduction in root dry weight at 122 ppm·h.	Hogsett et al. (1985a)
Slash pine	200 to 1,000 ppm·h	28 mo	OTC	Litterfall	Twice as much litterfall at ozone above 220 ppm·h.	Byres et al. (1992)
Slash pine	179 to 443 ppm·h 24-h SUM00 multiples of ambient	28 mo	OTC	Leaf area	Reduced up to 33% by 443 ppm·h.	Dean and Johnson (1992)

[a]See Appendix A for abbreviations and acronyms.
[b]Means are seasonal means unless specified. Maximums are 1-h seasonal maxima unless otherwise specified. Cumulative exposures are SUM00 unless otherwise specified, accumulation based on 24 h/day unless otherwise noted.
[c]OTC = open-top chamber with plants in ground unless specified in pots; GC = controlled-environment growth chamber or CSTR; GH = greenhouse; F = field.
[d]The effect reported in the study that is a measure of growth, yield, or productivity.
[e]Effect measured at specified ozone concentration, over the range specified under concentration, or predicted (if specified) to occur based on relationships developed in the experiment.

Multiple-year OTC exposures of loblolly pine have resulted in decreased foliar weight, partly through accelerated abscission, and decreased root surface area in the first year following exposure to a 2.5-times-ambient O_3 treatment (0.10 ppm 12-h seasonal average, 318 ppm·h) (Qiu et al., 1992). In a 2-year study, Kress et al. (1992) found that fascicle length and number of early season needle flushes decreased linearly with increasing O_3, but the reverse was true in flushes produced later in the season. This may occur only in seedlings that produce more than two leaf flushes per year. Foliage retention decreased with O_3, and fewer fascicles were retained on trees exposed to ambient concentrations of O_3 (12-h seasonal mean of 0.045 ppm averaged over 2 years). Shafer and Heagle (1989) exposed seedlings of four families of loblolly pine to O_3 over three growing seasons and, based on their data, predicted growth suppressions of above ground plant parts of 0 to 19% (depending on the sensitivity of the family) at a 12-h seasonal mean of 0.05 ppm, after 2 years; after 3 years, suppressions of 13% were predicted in the most sensitive family. Cumulative effects of multiple-year exposures were not apparent from the above study, but no measures of root growth, which has been reported to be affected in other species (Andersen et al., 1991; Edwards et al., 1992a; Temple et al., 1993), were reported. Edwards et al. (1992a) also conducted a 3-year exposure and found a 4% reduction in whole plant biomass after exposure to a 7-h seasonal concentration of about 0.050 ppm. An 8% reduction was associated with a 7-h concentration of about 0.10 ppm. Growth reductions occurred in both above- and belowground plant parts.

Many studies with loblolly pine have used multiple families with a range of reported tolerance to O_3 (Adams et al., 1988, 1990b; Kress et al., 1992; Qiu et al., 1992; Shafer and Heagle, 1989; Wiselogel et al., 1991). These studies have demonstrated the range of response, from tolerant to sensitive, in the species. Adams et al. (1990b) suggest that resistance to natural stresses, such as drought, may be linked to tolerance to O_3, thereby affecting the response of the species to multiple stresses.

The response of slash pine to O_3 also has been characterized. Dean and Johnson (1992) found leaf area to be reduced by O_3 in all three growing seasons studied, with an intensification of the effect each year at an O_3 exposure of about 0.03 to 0.04 ppm (12-h seasonal means) or 77 to 216 ppm·h (SUM00). Leaf litterfall also was increased by O_3 (Byres et al., 1992a,b). Volume increment of the trees was affected, with an increased sensitivity to simulated acid rain in trees exposed to twice the ambient concentration. Hogsett et al. (1985a) found reduced height (22%), diameter (25%), top (18%), and root growth (39%) in slash pine exposed to a 7-h seasonal mean of 0.076 ppm, with a maximum concentration of 0.094 ppm. From these studies, it is clear that slash pine is relatively sensitive to O_3 on an annual basis.

Hogsett et al. (1989) report the results of exposing five western conifers to O_3 at a seasonal 7-h mean concentration of 0.067 or 0.071 ppm (SUM06 for 134 days was 49.5 and 63 ppm·h, respectively; SUM00 was 140 and 153 ppm·h, respectively). Ponderosa pine and western hemlock had reduced needle, stem, and root dry weight after 134 days of exposure. Douglas fir (*Pseudotsuga menziesii*) and western red cedar (*Thuja plicata* D. Don) were not different from the CF air control, but Douglas fir showed consistent decreases in weight of plant components. Lodgepole pine was not affected by either O_3 treatment. Carry-over effects were observed in bud elongation in the following spring in lodgepole pine, ponderosa pine, and hemlock. Andersen et al. (1991) also observed reduced root dry weight in ponderosa pine after exposure to SUM00 of 122 or 169 ppm·h during a 120-day growing

season. In addition, they observed a reduction in the weight of newly formed roots the following spring, possibly due to reduced levels of root starch.

In a 3-year field study, Temple et al. (1993) and Beyers et al. (1992) found that ponderosa pine trees exposed to a 24-h seasonal mean of 0.087 ppm had a 48 and 70% loss of 2- and 3-year-old needles, respectively. Radial stem growth and coarse root growth also were reduced but not as severely as needle weight (due to abscission). After three seasons of exposure, current-year needles in elevated O_3 treatments had a higher photosynthetic performance than those in filtered air. The compensation was apparently due to higher foliar nitrogen in O_3-exposed needles, a product of redistribution of nitrogen before abscission of needles. Cumulative responses would suggest that, eventually, reductions in growth of the trees would occur at lower concentrations of O_3.

A number of field studies have been conducted in North America in which an attempt was made to relate air quality to growth or injury of forest trees. Two field studies have correlated radial growth with visible injury in ponderosa and Jeffrey pine in California (Peterson and Arbaugh, 1988; Peterson et al., 1987). An 11% reduction in radial growth was measured in symptomatic Jeffrey pine, compared to trees that did not show symptoms of O_3 injury, but no reduction could be demonstrated in ponderosa pine; however, the authors point out that the trees they measured were not under competitive stress, which might alter their response.

The response of evergreen trees varies widely, depending on species and genotype within species. It is clear, however, that major forest species, such as ponderosa, loblolly, and slash pine are sensitive to O_3 (depending on length of exposure, based on seedling studies) at or slightly above the concentrations of O_3 (0.04 to 0.05 ppm) that occur over wide areas of the United States. Furthermore, because of the long life span of these trees, including those that have not been reported sensitive to O_3, there is ample opportunity for a long-term, cumulative effect on growth of the trees. Most of the experiments are conducted over only 2% or less of the life expectancy of the tree; an equivalent exposure in field crop plants would be 2 to 3 days. Consideration also must be given to the fact that most of these trees grow as part of mixed forests, in competition with many other species. Small changes in growth might be translated into large changes in stand dynamics, with concomitant effects on the structure and function of the ecosystem.

5.6.5 Assessments Using Ethylene Diurea as a Protectant

A chemical protectant, EDU (*N*-[2-(2-oxo-1-imidagolidinyl)ethyl]-*N'*-phenylurea), has been used to study the response of plants to O_3 without attempting to control the concentration of the pollutant during the exposure (Table 5-31) (U.S. Environmental Protection Agency, 1986).

Disadvantages of the use of OTCs for assessing the effects of O_3 on the growth of plants include relatively high cost, the need for electrical power, and potential effects of the chambers themselves on the growth of the plants. In many cases, no chamber effects can be detected, and because most studies compare against a control, chamber effects would have a minimal effect on interpretation of results. Although, the number of experiments conducted with OTCs has led to a firm understanding of plant response to a chamber environment, the possibility of interactions with treatment cannot be ruled out. The use of EDU is attractive due to low cost and ease of application; however, it is essential to establish the correct dosage for protection from O_3, without direct effects of EDU on the plant, and an estimate of

Table 5-31. Effects of Ethylene Diurea (EDU) on Ozone Responses[a]

Crop/Species	EDU Application	O_3 Exposure	Effects of EDU	Reference
White bean	Spray to runoff, 2,000 ppm	Field; 34 h > 0.08 ppm	Reduced O_3 injury, 38%; delayed defoliation; increased yield, 24%.	Temple and Bisessar (1979)
	Spray to runoff, 2,000 ppm	Field; hours > 0.08 ppm = 518 ppm·h	Reduced O_3 injury, 20 to 80%; increased yield up to 35%.	Toivonen et al. (1982)
	Soil drench, 500 ppm, 0.5 L/pot	Greenhouse (charcoal-filtered)	No effect on growth.	Brennan et al. (1990)
	Soil drench, 500 ppm, 4 L/6 m row	Field; 78 h > 0.12 ppm (0.2 ppm max)	Reduced O_3 injury up to 50%; retarded maturation.	Brennan et al. (1990)
Corn	Spray to runoff, 500 ppm	Field (no details)	19% yield reduction.	Heggestad (1988)
Cotton	Spray to runoff, 500 ppm	Greenhouse (no details)	Increased yield in nonfiltered air; reduced yield in filtered air.	Heggestad (1988)
Potato	Spray to runoff, 1.1 kg/ha, five applications	Field; > 0.08 ppm on 18 days (0.138 ppm max)	Reduced O_3 injury, 50%; increased tuber weight, 35%.	Bisessar (1982)
	Soil drench, 6.7 kg/ha, four applications	Field; 282 h > 0.08 ppm	Reduced O_3 injury; increased tuber weight, 20 to 30%.	Clarke et al. (1990)
Radish	Soil drench, 100 mg/L, 2 L/m of row	Field; 0 h > 0.1 ppm, hours > 0.05 ppm = 0.76 ppm·h	No response to O_3. Reduced growth rates at low O_3 exposures.	Kostka-Rick and Manning (1992a)
	Soil drench, up to 800 mg/L, 100 mL/pot	Greenhouse, < 0.025 ppm	Increased shoot growth at <300 mg/L; reduced shoot growth at >300 mg/L. Reduced hypocotyl growth at all EDU levels.	Kostka-Rick and Manning (1993)
	Soil drench, up to 400 mg/L, 100 mL/pot	Greenhouse, 0.075 ppm/7 h, 6 days/week, with one weekly peak to 0.14 ppm	Complete protection against O_3 injury at 100 mg/L.	Kostka-Rick and Manning (1993)

Table 5-31 (cont'd). Effects of Ethylene Diurea (EDU) on Ozone Responses[a]

Crop/Species	EDU Application	O_3 Exposure	Effects of EDU	Reference
Radish (cont'd)	Soil drench, 150 mg/L, 60 mL/pot	Greenhouse, 0.07 ppm/7 h, 5 days/week, with two weekly peaks to 0.12 ppm	Reduced O_3 injury, 90 to 100%; less reduction in hypocotyl weight.	Kostka-Rick and Manning (1992b)
Soybean	Soil drench, 500 ppm, 0.5 L/pot	Greenhouse; 0.2 ppm, 6 h/day, 2 days	Reduced O_3 injury, 80 to 90%.	Brennan et al. (1987)
	Soil drench, 500 ppm, 4 L/6 m row	Field; 78 h > 0.12 ppm (0.2 ppm max)	No effect on loss of chlorophyll; no effect on seed weight.	Smith et al. (1987) and Brennan et al. (1990)
Tobacco	Spray to runoff, 1 kg/ha, seven applications	Field; > 0.08 ppm on 2 days	Increased growth, 22%.	Bisessar and Palmer (1984)
Beech	Stem injection 1 g/L; 0.25 mL	OTC; ambient and ambient +0.08 ppm, 8 h/day	No consistent effect.	Ainsworth and Ashmore (1992)
Black cherry	Spray to runoff, 1,000 ppm, seven applications per year	Field; 75 h > 0.08 ppm (over 4 years)	Twofold increase in growth.	Long and Davis (1991)
Other woody species:				
Red maple	Spray to runoff, 500 ppm or soil drench, 500 or 2,000 ppm, 250 mL/pot	Up to 0.95 ppm, 3 h	Reduced O_3 injury.	Cathey and Heggestad (1982)
Paper birch			Reduced O_3 injury.	
White ash			Reduced O_3 injury.	
Honey locust			Reduced O_3 injury.	
Golden-rain			Reduced O_3 injury.	
London plane			Reduced O_3 injury.	
Lilac			Reduced O_3 injury.	
Basswood			Reduced O_3 injury.	

[a]See Appendix A for abbreviations and acronyms.

the level of protection from O_3 achieved (Kostka-Rick and Manning, 1992a,b, 1993). Ethylene diurea is known to be phytotoxic, so studies under controlled O_3 conditions to establish an effective level of protection without phytotoxicity are essential before EDU can be used as an assessment tool.

Previous studies with EDU led to the conclusion, as did experiments with OTCs, that ambient concentrations of O_3 were sufficient to reduce crop yields (U.S. Environmental Protection Agency, 1986). If hourly O_3 concentrations exceeded 0.08 ppm for 5 to 18 days during the growing season, yields of crops might be reduced 18 to 41% (U.S. Environmental Protection Agency, 1986).

Inspection of Table 5-31 shows that in many cases there were clear-cut reductions in O_3-induced injury and increases in yield resulting from the application of EDU. However, the conflicting results for field-grown soybean indicated that, at the rate of EDU application used, no beneficial effects could be demonstrated. Similarly, experiments with corn and cotton suggest that any possible effects of O_3 may have been confounded by direct effects of EDU on growth.

A few studies using EDU have been conducted since 1986. Kostka-Rick and Manning (1992a,b, 1993) conducted studies to determine the direct effects of EDU on growth and to develop an understanding of dose-response to EDU itself. Their studies used EDU and radish (*Raphanus sativus*) in the presence or absence of a controlled O_3 fumigation in a greenhouse and found that the chemical did suppress O_3-induced reductions in belowground plant organs; it also protected the plants from foliar injury. The EDU itself did not cause effects on growth at a concentration of 150 mg L^{-1} applied as a 60-mL drench to each plant, a dosage much lower than often has been used (e.g., Long and Davis, 1991; Smith et al., 1987; discussed below). Kosta-Rick and Manning emphasize that it is essential to establish the appropriate dose for the species under consideration. Armed with this background, the investigators used EDU in a field study and found an O_3-induced decrease in the relative growth rates of sink organs of field-grown radish plants above a threshold level of about 0.052 to 0.058 ppm (7-h daily mean), an exposure that is near ambient O_3 concentrations.

Ethylene diurea also has been used to estimate the effect of O_3 on field-grown soybean in New Jersey (Smith et al., 1987; Brennan et al., 1990). In this case, the researchers did not establish the appropriate dose level for O_3 protection, as was done by Kostka-Rick and Manning. No differences in yield were found, and the authors concluded that O_3 does not impact soybean yield of the tested cultivars in New Jersey. However, they did not demonstrate that EDU was an effective protectant at the concentrations used and on the cultivars grown.

In a similar study, potato yields were measured and related to foliar injury in EDU-treated and nontreated plots over a 4-year period (Clarke et al., 1990). The cumulative O_3 dose ranged from 45 to 110 ppm·h, depending on the year, producing a range of foliar injury from 1 to 75%. The authors found that significant differences in yield between EDU-treated and control plants occurred only when foliar injury on untreated plants was 75% of leaf area. No level of protection, other than from foliar injury, could be assessed.

In a 3-year study of potted green ash, no significant effects on growth were measured using EDU (2 years) or by comparison of filtered and NF air in OTCs (1 year) (Elliot et al., 1987). Foliar injury was observed only late in the season of the first year in the NF chambers.

An effort by Ensing et al. (1986) to assess the impact of O_3 on yield of peanut in Ontario found that year-to-year variation was greater than that they could account for either by correlation of O_3 concentration with yield of test plots or by EDU treatment. They conclude that a correlative approach to assessing losses due to O_3 will not work.

Finally, a 4-year study of black cherry using EDU as a protectant was conducted by Long and Davis (1991). They found significant effects with a 47% reduction in aboveground biomass compared to EDU-treated trees. The authors do not believe the difference was due to a stimulation in growth due to nitrogen in the EDU, but they did not conduct studies, as recommended by Kostka-Rick and Manning, to characterize the EDU system for black cherry.

In summary, the EDU method for assessing the impact of O_3 is promising, particularly for remote areas or as a validation tool for existing crop-loss models. The system must be carefully characterized, however, as pointed out by many of its users.

It should be noted that, in spite of the promise shown by EDU as a field protectant over many years, it has not been developed commercially and, until recently, was unavailable for further experimentation.

5.6.6 Summary

Several conclusions were drawn from the various approaches used to estimate crop yield loss. In 1986, U.S. Environmental Protection Agency (1986) established that 7-h/day growing season mean exposures to O_3 concentrations above 0.05 ppm were likely to cause measurable yield loss in agricultural crops. At that time, few conclusions could be drawn about the response of deciduous or evergreen trees or shrubs because of the lack of information about the response of such plants to season-long exposures to O_3 concentrations of 0.04 to 0.06 ppm and above. However, the 1978 and 1986 criteria documents (U.S. Environmental Protection Agency, 1986) indicate that the limiting values for foliar injury to trees and shrubs was 0.06 to 0.10 ppm for 4 h. Since 1986, considerable research has been conducted, and the sensitivity of many tree species has been established.

Based on research published since U.S. Environmental Protection Agency (1986), a number of conclusions can be drawn.

(1) An analysis of 10 years of monitoring data from more than 80 to almost 200 nonurban sites in the United States established ambient 7-h growing season average concentrations of O_3 for 3 or 5 mo of 0.051 to 0.060 ppm and 0.047 to 0.054 ppm, respectively. The SUM06 exposures ranged from 24.8 to 45.2 ppm·h for 3 mo, and 32.7 to 58.0 ppm·h for 5 mo (Tingey et al., 1991).

(2) The results of OTC studies that compare yields at ambient O_3 exposures with those in filtered air and retrospective analyses of crop data summarized in this section establish that the current ambient (0.04 to 0.05 ppm) concentrations of O_3 at some sites are sufficient to reduce the yield of major crops in the United States. The results of research since 1978 do not invalidate the conclusions of the U.S. Environmental Protection Agency (1978, 1986) that visible injury due to O_3 exposures reduces the market value of certain crops and ornamentals where leaves are the product (spinach [*Spinacea oleracea*], petunia, geranium [*Pelargonium hotortorum*], and poinsettia [*Euphorbia*

pulcherrima] for instance), and that such injury occurs at O_3 concentrations (0.04 to 0.10 ppm) that presently occur in the United States.

(3) A growing season SUM06 exposure of 26.4 ppm·h, corresponding to a 7-h growing season mean of 0.049 ppm and a 2HDM of 0.094 ppm may prevent a 10% loss in 50% of the 54 experimental cases analyzed by Tingey et al. (1991) and Lee et al. (1994a,b). A 12-h growing season mean of 0.045 should restrict yield losses to 10% in major crop species (Lesser et al., 1990).

(4) Concentrations of O_3 and SUM06 exposures, depending on duration, that occur at present in the United States are sufficient to affect the growth of a number of trees species. Given the fact that multiple-year exposures may cause a cumulative effect on the growth of some trees (Simini et al., 1992; Temple et al., 1992), it is likely that a number of species currently are being impacted, even at ambient exposures (0.04 to 0.05 ppm).

(5) Exposure-response functions for 51 cases of seedling response to O_3 (Hogsett et al., 1995), including 11 species representing deciduous and evergreen growth habits, suggest that a SUM06 exposure for 5 mo of 31.5 ppm·h would protect hardwoods from a 10% growth loss in 50% of the cases studied. A SUM06 exposure of 42.6 ppm·h should provide the same level of protection for evergreen seedlings. It should be noted that these conclusions do not take into the account the possibility of effects on growth in subsequent years, an important consideration in the case of long-lived species.

(6) Studies of the response of trees to O_3 have established that, in some cases poplars (*Populus*) and black cherry, for instance, trees are as sensitive to O_3 as are annual plants, in spite of the fact that trees are longer lived and have lower rates of gas exchange, and, therefore, a lower uptake of O_3.

(7) The use of the chemical protectant, EDU is of value to establish O_3-related losses in crop yield and tree growth, providing care is exercised in establishing the appropriate dosage of the compound to protect the plants without affecting growth. Ethylene diurea cannot be used to predict the response of plants at concentrations greater than those that exist in ambient air.

5.7 Effects of Ozone on Natural Ecosystems

5.7.1 Introduction

Ozone is a regionally distributed phytotoxic air pollutant capable of changing the chemical environment of forests. It is the only gaseous air pollutant capable of exposing a large region without a leaving a permanent trace of its presence. Ozone molecules are ephemeral. They decompose rapidly to oxygen and free radicals and leave no residuals; therefore, O_3-caused stresses are frequently difficult to determine (Taylor and Norby, 1985; Garner, 1991).

Ozone stresses can be acute, chronic, or both. Trees may experience O_3 exposures for minutes, hours, a few days, or weeks. In addition, exposures usually occur more than once during a growing season. During an episode, O_3 trajectories may cover very large areas. Concentrations can increase as the air trajectories move across the country and pass over new sources of O_3 (Wolff et al., 1977a,b,c, 1980; Wolff and Lioy, 1980). Acute

episodic exposures (short-term high concentrations) may be experienced several times in a year. During chronic exposures, low concentrations may be experienced continuously for a major portion of the life of a plant. Forest trees, shrubs, and other perennial plants must cope with the cumulative effects of several acute episodes; chronic, long-term exposures; or both. Trees may respond rapidly; for example, the needles of sensitive eastern white pine exhibit visible injury symptoms within days after exposure to high O_3 concentrations (Garner, 1991). In most instances, however, responses are subtle and not observable for many years because trees adapt and respond to cumulative stresses by differential growth, which is the result of altered carbon allocation (Waring and Schlesinger, 1985). Trees usually can recover when the stresses are removed, depending on the length of exposure.

Ozone concentrations and the effects, past and present, of exposure to O_3 on ecosystems in the San Bernardino Mountains and the Sierra Nevada Mountains of California and in the Appalachians Mountains of the eastern United States are presented in the pages that follow. The final section relates known ecosystem responses to stress and presents possible reasons why the effects on the ecosystem components in the two regions resulted in different responses. How plants respond to O_3 exposures and may compensate for stresses has been pointed out in the section on mode of action (Section 5.3). The importance of genetic variability in plant response and plant competition, as well as the multiple biological and physical factors that may modify plant response and, in some cases, cause stress, have been discussed in factors that modify plant response (Section 5.4). The discussion regarding modifying factors is of particular importance in understanding ecosystem response to stresses because they are much more likely to be encountered by plants growing in their natural habitats. Figure 5-30 outlines how plant response can lead to ecosystem response.

The responses to a variety of O_3 concentrations and exposure durations of various species of deciduous trees and shrubs (Table 5-27) and evergreen trees (Table 5-30) under experimental conditions have been presented in the previous section. The studies cited in the tables in the previous section, whether conducted in chambers, greenhouses, or OTCs, suggest that all sensitive plants will respond within hours to O_3 concentrations above 0.06 ppm. In general, depending on the length of exposure, the number and height of peaks, and the sensitivity of the vegetation, data from the field supports this contention. This section places the response of the individual trees, shrubs, and other perennial plants in the ecosystem context. The responses of forest ecosystems to pollutant exposure have received more study than unmanaged ecosystems of other biomes (grasslands, shrublands, or deserts), therefore, the following discussion relies mainly on forest ecosystems for examples.

5.7.2 Ecosystem Characteristics

Ecosystems are composed of populations of "self-supporting" and "self-maintaining" living plants, animals, and microorganisms (producers, consumers, and decomposers) interacting with one another and with the nonliving chemical and physical environment within which they exist (Odum, 1989; U.S. Environmental Protection Agency, 1993). Ecosystems respond to stresses through their constituent organisms. The response of plant species and populations to environmental perturbations depends on their genetic constitution (genotype), their life cycles, and the microhabitats in which they are growing. Stresses such as the changes in the physical and chemical environment of plant populations apply new and additional selection pressures on individual organisms (Treshow, 1980). The

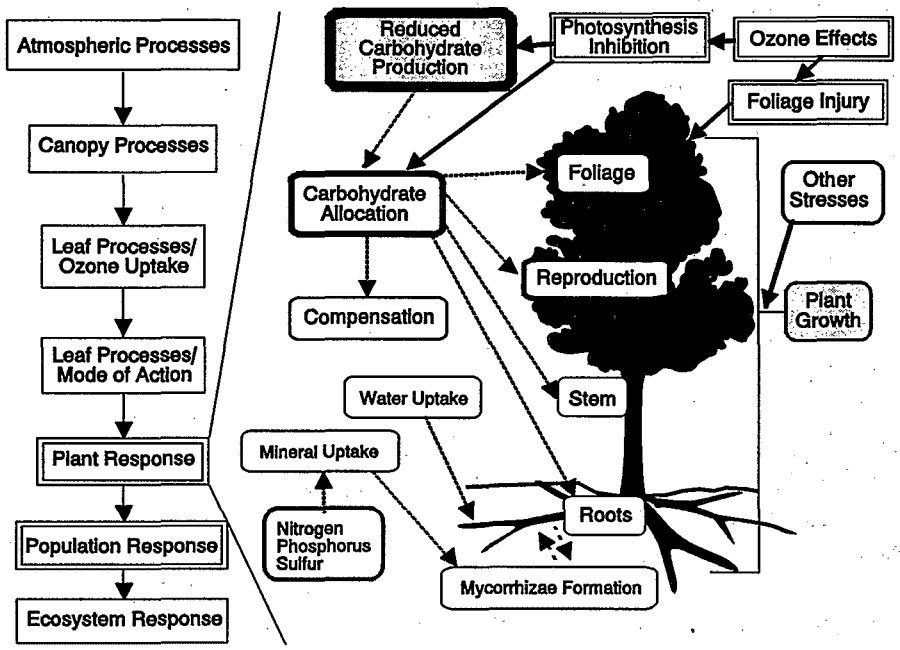

Figure 5-30. Effects of ozone (O_3) on plant function and growth. Reduced carbohydrate production decreases allocation and resources needed for plant growth processes. Individual plant responses must be propagated hierarchically through the more integrative levels of population and community to produce an ecosystem response. Solid black arrows indicate the effects of O_3 absorption; stippled arrows indicate affects on plant functions. Double border indicates site of response; darkened border indicates site of impact.

changes that occur within plant communities reflect these new and different pressures. A common response in a community under stress is the elimination of the more sensitive populations and an increase in abundance of species that tolerate or are favored by the stress (Woodwell, 1970; Guderian et al., 1985).

Ecosystems usually have definable limits within which the integrated functions of energy flow, nutrient cycling, and water flux are maintained (Odum, 1993). Their boundaries, and the organisms that live within them, are determined by the environmental conditions of that particular habitat, area, or region. Structurally complex communities, they are held in an oscillating steady state by the operation of a particular combination of biotic and abiotic factors. They may be large or small (e.g., fallen logs, forests, grasslands, meadows, old uncultivated fields, ponds, lakes or rivers, estuaries, oceans, the earth) (Odum, 1971). Together, the environment, the organisms, and the physiological processes resulting from their interactions form the life-support systems that are essential for the existence of any species on earth, including man (Odum, 1993).

Human existence on this planet is dependent on ecological systems and processes. Natural ecosystems traditionally are spoken of in terms of their structure and functions. Ecosystem structure includes the species (richness and abundance) and their mass and

arrangement in an ecosystem. This is termed an ecosystem's standing stock—nature's free "goods" (Westman, 1977; U.S. Environmental Protection Agency, 1978, 1986, 1993). Society reaps two kinds of benefits from the structural aspects of an ecosystem: (1) products with market value such as fish, minerals, forest products and pharmaceuticals, and genetic resources of valuable species (e.g., plants for crops and timber and animals for domestication); and (2) the use and appreciation of ecosystems for recreation, aesthetic enjoyment, and study (Westman, 1977; U.S. Environmental Protection Agency, 1978, 1986, 1993).

More difficult to comprehend, but of equal or greater importance are the functional aspects of an ecosystem. Ecosystem functions are characterized by the way in which components interact. They are the dynamics of ecosystems—nature's free "services". The benefits imparted to society include absorption and breakdown of pollutants, cycling of nutrients, binding of soil, degradation of organic waste, maintenance of a balance of gases in the air, regulation of radiation balance, climate, and the fixation of solar energy. These, in short, are the functions that maintain clean air pure water, a green earth, and a balance of creatures, the functions that enable humans to obtain the food, fiber, energy, and other materials for survival (Westman, 1977). The majority of the free services are performed by the microorganisms that constitute as many as half of all living creatures on the earth but are seldom recognized.

The term "ecological risk" highlights the importance of ecosystems to human existence. Ecosystems change dramatically throughout time, have no optimal condition, and are only healthy when compared to some desired state specified by humans (Lackey, 1994). The importance of ecosystems to human existence is presented in more detail in the nitrogen oxides (NO_x) criteria document (U.S. Environmental Protection Agency, 1993).

5.7.3 Effects of Exposure to Ozone on Natural Ecosystems

5.7.3.1 The San Bernardino Forest Ecosystem—Before 1986

The mixed-conifer forest ecosystem in the San Bernardino Mountains of Southern California is one of the most thoroughly studied ecosystems in the United States. Chronic O_3 exposures over a period of 50 or more years has resulted in major changes in the San Bernardino National Forest ecosystem. The primary effect was on the more susceptible members of the forest community, individuals of ponderosa and Jeffrey pine, such that they were no longer able to compete effectively for essential nutrients, water, light, and space. As a consequence of altered competitive conditions in the community, there was a decline in the sensitive species, permitting the enhanced growth of more tolerant species (Miller et al., 1982; U.S. Environmental Protection Agency, 1978, 1986). The results of the studies of the San Bernardino Forest ecosystem were reported in both the 1978 and 1986 criteria documents (U.S. Environmental Protection Agency, 1978, 1986). The information summarized below is from these two documents.

An inventory of the forest was begun in 1968 and conducted through 1972 to determine the results of more than 30 years of exposure to O_3. Based on that inventory and accompanying studies, the conclusions reached are presented in Table 5-32. Data from the inventory indicated that, during 5 mo/year from 1968 through 1972, trees were exposed to O_3 concentrations greater than 0.08 ppm for more than 1,300 h. Concentrations rarely fell below 0.05 ppm at night near the crest of the mountain slope (elevation approximately 5,500 ft [Miller, 1973]). The importance of altitude in plant response was discussed in the

Table 5-32. San Bernardino Forest—Status 1972

1. Ponderosa and Jeffrey pine suffered the most injury. Mortality of one population of ponderosa pine ($n = 160$) was 8% between 1969 and 1971 ($p = 0.01$); in a second population ($n = 40$), mortality was 10% between 1968 and 1972. White fir populations suffered slight damage, with scattered individual trees showing severe symptoms. Sugar pine, incense cedar, and black oak exhibited only slight foliar injury from oxidant exposure.
2. A substantial shift occurred in ponderosa pines from the "slight injury" category in 1969 to the "moderate injury" category in 1971, indicating that there was continuing oxidant stress and that the selective death of ponderosa pines was occurring.
3. Suppression of photosynthesis in seedlings was observed (Miller et al., 1969). In ponderosa pine saplings, needles shortened by exposure to oxidants returned to normal length when the seedlings were moved to O_3-free air from 1968 to 1973 (Miller and Elderman, 1977).
4. Bark beetles were judged to be responsible for the death of weakened trees in the majority of cases. Elimination of ponderosa pine from the mixed-conifer forest was postulated to occur in the future if the rate of bark beetle attack were to continue unabated (Cobb and Stark, 1970).
5. Aerial portions of O_3-injured pine trees showed a decrease in vigor that was associated with deterioration of the feeder root system (Parmeter et al., 1962).
6. Seed production was decreased in injured pines. Ordinarily, trees 25 to 50 in. diameter at breast height produce the most cones, but they were also the most sensitive to oxidants (Luck, 1980).
7. Under-story plant species sensitive to oxidant pollution may already have been removed by air pollution stress at the time of these early studies (Miller and Elderman, 1977).

Source: U.S. Environmental Protection Agency (1986).

1986 criteria document (U.S. Environmental Protection Agency, 1986) and also is discussed in Chapter 4 of this document. The monthly averages of the daily maxima of total oxidant concentrations for the 5 years of the study are given in Figure 5-31. The highest single daily maximum oxidant concentration of 0.58 ppm occurred in June 1970 between 4:00 and 9:00 p.m., PST (Miller, 1973).

The survey cited above indicated the need for further information. To more accurately determine the effects of the 30 years of exposure to O_3 of the San Bernardino Forest ecosystem, an interdisciplinary research team designed a study to answer the following questions: how do organisms and biological processes of the conifer forest respond to different levels of chronic oxidant exposure? and how can these responses be interpreted within an ecosystem context?

Included in the study plan were the following ecosystem processes: carbon (energy) flow (the movement of CO_2 into the plants, its incorporation into carbohydrates, and then its partitioning among consumers, decomposers, litter, and soil); the movement of water in the soil-plant-atmosphere continuum; mineral nutrient flow through the green plant, litter, and soil-water compartments; and the shift in diversity patterns in time and space, as represented by changes in age, structure, and density in the composition of tree species in communities.

The major abiotic components studied were water (precipitation), temperature, light, mineral nutrients (soil substrate), and oxidant pollution. The biotic components studied

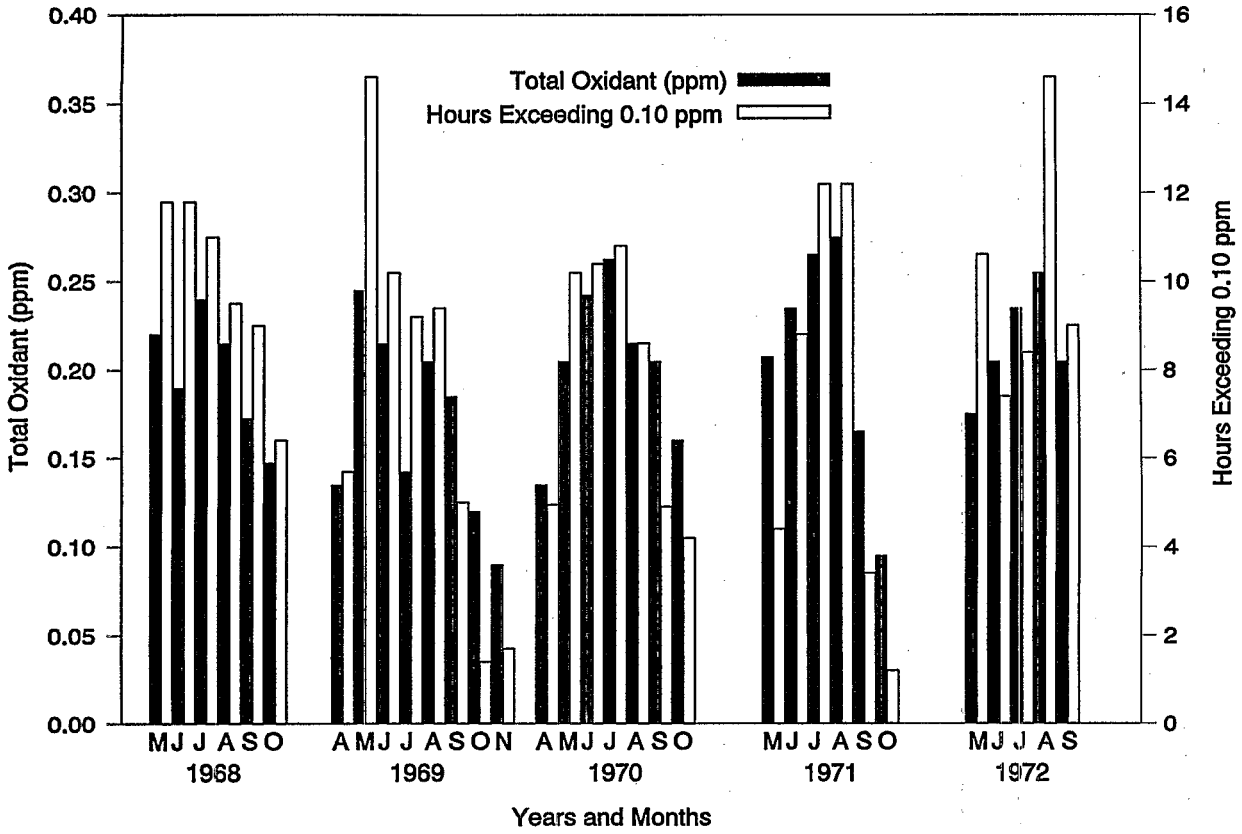

Figure 5-31. Total oxidant concentrations at Rim Forest (5,640 ft) in Southern California during May through September, 1968 through 1972. Values of total oxidant are averages of daily maxima for a month. The number of hours in which total oxidant exceeded 0.10 ppm also was recorded for the 5-year period.

Source: Miller (1973).

included producers (an assortment of tree species and lichens), consumers (wildlife, insects, and disease organisms), and decomposers, (populations of saprophytic fungi responsible for the decay of leaf and woody litter) (U.S. Environmental Protection Agency, 1978, 1986).

During the period of the study, 1973 to 1978, average 24-h O_3 concentrations ranged from a background of 0.03 to 0.04 ppm in the eastern part of the San Bernardino Mountains to a maximum of 0.10 to 0.12 ppm in the western part during May through September. Hourly average concentrations for 1975 (measured by ultraviolet [UV]) indicated that O_3 buildup began around 10 a.m. and reached a maximum at all six monitoring stations in all months (May through September) at around 4 p.m. For example, at the Rim Forest-Sky station, where the highest concentrations usually were recorded, the 1-mo average of hourly values ranged from 0.07 to 0.10 ppm at 10 a.m. and from 0.15 to 0.22 at 4 p.m. The highest concentrations occurred in June, July, and August, and the lowest were observed in September. The total number of hours with concentrations of 0.08 ppm or more during

June through September was never less than 1,300 h per season during the first 7 years (1968 through 1974) of the study (Miller and Elderman, 1977). In addition to total oxidant, PAN and NO_2 concentrations were measured. Peroxyacetyl nitrate injury symptoms could not be distinguished from O_3 symptoms on herb-layer plant species while NO_2 remained at nontoxic concentrations (Miller et al., 1982; U.S. Environmental Protection Agency, 1978, 1986).

The study indicated that the major changes in the ecosystem began with injury to ponderosa and Jeffrey pine. Ponderosa pine was the most sensitive of the trees to O_3, with Jeffrey pine, white fir, California black oak (*Quercus kelloggii*), incense cedar (*Calocedrus decurrens*), and sugar pine (*Pinus lambertiana*) following in decreasing order of sensitivity. Foliar injury on sensitive ponderosa and Jeffrey pine was observed when the 24-h average O_3 concentrations were 0.05 to 0.06 ppm (Miller et al., 1982). Foliar injury, premature senescence, and needle fall decreased the photosynthetic performance of stressed pines and reduced the production of carbohydrates needed for use in growth and reproduction by the trees. Nutrient availability to the trees also was reduced by the trees retention of smaller amounts of green foliage (Miller et al., 1982). Decreased carbohydrate resulted in a decrease in radial growth and height of stressed trees (McBride et al., 1975; Miller and Elderman, 1977).

A reduction in available carbohydrate also influenced tree reproduction. Injured ponderosa and Jeffrey pines older than 130 years produced significantly fewer cones per tree than uninjured trees of the same age (Luck, 1980). Tree-ring analysis indicated declines in ring-width indices for many trees. Stand thinning, however, reversed the trend (Miller et al., 1982).

Summarized, the responses of individual conifers sensitive to O_3 include visible foliar injury; premature needle senescence; reduced photosynthesis; reduced carbohydrate production and allocation; reduced plant vigor; and reduced growth or reproduction, or both (Miller et al., 1982).

The ecosystem components most directly affected by O_3 exposure were tree species, the fungal microflora of conifer needles, and the foliose lichens growing on tree bark. Injury to or changes in the functioning of other living ecosystem components affected, either directly or indirectly, the processes of carbon (energy) flow, mineral nutrient cycling, water movement, and changed vegetational community patterns (Miller et al., 1982). Early senescence and abscission resulted in accumulation of pine needles into a thick layer under the stands of O_3 injured trees and changed decomposition patterns, which changed successional patterns of the fungal microflora as well. Altering the taxonomic diversity and population density of the microflora that normally develop on needles while they are on the tree influenced the relationship of the microflora with the decomposer community. Change in the type of fungi on needles weakened the decomposer community and slowed the rate of decomposition (Bruhn, 1980). Nutrient availability was influenced by the carbon and mineral nutrients accumulated in the heavy litter and thick needle layer under stands with the most severe needle injury and defoliation.

A comparison of species of lichens found on conifers during the years 1976 to 1979 with collections from the early 1900s indicated a 50% reduction in species in the more recent period. Marked morphological deterioration of the common species *Hypogymnia enteromorpha* was documented in areas of high oxidant concentrations (Sigal and Nash, 1983).

Biotic interactions associated with predators, pathogens, and symbionts were influenced by changes in the energy available to the trees. The decrease in vigor and lack of ability to recover from O_3 injury associated with reduced carbohydrates made the ponderosa pines more susceptible to attack by predators and pathogens (Stark and Cobb, 1969). Dahlsten and Rowney (1980) have pointed out that oxidant-weakened pines can be killed by fewer western pine beetles than are required to kill healthier trees. In stands with a high proportion of O_3-injured trees, a given population of western pine beetles therefore could kill more trees. James et al. (1980a,b) observed that the root rot fungus, *Heterobasidium annosum*, increased more rapidly because freshly cut stumps and roots of weakened trees were more vulnerable to attack (U.S. Environmental Protection Agency, 1986).

Changes in the plant populations that alter communities and forest stands also can affect the animal populations. Production of fewer cones, seeds, and fruits reduces the food available to small vertebrates living in the ecosystem (U.S. Environmental Protection Agency, 1978). The continuum of ecosystem responses associated with increasing pollutant stress (presented in Table 5-33) are reflected in the response of the San Bernardino mixed forest ecosystem (Garner et al., 1989; U.S. Environmental Protection Agency, 1986). The influence of pollutants on the processes of carbon production and allocation are presented in continuum Stage II, Table 5-33.

Table 5-33. Ecosystem Response to Pollutant Stress

	Continuum of Vegetation Responses	Continuum of Ecosystem Responses
0	Anthropogenic pollutants insignificant.	Unaffected; systems pristine.
I	Pollutant concentrations low; no measurable physiological response.	Ecosystem functions unaffected; pollutants transferred from atmosphere to organic or available nutrient compartments.
II	Pollutant concentrations injurious to sensitive species: (1) Reduced photosynthesis, altered carbon allocation, and reduced growth and vigor; (2) Reduced reproduction; (3) Predisposition to entomological or microbiological stress.	Altered species composition; populations of sensitive species decline; some individuals are lost. Their effectiveness as functional ecosystem members diminishes; they could be lost from the system. Ecosystem reverts to an earlier stage.
III	Severe pollution stress. Large plants of sensitive species die. Forest layers are peeled off; first trees and tall shrubs, then, under the most severe conditions, short shrubs and herbs.	(1) Simplification, basic ecosystem structure changes, becomes dominated by weedy species not previously present. (2) Reduced stability and productivity; loss of capability for repairing itself. Runoff increases and nutrient loss and erosion accelerates; a barren zone results. Ecosystem collapses.

Source: Garner et al. (1989); adapted from Bormann (1985); Kozlowski (1985); Smith (1974).

5.7.3.2 The San Bernardino Forest Ecosystem—Since 1986

Monitoring of O_3 trends in the South Coast Air Basin of Southern California, the source of pollutants transported to the mixed-conifer forests of the San Bernardino Mountains, resulted in the conclusion that the air quality had improved substantially between 1976 and 1984.

Between 1976 and 1991 the weather-adjusted O_3 data for the May through October "smog season" indicates that the number of Basin days exceeding 0.12 ppm, 1-h average, have declined at an average annual rate of 2.27 days/year, whereas the number of days with episodes greater than 0.2 ppm, 1-h average, have declined at an average annual rate of 4.70 days/year over the same period. The total days per year with concentrations greater than 0.12 ppm was as high as 159 in 1978, with the lowest number being 105 days in 1990 (Davidson, 1993). The 1974 to 1988 trends of the May through October hourly average and the average of monthly maximum O_3 concentrations for Lake Gregory, a forested area in the western section of the San Bernardino Mountains, also have shown a gradual decline (Miller et al., 1989a). Similarly, for the same period, there was an improvement shown in the injury index used to describe chronic injury to the crowns of ponderosa and Jeffrey pines in 13 of 15 plots located on the gradient of decreasing O_3 exposure in the San Bernardino Mountains (Miller et al., 1989a). The two exceptions were plots located at the highest exposure end of the gradient. The basal area increase of ponderosa pines was generally less than competing species at 12 of the 13 plots evaluated. The total basal area for each species as a percent of the total basal area for all species indicates that ponderosa and Jeffrey pines in plots with slight to severe crown injury lost basal area in relation to competing species that are more tolerant to O_3, namely, white fir, incense cedar, sugar pine, and California black oak (Figure 5-32).

In effect, stand development had been reversed (i.e., the development of the normal fire climax mixture dominated by fire-tolerant ponderosa and Jeffrey pines was altered). The accumulation in the understory of a greater number of stems of more O_3-tolerant species resulted in the formation of a fuel ladder that jeopardized the remaining overstory trees in the event of a catastrophic fire. The O_3-tolerant species, because of thinner bark and branches growing close to the ground, are inherently more susceptible to fire injury. The important question for the future at that time was whether the declining O_3 exposure eventually would allow ponderosa and Jeffrey pine to resume dominance in basal area.

The possible interactive effects of nitrogen and O_3 on the forests of the San Bernardino Mountains has come under consideration more recently. For some time, there has been a concern that O_3 is not the only pollutant in the photochemical mixture that may be causing lasting changes in the mixed-conifer forest ecosystem. A multidisciplinary study to investigate the possibility of the combined impacts on ecosystem processes from chronic O_3 injury and both wet and dry deposition of acidic nitrogen compounds has been under way since 1991 at Barton Flats in the San Bernardino Mountains. The database includes frequent measurements of stomatal conductance in relation to weather and O_3 exposure.

The NO_x criteria document (U.S. Environmental Protection Agency, 1993) explored the possible effects of increased nitrogen on litter content and decomposition. That discussion is presented here.

Increases in the nitrogen litter content and in litter decomposition rates and an alteration in nitrogen cycling have been observed in the more highly polluted areas when compared with moderately polluted and low-polluted areas of the San Bernardino Mountains

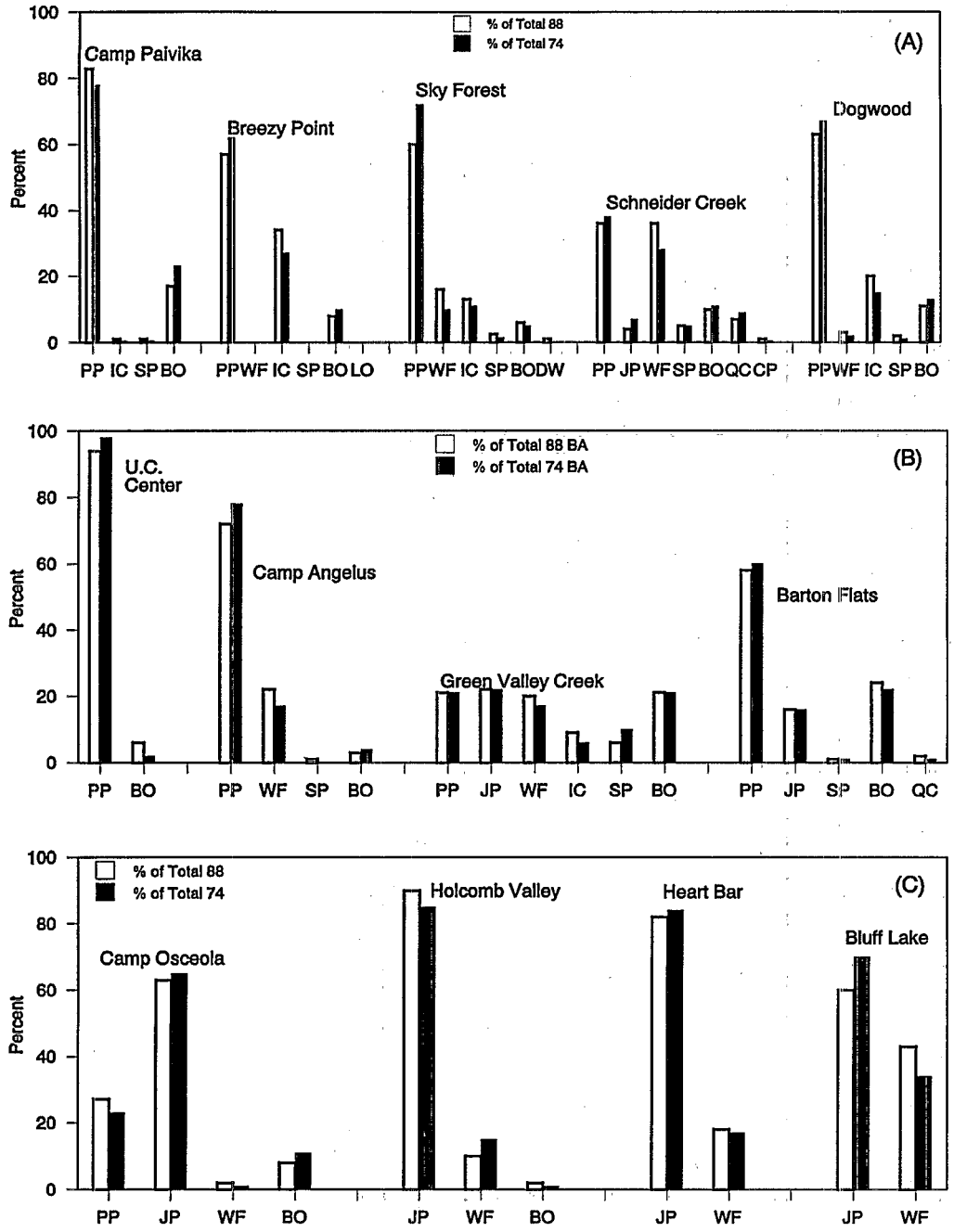

Figure 5-32. Total basal area for each species as a percent of the total basal area for all species in 1974 and 1988 on (A) plots with severe to moderate damage, (B) plots with slight damage, and (C) plots with very slight damage or no visible symptoms. PP = Ponderosa pine, IC = Incense cedar, SP = Sugar pine, BO = Black oak, WF = White fir, LD = Libocedrus decurrens, DW = Dogwood, QC = Quercus chrysolepis, CP = Coulter pine, and JP = Jeffrey pine.

Source: Miller et al. (1991).

(Fenn and Dunn, 1989). A pollutant concentration gradient was observed to exist, with 24-h O_3 concentrations at the high sites in the west averaging 0.1 ppm or more, moderate sites ranging from 0.06 to 0.08 ppm, and low sites in the east averaging 0.05 ppm or less (Fenn, 1991). Nitrogen and sulfur compounds also occur in the pollutant mixture to which the mountains downwind of the Los Angeles Basin are exposed (Bytnerowicz et al., 1987a,b; Solomon et al., 1992). A nitrogen deposition gradient from west to east parallels the decreasing O_3 gradient. Deposition of nitrogen exceeds that of sulfur (Fenn and Bytnerowicz, 1993). Annual average HNO_3 concentrations in 1986 ranged from 1.2 ppb near the Southern California coast to 2.7 ppb in the San Gabriel Mountains (Solomon et al., 1992).

The litter layers under trees severely injured by O_3 are deeper than those under trees less severely injured (Fenn and Dunn, 1989). A comparison study of decomposition rates of the undecomposed surface layer of needle litter indicates that litter in the more polluted areas in the west decomposed at a significantly ($p = 0.01$) faster rate than did litter from moderate to low pollution levels (Fenn and Dunn, 1989; Fenn, 1991). Nitrogen content of litter was greatest at the high pollution sites and was positively correlated with the litter decomposition rate. The higher nitrogen and lower calcium contents of the litter suggest that litter in the western plots originated from younger needles than those at the less polluted sites, possibly due to O_3-induced needle abscission. Fungal diversity was also greater in the litter from the western San Bernardino Mountains (Fenn and Dunn, 1989).

When the factors associated with enhanced litter decomposition were investigated, it was found that nitrogen concentrations of soil, foliage, and litter of ponderosa and Jeffrey pine were greater in the plots where pollution concentrations were high than in moderately polluted or low-pollution sites. This was also true for sugar pine and for incense cedar, two O_3-tolerant species. The rate of litter decomposition for sugar pine, incense cedar, and Ponderosa pine species was greatest at the high-pollution sites. Therefore, the increased rate of litter decomposition in the high-pollution plots does not appear to be related to O_3 sensitivity or premature needle abscission, but, instead, it is associated with higher levels of nitrogen in the soils (Fenn, 1991). Foliage and litter nitrogen is higher in high-pollution sites when compared with moderate- or low-pollution sites.

At the present time, data dealing with the response of trees or other vegetation to the combined stresses of O_3 exposure above ground and nitrate deposition through the soil are sparse. Tjoelker and Luxmoore (1991), however, have assessed the effects of soil nitrogen availability and chronic O_3 stress on carbon and nutrient economy in 1-year-old seedlings of loblolly pine and yellow poplar. Elevated O_3 concentrations altered biomass partitioning to needles of the current year. Ozone concentrations of 0.108 ppm reduced the biomass of current-year needles in loblolly pine seedlings grown at the highest (172 μg/g) nitrogen supply by 20%, but not those grown with a low (59 μg/g) supply of nitrogen. The interaction between O_3 and nitrogen suggests that plants grown with a high nitrogen supply are more sensitive to chronic O_3 stress in terms of biomass reduction (Tjoelker and Luxmoore, 1991). Similar results in the growth of domestic radish were obtained by Pell et al. (1990). Brewer et al. (1961) and Harkov and Brennan (1980) observed increased foliar injury when plants were grown with an adequate nitrogen supply (U.S. Environmental Protection Agency, 1986).

5.7.3.3 The Sierra Nevada Mountains

The continued presence over the years of O_3 concentrations injurious to trees in the San Bernardino Mountain forests and the knowledge that O_3 is a regionally dispersed gaseous air pollutant led to concern that other forests in California, and possibly other western states as well, were being exposed to injurious concentrations. Summary statistics for the 1980 to 1988 growing season (May through October), using data from O_3-monitoring sites in or near Western forests, substantiated the concern. Growing season (May through October) means, percentiles and percent occurrence of hourly O_3 concentrations above 0.06, 0.08, 0.10, and 0.120 ppm for all O_3 sites near Western forests are presented in Table 5-34 (Böhm, 1992). The lowest O_3 concentrations with little hourly variation were experienced at sites far from urban or point sources. Sites on the fringe of urbanized centers or valleys, on the other hand, experienced patterns with some variation in hourly concentrations; the higher concentrations usually occurred during the late afternoon. Forests located on the rims of valleys with large urban areas experienced O_3 concentrations >0.10 ppm. Yosemite and Sequoia National Parks, which receive pollutants transported from highly urbanized areas, had 24-h means ranging from 0.036 to 0.085 ppm on 75% of summer days, whereas Lake Gregory had a growing season mean of 0.073 ppm. During 49% of the summer days, means of diurnal patterns ranged from 0.085 to 0.100 ppm, decreasing with altitude and distance from the source (Böhm, 1992). The San Bernardino National Forest was exposed to O_3 levels >0.10 ppm during all seasons. Ozone concentrations tended to decrease with altitude and distance from the source.

There is little evidence of O_3 injury in forests in the western United States outside of California, even near urban sites. Growing season means near forests ranged between 0.012 and 0.022 ppm in Washington, between 0.028 and 0.037 ppm in Utah, and between 0.032 and 0.058 ppm in Colorado (Table 5-34; Böhm, 1992; Böhm et al., 1995).

The Sierra Nevada, the largest forested area in the world documented to have visible injury from high O_3 exposures, is an area approximately 300 miles long (Peterson and Arbaugh, 1992). Since 1991, there has been an annual survey of the amount of crown injury by O_3 to the same trees in approximately 33 sample plots located in the Sierra Nevada. These include Tahoe, Eldorado, Stanislaus, Yosemite, Sierra, Sequoia, and San Bernardino National Forests and Yosemite and Sequoia-Kings Canyon National Parks.

Dominant tree species in the area are ponderosa and Jeffrey pine, white fir, sugar pine, incense cedar, Douglas fir, and California black oak, and the giant sequoia (*Sequoiadendron giganteum*) is locally common (Peterson and Arbaugh, 1992).

Foliar O_3 injury to ponderosa and Jeffrey pine was first documented in the Sierra Nevada Mountains of California in the early 1970s (Miller and Millecan, 1971). Monitoring of visible injury to ponderosa pine on national forest land in the western Sierra Nevada, however, was not begun until 1975 (Duriscoe and Stolte, 1989). Results of the monitoring in the Sierra and Sequoia National Forests showed that there was an increase in chlorotic mottle of pines in the plots from approximately 20% in 1977 to approximately 55% in 1988, and an increase in severity of injury was observed as well.

In general, the results of this study document the regional nature of the O_3 pollution problem originating primarily from the San Jaoquin Valley Air Basin, as well as from the San Francisco Bay Air Basin further to the west. Oxidant air pollution is transported southward in the San Jaoquin Valley Air Basin until it reaches the southern boundary of the air basin, the Tehachipi Mountains. Because of this barrier, polluted air

Table 5-34. Growing Season (May Through October) Summary Statistics for Ozone Monitoring Sites in or Near Forests for the Period 1980 through 1988 (Percentiles and means were generated using the entire data set [1980 through 1988; May through October].)

Site[b]	Elevation (m)	Percent Data Capture	Percentiles (ppb)								Percent Hours[a]			
			5	10	25	50	Mean ± SD[c]	75	90	95	≥60	≥80	≥100	≥120
Aptos, CA	78	100	0	10	10	20	25.1 ± 15	30	40	50	3	0	0	0
Ash Mountain, CA (AIRS)	526	50	20	30	50	60	64.1 ± 26	80	100	110	64	36	12	2
Ash Mountain, CA (NPS)	610	57	20	30	47	61	62.9 ± 24	80	93	100	59	29	8	1
Azusa, CA	185	93	0	0	0	20	43.3 ± 56	70	130	160	28	22	17	12
Banning, CA	722	98	10	10	20	40	49.6 ± 35	70	100	120	35	19	11	6
Bishop, CA	1,260	84	0	10	20	30	31.5 ± 16	40	50	60	7	0	0	0
Burbank, CA	170	95	0	0	0	20	36.3 ± 45	50	100	130	25	18	12	8
Camp Mather, CA	1,432	33	22	26	36	46	47.5 ± 16	59	70	76	24	3	0	0
Carmel Valley, CA	131	86	10	10	20	30	28.4 ± 14	40	50	50	4	1	0	0
Fresno County, CA	1,723	85	20	20	30	40	44.9 ± 17	60	70	80	26	5	0	0
Lake Gregory, CA	1,397	93	10	20	40	60	72.5 ± 49	100	140	170	55	37	26	18
Lassen NP, CA	1,788	36	17	21	28	36	37.8 ± 14	46	58	64	9	0	0	0
Kaweah, CA (AIRS)	1,901	35	10	20	40	60	59.7 ± 26	80	90	100	57	32	8	1
Kaweah, CA (NPS)	1,890	58	21	30	41	56	56.3 ± 21	71	83	90	44	15	2	0
Mammoth Lakes, CA	2,395	92	20	30	40	50	46.6 ± 16	60	70	70	30	5	0	0
Monterey, CA	23	86	10	10	20	30	27.3 ± 12	30	40	50	1	0	0	0
Ojao, CA	233	87	10	10	20	40	42.3 ± 26	60	80	90	30	12	3	1
Pasadena, CA	255	89	0	0	10	20	47.8 ± 58	70	130	170	30	24	18	14
Pinnacles NM, CA	355	66	10	16	26	41	42.8 ± 22	58	72	80	22	5	1	0
Redwood NP, CA	233	49	8	10	15	22	22.0 ± 0.09	28	34	39	0	0	0	0
San Bernardino, CA	320	80	0	0	0	30	50.2 ± 57	80	140	170	35	28	21	15
Santa Barbara, CA	25	96	0	10	20	30	32.2 ± 19	40	60	60	11	2	0	0
Santa Barbara County, CA	12	96	0	10	20	30	31.5 ± 20	40	60	70	13	2	1	0
Santa Monica Mountains, CA	191	55	0	2	10	30	39.6 ± 35	59	86	110	25	13	7	4
Scotts Valley, CA	171	79	0	0	10	20	22.4 ± 18	30	50	50	5	1	0	0
South Lake Tahoe, CA	1,907	88	10	20	20	40	37.8 ± 17	50	60	60	18	1	0	0
Ventura County, CA	1,600	83	0	10	20	40	36.2 ± 22	50	60	70	18	5	1	0
Wawona Valley, CA	1,280	66	9	15	27	42	44.0 ± 23	61	76	83	26	7	1	0

Table 5-34 (cont'd). Growing Season (May Through October) Summary Statistics for Ozone Monitoring Sites in or Near Forests for the Period 1980 through 1988 (Percentiles and means were generated using the entire data set [1980 through 1988; May through October].)

Site[b]	Elevation (m)	Percent Data Capture	Percentiles (ppb)								Percent Hours[a]			
			5	10	25	50	Mean ± SD[c]	75	90	95	≥60	≥80	≥100	≥120
Yreka, CA	809	80	0	0	10	20	25.9 ± 18	40	50	60	6	0	0	0
Clackamas County, OR	174	94	4	8	14	23	25.3 ± 16	33	45	55	4	1	0	0
Columbia County, OR	6	88	1	4	11	20	21.3 ± 14	29	39	46	2	0	0	0
Crook County, OR	1,372	90	20	25	30	35	36.5 ± 0.09	40	50	55	2	0	0	0
Eugene, OR	187	77	1	3	9	18	21.4 ± 17	30	42	52	3	1	0	0
Marion County, OR	102	94	1	1	7	18	20.3 ± 16	30	41	50	3	1	0	0
Medford, OR	503	93	1	3	9	22	24.8 ± 18	37	50	59	5	1	0	0
Cedar River, WA	210	82	11	14	19	28	31.4 ± 17	39	53	64	7	2	0	0
King County, WA	22	90	0	0	0	10	14.9 ± 17	20	40	50	3	1	0	0
Olympic NP, WA (DOE)	100	85	0	0	10	20	16.3 ± 11	20	30	40	0	0	0	0
Olympic NP, WA (NPS)	125	26	0	1	1	2	4.8 ± 0.07	3	17	23	0	0	0	0
Pack Forest, WA	24	80	10	10	20	30	30.0 ± 18	40	50	70	8	3	1	0
Pierce County, WA	14	85	0	0	0	10	15.1 ± 16	20	40	40	2	0	0	0
Port Angeles, WA	30	71	0	0	1	2	8.4 ± 10	10	20	30	0	0	0	0
Snohomish County, WA	120	83	0	0	0	10	17.0 ± 15	30	40	40	2	0	0	0
Spokane, WA	584	74	0	0	10	20	20.9 ± 16	30	40	50	20	0	0	0
Stampede Pass, WA	1,217	80	20	20	30	30	35.2 ± 14	40	50	60	7	0	0	0
Apache-Sitgreaves, AZ	2,462	94	25	30	35	40	42.3 ± 12	50	60	65	12	1	0	0
Cochise County, AZ	1,401	56	13	17	26	37	37.4 ± 15	49	58	63	8	0	0	0
Flagstaff, AZ	2,117	77	17	24	34	44	43.7 ± 15	53	62	67	13	1	0	0
Grand Canyon NP, AZ	2,073	56	17	20	23	27	29.4 ± 0.09	33	43	46	0	0	0	0
Pima County, AZ	695	86	1	2	10	28	29.8 ± 22	46	60	68	10	1	0	0
Prescott, AZ	1,673	69	5	9	16	30	29.9 ± 16	43	52	55	2	0	0	0
Saguaro NM, AZ	933	66	19	22	30	38	38.8 ± 14	47	57	63	8	1	0	0
Douglas County, NV	1,951	60	6	11	20	35	35.5 ± 19	49	62	69	12	1	0	0
Reno, NV	1,280	92	0	0	10	30	28.4 ± 20	40	50	60	10	1	0	0
Boulder County, CO	1,635	95	8	14	24	35	36.0 ± 18	47	60	69	11	2	0	0
Colorado Springs, CO	1,842	88	0	2	10	25	26.3 ± 18	40	51	57	4	0	0	0

Table 5-34 (cont'd). Growing Season (May Through October) Summary Statistics for Ozone Monitoring Sites in or Near Forests for the Period 1980 through 1988 (Percentiles and means were generated using the entire data set [1980 through 1988; May through October].)

Site[b]	Elevation (m)	Percent Data Capture	Percentiles (ppb)								Percent Hours[a]			
			5	10	25	50	Mean ± SD[c]	75	90	95	≥60	≥80	≥100	≥120
Colordao NM, CO	1,750	30	30	32	37	42	44.1 ± 14	48	54	57	2	0	0	0
Denver, CO	1,591	96	0	2	7	19	21.9 ± 18	33	47	55	3	1	0	0
Great Sand Dunes, CO	2,487	54	24	27	33	39	38.4 ± 0.09	44	49	52	1	0	0	0
Larimer Country, CO	1,522	90	1	4	14	27	27.9 ± 18	40	52	59	5	0	0	0
Rocky Mountains NP, CO	2,743	49	25	31	38	46	46.0 ± 12	54	60	65	10	1	0	0
Arches NP, UT	1,567	32	28	31	36	43	42.8 ± 0.09	49	54	58	4	0	0	0
Bountiful, UT	1,335	87	8	14	25	38	38.3 ± 20	49	62	72	12	3	1	0
Logan, UT	1,382	45	8	12	20	32	32.5 ± 15	45	52	58	4	0	0	0
Ogden, UT	1,314	97	0	1	9	30	29.8 ± 22	46	58	65	8	1	0	0
Provo, UT	1,402	72	2	5	14	29	32.1 ± 22	49	62	68	12	2	0	0
Salt Lake, UT	1,305	87	2	4	11	28	30.4 ± 22	45	59	70	10	3	1	0
Albuquerque, NM	1,585	89	1	5	15	29	29.8 ± 19	43	55	61	6	1	0	0
Yellowstone NP, WY	2,484	58	15	19	27	36	35.4 ± 12	44	51	55	2	0	0	0

[a]Percent hours are normalized to represent the average occurrence of ozone levels during May through October. Percent data capture = number of valid hours/4,416 × 100, where 4,416 is the total number of hours during the period May through October.
[b]Site abbreviations: NPS = National Park Service, NM = National Monument, DOE = Department of Energy.
[c]SD = Standard deviation.

Source: Modified from Böhm (1992).

masses circulate back northward. This circulation cell causes higher O_3 levels to be adverted to the southernmost sites, the Sequoia National Forest and the Sequoia-Kings Canyon National Park. Mean hourly average concentrations in the Sierra Nevada during 1987 ranged from 0.018 to 0.076 ppm, with annual hourly maxima of 0.11 to 0.17 ppm. An O_3 exposure gradient with highest concentrations in the south and lowest in the north was observed. Associated with the gradient, injury is most severe at the southern end of the range and least severe in the north (Peterson et al., 1991).

The studies cited above reported visible O_3 injury only to the trees in the Sierra Nevada forests. To evaluate growth changes in O^3-stressed ponderosa and Jeffrey pine, Peterson and his coworkers, beginning in 1985, conducted the largest investigation of regional tree growth in the western United States (Peterson et al., 1987; Peterson and Arbaugh, 1988, 1992; Peterson et al., 1991). Using cores to determine whether growth reductions had occurred, they randomly sampled both trees with visible O_3 injury symptoms and asymptomatic trees. Major decreases in growth occurred for both symptomatic and asymptomatic trees during the 1950s and 1960s. The percentage of trees exhibiting growth decreases at any given site never exceeded 25% in a given decade (Peterson et al., 1991). The mean annual radial increment of trees with symptoms of O_3 injury was 11% less than trees at sites without O_3 injury. Trees larger than 40 cm in diameter and trees older than 100 years showed greater decreases in growth than did smaller and younger trees. Differences in growth between injured and uninjured trees were prominent after 1965 (Peterson et al., 1987).

The region-wide survey (Peterson et al., 1991) of ponderosa pine provides a useful backdrop for reporting a number of other studies or surveys in the Sierra Nevada that were more narrowly focused. Another tree ring analysis and crown injury study concentrated on Jeffrey pines in Sequoia-Kings Canyon National Park (Peterson et al., 1989). This study suggested that decreases of radial growth of large, dominant Jeffrey pines growing on thin soils with low moisture holding capacity and direct exposure to upslope transport of O_3 amounted to as much as 11% in recent years when compared with similar trees without symptoms.

Both a network of permanent plots established in 1980 and cruise surveys have been employed in Sequoia-Kings Canyon and Yosemite National Parks to determine the spatial distribution and temporal changes of injury to ponderosa and Jeffrey pine within the parks (Duriscoe and Stolte, 1989). In Sequoia-Kings Canyon, O_3 injury to individual trees and the mean number of trees injured in each plot increased from 47% for 1980 to 1982 to 79% for 1984 to 1985. Foliar injury was the most common response among the 28 plots studied. Ozone injury tends to decrease with the increasing elevation of plots. The O_3 concentrations associated with the highest levels of tree injury in the Marble Fork drainage of the Kaweah River, at approximately 1,800 m elevation, are hourly averages peaking frequently at 80 to 100 ppb but seldom exceeding 120 ppb.

During a cruise survey in 1986 (Duriscoe and Stolte, 1989) to identify the partial distribution of injury, there were 3,120 ponderosa or Jeffrey pines evaluated for O_3 injury in Sequoia-Kings Canyon and Yosemite National Parks. Approximately one-third of this number were found to have some level of chlorotic mottle. At Sequoia-Kings Canyon, symptomatic trees comprised 39% of the sample (574 of 1,470), and, at Yosemite, they comprised 29% (479 of 1,650). Ponderosa pines generally were injured more severely than Jeffrey pines.

In Sequoia-Kings Canyon, observations at field plots showed that giant sequoia seedlings developed O_3 injury symptoms at both ambient O_3 concentrations and 1.5 × ambient O_3 (0.08- to 0.1-ppm hourly peaks) in OTCs during the 8 to 10 weeks following germination (Miller et al., 1994). Field-plot observations of seedling health and mortality in natural giant sequoia groves over a 4-year period showed that seedling numbers were reduced drastically from drought and other abiotic factors. Any variable, such as O_3, that could stress seedlings sufficiently to reduce root growth immediately after germination could increase vulnerability to late summer drought. Significant differences in light-compensation point, net assimilation at light saturation, and dark respiration were found between seedlings in CF air treatments and 1.5 × ambient O_3 treatments (0.08- to 0.1-ppm hourly peaks) (Grulke et al., 1989). One interpretation of these results is that O_3 could be a new selection pressure during the regeneration phase of giant sequoia, possibly reducing genetic diversity.

The Lake Tahoe Basin is located at the northern end of the Sierra Nevada (near Eldorado National Forest) (Peterson et al., 1991). Because it is an air basin unto itself, the air quality situation is distinct from other Sierra Nevada sites. Ozone injury was first reported for the area in the late 1970s. In 1987, a survey of 24 randomly selected plots in the basin included a total of 360 trees, of which 105 (29.2%) had some level of foliar injury (Pedersen, 1989).

The radial growth response of big cone Douglas firs (*Pseudotsuga macrocarpa*) to long-term O_3 exposure was studied throughout the range of these firs in the San Bernardino Mountains of Southern California. Big cone Douglas fir is found in the mountain ranges of Southern California and northern Baja California, Mexico. In the San Bernardino Mountains, the species grows in canyons and on dry slopes at elevations from 700 to 2,200 m and, in association with canyon live oak (*Quercus chrysolepis*), throughout the chaparral and lower elevation mixed-forest communities. Big cone Douglas fir is usually rated as less sensitive than ponderosa or Jeffrey pine; however, injury symptoms resulting from elevated O_3 exposures have been seen (Peterson et al., 1995).

Dendroecological analyses indicate that growth rates have decreased considerably since 1950 (Peterson et al., 1995). Differences in basal area indices for 1913 to 1950 were compared with those for 1951 to 1988 to determine whether there were growth changes associated with increased air pollution during the latter period. More than 80% of all trees had reduced growth. Trees growing in regions of high O_3 exposure had the largest growth decreases, with approximately 30% of those growing under these conditions having reductions greater than 50%, and 60% having reductions greater than 20%. Fewer than 10% of the trees in any O_3 exposure area had growth increases greater than 25%. Based on their study, the authors conclude that, although O_3 does not have the same level of impact on these trees as it does on ponderosa and Jeffrey pine, reduced needle retention and lower recent growth rates could indicate increased O_3 stress (or O_3 stress mediated by climate) in big cone Douglas fir. Long-term monitoring of this species could provide an early warning of additional injury caused by air pollution in forest ecosystems of Southern California (Peterson et al., 1995).

Site Variables Affecting Ozone Response in the California Ecosystems

Structural changes in forest stands are highly related to their position or site on the landscape. Site variables can be defined at regional and local levels. For example, the regional level is defined in California by the location of forested mountain slopes and

summits in relation to polluted urban air basins. In both the Sierra Nevada and the San Bernardino Mountains in California the greatest tree injury is found on ridges that overlook the polluted air basins. The polluted air masses are transported up-slope or up-canyon in terrain that is usually sunlit in the afternoon and early evening, thus the thermal convection on warm slopes is a major means by which O_3 and associated pollutants are delivered to the first forested ridges. Both vertical mixing and horizontal diffusion into cleaner air results in a distinct gradient of decreasing O_3 concentration in more distant forest stands. Two such gradients have been described in the San Bernardino Mountains (Miller et al., 1986). Along the longer, west-to-east orientation axis of the mountain range, 24-h average O_3 concentrations for the highest summer months ranged from 0.09 to 0.140 ppm nearest the polluted South Coast Air Basin to 0.04 to 0.05 ppm at a downwind distance of 35 to 40 km. In the more narrow, south-to-north direction, the same concentration gradient is seen over a much shorter distance of 5 to 8 km because of a more rapid transition to the warm desert influence, which causes mixing and dilution (Miller et al., 1972). Accordingly, O_3 injury to sensitive vegetation ranges from severe to none over these distances.

In the Sierra Nevada Mountains, a gradient of decreasing injury is observed from west to east and south to north (Peterson and Arbaugh, 1992). But the worst level of chronic injury is generally much less than observed in the San Bernardino Mountains.

With respect to localized site variables, there is evidence from repeated surveys in Sequoia-Kings Canyon National Parks that the percent of trees injured and the severity of foliar injury both increased with decreasing elevation in the 1,500- to 2,500-m zone on generally west-facing slopes adjacent to the polluted San Joaquin Valley Air Basin (Stolte et al., 1992). In Sequoia-Kings Canyon National Parks, radial growth reductions in Jeffrey pine with foliar injury by O_3 were documented only for large, dominant trees growing on shallow soils (Peterson et al., 1987). Soil moisture availability is generally lower on such sites. One hypothesis for explaining radial growth decline on these sites and not on more favorable sites with greater moisture-holding capacity is that O_3 defoliation in favorable moisture years and water stress in dry years integrate sequentially to suppress growth.

In the San Bernardino Mountains, radial growth of ponderosa and Jeffrey pines in plots along the decreasing O_3 gradient was not well correlated with level of chronic injury but was better correlated with soil-moisture-holding capacity. Within a single plot with relatively uniform moisture availability there was a good correlation between increased radial growth and a decreasing level of chronic O_3 visible injury to crowns.

5.7.3.4 The Appalachian Mountains—Before 1986

Oxidant-induced injury on vegetation in the Applachian Mountains has been observed for many years but has not produced the same ecosystem responses as vegetational injury in the San Bernardino Mountains. Results of studies in the eastern United States were reported in the 1986 criteria document and are summarized in the following passages (U.S. Environmental Protection Agency, 1986). Needle blight of eastern white pine was first reported in the early 1900s, but it was not known until 1963 that the needle blight was the result of acute and chronic O_3 exposure (Berry and Ripperton, 1963). In the 1950s, the U.S. Forest Service studied the decline of eastern white pine in an area covering several hundred square miles on the Cumberland Plateau in Tennessee and concluded that atmospheric constituents were the causes of this decline (Berry and Hepting, 1964; Garner et al., 1989; Garner, 1991).

Growth reductions in trees growing on the Cumberland Plateau of eastern Tennessee were studied by Mann et al. (1980) and McLaughlin et al. (1982). A steady growth decline in annual-ring increment was observed during the years 1962 through 1979. Reductions of 70% in average annual growth and of 90% in average bole growth were observed in sensitive trees, when compared to the growth of tolerant and intermediate trees. Tolerant trees, when compared to trees of intermediate sensitivity, consistently showed a higher growth rate (from 5 to 15%) than did intermediate trees for the 1960 to 1968 interval, similar growth rate from 1969 through 1975, and a reduction in growth (5 to 15%) for the period 1976 through 1979. The decline was attributed to chronic O_3, which frequently exceeded 1-h average concentrations of 0.08 ppm. Maximum 1-h concentrations ranged from 0.12 to 0.30 ppm for the years 1975 to 1979 (U.S Environmental Protection Agency, 1986).

McLaughlin et al. (1982) observed that the decline in vigor and the reduction in growth in trees and the production of carbohydrates (carbon flow) were associated with the following sequence of events and conditions: premature senescence of mature needles at the end of the growing season; reduced carbohydrate storage capacity in the fall and reduced resupply capacity in the spring to support new needle growth; increased reliance of new needles on self-support during growth; shorter new needles, resulting in lower gross photosynthetic productivity; and higher retention of current photosynthate (carbohydrate) by foliage, resulting in reduced availability for transport for external use, including repair of chronically stressed tissues of older needles (U.S. Environmental Protection Agency, 1986).

Despite the early field observations of Berry (1961) and Berry and Ripperton (1963), no concerted effort was made to determine the effects of O_3 on vegetation in the Appalachian Mountains until the 1970s, when, between April 1975 and March 1976, Skelly and his coworkers began monitoring total oxidant concentrations and recording associated injury to eastern white pine in three rural Virginia sites. Injury was observed in the Jefferson and George Washington National Forests and throughout the Blue Ridge Mountains, including areas in the Shenandoah National Park and along the Blue Ridge Parkway in Virginia and North Carolina (Hayes and Skelly, 1977; Skelly et al., 1984). Taylor and Norby (1985), in their analysis of the 4-year monitoring data of Skelly et al. (1984), point out that there were an average of five episodes (any day with a 1-h mean O_3 concentration >0.08 ppm) during the growing season in this area. Episodes lasted from 1 to 3 days.

In studies conducted in the Blue Ridge Mountains of Virginia, Benoit et al. (1982) used annual-ring increments to evaluate the possible effects of oxidant air pollution on the long-term growth on eastern white pine of reproducing age. Reductions in overall growth of eastern white pine trees classified as tolerant, intermediate, and sensitive to O_3 exposure were observed. Comparison of growth from 1974 to 1978 with that for 1955 to 1959 indicated decreases of 26, 37, and 51% for tolerant, intermediate, and sensitive trees, respectively. No significant changes in seasonal precipitation had occurred during the 1955 to 1963 period or the 1963 to 1978 period; therefore, the significant reduction in radial growth was assumed by the authors to be the result of cumulative O_3 stress and reduced photosynthetic performance due to oxidant injury. Monitoring of O_3 indicated monthly average concentrations of 0.05 to 0.07 ppm on a recurring basis, with episodic 1-h peaks frequently in excess of 0.12 ppm for the latter time period (Benoit et al., 1982; U.S. Environmental Protection Agency, 1986). Duchelle et al. (1982), monitoring in the same area, reported peak hourly averages >0.08 ppm for the months of April through September

in 1979 and 1980. As early as 1979, Skelly (1980) concluded that the most sensitive eastern white pines were injured so severely by oxidant exposure that they probably were being removed from the population. It was estimated that, of the population, 22% were tolerant, 67% were intermediate, and 11% were sensitive.

In the previous O_3 document (U.S. Environmental Protection Agency, 1986), Duchelle et al. (1982, 1983) reported that exposing native tree seedlings and herbaceous vegetation in the Big Meadows area of the Shenandoah National Park in the Blue Ridge Mountains of Virginia to ambient O_3 reduced both the growth of the native trees other than eastern white pine and the productivity of the native herbaceous vegetation found growing in forested areas. Comparison of growth of seedlings in open plots or OTCs with CF air revealed that growth was suppressed in wild-type seedlings of tulip poplar, green ash, sweet gum (*Liquidambar styraciflua*), black locust (*Robinia pseudoacacia*), eastern hemlock (*Tsuga canadensis*), Table Mountain pine (*Pinus pungens*), Virginia pine (*Pinus virginiana*), and pitch pine, usually without visible foliar injury symptoms. Open-top chambers were operated continuously from May 9 until October 9 during 1979 and from April 24 until September 15 in 1980 (U.S. Environmental Protection Agency, 1986). Common milkweed and common blackberry (*Rubus allegheniensis*) were two species of native vegetation that exhibited visible injury symptoms (Duchelle and Skelly, 1981). Monthly 8-h average O_3 concentrations ranged from 0.035 to 0.065 ppm, and peak hourly concentrations from 0.08 to 0.13 ppm (Skelly et al., 1984; U.S. Environmental Protection Agency, 1986). Common milkweed and common blackberry represented natural vegetation sensitive to O_3 exposure (Duchelle and Skelly, 1981; U.S. Environmental Protection Agency, 1986).

Forest ecosystems at high altitudes experience higher total exposures because of the prolonged duration of elevated O_3 at high altitudes (see Section 5.4; Wolff et al., 1987; Winner et al., 1989; U.S. Environmental Protection Agency, 1986). Although daily maximum and mid-day O_3 concentrations are similar at different altitudes, the dosage increases with height. Ozone is depleted rapidly at night near the earth's surface below the nocturnal inversion layer; however, mountainous sites above the nocturnal inversion layer do not experience this depletion. Therefore, the total exposure to O_3 in mountainous areas can be much higher than that in nearby valleys (Berry, 1964; Garner et al., 1989). Maximum O_3 concentrations observed at elevated mountain sites often occur at night; in addition, higher elevations are often exposed to sustained or multiple peak concentrations of O_3 within a given 24-h period. High morning concentrations occur at a time when stomatal conductance is high and photosynthetic activity is greatest. The cumulative effects of O_3 uptake, therefore, could be severe. These considerations need to be taken into account when assessing the exposure-response relationships of forest ecosystems at high altitudes (Wolff et al., 1987; Garner et al., 1989; Garner, 1991).

The field observations cited above indicate that oxidant-induced injury to vegetation has been occurring in the Appalachian Mountains for many years. By the time intensive studies were begun in Pocahontas County, WV, in 1957, to determine the cause of "emergence tipburn", many people living in the area had been reporting casual observations of the phenomenon for over 20 years. Emergence tipburn, also known as needle blight, of eastern white pine was observed first in the early 1900s, however it was not shown to be the result of acute or chronic O_3 exposure until 1963 (Berry and Ripperton, 1963).

Although vegetation injury resulting from O_3 exposure had been observed in New Jersey in the 1940s, its cause was not recognized until 1960 (Daines et al., 1960). Ozone was first recognized as a causal factor of foliar injury when Heggestad and Middleton (1959)

reported that weatherfleck of tobacco was the result of O_3 exposures. Concentrations of 0.38, 0.43, and 0.5 ppm were measured at Beltsville by newly developed Mast meters during 1958. (The concentrations cited are approximately 0.1 ppm higher than those measured more recently. Calibration of the then new Mast meters was sometimes a problem [Garner, 1991]).

Regular oxidant monitoring stations were first established east of the Mississippi River in 1962. Valid oxidant data, however, was not available until 1964, and then only for the cities of Chicago, Cincinnati, St. Louis, Philadelphia, Washington, DC, and Denver (National Air Pollution Control Administration, 1968). Maximum oxidant concentrations recorded between 1964 and 1967 indicated that Cincinnati had 10 days >0.15 ppm; Philadelphia, 60 days >0.06 and 13 days >0.15 ppm; and Washington, DC, 65 days >0.10 ppm and 7 days >0.15 ppm (U.S. Environmental Protection Agency, 1986). Berry and Ripperton (1963) reported the presence of oxidant concentrations above 0.10 ppm during 1961 and 1962 in West Virginia and in North Carolina as far east as Raleigh. These data indicate that O_3 concentrations sufficient to injure vegetation regularly were present from the Midwest to the east coast (Garner et al., 1989; Garner, 1991).

In retrospect, it is apparent that O_3 episodes in the eastern United States have not been unusual. Taylor and Norby (1985) analyzed the 4-year monitoring data of Skelly et al. (1984) and concluded that episodes in which the 1-h O_3 concentration was >0.08 ppm were experienced, on average, five times during the growing season. Episodes when peak O_3 concentrations exceeded 0.10 ppm in the southern Appalachian Mountains were recorded during 1975 (Hayes and Skelly, 1977) and 1979 through 1982 (Skelly, 1980). Injury to eastern white pine at three rural sites in Virginia from July 1 to 5, 1975, was associated with a high pressure over the Great Lakes and a low, Hurricane Amy, off the Atlantic coast. Air parcels bearing O_3 moved in from the Northeast and Midwest into Virginia. The episode dissipated when the cold from the Midwest moved across Virginia into the Atlantic Ocean (Hayes and Skelly, 1977). More recent O_3 episodes in the same area have been associated with meteorological phenomena similar to the one mentioned above (Skelly et al., 1984; Garner et al., 1989).

Ozone episodes for the eastern United States also were recorded during 1976 and 1977. Typical episodes were associated with high-pressure systems that originated in Canada, moved southeastward into the Midwest, and then eastward to the Atlantic coast. For example, an episode covering most of a 20-state area occurred April 12 to 23, 1976. During this episode, O_3 concentrations in excess of 0.08 ppm occurred simultaneously from the Midwest to the Atlantic coast and into the northeastern United States. Ozone trajectories extended from Ohio to New Jersey (Wolff et al., 1977a,b,c; Garner, 1991). Additional studies indicated that two other episodes exhibiting trajectories similar to the one described above took place in August 1976. These episodes included an area extending from West Virginia across Virginia in the south and north to Maine. Maximum concentrations measured in the trajectories during the two August episodes were 0.20 ppm (Wolff et al., 1980; Garner, 1991).

In 1977, there were three episodes: (1) July 12 to 21, (2) July 21 to 24, and (3) July 26 to 30 (Wolff et al., 1980). The first episode, unlike the ones the previous year, originated in the Texas-Louisiana area. Air parcels traveled northeastward to the lower Midwest and then to the Atlantic coast, extending an "ozone river" from the gulf coast of Texas to Louisiana to the northeast Atlantic coast, exposing the entire area to concentrations averaging 0.12 to 0.13 ppm. The second and third episodes, like the 1976 episodes,

originated in Canada. Because the southern part of the first episode persisted at the time the second and third episodes began, O_3 from the south was pulled into the Midwest, and the region from the Texas gulf coast eastward to the Atlantic coast continued to be exposed to the high concentrations. These episodes simultaneously exposed nearly two-thirds of the United States (Wolff and Lioy, 1980; Garner, 1991).

Long-range transport of O_3 need not begin in Canada, the Midwest or Texas. Fankhauser (1976) reported the transport of O_3 in a giant loop stretching from New York City, Philadelphia, Baltimore, and Washington, DC, west through Virginia and Ohio and back to Wheeling, WV, to the Pittsburgh, PA, area. This path continued for 4 to 5 days in September, 1972. Earlier, in May 1972, a stagnant high and a slow-moving low transported air parcels from the Chicago and Pittsburgh areas to Miami, FL (Garner et al., 1989; Garner, 1991).

The foregoing discussion not only depicts the episodic nature of O_3 exposures, but also points out the fact that the major portion of the United States east of the Mississippi River has been exposed frequently to phytotoxic O_3 concentrations. Taylor and Norby (1985) estimate the probability is 80% that any given O_3 episode in the Shenandoah forest will persist for at least 3 days. This information concerning the effects of O_3 exposure is summarized from the 1986 criteria document (U.S. Environmental Protection Agency, 1986).

5.7.3.5 The Appalachian Mountains and the Eastern United States—Since 1986

Changes in growth, decline, and mortality of certain tree species have been reported for high-elevation forest ecosystems from Maine, New Hampshire, Vermont, and New York, south to North Carolina and Tennessee. Studies indicate that the decrease in growth of forest trees began during the late 1950s or early 1960s (Adams et al., 1985; Benoit et al., 1982; Johnson et al., 1984; Phipps and Whiton, 1988; Garner et al., 1989; Garner, 1991). The extent of decrease in growth and of dieback and mortality, and the factors that precipitated them, are subject to controversy (Garner, 1991; Garner et al., 1989; Taylor and Norby, 1985). Many hypotheses, including O_3 exposure, have been advanced as possible causes. The problem, as pointed out by Woodman and Cowling (1987), is establishing causation. Rigorous proof is needed, but only circumstantial evidence is available. Because the growth reductions began so many years ago, long-term historical data regarding forest structure and composition is lacking (Garner, 1991; Garner et al., 1989). An additional factor that makes causation difficult to determine is that mature ecosystems are not completely stable, but maintain themselves in an oscillating steady state (Kozlowski, 1985). No long-term studies of the effects of tree decline and mortality on ecosystems similar to those dealing with the exposure and response of the San Bernardino mixed-forest ecosystem in California have been made in the East.

Surveys made in 1982, but mentioned only briefly in the previous criteria document (U.S. Environmental Protection Agency, 1986) give quantitative evidence of a marked dieback and large reductions in basal area and in density of red spruce in the high-elevation forests of New York, Vermont, and New Hampshire (Johnson and Siccama, 1983). Red spruce is the most characteristic species of subalpine forests that occupy the higher peaks and ridges of the Appalachian Mountains from Maine to North Carolina and Tennessee. A co-dominant species in the North is balsam fir (*Abies balsamea*), whereas Fraser fir, a closely related species, is co-dominant in the South (Adams et al., 1985). A detailed description of the red spruce decline in the eastern United States and possible causes and studies conducted to determine the causes can be found in Eager and Adams

(1992). In the summary chapter of that text, Johnson et al. (1992) write that they "are in a position to state and support with field and laboratory data that regional scale air pollution has played a significant role in the decline of red spruce in the eastern United States." Ozone usually is considered the only regional air pollutant. In this instance, however, the authors are referring to NO_x and SO_x, the precursors of acidic deposition. Studies evaluating the direct effects of O_3 on red spruce have found little evidence for a significant effect (McLaughlin and Kohut, 1992). Recent studies evaluating the responses of red spruce and loblolly pine to acidic precipitation and O_3 indicate that high-elevation red spruce forests could be impacted by acidic deposition enhancing soil acidification, mobilization of aluminum ions (Al^{3+}), and reducing the availability of important base cations (Edwards et al., 1995).

In the Southeast, the decline and mortality of Fraser fir in the Great Smoky Mountains National Park, and, in North Carolina, the Plott Balsam Mountains and the Black Mountains, which include Mt. Mitchell, have been attributed to infestation by the balsam wooly adelgid (Hain and Arthur, 1985). The west-facing slope of Mt. Mitchell showed the greatest injury. During a 20-year experimental study of Fraser fir growing in the Smoky Mountains National Park, balsam wooly adelgids killed almost all of the canopy trees and reduced the basal area in two plots established in the 1960s. Red spruce basal area in these plots remained about the same for the same period. The report does not mention whether atmospheric pollutants were monitored, nor does it discuss possible pollutant-pathogen interaction or possible predisposition (Busing et al., 1988).

Other studies on Mt. Mitchell, however, do not attribute the death of Fraser fir solely to the balsam wooly adelgid, but suggest that atmospheric deposition and multiple pollutant stresses also had a role in tree mortality. These studies cite exposure to gaseous air pollutants, particularly O_3, and cloud-water deposition of acidic substances among possible stresses that have increased host susceptibility to attack by the balsam wooly adelgid (Hain and Arthur, 1985; Aneja et al., 1992). Ozone levels for the area have ranged from 0.01 to 0.150 ppm, with the highest concentrations occurring early in the summer (Aneja et al., 1992).

Other than the studies of tree death in the specific regions cited above, the studies in the Appalachian Mountains have been field surveys made to identify possible O_3-related foliar injury symptoms on native vegetation and experimental exposures to verify the symptoms and to determine O_3 response of individual forest tree species and other native vegetation, usually using OTCs. Unfortunately, some of the studies exposing individual forest tree species cannot be used because the concentrations at which exposure occurred are given as ambient plus 1, plus 1.5, or plus 2, etc. The actual ambient concentration at the time of exposure is never mentioned in the paper. A few studies use an index, again without stating the O_3 concentration and duration of exposure from which the index was derived. These papers are of little scientific value in this discussion because the actual concentrations and duration of exposures at which vegetational injury occurred cannot be determined.

Data from the Forest Inventory Analysis timber inventory taken between 1972 and 1982, revealed that the annual growth rate of most southern yellow pines (loblolly, pitch, shortleaf, and slash) under 16 in. in diameter had declined by 30 to 50% throughout the Piedmont and mountain areas of the Southeast since measurements were made during the survey of 1957 to 1966 (Sheffield et al., 1985). Ozone has been suggested as a possible cause; however, verification of growth effects on mature trees has been lacking (McLaughlin and Downing, 1995).

Additional studies of the forest condition were conducted by the United States Forest Service. Millers et al. (1989) reviewed the information on tree mortality that has occurred in the eastern hardwood forest during the last century to determine whether a relationship exists between the patterns of mortality and the patterns of atmospheric pollution. The authors suggest "that the apparent increase in the decline and mortality of many hardwood species during the last few decades may be due to intensification of reporting and to the maturation of the forest itself." Most of the mortality observed was attributed to abiotic and biotic stress factors such as weather, silviculture, and injury by insects and diseases. Although there is evidence of injury to hardwoods from point-source pollutants such as smelters and to eastern white pine from O_3, there is no conclusive evidence of an association between patterns of hardwood mortality and regional atmospheric pollution (Millers et al., 1989). Millers et al. (1989) point out, however, that historical data on atmospheric deposition are not readily available to compare with historical data on mortality.

Twardus et al. (1993) describe forest conditions in twenty states within the Northeastern United States. Information on forest health in this report was obtained from the Cooperative Forest Health Program, from the Forest Inventory and Analysis Surveys conducted by the U.S. Department of Agriculture Forest Service between 1971 and 1993, and from the Northern Forest Health Monitoring Program. They state that "there continues to be no evidence of large, regional-scale declines in forest ecosystem health as determined by observation of visible crown indicators on trees, e.g., crown dieback, crown density, and foliage transparency." Symptoms of exposure to O_3 were noted on sensitive plants on 10 of 98 plots where bioindicator plants were located.

Recently, McLaughlin and Downing (1995) completed a 5-year study of the interactive effects of ambient O_3 and climate on the growth of mature loblolly pines. Ozone, temperature, and moisture stress often correlate well with each other in the southeastern United States because hot, dry years often are associated with air stagnation systems that result in regional O_3 episodes. Tree growth rates, as measured by annual circumference increase per tree for two drier upland sites (16 trees) and a wetter more fertile stand near a stream bottom (18 trees), were compared. Short-term changes in stem circumference of 24 to 34 mature trees were measured at 138 intervals during five growing seasons (May through October) using a sensitive dendrometer band system. During the period of the study, widely variable temperature, rainfall, and O_3-exposure conditions and growth rates that varied by 75% across the years were observed. Growth rates were consistently influenced by 3-day average O_3 exposures $\geq$40 ppm during the period from 0900 to 2000 hours (9:00 a.m. to 8:00 p.m.). McLaughlin and Downing (1995) stated that their model, which combined 5 years of growth data, suggested that the high-frequency effects of the 0.30 ppm-h increase in mean daily O_3 exposure in the most polluted year (1988), when compared to the cleanest year (1989), would reduce stem growth by approximately 7% in a relatively moist year and by almost 30% in a moderately dry year. They conclude that both episodic and chronic alterations of stem growth in mature trees are associated with ambient levels of O_3. Episodic reductions are related directly to O_3 exposure, whereas chronic alterations reflect the interaction of O_3 exposures and climatic stresses.

The surveys described below, specifically those made in the Shenandoah National Park, indicate that the injury to native vegetation reported by Hayes and Skelly (1977), Skelly (1980), Benoit et al. (1982), and Duchelle et al. (1982) continues to occur. This is cause for concern because the 48 national parks, including the Great Smoky Mountain and Shenandoah National Parks, are designated as Class I areas under the amended Clean Air Act

(U.S. Code, 1991). Air pollution effects on resources in Class I areas constitute an unacceptable adverse impact if such effects diminish the national significance of the area, impair the quality of the visitor experience, or impair the structure and functioning of the ecosystem (Fox et al., 1989; Chappelka et al., 1992). Factors considered in determining if an effect is unacceptable include the frequency, magnitude, duration, location, and reversibility of the impact.

In a survey of eastern white pine stands in the southern Appalachians, 50 white pines were examined for foliar symptoms (chlorotic mottle) believed to be caused by O_3 at each of 201 sites distributed on a 24 × 24-km grid across the natural range of the species in South Carolina, Tennessee, Virginia, North Carolina, Kentucky, and Georgia (Anderson et al., 1988). The survey was conducted from September through November 1985. The percentage of stands with at least one symptomatic tree was highest in Kentucky (77%), followed by Tennessee (31%), and lowest in Georgia (10%). The mean percentage of symptomatic trees per plot for all six states was 27%. The mean volume difference of 48 pairs of symptomatic and nonsymptomatic trees was 49% less for symptomatic trees. Elevation and percent slope were not correlated with occurrence of symptomatic trees, but most symptomatic trees were found on southwest-facing slopes. Plantations had a higher percentage of symptomatic trees than did natural stands. Ozone exposure concentrations were not reported, but it may be possible to make estimates of exposure using data from the nearest O_3 monitoring sites.

Shenandoah and Great Smoky Mountains National Parks are contained within the survey area investigated by Anderson et al. (1988). Winner et al. (1989) surveyed 7 to 10 individuals of five native species at 24 sites in Shenandoah National Park. These species included tulip poplar, wild grape (*Vitis sp.*), black locust, virgin's bower (*Clematis virginiana*), and milkweed. Visible foliar injury due to O_3 was most prevalent on milkweed species (up to 70%), whereas the remaining species had injury approaching 20%. In each case, the level of foliar injury increased with the elevation of the sites. The summer monthly 24-h mean O_3 concentrations at Blacksburg, Rocky Knob, Salt Pond, and Big Meadows did not exceed 0.06 ppm, and foliar injury still was observed.

Another survey made during August to September, 1991, in the Shenandoah National Park included black cherry, yellow poplar, and white ash; and, in the Great Smoky Mountains National Park, black cherry, sassafras (*Sassafras albidum*), and yellow poplar (Chappelka et al., 1992). Black cherry exhibited symptoms in both parks. In the former, the percentage of leaves injured ranged from 18 to 40, whereas, in the latter, the range was 8 to 29% in 1991. Black cherry at Cove Mountain in the Great Smoky Mountains National Park exhibited the highest percentage of symptomatic trees (97%). This site also had the highest number of hours exceeding 0.08 ppm. The majority of occurrences of concentrations exceeding 0.08 ppm occurred during evening hours. Chappelka et al. (1992) suggest that some of the variability in foliar injury response of hardwood species to O_3 in the Shenandoah and Great Smoky Mountains National Parks is due to elevation and microsite conditions, including proximity to streams.

During surveys made in the summers of 1987 through 1990, a total of 95 different plant species, approximately 6% of those growing in Smoky Mountain National Park, exhibited possible foliar injury symptoms attributable to O_3 exposures (Neufeld et al., 1992). Plant species exhibiting foliar injury varied from herbaceous herbs, a grass and a fern, to woody deciduous angiosperms and nine species of evergreens, of which six were conifers. Species exhibiting field symptoms included the native trees (black cherry; sycamore; tulip

poplar; black locust; sweet gum; eastern hemlock; and Virginia, Table Mountain, and pitch pines) and herbaceous plants, such as virgin's bower, wild grape, and tall milkweed, all plants previously reported by Duchelle and Skelly (1981) and listed in the previous criteria document (U.S. Environmental Protection Agency, 1986) as being sensitive to O_3 exposures. Ozone concentrations during the period of the surveys did not exceed 0.12 ppm. The observation was made that plants growing at the highest elevations experienced higher maximum and higher minimum concentrations and were exposed to 50% more O_3.

To verify the foliar injury observed in the field as being due to O_3, 39 species, 28 of them with field injury symptoms, had been fumigated experimentally in OTCs at the time of publication. Exposures resulted in injury symptoms on 25 of the 28 species that exhibited injury in the field (Neufeld et al., 1992).

Surveys also were made in a Class I areas in New Hampshire and Vermont during the years 1988 to 1990 (Manning et al. 1991; Lefohn and Manning, 1995). Ozone injury was extensive on vegetation growing in open-top and ambient air experimental plots in both states in 1988, when O_3 concentrations were unusually high. The incidence and intensity of O_3 injury symptoms were considerably less in 1989, whereas, in 1990, injury symptoms were evident on all plants. Based on the studies, it was determined that black cherry, milkweed, white ash, white pine, and two species of blackberry were all reliable biological indicators of ambient O_3 exposure (Manning et al., 1991).

The above surveys indicated that although there has been evidence of widespread injury to native trees and other vegetation from exposure to O_3, the amount of injury has not been great enough for it to be transferred from the tree level to the stand level. Undoubtedly, there has been selection for and removal of the most sensitive tree species of eastern white pine, for example. However, the numbers of sensitive individuals in a stand have not been great enough to make a visible impact on the forest. Simulations suggest that, in forests with mixed species of uneven-aged stands, long-term responses are likely to be shifts in species composition rather than widespread degradation (Taylor and Norby, 1985; U.S. Environmental Protection Agency, 1986).

5.7.3.6 Rhizosphere and Mycorrhizal-Plant Interactions

The importance of the below-ground ecosystem largely has been overlooked when evaluating ecological responses to oxidant exposure. Although the soil system is part of the larger terrestrial ecosystem, it is a system that operates independently and, therefore, is itself an ecosystem (Richards, 1987). Although above-ground components of the terrestrial ecosystem are dominated by producers, the below-ground system is composed primarily of consumers. Thus, the below-ground system is dependent on the above-ground system for inputs of energy-containing substrates. Bacteria, fungi, protozoa, nematodes, microarthropods, earthworms, and enchytraeids all serve various functions in maintaining biological, physical and chemical characteristics of soil, and all are dependent on plant residues for their maintenance. Although the uniqueness of the below-ground ecosystem needs to be recognized, the interdependence between the above- and below-ground systems cannot be over emphasized.

Mycorrhizal fungi are an integral part of the below-ground ecosystem of terrestrial plant communities and are of great importance for vegetational growth. The 1986 criteria document (U.S. Environmental Protection Agency, 1986) discussed mycorrhizae-plant interactions and their importance in some detail. Mycorrhizae are formed on the roots of the vast majority of terrestrial plants and contribute substantially to ecosystem function (Allen,

1991; Harley and Smith, 1983). Fungi invade the roots of terrestrial plants and transform them into mycorrhizae or "fungus roots". The fungus and the host plant live together in an association beneficial to both organisms. Most terrestrial plants cannot adequately take up soil nutrients and water and achieve optimum growth and reproduction without mycorrhizae (Hacskaylo, 1973; Ho and Trappe, 1984; Allen, 1991). Mycorrhizal fungi increase the solubility of minerals, improve the uptake of nutrients for host plants, protect their roots against pathogens, produce plant growth hormones, and transport carbohydrate from one plant to another (Hacskaylo, 1973). In exchange, the roots of the host plant provide the fungi with simple sugars (Hacskaylo, 1973; Krupa and Fries, 1971). The fungus-plant root relationship is particularly beneficial to plants growing on nutrient-poor soils.

Ozone stress reduces photosynthesis and growth, and roots often are more affected than shoots (Figure 5-33; Winner and Atkinson, 1986; McCool and Menge, 1984; Blum and Tingey, 1977; Manning et al., 1971b; Tingey and Blum, 1973; Hogsett et al., 1985a; Tingey et al., 1976b; Spence et al., 1990; McLaughlin et al., 1982). It has been shown to affect both leaf senescence and root production in plants, thereby disrupting carbon availability for maintenance of the below-ground system (Gorissen et al., 1991b; Andersen and Rygiewicz, 1991), and to alter mycorrhizal colonization and compatibility (Stroo et al., 1988; Reich et al., 1986a; Simmons and Kelly, 1989).

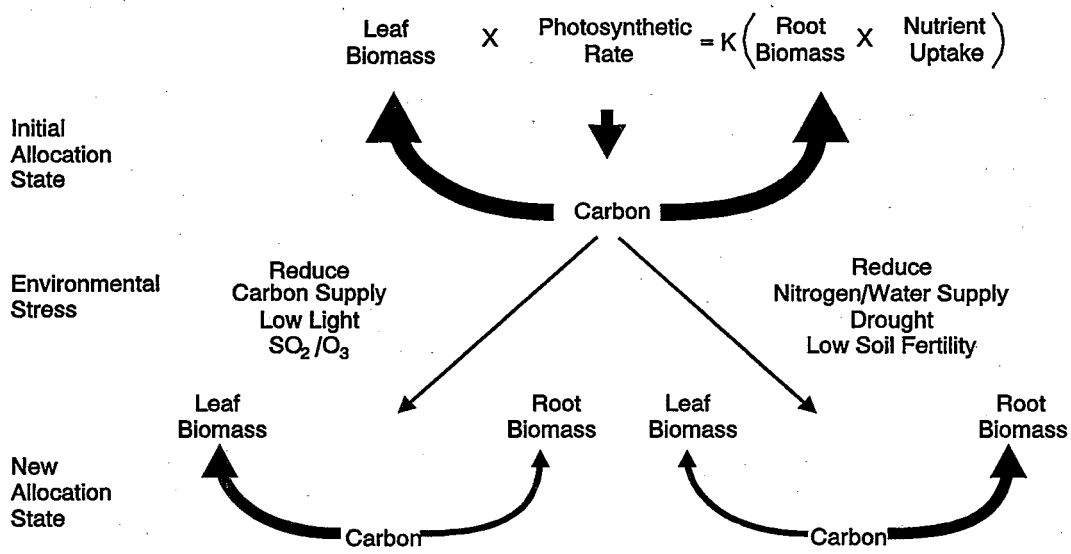

Figure 5-33. Impact of a reduced supply of carbon to the shoot, or water and nitrogen to the roots, on subsequent allocation of carbon.

Source: Winner and Atkinson (1986).

Mycorrhizae are sensitive to the capacity of the plant to translocate carbohydrate compounds to the roots. Studies have shown that simple sugars provided by plant roots are utilized readily by mycorrhizae and enhance fungal inoculation (McCool and Menge, 1984;

Hacskaylo, 1973). Ozone has the capability of disrupting the association between the mycorrhizal fungi and host plants by inhibiting photosynthesis and reducing the amount of sugars available for transfer from the shoot to the roots (see Figure 5-3). Reduction in the roots of available sugars can reduce mycorrhizal formation and root growth as well (Andersen et al., 1991). Berry (1961) examined the roots of eastern white pine injured by O_3 and observed that healthy trees had almost twice the percentage of living feeder roots as trees with O_3 injury. In the San Bernardino Forest in California, Parmeter et al. (1962) observed that the feeder roots system of ponderosa pine exposed to O_3 showed marked deterioration (U.S. Environmental Protection Agency, 1986).

Some studies of the effects of O_3 on tree species that include the investigation of the effects on ectomycorrhizal associations and have been discussed in a series of articles (Shafer and Schoeneberger, 1991). Selected studies are summarized in Table 5-35. The understanding of oxidant effects on root symbioses has not changed substantially since 1986 (U.S. Environmental Protection Agency, 1986); however, the understanding of the importance of symbiotic organisms in ecosystem function has improved. The basic hypothesis on mechanisms remains the same (i.e., effects are mediated through host carbohydrate metabolism) because oxidants do not penetrate the soil more than a few centimeters. Most of the research has been conducted on individual plant species, usually as seedlings, in controlled environments. Although the role of mycorrhizae in community structure has been recognized, it has not specifically been addressed experimentally.

Other studies have refined the understanding of oxidant stress effects on roots. In Douglas fir, root/soil respiration was reduced significantly during the first 1 to 2 weeks after exposure to O_3 or SO_2, followed by a recovery period that resulted in similar total respiratory release between treatments and controls (Gorissen and van Veen, 1988; Gorissen et al., 1991a). Total allocation to roots did not appear to be reduced, but O_3 apparently reduced translocation to roots in that respiration of ^{14}C was suppressed. Edwards (1991) found that root and soil respiration were reduced in loblolly pine seedlings exposed to O_3 levels ranging from 0.07 to 0.11 ppm (7-h mean) compared to seedlings exposed to levels below ambient (0.02 to 0.04 ppm). Nouchi et al. (1991) found that O_3 at 0.1 ppm reduced root respiration by 16% in domestic rice (*Oryza. sativa*) after 1 week of exposure. However, exposure to 3 to 7 weeks of 0.1 ppm O_3 resulted in elevated levels of root respiration.

The effects of O_3 on carbohydrate allocation to roots and subsequent shifts in biomass allocation have been examined (Cooley and Manning, 1987; Kostka-Rick and Manning, 1992a; Karnosky et al., 1992b; De Temmerman et al., 1992; Qui et al., 1992; Sharpe et al., 1989; Gorissen and van Veen, 1988; Gorissen et al., 1991a; Spence et al., 1990). Gorissen et al. (1991b) also studied the effects of O_3 exposure on Douglas fir inoculated with the fungi *Rhizopogon vinicolor* and *Lactarius rufus* and watered with ammonium sulfate. The investigators found greater needle retention of ^{14}C-labeled compounds in the new needles of O_3-treated plants, and a trend towards fewer ^{14}C-labeled substrates recovered in roots and root/soil fractions. Short-term transport of ^{11}C-labeled substrates were followed throughout loblolly pine (Spence et al., 1990). A 45% reduction in transport of photosynthates to roots occurred in O_3-treated plants compared to controls. Collectively, the studies have shown a general trend of diversion of carbohydrate from roots and retention in the photosynthetically active portions of plants. A reduction in allocation to roots can be associated with a change in the availability of carbohydrate for maintenance of root symbioses.

Table 5-35. Interactions of Ozone and Forest Tree Ectomycorrhizae[a]

Host Plant	Mycorrhizae	Exposure Conditions	Effect of O_3 on Mycorrhiza	Reference
Loblolly pine	*Pisolithus tinctorius*	OTC, field	Reduced root infection	Adams and O'Neill (1991)
	Not stated	OTC, field, 3 years	Reduced root infection	Edwards and Kelly (1992)
	Not stated	CEC	No effect	Mahoney et al. (1985)
	Not stated	CSTR	Reduced root infection	Meier et al. (1990)
Scots pine	Ten species	Open air, field, 3 years	No significant effects	Shaw et al. (1992)
White pine	*Pisolithus tinctorius*	CEC	Reduced root infection	Stroo et al. (1988)
Norway spruce	Six species	Open air, field, 3 years	No significant effects	Shaw et al. (1992)
	Four species	CEC	No consistent effects	Blaschke and Weiss (1990)
Paper birch	*Pisolithus tinctorius*	CEC	No effects	Keane and Manning (1988)
Red oak	Not stated	CEC and OTC	Significant increase	Reich et al. (1985)

[a]See Appendix A for abbreviations and acronyms.

The effects of O_3 on mycorrhizal colonization have varied depending on the experimental conditions and the species used. Stroo et al. (1988) studied the effects of O_3 on mycorrhizal infection in eastern white pine seedlings grown for 4 mo in several soils. Results varied by soil type and nitrogen availability; however, in several soils, the number of mycorrhizal short roots increased slightly at low O_3 levels and decreased significantly at higher O_3 concentrations. Reich et al. (1986a) found similar results in eastern white pine and red oak and concluded that O_3 may stimulate mycorrhizal infection at low O_3 concentrations. Simmons and Kelly (1989) observed a trend of greater mycorrhizal short roots in loblolly pine seedlings exposed to subambient O_3 treatment than those exposed to ambient or twice ambient O_3 levels, but the results were not statistically significant. In another study with two families of loblolly pine, Adams and O'Neill (1991) found that mycorrhizal colonization tended to increase with O_3 during the first 6 weeks of exposure and decrease with O_3 after 12 weeks of exposure. Meier et al. (1990) found a decrease in ectomycorrhizal root tips and percentage of feeder roots in loblolly pine seedlings. Keane and Manning (1988) found significant interactions among O_3, soil type, and pH; however, the direct effects of O_3 were difficult to elucidate. Collectively, these results suggest that O_3 does impact colonization of roots by mycorrhizal fungi; however, the results illustrate the variability in response due to such factors as soil condition, duration of experiment, and timing of measurements.

Altered root carbohydrate allocation resulting from O_3 exposure can affect host-fungus compatibility (Edwards and Kelly, 1992; Simmons and Kelly, 1989). Combined effects of O_3, rainfall acidity, and soil magnesium status on growth and ectomycorrhizal colonization of loblolly pine has been studied (Simmons and Kelly, 1989). Although variation was high, there was a trend towards altered species composition and reduced mycorrhizal infection in O_3-treated seedlings. Edwards and Kelly (1992) found high variability in morphotype (morphologically different) frequency in response to O_3 treatments in loblolly pine and noted changes in morphotype frequency over the 3-year study that suggested fungal succession had occurred. Fungal succession and the effects of oxidant stress on normal successional patterns are poorly understood. Shaw et al. (1992), using an open-field exposure system, found no differences in morphotype frequency or fruit-body succession in response to O_3 treatments.

The availability of current photosynthate for root growth is reduced under O_3 stress, and maintenance of below-ground processes dependent on roots for their carbon substrates may be affected. Mycorrhizae alter the size, quality, and retention time of carbon-pools below ground. As noted in the previous section, a 45% reduction in transport of photosynthates to roots occurred in O_3-treated loblolly pine (Spence et al., 1990). Ozone reduces concentrations of root carbohydrates (Jensen, 1981; Tingey et al., 1976b; Meier et al., 1990; Andersen et al., 1991). Starch in roots was reduced significantly in ponderosa pine by the end of one growing season of O_3 exposure (Tingey et al., 1976b). Reductions in coarse and fine root starch concentrations persisted over the winter in O_3-treated ponderosa pine and were lower during shoot flush in subsequent years (Andersen et al., 1991). In this study, lower starch concentrations in O_3-treated seedlings were associated with suppressed growth of new roots. The consequences of a reduction in carbon allocation below ground include reduced substrate availability for soil flora and fauna; altered soil physical characteristics, such as total organic matter and aggregation; and altered soil chemical characteristics including cation exchange capacity.

Premature leaf senescence has been observed in plants exposed to O_3 stress (U.S. Environmental Protection Agency, 1986). Premature senescence affects the belowground ecosystem by reducing canopy photosynthesis and carbon availability for transport to the belowground system and by increasing leaf litter inputs to the forest floor (Miller, 1984; Fenn and Dunn, 1989). The result is increased flux of nutrients, especially nitrogen, below ground, due to oxidant exposure.

The increased flux of nitrogen due to premature needle senescence in oxidant-exposed plants may act to disrupt the nutrient flow of the ecosystem. Allocation of carbon resources throughout a plant is based on a priority scheme that is driven by carbon and nutrient availability (Waring and Schlesinger, 1985). When soil nutrient levels are high, allocation to the shoot is favored over the roots (Figure 5-32). By shifting carbon allocation to organs in this fashion, plants can adjust to shifts in resource availability in their environment. Oxidant stress alters typical allocation schemes and, in the process, may impair the plant's ability to cope with drought or other stresses. In addition, reductions in allocation to roots can alter root-system size, architecture, and spatial arrangement, which, in turn, can influence populations of soil organisms.

Bacteria and fungi are particularly important in nutrient cycles and act to immobilize nitrogen, carbon, phosphorus, and other nutrients in the biomass. The turnover of these nutrient pools is relatively short because bacterial and fungal predators act to release these nutrients. The majority of plant-available nitrogen during the growing season comes from these predatory interactions in the soil (Kuikman et al., 1990; Ingham et al., 1985), emphasizing their importance in the maintenance of terrestrial ecosystems. Currently, there are no data available on the effect of O_3 on soil fauna.

In summary, mycorrhizal fungi are essential for optimal plant growth. Mycorrhizal fungi increase the solubility of minerals, improve the uptake of nutrients for the host plants, and protect plant roots against pathogens. In turn, the plant roots furnish the fungi with simple sugars that readily are utilized by the fungi and enhance their ability to form mycorrhizae. Mycorrhizae are sensitive to the capacity of the plant to translocate these carbohydrates to the roots. Ozone, by inhibiting photosynthesis, reduces the production of sugars available for transport to the roots. Reduction of sugars in the roots can reduce formation of mycorrhizae and root and tree growth as well.

5.7.4 Ecosystem Response to Stress

5.7.4.1 Introduction

Mature forest ecosystems are seldom stable. They are complex, dynamic communities of living and dead trees interacting among themselves; with populations of native forest floor plants; and with an array of microorganisms, insect pests, and environmental, human, and other factors to continuously shape and reshape the community over time (Manion and Lachance, 1992). Forest communities are held in steady state by the operation of a particular combination of biotic and abiotic factors. Stresses that alter or remove any of the factors can alter the community and change the ecosystem (Kozlowski, 1980; Garner et al., 1989).

Growth of new trees and other vegetation requires the expenditure of energy in the form of carbon compounds. Plants accumulate, store, and use carbon compounds to build their structure and to maintain their physiological processes. Carbon dioxide absorbed from the atmosphere is combined in plant leaves with water from the soil to produce the carbon

compounds (sugars) that provide the energy utilized by trees for growth and maintenance (Figure 5-34; Waring and Schlesinger, 1985). Patterns of carbon allocation to roots, stems, and leaves directly influence growth rate. The strategy for carbon allocation changes during the life of a plant, as well as with different environmental conditions (Figure 5-33; Winner and Atkinson, 1986). Mature trees have a higher ratio of respiration to photosynthetic tissue (Cregg et al., 1989). Even small changes in photosynthesis or carbon allocation can profoundly alter the structure of a forest (Waring and Schlesinger, 1985). Impairment of the process of photosynthesis shifts carbon allocation from growth and maintenance to repair and increased respiration and can result in resource imbalances. The significant changes observed in the San Bernardino Forest ecosystem were possible outcomes of the combined influences of O_3 on carbon, water, and nutrient allocation (McLaughlin, 1994).

Intense competition among plants for light, water, nutrients, and space, along with recurrent natural climatic (temperature) and biological (herbivory, disease, or pathogen) stresses, can alter the species composition of communities by eliminating those individuals sensitive to specific stresses, a common response in communities under stress (Woodwell, 1970; Guderian, 1985). Individual organisms within a population vary in their ability to withstand the stress of environmental changes. The range of variability within which these organisms can exist and function determines the ability of the population to survive. Those organisms able to cope with stresses survive and reproduce. Competition among different species results in succession (community change over time) and ultimately produces ecosystems composed of populations of plant species that have a capacity to tolerate the stresses (Kozlowski, 1980). Pollutant stresses, such as those caused by exposure to O_3, are superimposed on the naturally occurring competitional stresses mentioned above (see also Section 5.4). Communities, due to the interaction of their populations, respond to pollutant stresses differently from individuals (U.S. Environmental Protection Agency, 1993). Air pollutants are known to alter the diversity and structure of plant communities (Guderian et al., 1985). The extent of change that may occur in a community depends on the condition and type of community, as well as on the pollutant exposure.

The plant processes of photosynthesis, nutrient uptake, respiration, translocation, carbon allocation, and growth are directly related to the two essential ecosystem functions of energy flow and nutrient cycling. Altering the above processes can alter energy flow and nutrient cycling and impact ecosystems (Smith, 1992). Response of forest ecosystems to stress are growth-related processes that begin within individual trees and progress to increasing levels of integration and complexity (Figure 5-35; McLaughlin, 1994). Cytological and biochemical changes within a tree can impact physiological functions and alter the tree's growth and productivity. Plants acclimate to changing environmental stresses through both short- and long-term physiological responses, as well as through structural and morphological modifications (Dickson and Isebrands, 1991). When there are many sensitive individuals, the forest structure is changed. As indicated above, response begins with the interaction of the individual and its environment, progresses to the population and its environment, and then to the biological community and its environment (Billings, 1978).

In unpolluted atmospheres, the number of species in an ecosystem usually increases during succession. Productivity, biomass, community height, and structural complexity increase in the early stages of development. Severe stresses, on the other hand, divert energy from growth and reproduction to maintenance and repair and alter succession (Waring and Schlesinger, 1985). In addition, biomass accumulation and production decrease,

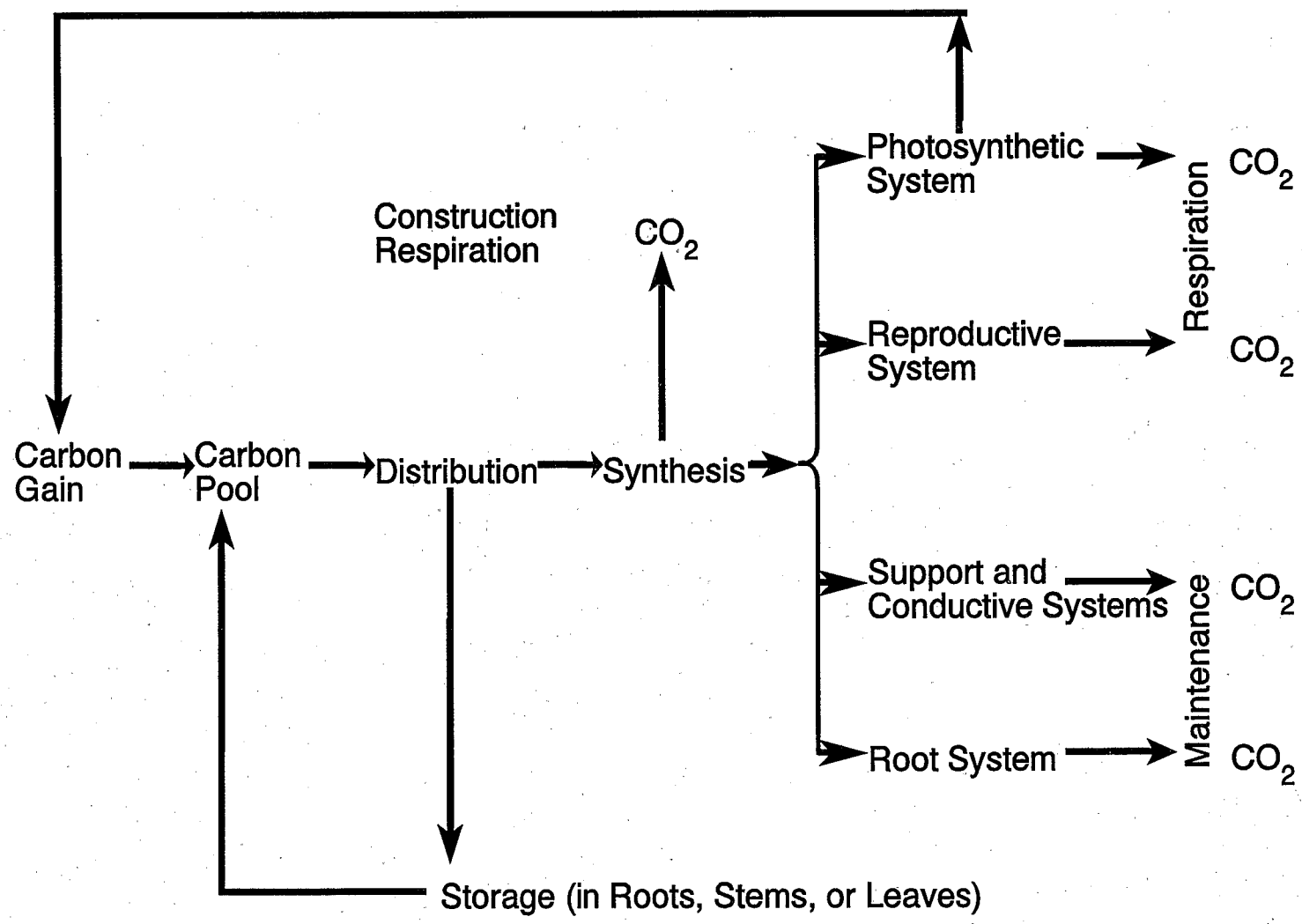

Figure 5-34. Carbon uptake through photosynthesis is made available to a general pool of carbohydrates used in construction and maintenance of various tissues. Carbohydrates may be shifted from one category to another, depending on environmental conditions.

Source: Waring and Schlesinger (1985).

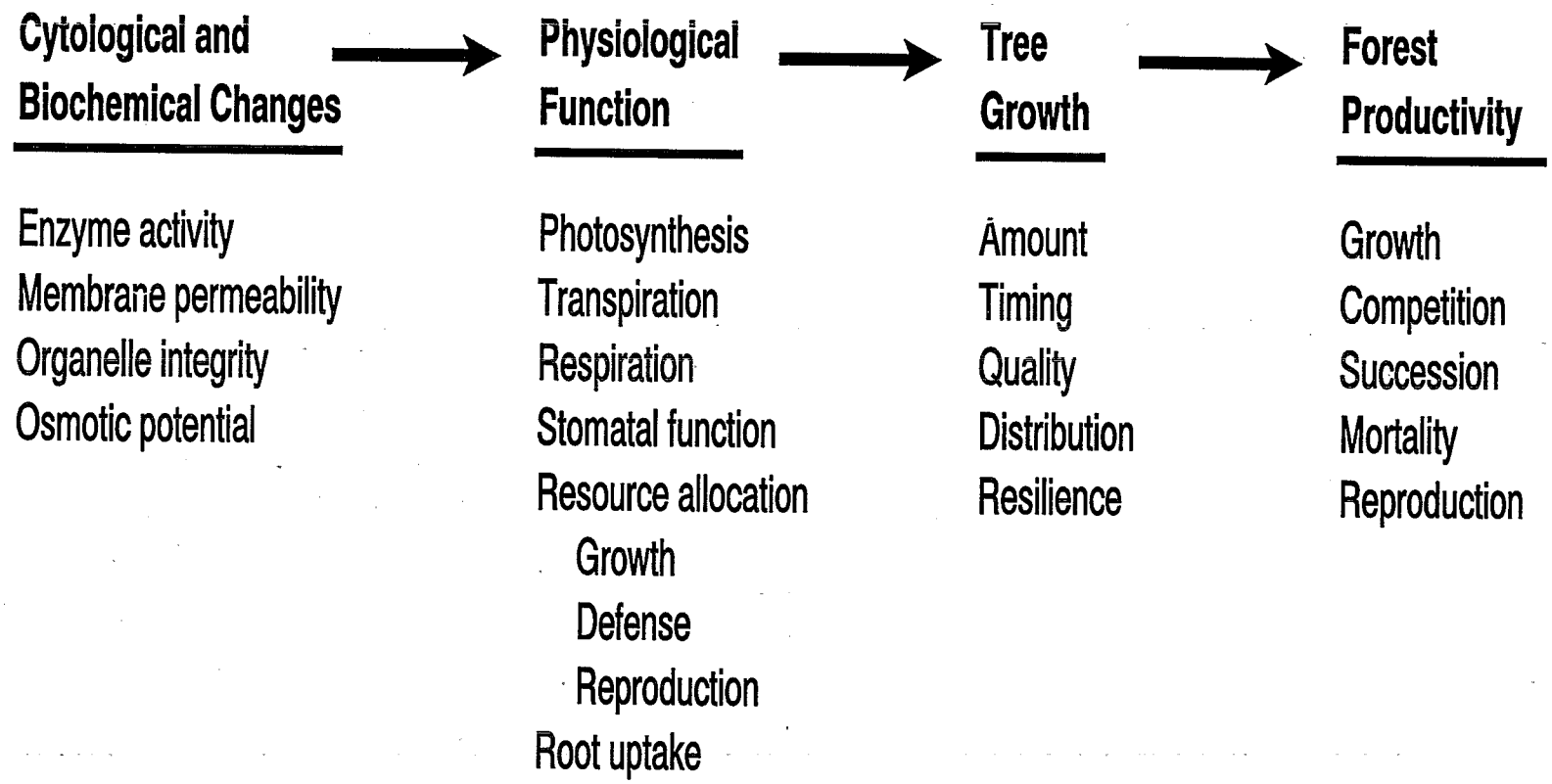

Figure 5-35. Organizational levels at which air pollutants have been shown to affect the growth-related process of forest trees.

Source: McLaughlin (1994).

Table 5-36. Interaction of Air Pollution and Temperate Forest Ecosystems Under Conditions of Intermediate Air Contaminant Load

Forest Soil and Vegetation: Activity and Response	Ecosystem Consequence and Impact
1. Forest tree reproduction, alteration, or inhibition	1. Altered species composition
2. Forest nutrient cycling, alteration a. Reduced litter decomposition b. Increased plant and soil leaching and soil weathering c. Disturbance of microbial symbioses	2. Reduced growth, less biomass
3. Forest metabolism a. Decreased photosynthesis b. Increased respiration c. Altered carbon allocation	3. Reduced growth, less biomass
4. Forest stress, alteration a. Phytophagous insects, increased or decreased activity b. Microbial pathogens, increased or decreased activity c. Foliar damage increased by direct air pollution influence	4. Altered ecosystem stress: increased or decreased insect infestations; increased or decreased disease epidemics; reduced growth, less biomass, altered species composition

Source: Smith (1990).

and structural complexity, biodiversity, environmental modification, and nutrient control are reduced (Bormann, 1985). With maturity, energy utilization in ecosystems shifts from production to maintenance (Odum, 1993) (see Figure 5-34). When catastrophic disturbances or injury, whether from natural (e.g., fire, flood, windstorm) or anthropogenic stresses (e.g., O_3), alter the species composition (biodiversity) of a forest sufficiently to disrupt food chains and to modify rates of energy flow and nutrient cycling, succession reverts to an earlier, less complex stage. The effects of stresses on ecosystems, unless the effects are catastrophic disturbances, are frequently difficult to determine (Kozlowski, 1985; Garner et al., 1989). In a mature forest, a mild disturbance becomes part of the oscillating steady state of the forest community or ecosystem. Responses to catastrophic disturbances, however, as a rule, are readily observable and measurable (Garner, 1994). How changes in plant processes attributed to O_3 exposure affect forest ecosystems is discussed in the following text.

5.7.4.2 Forest Ecosystems

The primary responses of a forest ecosystem to sustained exposure of O_3 are reduced growth and biomass production (Table 5-36; Smith, 1990). Exposure to O_3 inhibits photosynthesis and decreases carbohydrate production and allocation, and, as has been

discussed previously, decreased allocation to the roots interferes with mycorrhizae formation and nutrient uptake (Figure 5-33). The resulting loss in vigor affects the ability of trees to compete for resources and makes them more susceptible to a variety of stresses (Table 5-36; see also Sections 5.3, 5.6.4.2, 5.6.4.3, and 5.7.3.1). Responses of seedlings under experimental conditions indicate that reductions in growth occur at O_3 concentrations of 0.06 ppm or greater (Table 5-30). Cregg et al. (1989) state that information on seedling response must be used with caution. The environments in which seedlings and trees grow are substantially different due to differences in rooting depth and canopy structure. Trees have the potential to significantly alter their environments through shading, whereas seedlings do not. In the San Bernardino Forest, mortality of canopy trees leads to replacement by trees (white fir, incense cedar, sugar pine, and black oak) more tolerant to O_3 and reduced ecosystem structure. Reduction in structure altered nutrient cycling and energy flow and affected the functioning of other ecosystem components (see Section 5.7.3.1). Ozone concentrations capable of causing injury to forest trees and affecting forest processes continue to occur both in the West and the East. Although reports have described the presence of sensitive species in other U.S. forests; only the San Bernardino Forest has been severely impacted by exposure to O_3. Why this is the case is impossible to answer definitively because of the absence of data. Evidence obtained from many studies of a variety of ecosystems over the years indicates that ecosystems, in response to pollution or other disturbances, follow definite patterns that are similar even in different ecosystems (Woodwell, 1970). It is possible, therefore, to predict broadly the basic biotic responses to the disturbance of an ecosystem. These responses are reduction in standing crop (trees), inhibition of growth or reduction in productivity, differential kill (removal of sensitive organisms at the species and subspecies level), food chain disruption, successional setback, and changes in nutrient cycling (U.S. Environmental Protection Agency, 1978).

The effects of the stresses associated with O_3 exposure that have developed over the years in the San Bernardino Forest ecosystem are similar to those listed in the previous paragraph.

The extent of injury that an ecosystem will experience from O_3 exposure is determined by the severity and extent of individual response. Leaf injury, as has been stated previously, is usually the first visible indication of O_3 exposure. Structural effects develop when physiological processes within individual plants are disrupted severely (see Table 5-37 and Figure 5-35). With ecosystem responses such as those seen in the San Bernardino Forest, four levels of biological organization beginning with the individual organism are altered (see Table 5-37; Sigal and Suter, 1987). Taylor and Norby (1985) discuss the possible effects on ecosystems at the individual population and community levels. Alteration of functional properties (ecosystems functions) results in structural dysfunction. Stresses, whose primary effects occur at the molecular or cellular physiology level in the individual, must be scaled progressively up through more integrative levels of organ physiology (e.g., leaf, branch, root) to whole plant physiology, stand dynamics, and then to the landscape level to produce ecosystem effects (Figure 5-36; Table 5-37). Particularly, this is true if the stress is of low-level because only a small fraction of stresses at the molecular and cellular level become disturbances at the tree, stand, or landscape level. The processes of energy flow and nutrient cycling must be altered if ecosystems are to be affected. Insect defoliation, for example, may reduce severely the growth of one or several branches, whereas the growth of the tree appears not to be affected (Hinckley et al., 1992).

Table 5-37. Properties of Ecological Systems Susceptible to Ozone at Four Levels of Biological Organization

Level of Organization Properties	Structural Properties	Functional Properties
Organism	Leaf area and distribution Biomass and allometry	Photosynthesis, respiration Nutrient uptake and release Carbon allocation
Population	Age and size structure Population density Genetic composition Spatial distribution Dispersion (spatial pattern)	Natality (reproduction, mortality) Competition Productivity
Community	Species composition (diversity) Trophic levels and food webs Physical structure (leaf-area index)	Redundancy and resilience Succession (the integration of all species processes such as competition and predation)
Ecosystem	Biomass Element pools Soil properties	Ecosystem productivity Nutrient cycling Hydrologic cycling Energy flow

Source: Adapted from Sigal and Suter (1987).

Variability and compensation are two properties important in determining the effect a stress at one hierarchial level will have on a higher level of organization (see Sections 5.3 and 5.4). Variability in individual response to stress can be the result of each individual being genetically different (See Section 5.4). Individual trees do not respond equally to O_3 exposure. Ponderosa, Jeffrey, and eastern white pine all have been observed to have sensitive, intermediate, and tolerant varieties based on the degree of response. Variability in exposure-response also can be influenced by the movement of O_3 from the leaf surface through the stomata to the metabolic site of action in the leaf interior (Taylor and Hanson, 1992). The stomatal conductance also influences this action.

Variation in age and stage of growth of the organism also can determine response to O_3 exposure (see Section 5.3). Variability in response between seedlings, saplings, and canopy black cherry trees at a site in north-central Pennsylvania was observed by Fredericksen et al. (1994). Physiological, phenological, and morphological differences among seedlings, saplings, and canopy trees were associated with altered O_3 uptake and differential response. Leaves at different crown positions of larger trees exhibited differences in leaf physiology and O_3 uptake. Seedling uptake of O_3 and apparent sensitivity per unit leaf area was greater, based on foliar injury symptoms; however, the relative

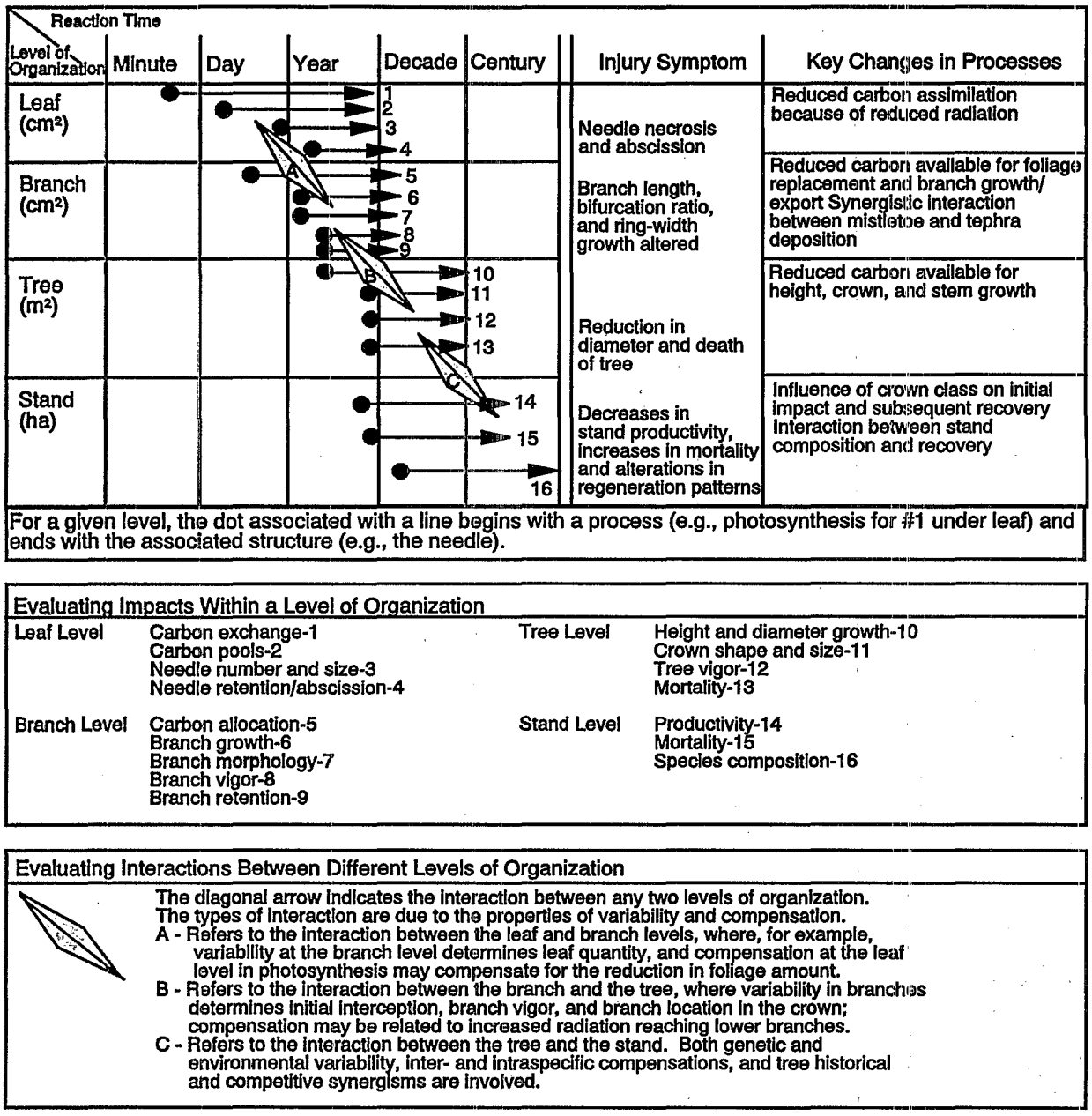

Figure 5-36. Effects of environmental stress on forest trees are presented on a hierarchial scale for the leaf, branch, tree, and stand levels of organization. The evaluation of impacts within a level of organization are indicated by horizontal arrows. The evaluation of interactions between different levels of organization are indicated by diagonal arrows.

Source: Hinckley et al. (1992).

exposure of seedlings to O_3 was reduced by an indeterminate growth habit because the majority of their leaves were produced after shoot growth had ceased for sapling and canopy trees. Therefore, their relative uptake was reduced on a whole-crown basis over the growing season and cumulative exposure. Lower crown leaves of saplings and canopy trees appear to be more sensitive to O_3 than upper crown leaves, despite lower uptake, which possibly is due to low availability of photosynthate for anti-oxidant defense and repair of injured leaf cell membranes. Shade leaf morphological characteristics also may play a significant role. The above study describes the interaction of multiple factors that determine O_3 uptake and potential response and illustrates the complexity involved in scaling responses from controlled studies of open-grown seedlings to larger forest trees (Fredericksen et al., 1994).

Compensation in response to stress involves the capacity of the individual to adapt to the stress. Some plants compensate physiologically by detoxifying the O_3 entering the leaves. Other plants compensate by altering the root-shoot ratio. They reallocate carbohydrates to the source of injury in the leaves instead of to the roots, as do uninjured trees (see Figure 5-33 and Section 5.1.3). At the stand level, the slower growth of some trees may be compensated for by the relatively faster growth of others that are experiencing reduced competition so that the overall growth of the stand is not affected (Hinckley et al., 1992). These properties, when taken together, will determine the extent and the rate at which stress at one hierarchical level will impact the next highest level. A framework of hierarchical scales (Figure 5-36) was developed by Hinckley et al. (1992) to provide a means by which the effects of the eruption of Mount St. Helens on forest trees could be followed and understood. This framework is also applicable for use when considering O_3 effects and can be used to explain the difference between the response of the San Bernardino Forest ecosystem and the forests in the eastern United States. As pointed out above, variability and compensation determine the severity of the response of the individual.

Variability and compensation also occur at the population level, all populations do not respond equally (Taylor and Pitelka, 1992). Plant populations can respond in four different ways: (1) no response, the individuals are resistant to the stress; (2) mortality of all individuals and local extinction of the extremely sensitive population (the most severe response); (3) physiological accommodation, growth, and reproductive success of individuals are unaffected because the stress is accommodated physiologically; and (4) differential response, members of the population respond differentially, with some individuals exhibiting better growth and reproductive success due to genetically determined traits (Taylor and Pitelka, 1992). Differential response results in the progressive elimination over several generations of the sensitive individuals and a shift in the genetic structure of the population toward greater resistance (microevolution). Physiological accommodation or microevolution, with only the latter affecting biodiversity, are the most likely responses for exposure to chronic stress (i.e., stresses that are of intermediate-to-low intensity and of prolonged duration). The primary effect of O_3 on the more susceptible members of the plant community is that the plants can no longer compete effectively for essential nutrients, water, light, and space, hence are eliminated. The extent of change that can occur in a community depends on the condition and type of community, as well as the exposure (Garner, 1994). Forest stands differ greatly in age, species composition, stability, and capacity to recover from disturbance. For this reason, data dealing with the responses of one forest type may not be applicable to another forest type (Kozlowski, 1980).

In the eastern United States, ecosystem reduction in structure and diversity has never reached the proportions seen in the San Bernardino Mountains. Visible tree decline

has been observed only in the southern Appalachian Mountains, particularly Mt. Mitchell, and on Camel's Hump, VT. The actual cause of decline and mortality of trees in these areas is a matter of question.

Among the hypotheses suggested for the absence of major changes in the eastern forests is a difference in the O_3 exposures experienced by the eastern forests when compared with the San Bernardino Forest, a region noted for high O_3 levels. Lefohn et al. (1994) characterized and compared O_3 concentrations measured from 1988 to 1992 at Bearden Knob and Parsons, located in a remote forested region of north-central West Virginia, with other sites in the region. It was observed that 1988, when compared with 1992, was a year with very high O_3 exposures. At almost all sites in 1992, few hourly concentrations were $\geq$0.10 ppm, whereas in 1988, several sites had 100 or more average concentrations $\geq$0.10 ppm. These concentrations were found at both high- and low-elevation sites. In 1992, Bearden Knob, a high-elevation site, experienced a flat diurnal pattern, whereas Parsons, the nearby low-elevation site, experienced a varying diurnal pattern, an indication that O_3 was being scavenged. Horton Station, a high-elevation site in southwestern Virginia, in 1992, experienced 25 episodes with hourly average concentrations near 0.05 ppm for 8 h or longer, 18 episodes with hourly average concentrations near 0.06 ppm, and three episodes with concentrations at or near 0.07 ppm. For the same period, Bearden Knob experienced 31 episodes of 8 h or longer for average hourly concentrations near 0.05 ppm, 13 episodes at or near 0.06 ppm, and 3 episodes at or near 0.07 ppm.

Maximum hourly average concentrations from April to October during 1988 were 0.145 ppm at or near Horton Station, compared with the 0.29 ppm received by the San Bernardino National Forest. Horton Station was exposed to 2,758 hourly concentrations between 0.05 ppm and 0.087 ppm, whereas the San Bernardino site received 2,027 h. The latter site had more concentrations above 0.10 ppm; therefore, it received fewer exposures between 0.05 and 0.087 ppm. It was suggested that the extreme growth reduction and injury that has been observed in the San Bernardino area over the years, when compared with the absence of such injury in the Horton Station area, could be attributed solely to the higher number of hourly average concentrations exceeding 0.10 ppm at the former site (Lefohn et al., 1994). Factors not considered were differences in sensitivity, stand composition, and the ability to compensate for the stress, as well as site variables.

These factors definitely apply in the Appalachian Mountains and, to a degree, in the Sierra Nevada, where the sensitive individuals and composition of the forests vary from the San Bernardino Mountains. The forests of the Appalachian Mountains are known to be more biologically diverse than western forests. Only in the San Bernardino Forest did the removal of sensitive trees reach the population level. Population dynamics impact the ecosystem functions of energy flow and nutrient cycling.

Eastern white pine responses to O_3 exposures were classified into three sensitivity levels ([1] sensitive, [2] intermediate, and [3] tolerant) by both McLaughlin et al. (1982) and Benoit et al. (1982). Black cherry also has been observed to have three sensitivity levels. None of these trees can be termed canopy dominants. In addition to conifers, the forest canopy includes varieties of oaks that are not as sensitive to O_3 exposures. Species removal, therefore, has not affected the eastern forests as did removal of ponderosa and Jeffrey pine from the San Bernardino Forest. Taylor and Norby (1985) have pointed out that the nature of community dynamics, particularly in mixed species, and stands with trees of uneven age play important roles in forest response. Shifts in species composition are more likely responses to stresses such as O_3 than to community degradation. The removal of the

codominant American chestnut (*Castanea dentata*) in the first half of this century caused no major change in the tree species relationships in the Appalachian forests. The effects of O_3 exposure also will probably not cause major change.

The previous O_3 document (U.S. Environmental Protection Agency, 1986) concluded that none of the tree species shown to be injured by O_3 play a dominant role in the Blue Ridge Mountain ecosystem. Therefore, the removal of any of these species probably would not have the impact that the decline and death of ponderosa and Jeffrey pine have had on the San Bernardino Forest ecosystem. This same conclusion applies today.

5.7.5 Summary

Ecosystems are composed of populations of "self-supporting" and "self-maintaining" living plants, animals, and microorganisms (producers, consumers, and decomposers) interacting with one another and with the nonliving chemical and physical environment within which they exist (Odum, 1989; U.S. Environmental Protection Agency, 1993). Mature ecosystems are seldom stable. Structurally complex communities, they are held in an oscillating steady state by the operation of a particular combination of biotic and abiotic factors, and they must respond and adapt continually to changing environments (Kozlowski, 1985).

Ecosystem response to stress begins with individuals (Figures 5-34, 5-35, and 5-36). Growth of trees and other vegetation requires the expenditure of energy in the form of carbon compounds. Carbon compounds are accumulated, stored, and used by plants to build their structure and maintain their physiological processes (Figure 5-34). Carbon dioxide absorbed from the atmosphere, combined with water from the soil in plant leaves during photosynthesis, provides the energy in the form of carbon compounds (sugars) utilized by trees for growth and maintenance (Figure 5-34; Waring and Schlesinger, 1985). Patterns of carbon allocation to roots, stems, and leaves directly influence growth rate. The strategy for carbon allocation may change during the life of a plant, as well as with different environmental conditions (Figure 5-33; Winner and Atkinson, 1986). Trees acclimate to changing environmental stresses through both short-term and long-term physiological responses and structural and morphological modifications (Dickson and Isebrands, 1991). Even small changes in photosynthesis or carbon allocation can alter profoundly the structure of a forest (Waring and Schlesinger, 1985). Impairment of the processes of photosynthesis shifts carbon allocation from growth and maintenance to repair, increases respiration, and can result in resource imbalances. The significant changes observed in the San Bernardino Forest ecosystem were a possible outcome of the combined influences of O_3 on carbon, water, and nutrient allocation (McLaughlin, 1994).

Mycorrhizae are an extremely important, but unheralded, component of all ecosystems. The majority of plants depend on them for the uptake of mineral nutrients from the soil. Their absence from the roots of plants has been shown to have a detrimental impact on plant growth. Decreased carbohydrate production and reduced allocation to the roots due to O_3 exposure affect the formation of mycorrhizae and impact plant growth. Exposure to O_3, therefore, affects plant growth both above and below ground.

Intense competition among plants for light, water, nutrients, and space, along with recurrent natural climatic (temperature) and biological (herbivory, disease, pathogens) stresses, can alter the species composition of communities by eliminating those individuals sensitive to specific stresses, which is a common response in communities under stress

(Woodwell, 1970; Guderian, 1985). Those organisms able to cope with stresses survive and reproduce. The effects of stresses on ecosystems, unless the effects are catastrophic disturbances, are frequently difficult to determine (Kozlowski, 1985; Garner et al., 1989). In a mature forest, a mild disturbance becomes part of the oscillating steady state of the forest community or ecosystem. Responses to catastrophic disturbances, however, as a rule, are readily observable and measurable and return ecosystems to a less complex stage (Garner, 1994).

Ecosystem responses are hierarchical. The extent of injury that an ecosystem can experience from exposure to O_3 will be determined by the severity of the effect on individual members of a population. Stresses, whose primary effects occur at the molecular or cellular physiology level of an individual, must be propagated progressively through the more integrative levels, from the leaf, branch, or root, to whole plant physiology, to stand dynamics, and, ultimately, to the ecosystem (see Figures 5-35 and 5-36). Only a small fraction of the stresses at the molecular, cellular, or leaf level leads to disturbances at the tree, stand, or ecosystem level. Variability in response to stress at both the individual and the population level and the ability to compensate for the stress determine the hierarchical extent of the response.

The mixed-conifer forest ecosystem in the San Bernardino Mountains of Southern California is one of the most thoroughly studied ecosystems in the United States. The changes observed in the mixed-conifer forest ecosystem exemplify those expected in a severely disturbed ecosystem. Chronic O_3 exposures over a period of 50 or more years resulted in major changes in the San Bernardino National Forest ecosystem by influencing forest processes. The primary effect was on the more susceptible members of the forest community, individuals of ponderosa and Jeffrey pine, in that they were no longer able to produce the energy required to compete effectively for essential nutrients, water, light, and space. As a consequence of altered competitive conditions in the community, there was a decline in the sensitive species, permitting the enhanced growth of more tolerant species (Miller et al., 1982; U.S. Environmental Protection Agency, 1978, 1986). Changes in the function of other ecosystems components directly or indirectly affected the processes of carbon (energy) flow, mineral-nutrient cycling, and water movement and changed community patterns. Biotic interactions associated with predators, pathogens, and symbionts were influenced by changes in available energy. The results of the studies of the San Bernardino Forest ecosystem were reported in both the 1978 and 1986 criteria documents (U.S Environmental Protection Agency, 1978, 1986). The more recent data from the San Bernardino Forest and from other ecosystems in California indicate that O_3 concentrations capable of injuring forest vegetation continue to occur, but at lower concentrations and with shorter durations. Therefore, vegetational injury has not been as great.

There is some indication from new data that O_3 may not have been the only stress encountered by the San Bernardino Forest ecosystem. Nitrate deposition gradients similar to those measured for O_3 suggest that possible soil-mediated exposures to nitrate could have been and continue to be combined with the foliage-mediated O_3 exposures as an additional stress. Research in this area is continuing.

Ozone concentrations capable of causing injury to trees in the Sierra Nevada Mountains have been occurring for many years. Injury to sensitive trees, however, has never reached the same proportions as in the San Bernardino Forest. Differences in forest stand composition (fewer conifers, more hardwoods), ability to compensate for stress, and site dynamics undoubtedly play roles in the forest response.

The forests of the Appalachian Mountains have been episodically exposed to O_3 concentrations capable of causing vegetational injury for many years. Visible injury to foliage of eastern white pine and reduction in growth have been associated with the exposures to concentrations >0.06 ppm lasting for several days. Black cherry also has been shown to be sensitive to O_3 exposures. Surveys of various regions, including the Smoky Mountain and Shenandoah National Parks, indicate that visible injury to a variety of different types of vegetation is continuing to occur. Neither eastern white pine nor black cherry are dominant canopy trees. Removal of sensitive individuals and the absence of changes in the population of these species have not resulted in any visible change in the forest ecosystems along the Appalachian Mountains, possibly because no changes in the ecosystem functions of energy flow or nutrient cycling have occurred. Decline and dieback of trees on Mt. Mitchell and Camel's Hump cannot be related solely to O_3 injury. Ongoing research is attempting to better understand the effects of O_3 exposure on individual plants and the effect, if any, on the ecosystems to which the plants belong.

5.8 Effects of Ozone on Agriculture, Forestry, and Ecosystems: Economics

5.8.1 Introduction

Evidence from the plant science literature cited in the 1986 O_3 Criteria Document (U.S. Environmental Protection Agency, 1986) and in the present document is unambiguous with respect to the adverse effects of tropospheric O_3 on some types of vegetation. For example, findings from EPA's multiyear NCLAN program provide rigorous corroboration of at least a decade of previous research that showed that O_3 at ambient levels caused physical damage to important species. Specifically, NCLAN established that ambient O_3 levels resulted in statistically significant reductions in yields for these crops. Literature reviewed in Section 5.6 of this document assesses the state of natural science findings regarding O_3 effects on crops, forests, and other types of vegetation in more detail.

Information on the benefits and costs of alternative policy options or states of the world (such as changes in air pollution) is of use to decision makers in a variety of settings. For example, economic information provides one means by which to choose from alternative policies or public investments. The role of cost-benefit analysis in federal rule making or standard setting was enhanced by President Reagan's Executive Order 12291 (February 19, 1981), which required that such calculations be performed on any rule or regulation promulgated by the federal government. President Clinton's Executive Order 12886 (October 4, 1993) reconfirmed the importance of economic information in the federal regulatory process. These executive orders provided the stimulus for a large increase in the use of economic analysis in evaluating federal actions, including environmental policies. Although the Clean Air Act and its amendments do not allow the use of cost-benefit analysis in the standard-setting process for primary (human health) effects, economic information has been introduced into the discussion of secondary or welfare effects. A number of economic studies addressing vegetation and other welfare effects have been performed in the last decade.

Assessments of the economic consequences of O_3 on vegetation reflect the state of natural science information on each vegetation category. The natural science evidence concerning effects of O_3 on individual tree species or plant communities is less secure than

for agricultural crops (see Section 5.6). As a result, most economic assessments focus on agricultural crops. The economics literature on effects of O_3 and other air pollutants on forest productivity is very sparse; the few assessments are confined to evaluations of assumed or hypothetical changes in output (e.g., board feet of lumber). The economic effects of O_3 on plant communities or ecosystems have not been measured in any systematic fashion.

This section reviews economic assessments across these vegetation categories. The discussion of economic valuation of ecosystem effects is limited to conceptual and methodological issues in performing such assessments, given the absence of empirical analyses in this category.

5.8.2 Agriculture

In view of the importance of U.S. agriculture to both domestic and world consumption of food and fiber, reductions in crop yields could adversely affect human welfare. The plausibility of this premise resulted in numerous attempts to assess, in monetary terms, the losses from ambient O_3 or the benefits of O_3 control to agriculture. Fourteen assessments of the economic effects of O_3 on agriculture were reviewed in the 1986 document (U.S. Environmental Protection Agency, 1986). Since the preparation of the 1986 document, there have been at least nine other studies published in the peer review literature that provide estimates of the economic consequences of O_3 on agriculture.

The 1986 document highlighted key issues in judging the validity of economic assessments that are applicable to post-1986 studies (i.e., how well the biological, aerometric, and economic inputs used in the assessment conform to specific criteria). First, the evidence on crop response to O_3 should reflect how crop yields will respond under actual field conditions. Second, the air quality data used to frame current or hypothetical effects of O_3 on crops should represent actual exposures sustained by crops at individual sites or production areas. Finally, the assessment methodology into which such data are entered should capture the economic behavior of producers and consumers as they adjust to changes in crop yields and prices that may accompany changes in O_3 air quality; should reflect accurately institutional considerations, such as regulatory programs and income support policies (e.g., provisions of federal "Farm Bill" legislation), that may result in market distortions; and use measures of well-being that are consistent with principles of welfare economics.

5.8.2.1 Review of Key Studies from the 1986 Document

Assessments of O_3 damages to agricultural crops reported in the 1986 document displayed a range of procedures for calculating economic losses, from simple monetary calculation procedures to more complex assessment methodologies that conform to some or all of the economic criteria above. As noted in the 1986 document, the simple procedures calculate monetary effects by multiplying predicted changes in yield or production resulting from exposure to O_3 by an assumed constant crop price. By failing to recognize possible crop price changes arising from yield changes and not accounting for potential producer responses, such assessments are flawed, except for highly restricted situations such as localized pollutant events. Conversely, some assessments provide estimates of the economic consequences of O_3 and other air pollutants that reflect producer-consumer decision-making processes, associated market adjustments, and some measure of distributional consequences between affected parties. The distinctions between studies based on naive or simple models

and those based on correct procedures is important at the regional and national levels, because the simple procedures may be biased and lead to potentially incorrect policy decisions.

Most (9 of 14) of the economic assessments reviewed in the 1986 document focused on O_3 effects in specific regions, primarily California and the Corn Belt (Illinois, Indiana, Iowa, Ohio, and Missouri). There have been a number of additional regional assessments since the 1986 document; most are non-peer-reviewed reports arising from consulting or contract research. This regional emphasis in the earlier literature may be attributed to the relative abundance of data on crop response and air quality for selected regions, as well as the importance of some agricultural regions, such as California, in the national agricultural economy. Most of the recent state or regional assessments are commissioned by state public utility commissioners or similar regulatory agencies and use variants of the simple "price times yield" approach, where yields are calculated from response functions arising from the NCLAN data. Although perhaps of use to public utility commissioners concerned with effects from single power plants or other localized sources, these studies generally contribute little to the assessment of pollution effects at the national level. (Most local or regional studies abstract from physical and economic interdependencies between regions, which limits their utility in evaluating secondary National Ambient Air Quality Standards [NAAQS].)

National studies that account for economic linkages between groups and regions can overcome some limitations of regional analyses. A proper accounting of these linkages, however, requires additional data and more complex models and frequently poses more difficult analytical problems. Thus, detailed national assessments tend to be more costly to perform. As a result, there are fewer assessments of pollution effects at the national level than at the regional level.

Two national studies reported in the 1986 document were judged to be "adequate" in terms of the three critical areas of data inputs. Together, they provided a reasonably comprehensive estimate of the economic consequences of changes in ambient air O_3 levels on agriculture. Because of their central role in the 1986 document, these two studies are reported in Table 5-38 and are reviewed briefly below.

In the first of these studies, Kopp et al. (1985 [cited as 1984 in the earlier document but subsequently published as a journal article in 1985]) measured the national economic effects of changes in ambient O_3 levels on the production of corn, soybeans, cotton, wheat, and peanuts. In addition to accounting for price effects on producers and consumers, the assessment methodology used is notable in that it placed emphasis on developing producer-level responses to O_3-induced yield changes (from NCLAN data available at the time) in 200 production regions. The results of the Kopp et al. study indicated that a reduction in O_3 from 1978 regional ambient levels to a seasonal 7-h average of approximately 0.04 ppm would result in a $1.2 billion net benefit in 1978 dollars. Conversely, an increase in O_3 to an assumed ambient concentration of 0.08 ppm (seasonal 7-h average) across all regions produced a net loss of approximately $3.0 billion.

The second study, by Adams et al. (1986a), was a component of the NCLAN program. The results were derived from an economic model of the U.S. agricultural sector that includes individual farm models for 63 production regions integrated with national supply and demand relationships for a range of crop and livestock activities. Using NCLAN data, the analysis examined yield changes for six major crops (corn, soybeans, wheat, cotton,

Table 5-38. Recent Studies of the Economic Effects of Ozone and Other Pollutants on Agriculture[a]

			Model Features				Results (Annual 1980 U.S. Dollars)			
Study	Region	Pollutant and Concentration	Price Changes	Output Substitutions	Input Substitutions	Quality Changes	Crops	Consumer Benefits	Producer Benefits	Total Benefits (Costs)
Garcia et al. (1986)	Illinois	Ozone, 10% increase from 46.5 ppb[b]	No	Yes	Yes	No	Corn, soybeans	None	$226 × 10^6$	$226 × 10^6$
Adams et al. (1986a)[d]	U.S.	Ozone, 25% reduction from 1980 level for each state[a]	Yes	Yes	Yes	No	Corn, soybeans, cotton, wheat, sorghum, barley	$1,160 × 10^6$	$550 × 10^6$	$1,700 × 10^6$
Kopp et al. (1985)[d]	U.S.	Ozone, universal reduction from 53 to 40 ppb[b]	Yes	Yes	Yes	No	Corn, soybean, wheat, cotton, peanuts	Not reported	Not reported	$1,300 × 10^6$
Shortle et al. (1988)	U.S.	Ozone, universal reduction from 53 to 49 ppb[a]	Yes	No	No	Yes	Soybeans	$880 × 10^6$	$-90 × 10^6$	$790 × 10^6$
Adams et al. (1986b)	U.S.	Acid deposition, 50% reduction in wet acidic deposition	Yes	Yes	Yes	No	Soybeans	$172 × 10^6$	$-30 × 10^6$	$142 × 10^6$
Kopp and Krupnick (1987)	U.S.	Ozone, 10% reduction from annual levels (1986 to 1990) for rural areas. Includes adjustments for 1985 Farm Bill.	Yes	Yes	Yes	No	Corn, cotton, soybeans, wheat	NA	NA	$2,500 × 10^6$ (sum of discounted values at 5%, 1986 to 1990)
Adams et al. (1989)	U.S.	Ozone, seasonal standard of 50 ppb with 95% compliance[c]; includes adjustments for 1985 Farm Bill.	Yes	Yes	Yes	No	Corn, soybeans, cotton, wheat, sorghum, rice, hay, barley	$905 × 10^6$	$769 × 10^6$	$1,674 × 10^6$
Adams and Rowe (1990)	U.S.	Increased UV-B radiation and associated increase of tropospheric O_3 (of 16%)	Yes	Yes	Yes	No	Soybeans (for UV-B) and all crops in Adams et al. (1989) for tropospheric O_3	NA	NA	$-830 × 10^6$ (for the increase in tropospheric O_3 only)

[a]All studies except Garcia et al. (1986) use NCLAN data to generate yield changes due to ozone; see Appendix A for abbreviations and acronyms.
[b]Seven-hour growing season geometric mean. Given a log-normal distribution of air pollution events, a 7-h seasonal ozone level of 40 ppb is approximately equal to an hourly standard of 80 ppb, not to be exceeded more than once a year Heck et al. (1982).
[c]Seven and 12-h growing season geometric mean. Analysis includes both fixed roll-backs (e.g., 25%) and seasonal standards (with variable compliance rates).
[d]Reported in the previous criteria document (U.S. Environmental Protection Agency, 1986).

sorghum, and barley) that together account for over 75% of U.S. crop acreage. The estimated annual benefits (in 1980 dollars) from O_3 adjustments are substantial, but make up a relatively small percentage of total agricultural output (about 4%). Specifically, in this analysis, a 25% reduction in O_3 from 1980 ambient levels resulted in benefits of $1.7 billion. A 25% increase in O_3 resulted in an annual loss (negative benefit) of $2.4 billion. When adjusted for differences in years and crop coverages, these estimates are close to the Kopp and Krupnick (1987) benefit estimates.

The Kopp et al. (1985) and Adams et al. (1986a) studies indicated that ambient levels of O_3 were imposing substantial economic costs on agriculture. However, both Kopp et al. (1985) and Adams et al. (1986a) were judged to suffer from several sources of uncertainty. These include the issue of exposure dynamics (7-h/day exposures from the NCLAN experiments versus longer exposure periods, such as 12-h exposures) and the lack of environmental interactions, particularly O_3-moisture stress interactions, in many of the response experiments. Also, the O_3 data in both studies are based on a limited set of the monitoring sites in the AIRS system, mainly sites in urban and suburban areas. Although the spatial interpolation process used for obtaining O_3 concentration data (Kriging) resulted in a fairly close correspondence between predicted and actual O_3 levels at selected validation points, validation for rural sites was limited (Lefohn et al., 1987a). The economic models, with their large number of variables and parameters and the underlying data used to derive these values, also were noted as potential sources of uncertainty, including the effects on economic estimates of market-distorting factors such as the federal farm programs. Concern over farm programs stems from the evidence that reductions in O_3 will increase yields and hence total production of some crops. If the crop is covered (eligible for deficiency payments) under the provisions of the farm program, then the total costs to the government (of the farm program) may *increase* as a result of reduced O_3 (McGartland, 1987). Thus, the benefits of the O_3 reduction may not be as great as estimated.

The 1986 criteria document concluded that these possible improvements in future assessments were not likely to alter greatly the range of agricultural benefit estimates for several reasons. First, the studies covered about 75 to 80% of U.S. agricultural crops (by value). For inclusion of the other 20% to change the estimates significantly would require that their sensitivities to O_3 be much greater than for the crops included to date. Second, model sensitivity analyses reported in past studies indicate that changes in plant exposure-response relationships must be substantial to translate into major changes in economic estimates. For example, it was believed unlikely that use of different exposure measures or inclusion of interaction effects would alter greatly the magnitude of the economic estimates. Third, it was believed that there were likely to be countervailing effects that would mitigate against large swings in the estimates (e.g., longer exposure periods may predict greater yield losses), but O_3-water stress tends to dampen or reduce the yield estimates. Finally, the document noted that potential improvements in economic estimates are policy-relevant only to the extent that they alter the relationship between total benefits and total costs of that policy. The possible exception to this generally optimistic assessment of the robustness of the estimates was inclusion of market-distorting factors (i.e., farm programs), an issue that is addressed in some of the post-1986 assessments reviewed below.

5.8.2.2 A Review of Post-1986 Assessments

The previous criteria document (U.S. Environmental Protection Agency, 1986) concluded that the O_3 assessments of economic benefits to agriculture by Kopp et al. (1985)

and Adams et al. (1986a) provided the most defensible evidence in the literature at that time of the general magnitude of such effects. These two studies, in combination with the underlying NCLAN data on yield effects, were judged to be the most comprehensive information available on which to evaluate the economic impact of O_3 on crops.

Seven national assessments performed since the last criteria document are reported in Table 5-38. Of these, all use defensible economic approaches to quantify dollar effects, where "defensible" is measured in terms of conforming to the criteria cited earlier. An evaluation of these studies in terms of the adequacy of critical plant science, aerometric, and economic data is presented in the table, along with estimates of benefits or damages associated with changes in O_3.

The concluding statements in the 1986 document are a benchwork against which to judge these seven national studies published since the last document. Most of the contemporary studies build on either Kopp et al. (1985) or Adams et al. (1986a); indeed, the motivation of some of the more recent studies is to test whether the problems noted above (such as exclusion of farm programs) are sufficient to alter the original estimates in a meaningful manner. A relevant question is whether these new studies provided any "surprises" in terms of magnitude of economic effects. These studies are summarized in Table 5-38.

In discussing these latest evaluations, there are several points that relate to the comparability of the evaluations with those of Kopp et al. (1985) and Adams et al. (1986a). First, all studies use NCLAN response data to generate yield effects (for inclusion in the respective economic models). In most cases, data used in the post-1986 assessments reflect improvements of earlier NCLAN data. Second, these studies may be characterized as second generation assessments. They build on the first generation of studies reported in the 1986 document by refining selected aspects of those earlier studies, including interactions with other stresses; use of aerometric data and assumptions that, in some cases, more closely follow the seasonal and regional characteristics of O_3 exposure (Adams et al., 1989); and effects of O_3 on quality of commodities (Shortle et al., 1988). Several of the studies use updated versions of the economic models in Adams et al. (1986a) and Kopp et al. (1985). In addition, some of the studies model the effects of government programs to judge the potential consequences of such distortions on economic estimates (Kopp and Krupnick, 1987; Adams et al., 1989). Third, there are differences in underlying aerometric assumptions; some studies include both O_3 and other environmental stresses (e.g., acid deposition, ultraviolet-B [UV-B], radiation); others reflect O_3 data for more recent time periods. Because ambient O_3 levels vary across years, the choice of year will influence the yield estimates and ultimately the economic estimates.

Common themes or findings from these (and earlier) O_3 and other air pollution studies have been summarized in two recent synthesis papers (Adams and Crocker, 1989; Segerson, 1991). The results of the post-1986 assessments in Table 5-38 and the recent synthesis papers corroborate the general findings of the 1986 document. Specifically, the agricultural effects of tropospheric O_3 at ambient levels impose economic costs to society (or conversely, that reductions in ambient O_3 result in societal benefits). The magnitude of the economic costs reported in the more recent studies is similar to the estimates in Kopp et al. (1985) and Adams et al. (1986a). Such a similarity is not surprising, given the points noted above concerning use of similar data and economic models.

One important recent finding pertains to farm programs. In each case, the inclusion of farm programs in the economic models resulted in modest changes (reductions)

in the economic benefits of O_3 control (due to increased farm program costs). As Segerson notes, however, it is not clear that these increased costs should be charged against the potential benefits of an O_3 regulatory standard but rather as an additional cost associated with the inefficiencies of the federal farm program. Even with the inclusion of farm programs and other elements, the general magnitude of further effects reported in the 1986 criteria document are reduced only by approximately 20%.

In addition to including farm programs, there are a couple of other notable additions to the assessment literature. One study (Adams et al., 1989) attempts to analyze economic benefits under a regulatory alternative involving a seasonal (crop growing season) O_3 exposure index measured as a 12-h mean, instead of hourly levels or percent changes from ambient reported in earlier studies. Specifically, a seasonal average of 50 ppb O_3 (measured as a 12-h seasonal average), with a 95% compliance level, is reported in Adams et al. (1989). The result (of a $1.7-billion benefit) is similar to the assumed 25% reduction across all regions reported by Adams et al. (1986a). At least one study also has combined environmental stresses (e.g., O_3, UV-B, radiation) in preforming economic assessments. Adams and Rowe (1990), using the same model as Adams et al. (1986a, 1989), report that a 15% depletion of stratospheric O_3 (which results in a 13% increase in tropospheric O_3) caused an economic loss of approximately $0.8 billion attributed to the tropospheric O_3 increase.

5.8.2.3 Limitations and Future Research Issues

The recent literature (post-1986) on economic effects of O_3 on agriculture supports the general conclusions drawn in the 1986 document. That is, ambient levels of O_3 are imposing economic costs on producers and consumers. As in earlier economic assessments, the validity of this finding is conditional on the quality of the supporting agronomic and aerometric data. In addition, there are at least three issues that are not addressed in the extant literature on the topic. First, the existing assessments do not consider the external costs of changes in agricultural production arising from changes in O_3 exposures (Sergerson, 1991). These costs are important if changes in O_3 result in changes in crop mixes or production practices, which in turn result in changes in soil erosion, fertilizer and pesticide runoff, or other agricultural externalities. For example, if reductions in O_3 increase the relative profitability of a crop that uses higher levels of chemical inputs, then some increase in chemical effluent may result. Given that some assessments suggest that such changes in crop mixes and production practices are likely to accompany O_3 changes, these costs/benefits need to be addressed.

A second issue not directly assessed in the current literature is the relationship between climate change and tropospheric O_3 effects. This relationship is important if global warming is expected to increase tropospheric O_3 levels. In addition, research indicates that global climate change will lead to a relocation of crops (Adams et al., 1990d). This relocation may change the vulnerability of crop species to O_3, given the spatial distribution of O_3 across the United States (i.e., increased crop production in areas of relatively low ambient O_3, such as the Pacific Northwest, implies lower O_3 damage).

A third issue involves the institutional setting in which agricultural production occurs. Several recent studies have assessed O_3 effects in the presence of federal farm programs. However, the United States and most industrialized economies are moving away from price supports, production quotas, and import restrictions, the traditional form of government intervention in agriculture. At the same time that these market distortions are

being removed, there is increasing government regulation of agricultural production practices to reduce agricultural externalities. Future assessments of O_3 effects may need to pay less attention to farm program effects and instead include other institutional features of U.S. agriculture.

5.8.3 Forests (Tree Species)

The plant science literature on O_3 and other air pollutant effects on tree species is evolving rapidly as a result of recent research initiatives by EPA and other agencies. The long-term nature of air pollution effects on perennial species creates challenges to plant scientists in sorting out the specific effects of individual stresses from among the many potential explanatory factors, such as O_3 (Skelly, 1989), and in measuring impacts of direct economic value, such as reductions in board-feet of lumber produced per unit of time.

To date, most natural science literature on forest species reports O_3 effects in terms of foliar injury or similar measures (Taylor and Hanson, 1992; Davis and Skelly, 1992b; Simini et al., 1992; Freer-Smith and Taylor, 1992). This emphasis on foliar effects (rather than on marketable yield) is similar to the state of science for agricultural crops prior to 1975. Such visible foliar effects information is of limited use in economic assessments. The exception is in measuring the economic value of aesthetic changes in a forest stock (see Crocker, 1985).

The lack of usable data concerning changes in marketed output, such as board-feet of lumber (or even changes in growth rates), has limited the number of economic assessments of O_3 effects on forests. The few studies that attempt to measure economic losses arising from O_3 or other pollutants circumvent the lack of plant science data by assuming various arbitrary reductions in forest species growth or harvest rates (Callaway et al., 1985; Haynes and Adams, 1992; Adams, 1986; Crocker and Forster, 1985). These studies are summarized in Table 5-39.

Although the economic estimates reported in Table 5-39 are comparable to those reported for agricultural crops (e.g., $1.5 billion for eastern Canada, $1.7 billion for eastern U.S. forests), the lack of defensible natural science data makes these studies suggestive, at best, of possible economic consequences of forest (tree species) effects of O_3 or other environmental stresses. In addition, the economic methodology used in the assessments varies, from simple price-times-quantity calculations (e.g., Crocker, 1980) to the use of large, econometric-based representations of the U.S. timber market (Haynes and Adams, 1992). With appropriate data, the Timber Assessment Market Model methodology laid out by Haynes and Adams holds promise for assessing the economic consequences of O_3 when requisite natural science data become available.

In summary, the plant science literature shows that O_3 adversely influences the physiological performance of tree species; the limited economic literature also demonstrates that changes in growth have economic consequences. However, the natural science and economic literature on the topic is not yet mature enough to conclude unambiguously that ambient O_3 is imposing economic costs. The output from ongoing natural science research on this topic will be important to the understanding of this potentially important class of effects.

Table 5-39. Studies of the Economic Effects of Ozone and Other Pollutants on Forests

Study	Pollutant/Coverage	Response and Air Quality Data	Economic Model	Annual Damages or Benefits of Control (billions of dollars)
Callaway et al. (1985)	All pollutants. Forest products (hardwood and softwood) in the eastern United States.	Assumes three arbitrary growth reductions (10, 15, and 20%) for hardwood and softwood tree species.	Spatial equilibrium models of softwood and hardwood stumpage and forest products industries in the United States.	−270 to 563 damage in 1984 dollars for assumed reductions in growth levels
Crocker (1980)	Acid deposition. Forest products and forest ecosystem service flows for eastern United States.	Assumes a 5% reduction in products due to acid deposition: assumes a pristine background pH of approximately 5.2	Naive; assumed changes in output multiplied by average value of those goods or services.	−1,750 damage in 1978 dollars from current levels of acid deposition
Crocker and Forster (1985)	Acid deposition. Forest products and forest ecosystem services for eastern Canada.	Assumes 5% reduction in forest productivity for all eastern Canadian forests receiving ≥10 kg/ha/year sulphate deposition.	Naive; assumed changes in output multiplied by average value of goods or services.	−1,500 damage in 1981 Canadian dollars from current levels of acid deposition
Haynes and Adams (1992)	Air pollutants, including acid precipitation. Losses estimated for eastern U.S softwoods.	None; paper demonstrates a methodology for assessing economic effects of yield (growth and inventory) reductions due to any course. Assumes losses from 6 to 21% for softwoods.	Econometric model of U.S. timber sector (Timber Assessment Market Model).	−1,500 to −7,200 in 1986 dollars

5.8.4 Valuing Ecosystem Service Flows

5.8.4.1 Background

Over the last 30 years, economists have developed a variety of techniques for assessing the value of nonmarket goods and services (recently surveyed by Braden and Kolstad [1991] and Smith [1993]). "Nonmarket" refers to those goods and services not priced and traded in markets. Although most applications are to natural resources and environmental assets, the concepts extend to a range of goods not usually traded in markets. Early applications focused primarily on commodities used directly by the consumer, such as outdoor recreation. Within the last decade, attention has shifted to estimating nonuse (or passive) values, such as what individuals are willing to pay to insure the existence of

species or unique natural settings. The values elicited with these techniques are being used in an increasing array of settings; however, their use is not without controversy.

Valuing complex ecological functions and the associated range of ecosystem service flows is relatively uncharted territory and raises a number of conceptual and practical issues. Some difficulties in valuing ecosystem services lie in the inability of ecologists to unambiguously define and measure ecosystem performance and endpoints (see Section 5.7). Other problems arise from the inability of economic science to measure adequately the consequences of long-term and complex phenomenon. A related problem is the difference in disciplinary perspectives between ecologists and economists. As a result, the current state-of-the-art for valuing ecosystem service flows is inadequate for benefit-cost assessments used in environmental regulatory processes. Improvement in valuation of ecosystem service flows will require increased interdisciplinary cooperation and research between ecologists and economists.

5.8.4.2 Nonmarket Valuation: Implications for Ecosystem Service Flows

Nonmarket valuation techniques consist of two basic types: (1) indirect approaches rely on observed behavior to infer values, and (2) direct approaches use a variety of survey-based techniques to directly elicit preferences for nonmarket goods and services. Both sets of techniques share a common foundation in welfare economics, where measures of willingness-to-pay (WTP) and willingness-to-accept (WTA) compensation are taken as the basic data for individual benefits and costs.

Indirect approaches, sometimes referred to as revealed preference approaches, rely on observed behavior to infer values. Examples include the travel-cost method, where the relationship between visits to a recreational site and travel expenditures to reach the site (the "price" of the site) is used to infer the value of the site, and the hedonic pricing method, which attempts to infer the value of environmental attributes (e.g., clean air) by comparing the value of a market good (such as residential housing) across neighborhoods with varying levels of air quality. Travel-cost methods encompass a variety of models ranging from the simple, single-site, travel-cost model, to regional and generalized models that incorporate quality indices and account for substitution across sites. Hedonic pricing methods encompass both land price (real estate) and wage models, which account for variations in prices or wages due to environmental attributes (e.g., air and water quality, noise, aesthetics, environmental hazards). The indirect approaches can measure only use values. Recent summaries of the indirect approaches can be found in Braden and Kolstad (1991), Mendelsohn and Markstrom (1988), Peterson et al. (1992), and Smith (1989, 1993).

Direct approaches to nonmarket valuation are survey-based techniques to directly elicit preferences. The hypothetical nature of these experiments requires that markets (private goods or political) be "constructed" to convey a set of changes to be valued. Although there are a number of variants on these constructed markets, the most common is the contingent valuation method (CVM).

Contingent valuation method can be viewed as a highly structured conversation (Smith, 1993) that provides respondents with background information concerning the available choices and specific increments or decrements in one or more environmental goods. Values are elicited directly in the form of statements of maximum WTP or minimum WTA compensation for the hypothetical changes in environmental goods. This method can be applied to both use and nonuse values. The flexibility of constructing hypothetical markets

accounts for much of the popularity of the technique. However, measurement of nonuse value has been the subject of considerable debate (Federal Register, 1993; McFadden, 1994).

There are numerous methodological issues associated with application of CVM, including the specification of the hypothetical environmental change, the elicitation format for asking valuation questions, the appropriate welfare measure to be elicited (i.e., WTP or WTA), and various types of response biases. Randall (1991) argues that, because of the importance of nonuse values, CVM is likely to be the primary tool for measuring the environmental benefits of biodiversity. Recent summaries of CVM can be found in Mitchell and Carson (1989) and Carson (1991).

5.8.4.3 Challenges in Linking Valuation Techniques to Ecosystem Service Flows

The need for and interest in values of nonmarket goods and services have arisen independently of concerns regarding ecosystem management and sustainability.
As environmental planning and management change to accommodate new issues, the need for de novo valuation studies may increase (e.g., standard Resources Planning Act [1974] values may be poor indicators of the economic benefits and costs produced by forest quality changes under alternative air pollution regimes). The process of developing a tractable framework for ecosystem management may require that valuation studies also co-evolve to aid critical management decisions. For example, explicitly linking valuation techniques to physical resource functions through bioeconomic models, remains an important research area (Adams et al., 1990c). Linking valuation measures, from both market and nonmarket studies, to indices of biological diversity is a fundamental challenge.

Ecologists have a traditional skepticism of attempts to assign monetary values to ecosystem functioning, due both to the inherent limitations of benefit-cost analysis and to the inadequacy of quantitative information about ecological and social factors (Westman, 1977; Higgs, 1987). Attempts to monetize environmental benefits also are seen as having an inherent "quantitative bias"; poorly understood ecological functions are neglected, whereas traditional commodities (e.g., outdoor recreation) receive full attention (Foy, 1990).

A further question is whether total economic value really captures total value. Economists make no claim that all values are being considered, only total economic value. A related question is whether complex ecological functions can be accurately expressed in monetary terms? Although the CVM has been applied to an impressive array of nonmarket goods, precise valuation of ecosystem services with CVM will require a precisely defined commodity. As researchers move from valuing single environmental endpoints or services to addressing more complex "bundles" of endpoints and services, it will be become more difficult to define the commodity in a CVM survey. This may prevent unambiguous estimation of such values.

5.8.4.4 Valuing Ecosystem Service Flows: Summary

Economists have a variety of valuation techniques to help guide policy choices concerning the effects of air pollution or other environmental change on environmental assets. Applying these techniques to ecosystem management issues and valuing the full range of ecosystem service flows is a new and, as yet, unresolved challenge. Many scholars, in both ecology and economics, are inherently skeptical of any economic valuation of the full complex of ecosystem services and, hence, turn toward other value indicators. The identified research agenda for valuing ecosystem service flows crosses traditional disciplinary boundaries (Russell, 1993). Interdisciplinary dialogue, cooperation, and the development of

a shared language are necessary for successfully designing future valuation experiments concerning ecosystem service flows and for determining the proper role for such valuation.

5.8.5 Summary

The 1986 criteria document (U.S. Environmental Protection Agency, 1986) contained a review of assessments of the economic consequences of O_3 on U.S. agriculture. This section has evaluated selected post-1986 literature on the same topic. In addition, the review has been expanded to include potential economic effects on forests and ecosystems.

Based on economic assessments and physical science data available at the time, the previous criteria document concluded that O_3 at ambient levels was imposing economic costs on society. The review of more recent (post-1986) literature on agriculture corroborates that earlier conclusion. Specifically, the recent literature, using the full set of NCLAN data and addressing some deficiencies in the pre-1986 assessments, confirms the findings of substantial economic losses from ambient O_3 concentrations.

The exact level of these economic effects is a function of cropping patterns, O_3 concentrations (both ambient and episodic), and the spatial and temporal characteristics of projected or observed O_3 levels. The current economic assessments represent improvements in the scientific understanding of O_3 effects on agriculture. However, the assessments of economic effects initially incident on the agricultural sector remain incomplete.

Only a few assessments consider the economic effects of O_3 on forest trees and on urban trees, shrubs, and ornamentals. These studies assess the economic effects of hypothetical changes resulting from O_3 or other stressors on forest productivity and aesthetics and are best viewed as measures of the potential effect of O_3 on these receptors. Improvements linking O_3 effects data to productivity and aesthetic effects will improve the utility of such economic analyses.

The economic effects of O_3 on ecosystems have not been addressed in the published literature. There is, however, an emerging interest in applying economic concepts and methods to the management of ecosystems. Economic techniques for valuing nonmarket goods and services hold the potential to value some ecosystem goods and services. Ecological research also is addressing the challenging conceptual and practical issues in understanding and managing ecosystem functions. Increased dialogue between the disciplines is needed before empirical analyses of the economic consequences of ecosystem management are feasible.

In summary, the state of science concerning O_3 economic effects on agricultural crops is sufficient to conclude that O_3 imposes costs on society. Conclusions regarding effects on forests and ecosystems must await the acquisition of additional data and possible refinements in ecological and economic methods.

5.9 Summary and Conclusions for Vegetation and Ecosystem Effects

5.9.1 Introduction

Review of the post-1986 literature has not altered the conclusions of the 1986 O_3 criteria document (U.S. Environmental Protection Agency, 1986) or its supplement (U.S. Environmental Protection Agency, 1992). In the 1986 criteria document, several general

conclusions were drawn from various experimental approaches: (1) current ambient O_3 concentrations (>0.04 ppm) in many areas of the country were sufficient to impair growth and yield of plants, (2) effects occur with only a few hourly occurrences above 0.08 ppm, (3) data cited in the 1978 O_3 criteria document (U.S. Environmental Protection Agency, 1978) indicate growth and yield effects for some species when the mean O_3 concentration exceeded 0.05 ppm for 4 to 6 h/day for at least 2 weeks, and (4) regression analyses of NCLAN data to develop exposure-response functions for yield loss indicate that at least 50% of the crops studied will exhibit a 10% yield loss at 7-h seasonal mean O_3 concentrations of 0.05 ppm or less. These conclusions remain valid today. The 1992 supplement reviewed the literature on the appropriate exposure index for expressing O_3 effects on vegetation, including evaluation of the roles of exposure duration and peak concentrations and the 7- and 12-h mean concentrations, and compared many possible exposure indices to summarize seasonal exposures related to yield loss. It was concluded that, in light of research that indicated the influential roles of episodic, peak concentrations and the duration of the exposure, the 7- or 12-h seasonal mean is not an appropriate index because it treats equally all concentrations and fails to consider exposure duration. Instead, the supplement (U.S. Environmental Protection Agency, 1992) recommended use of indices that cumulate all hourly concentrations during the growing season and preferentially weight the higher concentrations. Since 1988, a few experimental studies have addressed directly the roles of individual exposure components in order to develop a more appropriate exposure index. Also, however, results from several retrospective statistical analyses of NCLAN data have increased scientific confidence in the use of the peak-weighted, cumulative indices.

The post-1986 literature includes additional analyses of the NCLAN database and of several European crop-yield-loss studies that substantiate the O_3 effects observed in this country. Although there has been little increase in the information about the response of mature trees individually or in stands, new studies of forest tree seedlings have substantiated pre-1986 reports concerning the sensitivity of a number of species as seedlings. Seedling growth response of several species is altered at the O_3 concentrations (>0.08 ppm) experienced for hours to days in many areas of the United States. Studies of the effects of O_3 on mature trees in their natural habitats are limited. Literature on the roles played by various biotic and abiotic environmental factors in plant response to O_3 indicates the need for more research concerning the response of plants in natural ecosystems, where the interaction of species of various genotypes with a multitude of environmental influences dictates the eventual response of the species or community in question.

The species is the level of biological complexity for which the understanding of O_3 response is greatest. The focus of research for developing quantitative relationships between O_3 exposure and biological effects has emphasized the response of individual species for three reasons. First, single species studies are achievable experimentally, including ease of developing adequate experimental design and exposure technology. Second, in many instances, the plants are grown in monoculture (e.g., most crop plants, ornamentals, fruit and nut species, plantation forests), and the interspecific competition and plant diversity, which typify natural communities, are not issues. The environmental influences of a plant's growing environment (e.g., drought) that modify the exposure-response relationship can be observed more readily. Third, in systems that are comprised of a multitude of species (e.g., mixed forest stands, pastures, grasslands), it is important to understand the response of the individual components so that behavior of the system may be analyzed systematically. The underlying assumption is that understanding how a forest stand responds to O_3 requires

knowledge of the response of sensitive individuals of each species within that stand as a starting point. The interactions that typify the population and the community are subject to O_3 effects as well and may manifest themselves as a measurable effect some time later as a result of these interactions.

The potential for using individual plant (species) responses to an environmental stress (such as O_3 exposure) to predict population and community response may be limited (Woodward, 1992). Propagation of stress responses from a tissue or organ to the whole plant, the population, the community, or the ecosystem level can be influenced by interactions between plants and by feedback mechanisms at the different levels. Important components of such feedback are the mechanisms of homeostasis that involve injury repair (at the metabolic level) or various types of compensation (Tingey and Taylor, 1982). Compensation, which may occur at all levels of organization, from the subcellular to the ecosystem, invokes processes that counteract the detrimental effects of the stress. At the ecosystem level, an effect on the growth rate of a sensitive species may not be translated into a comparable effect on the growth rate of a population of the species, because of changes in the intensity of competition within a community (Woodward, 1992).

Currently, most of the knowledge of O_3 concerns effects on individual plants or their parts. Although some information exists on effects at the population level with some agricultural crops, little is known about how, and to what extent, effects may be propagated through the different hierarchical levels within natural and forest ecosystems.

5.9.2 Methodologies

Most of the currently available information dealing with the effects of O_3 exposure on crops and tree seedlings is the result of experimental fumigation studies. The type of fumigation study determines the applicability of the data. Ozone-fumigation, plant-response studies require fumigation of well-characterized vegetation to varying regimes. Variation in regimes may be achieved by controlled fumigation, chemical/mechanical fumigation exclusion, or natural O_3 gradients. Controlled fumigation systems are designed to maintain a modified gaseous atmosphere around a plant for a specified period of exposure in order to monitor plant responses to that modified atmosphere. All fumigation systems share some features in common, namely, general plant growth conditions (light, temperature, humidity, CO_2, and soil moisture) must be met, and differential concentrations of O_3 generated either artificially or naturally must be supplied to the vegetation and maintained during the exposure period. Exposure systems have been established in controlled environments, greenhouses, and the field. Controlled fumigation systems may range from cuvettes that enclose leaves or branches to a series of tubes with calibrated orifices spatially distributed over a field to emit gaseous pollutants to a plant canopy. Systems that exclude O_3 by mechanical or chemical means have been used, as have natural gradients.

Open-top chambers represent the best technology for determination of crop yield to O_3 at the present time. Concentration and duration of the gaseous exposures are well controlled and plants are grown under near-field-culture conditions; however, plot size is small when compared with a field, microclimate may influence plant sensitivity to O_3, and air quality after passage through the charcoal filter has not been widely characterized. Caution should be used when extrapolating results to field conditions. Exclusion methods, particularly those using chemicals such as EDU, are the least disruptive of ambient culture

conditions in the field; therefore, these approaches most closely estimate "real" crop losses to O_3. However, the mechanism by which EDU protects plants is unknown.

5.9.3 Species Response/Mode of Action

The mode of action of O_3 on plant species described in the 1986 criteria document (U.S. Environmental Protection Agency, 1986) still holds true. The plant leaf is the site of O_3 action, and the critical effect is on the plant's carbon budget (the amount of carbohydrate produced). Inhibition of photosynthesis limits carbohydrate production and allocation resulting in reduced biomass, growth, and yield and increases susceptibility to abiotic and biotic stresses.

Ozone exerts a phytotoxic effect only if a sufficient amount reaches the sensitive cellular sites within the leaf. To do this, it must diffuse from the atmosphere into the leaf through the stomata, which exert control on O_3 uptake. Ozone effects will not occur if the rate of O_3 uptake is low enough that the plant can detoxify or metabolize O_3 or its metabolites, or if the plant is able to repair or compensate for the effects. Cellular disturbances that are not repaired or compensated are expressed ultimately as visible injury to the leaf or effects on growth, yield, or both (Tingey and Taylor, 1982; U.S. Environmental Protection Agency, 1986). The effects of O_3 exposures on plants are cumulative. The level of O_3 concentration and length and number of exposures determine the extent of plant effects. Annual plant responses are determined by the number of exposures during a single growing season. For trees and other perennial plants the effects are determined by multiple exposures over a number of years.

Ozone is expected to reduce growth or yield only if it directly impacts the plant process that is limiting to plant growth (e.g., carbon produced), or it impacts another step sufficiently so that it becomes the step limiting plant growth (e.g., allocation of carbohydrates to roots and nutrient uptake becomes limiting to plant growth) (Tingey, 1977). Conversely, O_3 will not limit plant growth if the process impacted by O_3 is not growth limiting. This implies that not all effects of O_3 exposures on plants are reflected in growth or yield reductions. These conditions also suggest that there are combinations of O_3 concentration and exposure duration that the plant can experience that may not result in visible injury or reduced plant growth and yield (U.S. Environmental Protection Agency, 1986). However, subtle physiological effects that may not result in immediate growth reductions may result in increased plant susceptibility to other environmental factors (e.g., drought, fungal pathogens, insects, at these concentrations) and competition.

Studies since 1986 corroborate this understanding, adding information on the effect of O_3 on photosynthetic capacity, respiration, leaf dynamics, and the detoxification and compensatory processes. In particular, exposure to O_3 concentrations at or near current ambient levels (0.04 to 0.06 ppm) (see Section 5.6; Table 5-18), depending on their duration, can affect photosynthesis, but exposures of longer duration are necessary to produce growth responses, taking days to weeks, rather than hours, as in earlier studies with high concentrations (0.25 ppm or greater). The loss of leaves prematurely as a result of O_3 exposure has been observed in several species and is particularly important in coniferous trees. However, the mechanism of premature senescence is not understood. Both reduced photosynthetic capacity and reduced leaf area due to O_3-induced leaf loss contribute to the reduction in carbohydrate production by plants. In addition to leaf loss, reports of stimulation of production of new leaves and higher photosynthetic capacity of new leaves

represent compensation processes that operate in some species of trees. More information is needed to understand O_3 uptake at the canopy level and how plants integrate the effects of O_3. Some quantitative understanding of these processes is needed to be able to predict long-term effects of O_3 on tree species. Unfortunately, there is little experimental evidence to date regarding effects of long-term O_3 exposure on perennial plants. Few experimental studies of tree seedlings have extended exposures beyond one season, and only in a limited number of studies have observations of growth effects been extended into the following year, thus observing "carry-over" effects. These carry-over effects are significant to long-lived species such as trees because they affect the elongation of new spring shoots or root growth in the year following exposure to O_3. In at least one instance, this has been correlated with reduced storage carbohydrate in roots. Reduction in growth and productivity, a result of altered carbohydrate production and allocation, may appear only after a number of years or when carbohydrate reserves in the tree are severely depleted. To enable prediction of long-term effects of O_3 exposure in ecosystems, species response as a function of interactions with other species and the effects of abiotic and biotic environmental factors on these interactions both must be known.

5.9.3.1 Exposure Dynamics

The uptake of O_3 from the atmosphere is a complex process involving absorption of O_3 primarily through the leaves. Plant uptake is influenced by temporal and seasonal variation of exposures. Plant response is influenced by canopy structure, stomatal conductance, respite time between exposures, phenology, and environmental conditions (e.g., soil moisture and nutrient content). Studies both prior to and after the 1996 criteria document, indicate that the components of exposure (i.e., peak concentrations >0.10 ppm, frequency of occurrence, duration, temporal distribution of hourly O_3 concentrations during a growing season) play influential roles in plant response. Greater yield reductions in both annual and perennial crop species (e.g., bush beans and alfalfa) and greater biomass reductions in tree seedlings (e.g., ponderosa pine and aspen) have resulted from experimental episodic peak exposures than from equivalent exposures with either daily peak occurrences or nondiurnal, continuously elevated exposures. In addition to the temporal distribution of concentration, the distribution of O_3 exposure during the growing season, as related to plant phenology, is also important. Some phenotypic stages of growth (e.g., the time of pod-fill in beans and the period of starch storage in perennial species) are more sensitive to O_3 than are others. Thus, effects of early-season versus late-season exposure will vary depending both on the phenology of the plant species and the growth response measured. Another key to plant response is the timing of the exposure. Ozone uptake is greatest when stomatal conductance is highest; therefore, the greatest potential effect for O_3 exposures to produce an effect on plants occurs at that time. Neither peak nor mid-range concentrations occur at the same time. Plant effects are determined by which concentrations occur when stomatal conductance is highest. Associated with stomatal conductance is atmospheric turbulence; O_3 concentrations must reach leaf surfaces if they are to be taken up by a plant.

In most crop-exposure studies, in particular, those included in the NCLAN database, the exposure treatments used in developing response functions have been based on O_3 concentrations at the experimental site. Few studies have been designed specifically to study the effect of varying the types of exposure regimes on crop and tree seedling responses. Research results enable only the prioritization of components of the exposure in terms of their degree of influence on growth alterations. For example, peak or higher

concentrations are more effective than lower concentrations in altering growth when those peaks occur in the daytime when stomatal conductance is high. Episodic occurrences of high concentrations during daylight hours are more injurious according to experimental chamber studies by Musselman et al. (1983, 1986b, 1994) than are either the daily occurrences of the same peak value with the regime having the same total exposure value as the episodic regime, or regimes having no episodic occurrence of peaks and no rise and fall diurnal pattern to daily concentrations (i.e., "flat", but relatively moderate to high concentrations) and having the same total exposure value over a growing season as the episodic regime. The concentrations used in these chamber studies were all >0.10 ppm, exposures seldom experienced outside of California. Because of the variation in species' growth/yield response as a function of exposure dynamics (i.e., concentration, distribution, duration), it is important to have an exposure index that is biologically based (i.e., a measure of ambient O_3 concentration that is related to the measured biological effects).

5.9.3.2 Age and Size

The role of age and size in modifying tree response to O_3 is the single largest uncertainty in quantifying O_3 effects on tree species. To date, most of the biological effects data and all of the exposure-response functions for trees have been developed with seedlings and saplings. The implicit assumption is that seedling response is a good indicator of large-tree response. However, gas-exchange and water-use differences with tree size and age presumably would affect O_3 uptake and thus O_3 exposure response. Indeed, published reports indicate that O_3 sensitivity is related to the gas-exchange characteristics of the current life stage. Recent data indicate that, for some species (e.g., giant sequoia), seedling growth is affected more by O_3 than is growth in large trees, whereas, for other species (e.g., red oak), seedling growth is less affected than is growth in large trees. These observations of differences in O_3 growth response between seedlings and large trees follow the differences in leaf conductance with age for each of these two species.

Another factor related to tree and size is the occurrence of "carry-over effects" (i.e., the impact of O_3 on growth responses in the season following exposure). For example, reductions in root growth and starch concentration and in shoot elongation in the year following exposure have been reported for ponderosa pine and aspen. Carry-over effects are significant in determining long-term growth response in long-lived species exposed year after year to both O_3 and changing environments.

5.9.4 Factors That Modify Plant Response to Ozone

Plant response to O_3 exposure is modified by factors within and external to the plant species; cultivars and individuals within populations display variable response to O_3. The plant's response and the variation of that response is dictated by genetics and the plant's present and past environmental milieu. The environment includes biotic and abiotic factors of the species' growing environment, the temporal pattern of exposure concentrations, and the plant's phenotypic stage during exposure.

5.9.4.1 Genetics

The response of an individual plant within a species and at any age is affected both by its genetic makeup and the environment in which it is growing. The specific genes controlling O_3 response and involved in mechanisms of O_3 tolerance are largely unknown;

however, control of stomatal conductance and internal biochemical defense systems are among the most commonly postulated tolerance mechanisms. Ozone tolerance is generally thought to be controlled by multiple genes. The implications of genetic variation for managed and natural ecosystems are several-fold. First, the potential for natural selection for O_3 tolerance and associated loss of sensitive genotypes is regional in nature, unlike point-source pollution impacts that occur mainly on plant populations in the vicinity of the source. However, the intensity of O_3 selection is generally thought to be quite low, 0.3 or less (Taylor and Pitelka, 1992), across most U.S. areas. Second, although it is known that individual plants within a species vary in their O_3 tolerance, the physiological costs to tolerant plants in terms of carbohydrate assimilation (energy production) and allocation are not known. Tolerance mechanisms based on reduced stomatal conductivity in the presence of O_3 presumably would reduce the growth of tolerant plants. Similarly, tolerance mechanisms based on the productivity of antioxidant compounds would shunt plant resources away from growth to the production of the defense compounds. Third, exposure-response equations and yield-loss equations developed for a single or small number of cultivars, genotypes, families or populations may not represent adequately the response of the species as a whole. As a corollary to this, the sensitivity of responder genotypes can not be determined by measuring effects just in relation to mean O_3 concentrations.

5.9.4.2 Environmental Factors

Plant response to O_3 exposure can be modified by a number of biotic and abiotic factors in the plants' past and present growing environment. Understanding and, if possible, quantifying these modifications will reduce uncertainty in the estimates of species' exposure responses. Also important is an understanding of how exposure to O_3 can modify a plant's ability to integrate the effects of its environment. For example, exposure to O_3 has been shown to reduce a tree's ability to withstand winter injury due to freezing temperatures and also to increase the success of pest infestations.

Biotic factors in a plant's environment include pests, pathogens, and plants of the same or competing species. Although only a limited number of plant-insect systems have been studied, some insect pests appear to have a preference for and to grow better when feeding on plants that have been affected by O_3 exposure, but there is no evidence to suggest that O_3 may trigger pest outbreaks in plants. Because the effects of O_3 on the vast majority of plant-insect systems are unknown, quantitative assessment of such interactions on crops and natural vegetation is impossible. At best, it reasonably may be concluded that some insect pest problems will increase as a result of increased ambient O_3 levels. Indeed, this phenomenon was observed in the San Bernardino Forest study where injured ponderosa pines experienced an increase in bark beetle infestations at higher O_3 exposures.

Plant-pathogen interactions also appear to be affected by O_3. The suggestion that O_3 exposure tends to diminish diseases caused by obligate pathogens and to favor those diseases caused by facultative pathogens (Dowding, 1988) generally is supported by the limited evidence currently available. This suggests that continued exposure to O_3 may lead to a change in the overall pattern of the incidence and severity of specific plant diseases affecting crops and forest trees.

Abiotic environmental factors include, among other physical and chemical elements, solar radiation, wind/atmospheric turbulence, and air and soil moisture and temperature. Collectively, abiotic factors greatly affect plant growth because of their influence on the processes of photosynthesis, respiration, and transpiration. For agricultural

crops, water availability may be the most important of these interactions with O_3. There is consistent evidence that severe drought conditions tend to reduce the direct adverse effects of O_3 on growth and yield, and that ready soil water availability tends to increase the susceptibility of plants to O_3 injury. However, a lack of water should not be viewed as a potentially protective condition, because of the adverse effects of drought per se. Unlike the situation with annual crops, a limited amount of evidence suggests that prolonged exposure of perennial trees to O_3 may lead to greater water-use efficiency, which, in turn, would better enable the exposed trees to survive drought conditions.

The numerous chemical components in a plant's environment, including soil nutrients, agricultural chemicals, and other air pollutants, also potentially influence the plant's response to O_3 exposure. The nature of these interactions is largely unknown. Although many studies have been conducted on the effects of O_3 on plants in conjunction with other gaseous air pollutants such as SO_2 and NO_2, the data obtained in several of these studies is of academic interest only because of the unrealistic exposure scenarios used.

Because increased tropospheric O_3 is a component of global climate change, which is of growing concern within world communities, data on the interactions of O_3 with increased levels of CO_2 and UV-B radiation, elevated temperatures, and drought are beginning to appear. Initial data suggest that increased CO_2 levels may ameliorate the effects of O_3, but conclusive generalizations about the outcome of this interaction are not yet possible. Studies investigating the interaction of O_3 with UV-B exposure reveal no significant changes in O_3 effect on the growth and yield of soybean due to UV-B levels, although there are significant effects of O_3.

Although a number of studies have examined the interactions of O_3 with specific environmental factors, no quantitative database exists from which the effects of O_3 on species can be extrapolated across environments. The role of different growing environments in a species' O_3 exposure response and the effect of O_3 exposure on a species' ability to integrate its' environment remain uncertain.

5.9.5 Effects-Based Air Quality Exposure Indices

A measurement is needed that relates ambient O_3 exposures with the degree of plant response. The effects of O_3 on individual plants and the factors that modify plant response to O_3, however, as indicated in the previous sections, are complex and vary with species, environmental conditions, and soil and nutrient conditions. Due to the complexities of the processes associated with uptake and O_3 interactions with external physical and internal genetic factors that influence plant response, the development of exposure indices that characterize plant exposure and response in a quantifiable manner has been and continues to be a major problem.

Plant uptake of O_3 (either rate of uptake or cumulative seasonal uptake) is a critical factor in determining plant response. Ozone uptake is controlled by canopy conductance, stomatal conductance, O_3 concentration external to the leaf and gases emitted from the leaf through the stomata. Any factor that affects stomatal conductance (e.g., light, temperature, humidity, atmospheric chemistry, soil and nutrients, time of day, phenology, biological agents) will affect O_3 uptake and, consequently, plant response. Empirical functions for predicting stomatal conductance have been developed for particular species (Losch and Tenhunen, 1981) but have not been used in development of exposure indices.

The mode of action of O_3 on plants, as presented in Section 5.2, is a culmination of a series of biochemical and physiological processes that lead to alterations in plant metabolism. Ozone-induced injury is cumulative, the result of net reduction in photosynthesis, changes in carbohydrate allocation, and early leaf senescence, which lead to reduction in biomass formation and reduction in yield. Increasing O_3 uptake results in increasing reduction in biomass production and yield.

The optimum exposure index that relates well with plant response should incorporate, directly or indirectly, the factors described above; unfortunately, such an index has not yet been identified. Exposure indices that weight the hourly O_3 concentration differentially appear to be the best candidates for relating exposure with predicted plant response. Peak concentrations occur primarily during daylight hours, thus indices that provide differential weight to the peak concentrations give greater weight to daylight concentrations, when stomatal conductance is usually greatest, than to nighttime concentrations, when conductance is minimal. Peak concentrations do not occur throughout the day; therefore, the timing of the exposure is important in determining plant response.

Evidence from the Musselman et al. (1983, 1986b, 1994) and Hogsett et al. (1985b) experimental chamber studies that applied two or more different exposure regimes support the view that daytime peak concentrations and respite time are important in eliciting plant responses. Ozone effects on plants exposed to two (or more) regimes having equal total exposure were greater for exposures experiencing the higher peak concentrations, respite time of 2 to 6 days, or peak concentrations during period of maximum leaf expansion. This conclusion is consistent with the mode of action of O_3 on plants and with the conclusions in the previous EPA criteria document (U.S. Environmental Protection Agency, 1986) and its supplement (U.S. Environmental Protection Agency, 1992).

No studies have been designed specifically to evaluate the adequacy of the peak-weighted, cumulative indices. Consequently, it is not possible to discriminate among the various peak-weighted, cumulative indices based on experimental data. Functional weighting approaches, including allometric, sigmoid, or threshold weighting, have been suggested and, in earlier retrospective studies, compared, but there is no evidence to favor one approach over the other on the basis of statistical fits to the data. Generally, the peak-weighted, cumulative indices relate well with plant response and order the treatment means in monotonically decreasing fashion with increasing exposure, based on studies that apply two or more types of exposure regimes and when combining data from replicate studies of the same species.

Peak-weighted, cumulative indices appear to have major advantages over the mean (e.g., 7-h seasonal mean), peak indices (e.g., 2HDM), and the index that cumulates all hourly average concentrations (i.e., SUM00). Crop yield loss and biomass reduction are estimated better using the peak-weighted, cumulative indices than the 2HDM index; when duration of exposure is taken into consideration, peak-weighted, cumulative indices perform better than the seasonal mean indices. In addition, results have been published to indicate that the SUM00 index does not relate adequately exposure with biological effects because the index focuses on the lower hourly average concentrations.

The greater importance of cumulative peak concentrations (>0.10 ppm) when compared with cumulative mid-range concentrations (0.50 to 0.09 ppm) in eliciting plant response has been questioned. The data supporting the two viewpoints are not comparable because the response parameters used in these studies were different. Musselman et al. (1983, 1986b) and Hogsett et al. (1985b), whose studies have been cited as a basis for

emphasizing the importance of cumulative peaks, measured both foliar injury and growth reductions and were based on exposures in open-top or greenhouse chambers to concentrations higher than those usually encountered in the ambient air outside of California. The biological evidence for supporting the importance of mid-range concentrations is based on ambient air field exposures using plants sensitive to O_3 in which exposures, seldom if ever, exceeded 100 ppb. The conclusions of Krupa et al. (1993, 1994, 1995), Tonneijck and Bugter (1991), and Tonneijck (1994) must be interpreted with caution because they are based on data from Bel W3 tobacco and other O_3-sensitive indicator plants. Tonneijck and Bugter (1991) concluded that O_3 effects varied with species and climatic condition; therefore, O_3 injury on Bel W3 tobacco was not an adequate indication of ambient condition, nor was it an adequate indicator to determine the risk of O_3 to other plant species or to vegetation as a whole. It should be obvious that plants take up all O_3 concentrations present in the atmosphere, not just O_3 peaks. Cumulative effects result from all O_3 concentrations that enter the plant. Plants can not respond to peaks if there are none in the ambient air. When peaks occur at the time of greatest stomatal conductance, the effect of mid-range concentrations will not be observable.

When predicting the effects of O_3 on vegetation under ambient conditions using experimental exposure-response models, the types of exposure regimes used in the experiments should be taken into consideration. For example, NCLAN experiments contained peak hourly average concentrations in their regimes. Any exposure index based on the NCLAN experiments should take into consideration the presence of these peak concentrations. By doing so, the situation may be avoided where two sites that experience two distinct distributions of high hourly average concentrations but have the same value of cumulation (e.g., same SUM06 or W126 value) exhibit differing biological effects.

The concentration level for a cumulative, peak-weighted index was determined from the best available biological response data (i.e., the crop yield responses from NCLAN). The concentration level selected to prevent a particular yield loss will have associated with it any uncertainty inherent in the methodology employed in NCLAN studies, in particular, the modified ambient exposures of NCLAN protocol typified by a relatively large number of episodic occurrences of high concentrations. The episodic occurrence of high concentrations is typical of many, but not all, agricultural areas in the United States. Some regions of the country may have different exposure regimes, typified by the lack of a large number of high concentration occurrences but still having a high cumulative, weighted exposure index value. The particular concentration level determined to protect 50% of the crops studied from a 10% yield loss based on NCLAN data may over- or underestimate the yield loss from a different regime type. Lefohn and Foley (1992) and Musselman et al. (1994) have suggested that a multi-component index, combining a cumulative, weighted index and the number of occurrences of concentrations ≥ 0.10 ppm would capture more adequately both the plant exposure response and the air quality at the site, thus overcoming some of the uncertainty associated with selection of a concentration level from the NCLAN crop response data.

Other experimental approaches have been employed to demonstrate effects of ambient O_3 exposure (e.g., chemical protectants [EDU]) but are of limited value in determining an exposure index. The ambient exposure approach addresses one of the shortcomings of the NCLAN methodology, but the experimental designs can not provide a range of O_3 treatments necessary for statistical robustness, quantifying the effect of ozone on yields, and the results cannot be extrapolated beyond the site and year of the exposure study.

5.9.6 Exposure Response of Plant Species

5.9.6.1 Introduction

The Clean Air Act seeks to protect public welfare resources, including plants and natural ecosystems, from adverse effects of criteria pollutants, including tropospheric O_3. "Adverse effect" has been interpreted in the 1986 criteria document (U.S. Environmental Protection Agency, 1986) and its supplement (U.S. Environmental Protection Agency, 1992) to be equated with yield loss and impairment in the intended use of the plant. In the instance of crop species, for example, an adverse effect of O_3 is agronomic yield loss. Foliar injury also can be an adverse effect, especially when decreasing marketability of foliar crops (e.g., spinach, lettuce, cabbage) or reduced aesthetic value of ornamentals. These effects constitute yield loss with or without concomitant growth reductions. With tree species grown for timber, paper, or pulp, biomass loss (and therefore loss of forest productivity) can be quantified as being an adverse effect.

Diverse experimental procedures, ranging from field exposures without chambers to field exposures with OTCs to exposures in chambers under highly controlled laboratory conditions, have been used to study O_3 effects on crops and trees seedlings. In general, the highly controlled laboratory experiments are most useful for investigating specific responses and for providing a scientific basis for interpreting and extrapolating results. Such experiments are very important in increasing the understanding of the biological effects of air pollutants. To accurately assess the economic impacts of O_3 on crop yield or ecological impact of altered carbon partitioning in tree species, however, requires exposure methodology that provides a range of O_3 treatments sufficient for quantifying effects (i.e., exposure-response functions) and also provides growing conditions that closely match those in the plants' natural growing environment. Because the OTC methodology provides control over O_3 exposure treatments and still allows some replication of field conditions, as well as permits replication of studies from year to year, this has been the primary methodology used for developing the empirical database of O_3 effects on crop and seedling tree species during the last 15 years. Many of the studies reviewed in this document, as well as those in the 1986 O_3 criteria document (U.S. Environmental Protection Agency, 1986) utilized the OTC methodology, including the NCLAN studies (see Section 5.6.2) that were initiated by EPA in 1980 primarily to improve estimates of yield loss in the field and the magnitude of crop losses resulting from O_3 exposure. The NCLAN studies used numbers of treatments sufficient to permit robust statistical designs and the development of exposure-response functions. It is the largest database available for establishing a quantitative relationship between O_3 exposure and crop yield. Studies of tree seedlings also have been conducted utilizing OTCs as a means of exposing seedlings to a range of treatments, replicate treatments, and approximate field conditions. The exposure-response function for each species permits estimations and generalizations of biological response to O_3, unlike the multiple comparison approach.

There has been debate concerning the experimental designs, particularly the number, types of regimes, and exposure concentrations used in the NCLAN studies. The O_3 exposures utilized by the NCLAN program have been described as artificial regimes that do not mimic actual conditions. The exposure treatments were "modified ambient" (i.e., treatments were achieved by addition of some amount of O_3 above the ambient concentration). Another criticism of NCLAN studies was the alteration of the environment by the OTCs to the degree that exposure-response functions obtained using this methodology

can not be extrapolated to ambient environments. A study by Heagle and co-workers (1989a) of OTCs suggests that, although departures from field conditions can occur, "they allow control of pollutant concentrations with dynamics that compare closly to exposure dynamics in ambient air." For NCLAN studies, although it was noted that OTCs decreased mean wind velocity, altered light profiles, and eliminated the vertical gradient in O_3 concentration (less near the ground) that usually occurs in the canopy of plants grown in the ambient air, chamber effects were found not to enhance consistently the treatment differences or plant responses to O_3. Despite the criticisms of the NCLAN studies, there is no other database that matches it. Approximately, 90% of the available O_3 dose-yield response data comes from these studies (Heagle et al., 1989a).

5.9.6.2 Predicted Crop Yield Losses

The NCLAN studied the major agronomic crop species, including corn, soybean, wheat, cotton, bean, and alfalfa, as well as several other regionally important species; collectively, the species studied account for 70% of all cropland in the United States and for 73% of the nations's agricultural receipts. To predict crop yield loss due to O_3 exposure, two approaches to developing a composite exposure-response function for all crops from the NCLAN database were taken. The first approach predicted crop yield losses of up to 20% at a 12-h seasonal mean of 0.06 ppm and a 10% loss at a 12-h seasonal mean of 0.045 ppm. The second approach calculated separate regressions for studies with multiple harvests or cultivars, resulting in a total of 54 individual equations from the 31 NCLAN studies (average study duration of 74 days) and 12 crop species using three different exposure indices, and concluded that 50% of the crops would experience 10% yield loss at a 3-mo SUM06 concentration of 26.4 ppm-h (Table 5-22), a 7-h seasonal mean of 0.049 ppm, or a 2HDM of 0.094 ppm. (These are averaged yield losses for all species; losses for many of the crops would be higher at these concentrations.) The box-plot distribution of yield loss for the compiled studies, expressed as a SUM06, is shown in Figure 5-23A.

Results reported for European crop studies support the NCLAN analyses results. For example, in the European studies, wheat yields were reduced by up to 29%, depending on the O_3 exposure level and cultivars used, but in no instance did the exposure level exceed a 0.062 ppm 7-h seasonal mean. Spring rape yields were reduced by 9 to 26% at 8-h seasonal means of 0.03 to 0.06 ppm. Seasonal 7-h means of 0.045 ppm reduced bean yield by 17%.

Perennial crop exposure response, unlike annual crops, is complicated by the fact that such crops receive multiple-year exposures, and the effects of such exposures may be cumulative. Yields of multiple-year forage crops (e.g., alfalfa and forage mixtures), as with yields of single-season crops, are reduced at O_3 concentrations at or near ambient (0.04 to 0.06 ppm, 7- and 12-h mean) in many parts of the growing areas for these crops. The question of cumulative effects in perennial crops has been addressed only in one instance (a 2-year alfalfa study in Southern California), and, in this study, there was no indication of carryover effects from year to year.

5.9.6.3 Predicted Biomass Changes in Trees

Trees, depending on species and genotype, exhibit a wide range of responses to O_3 exposure. Ozone exposures alter gas exchange, early senescence and needle retention on conifers, carbohydrate allocation, root growth, total biomass production, and reproduction. The alteration by O_3 of photosynthetic performance and needle retention shifts carbon

allocation priorities and changes growth. In particular, root growth in tree seedlings is often reduced, whereas shoot growth is maintained. Root growth reductions can decrease mycorrhizal formation and water and nutrient uptake in seedlings and impede seedling establishment. Changes in carbon budgets due to O_3 exposures also can affect long-term changes in tree growth. Small changes (even less than 1 to 2% biomass loss per year) that may not be detectable statistically may be translated into large changes during the life span of the tree and may result in changes in stand dynamics when sufficient trees are affected, with concomitant effects on the structure and function of the ecosystem. The implication of these effects on long-lived species is significant. However, most of the experiments have been conducted on seedlings for 1 to 3 seasons, only 2% or less of the life span of the tree. Seedlings and mature trees have different carbon allocation use patterns. Mature trees have a significantly higher ratio of respiring to photosynthetic tissue. Carbohydrate reserves also differ between trees and seedlings. Extrapolation of information from seedlings to mature trees must be done with caution because the environments in which trees and seedlings grow differ substantially due to differences in rooting depth and canopy structures.

5.9.7 Effects of Ozone on Natural Ecosystems

Ozone is the only regionally distributed phytotoxic pollutant capable of changing the chemical environment of forests without leaving a permanent trace of its presence. Ozone molecules are ephemeral, decompose rapidly to oxygen and free radicals, and leave no residuals; therefore, stresses resulting from exposure to O_3 are frequently difficult to determine (Taylor and Norby, 1985).

Ozone exposures are episodic. Ozone may be transported for long distances and may cover very large areas during an episode. Concentrations can increase as O_3 trajectories move across the country and pass over new sources (Wolff et al., 1977a,b,c, 1980; Wolff and Lioy, 1980). Forest trees, shrubs, and other perennial plants often must cope with the cumulative effects of several acute or chronic episodes. Exposures may last for minutes, hours, days, or weeks. Trees may respond rapidly as, for example, when needles of eastern white pine exhibit visible injury symptoms within days after exposure to high (>0.08 ppm) O_3 concentrations (Garner, 1991). In most instances, however, responses are more subtle and not observable for many years because trees compensate, adapt, and respond to cumulative stress by differential growth, the result of altered carbon allocation (Waring and Schlesinger, 1985).

Ecosystems are complex, dynamic communities composed of populations of living plants, animals, and microorganisms (producers, consumers, and decomposers). Because they must continually respond and adapt to changing environments, mature ecosystems are seldom stable (Kozlowski, 1985). They are held in an oscillating steady state by the operation of a particular combination of biotic and abiotic factors. Ecosystems can change dramatically throughout time, have no optimal condition, and are only healthy when compared to some desired state specified by humans (Lackey, 1994). Ecosystem functions maintain clean air, pure water, a green earth, and a balance of organisms. These functions enable humans to obtain food, fiber, energy, and other material needs for survival (Westman, 1977).

Ozone concentrations capable of causing injury to forest ecosystems (0.06 ppm or higher of varying durations; see Section 5.7.3) continue to occur in the San Bernardino and

the Sierra Nevada Mountains and in the Appalachian Mountains from Georgia to Maine. Visible injury to forest trees and other sensitive vegetation in these areas has been observed.

The impact that an ecosystem can experience from exposure to O_3 will be determined by the severity of the effect on individual members of a population. Stresses, whose primary effects occur at the molecular or cellular physiology level of an individual, must be propagated progressively through the more integrative levels, from the leaf, branch, or root, to whole plant physiology and stand dynamics, and, ultimately, to the ecosystem (see Section 5.7.4; Hinckley et al., 1992; Figure 5-36). Variability and compensation in response to stress at both the individual and the population levels determine the hierarchical extent of the response. Other factors, in addition to compensation and variability in response to stress, that affect response in individuals and populations include the location of a site and environmental factors, such as air and soil moisture and temperature and genetic composition of the individuals of a population. Responses at the population level must alter the ecosystem functions of energy flow, water movement, and nutrient cycling to produce an ecosystem impact.

The primary responses of a forest ecosystem to sustained O_3 exposure are reduced growth and biomass production (Section 5.7.4; Figure 5-34; Table 5-36; Smith, 1990). In mature trees, most of the carbohydrate produced is utilized in maintenance (Figure 5-34). Exposure to O_3 inhibits photosynthesis and decreases carbohydrate production and allocation, and, as has been stated previously, decreases allocation to the roots and interferes with mycorrhizal formation and nutrient uptake. The resulting loss in vigor affects the ability of trees to compete for resources and makes them more susceptible to a variety of stresses (Section 5.7.4; Table 5-36; see also Sections 5.3 and 5.7.3.1). In the San Bernardino Forest, the only available study dealing with the effects of O_3 exposure on forest ecosystems, the sensitive canopy trees, ponderosa and Jeffrey pine, no longer were able to compete effectively for essential nutrients, water, light, and space. Altered competitive conditions in the plant community, resulting from a decrease in the most sensitive species, permitted the enhanced growth of more tolerant species, white fir, incense cedar, sugar pine, and black oak (Miller et al., 1982; U.S. Environmental Protection Agency, 1978, 1986). Although the primary effect was on the more susceptible members of the forest community, changes in the function of other ecosystem components directly or indirectly affected the processes of carbon (energy) flow, mineral-nutrient cycling, and water movement, leading to changes in community patterns. Changes in available energy influenced biotic interactions associated with predators, pathogens, and symbionts (mycorrhizae).

The forests of the Appalachian Mountains have been episodically exposed to O_3 concentrations capable of vegetational injury for many years. Visible injury to foliage and reduction in growth of sensitive eastern white pine has been associated with peak hourly concentrations ranging from 0.08 to 0.13 ppm. Black cherry, also has been shown to be sensitive to O_3 exposures. Surveys of various regions of the Appalachian Mountains, including the Smoky Mountain and Shenandoah National Parks, indicate that visible injury to a variety of different types of vegetation continues to occur. Neither eastern white pine nor black cherry are canopy trees. Removal of sensitive individuals and the absence of population changes of these species have not resulted in any visible change in the forest ecosystems along the Appalachian Mountains, possibly because, as stated earlier (Section 5.7.4; Figure 5-36), "only a small fraction of the stresses at the molecular, cellular, or leaf level become disturbances at the stand or ecosystem level" (Hinckley et al., 1992). Decline and dieback of trees on Mt. Mitchell and Camel's Hump cannot be related solely to

O_3 injury. Ongoing research is attempting to understand better the effects of O_3 exposure on vegetation in these areas and the effect, if any, on the ecosystems to which they belong.

Injury to sensitive trees in the Sierra Nevada also appear to be in the same category as stated above. Injury to individuals has not been propagated to the population level and has not altered ecosystem functions; therefore, no changes have taken place in the ecosystems in those mountains.

The previous O_3 document (U.S. Environmental Protection Agency, 1986) concluded that "none of the plant species shown to be injured by O_3 plays a dominant role in the Blue Ridge Mountain ecosystem. Therefore, the removal of any of these species would probably not have an impact that the decline and death of ponderosa and Jeffrey pine have had on the San Bernardino Forest ecosystem." This same conclusion applies today.

5.9.8 Economic Assessments

Based on economic assessments and scientific data available at the time, the previous criteria document (U.S. Environmental Protection Agency, 1986) concluded that O_3 at ambient levels was imposing economic costs on society. The review of more recent (post-1986) literature on agriculture corroborates that earlier conclusion. Specifically, the recent literature, using the full set of NCLAN data and addressing some deficiencies in the pre-1986 assessments, confirms the finding of economic losses from ambient O_3 concentrations.

The exact level of these economic effects is a function of cropping patterns, O_3 concentrations (both ambient and episodic), and the spatial and temporal characteristics of projected or observed O_3 levels. The current economic assessments represent improvements in the scientific understanding of O_3 effects on agriculture. However, the assessments of economic effects initially incident on the agricultural sector remain incomplete.

Only a few assessments consider the economic effects of O_3 on forest trees and on urban trees, shrubs, and ornamentals. These studies assess the economic effects of hypothetical changes resulting from O_3 or other stressors on forest productivity and aesthetics and are best viewed as measures of the potential effect of O_3 on these receptors. Improvements linking O_3 effects data to productivity and aesthetic effects will improve the utility of such economic analyses.

The effects of O_3 on ecosystems have not been addressed in the published literature. There is, however, an emerging interest in applying economic concepts and methods to the management of ecosystems. Ecological research also is addressing the challenging conceptual and practical issues in understanding and managing ecosystem functions. Economic research continues to develop, refine, and apply techniques for valuing market and nonmarket products and services that will be of help in estimating the economic effects of O_3 on ecosystems. Increased dialogue between the disciplines is needed before empirical analyses of the economic consequences of ecosystem management are feasible.

In summary, the state of science concerning O_3 economic effects on agricultural crops is sufficient to conclude that O_3 imposes costs on society.

5.10 Effects of Ozone on Materials

5.10.1 Introduction

Photochemical oxidants are capable of reacting with a number of man-made and natural materials. Nearly all materials-damage research on photochemical oxidants has focused on economically important or abundant materials that are susceptible to oxidant damage. These include elastomers (natural rubber and certain synthetic polymers), textile fibers and dyes, and, to a lesser extent, paints. Recent research has been conducted on culturally important materials, such as artists' paints and pigments. It has been shown that oxidants harden and embrittle elastomers, causing cracking and loss in physical integrity. Oxidant exposure weakens certain textile fibers (i.e., reduces the breaking strength and increases the rate of wear) and changes the color of some dyes. The effects of oxidants on paints are not defined well, but they may be similar to some of the effects on elastomers; damage from other gaseous pollutants, such as SO_2, and from natural damaging agents, such as sunlight, moisture, oxygen, and temperature fluctuations, tend to overshadow the role of ambient O_3 in causing paint damage.

The literature selected for review in this section includes research previously reported in the 1978 and 1986 criteria documents (U.S. Environmental Protection Agency, 1978, 1986) and a limited number of other references published before and after 1986. Because little recent work has been reported on the effects of ozone on materials, reference to older studies is necessary for completeness. This assessment of the effects on materials includes a review of the mechanisms of damage and protection; it also presents dose-response information from laboratory and field studies and evaluates previously reported economic assessments.

5.10.2 Mechanisms of Ozone Attack and Antiozonant Protection

5.10.2.1 Elastomers

Most elastomeric materials found in the marketplace are composed of unsaturated, long-chain organic molecules (i.e., the molecules contain carbon-carbon double bonds). Natural rubber and synthetic polymers and copolymers of butadiene, isoprene, and styrene account for the bulk of elastomer production for products such as automobile tires (Mueller and Stickney, 1970). These types of compounds are particularly susceptible to O_3 attack. In contrast, synthetic elastomers with saturated chemical structures, such as butyl rubber, polymers of silicones, ethylene, propylene, hypalon, and polyurethanes, have an inherent resistance to O_3 damage, but higher cost and limiting physical and chemical properties have constrained their use in outdoor environments.

Ozone is thought to attack elastomers by adding a chain of three oxygen atoms directly across the double bond, forming a five-membered ring structure (Mueller and Stickney, 1970). This structure quickly rearranges (via Criegee ozonolysis) to form a zwitterion and an aldehyde (see Figure 5-37). The aldehyde-zwitterion pair can be formed on either side of the point of chain scission. Subsequent reactions of the zwitterion lead to a permanently oxidized elastomer. Ozone damage in the form of cracking is a surface phenomenon. It is greatly accelerated by mechanical stress, which produces fresh surface area at crack boundaries. At very high concentrations and high mechanical stress, O_3 damage can result in a large number of surface microcracks that produce a frosted appearance and mechanical weakening (Crabtree and Malm, 1956). At pollutant

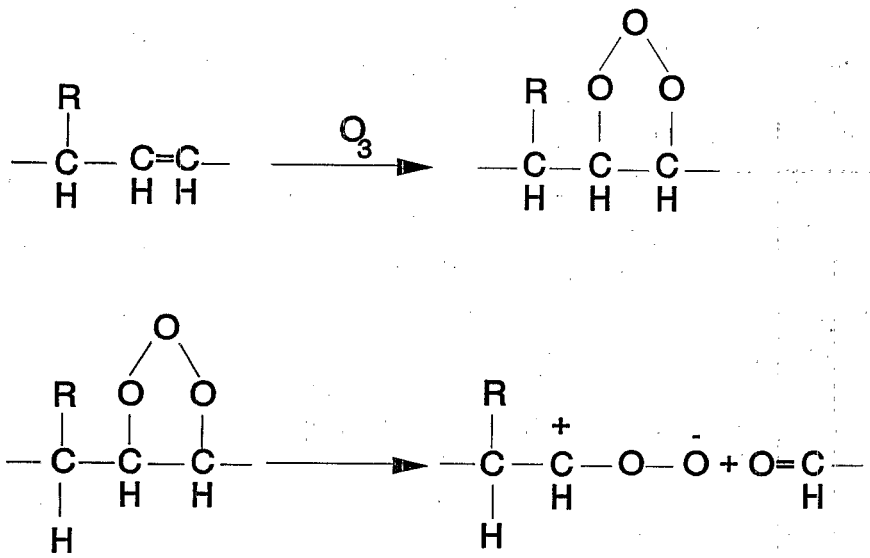

Figure 5-37. Postulated mechanism for damage to elastomers by ozone.

Source: Mueller and Stickney (1970).

concentrations normally encountered outdoors (and in many indoor environments), the elastomer hardens or becomes brittle and cracked, which results in loss of physical integrity.

According to Fisher (1957), work at the Rock Island Arsenal by R. F. Shaw, Z. T. Ossefa, and W. J. Tonkey in 1954 led to the development of effective antioxidant additives to protect elastomers from O_3 degradation. Subsequently, antiozonants generally were incorporated into elastomeric formulations during mixing, and their protection was effective, even when elastomers were stretched or flexed (Fisher, 1957; Mueller and Stickney, 1970).

Several theories (Andries and Diem, 1974) have been advanced to explain the mechanism of antiozonant protection. The two best supported theories are (1) the scavenger theory and (2) the protective film theory. The scavenger theory suggests that the antiozonant diffuses to the surface, where it reacts with the O_3 at a faster rate than with the carbon-carbon double bonds of the rubber, thereby protecting the rubber sacrificially. The protective film theory also includes diffusion to the surface, but assumes that the resulting layer is less reactive with O_3 than is the rubber and, thus, constitutes a protective layer.

The work of Razumovskii and Batashova (1970) on the mechanism of protective action by the antiozonant *N*-phenyl-*N′*-isopropyl-*p*-phenylenediamine (PIPP) is most consistent with the scavenger mechanism. These investigators showed that O_3 reacts preferentially with PIPP at a ratio of three O_3 molecules to one PIPP molecule.

Andries et al. (1979), using carbon-black-loaded natural rubber compounds, with and without antiozonants, attempted to distinguish among possible mechanisms with attenuated total reflectance spectroscopy and scanning electron microscopy. Their

experiments indicated that a combination of the scavenger and protective film mechanisms best explains antiozonant protection. Examination of the surface of the rubber samples with antiozonant showed that only ozonized antioxidant, not ozonized rubber, was present. This layer of ozonized antioxidant functioned as a relatively nonreactive film over the surface, preventing the O_3 from reaching and reacting with the rubber below.

Lattimer et al. (1984) conducted a series of experiments on cross-linked rubber (*cis*-polyisoprene and *cis*-polybutadiene) containing *N,N'*-di-(1-methylheptyl)-*p*-phenylenediamine antiozonant. They concluded that, although a number of O_3-rubber reactions and mechanisms are possible, these reactions do not become significant until the antiozonant is nearly completely consumed (i.e., the antiozonant preferentially reacts with the O_3). They concluded that the "scavenger-protective film mechanism" is primarily responsible for antiozonant protection.

In addition to reactive antiozonants, paraffinic and microcrystalline waxes are used to protect the elastomers in rubber products such as tires. The wax migrates to the surface of the rubber and forms a barrier against O_3 attack. Dimauro et al. (1979) studied the ability of 18 waxes to protect rubber against degradation from O_3. Dimauro found that no wax by itself provided an optimal level of protection; blending with a reactive antiozonant was required. The paraffinic waxes protected best at lower exposure temperatures, and the microcrystalline waxes were more effective at higher temperatures. Wax blends, which combine the best effects of each type of wax, offered the best protection over a wide range of temperature (Lake and Mente, 1992). It was found, however, that wax alone can be detrimental to dynamic O_3 resistance. Wax can induce localized stresses in the rubber that can lead to premature rubber failure under dynamic testing conditions.

5.10.2.2 Textile Fibers and Dyes

Cellulose-based, acrylic, and nylon fibers are affected by O_3 (Zeronian et al., 1971); however, it is difficult to distinguish O_3-induced damage from oxidation by molecular oxygen. Reduction in breaking strength and an increased rate of wear are the types of damage most commonly observed. As stated by Bogaty et al. (1952), however, for most uses of textile fibers, the action of O_3 is less important in affecting product lifetime than are physical abrasion, biological degradation, soiling, fashion, and other factors. Furthermore, most textiles are used and spend most of their life indoors, where O_3 concentrations are usually less than outdoor O_3 concentrations (Yocom et al., 1986). Accordingly, the economic significance of O_3 damage to textile fibers is relatively low, and the differences in the mechanisms of attack are not important.

Many textile dyes react with O_3. Figure 5-38 illustrates the reaction of Disperse Blue No. 3 with O_3 and with NO_x (Haylock and Rush, 1976). Ozone attacked the quinoid portion of the molecule, completely rupturing the ring system chromophore and oxidizing the dye to phthalic acid, which is colorless. Matsui et al. (1988) investigated the reactions of O_3 with aromatic azo compounds. Ozone was found to attack both the aromatic rings and the more electron-rich nitrogen atoms. Both the direct attack on the azo dye structure and the production of daughter products alter the original dye color.

The reactions between various dyestuffs and O_3 are influenced by the chemical nature of the fiber to which the dye is applied and the manner in which the dye is applied. Additional factors include the presence of protective agents; effects of temperature, air moisture, and other pollutants; and even the degree of strain of the base fiber caused by folding or creasing. In a study of O_3 fading of anthraquinone dyes on nylon, Haylock and

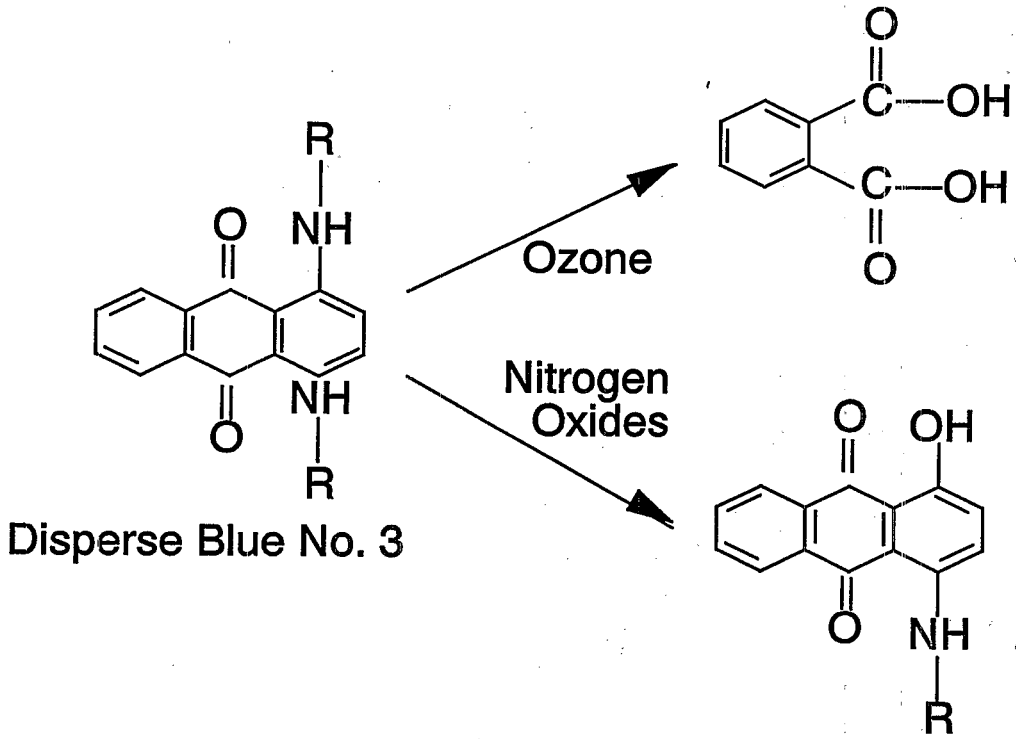

Figure 5-38. Reaction of anthraquinone dyes with ozone and with nitrogen oxides.

Source: Haylock and Rush (1976).

Rush (1976, 1978) found that fiber properties such as cross-section shape, draw ratio, and the degree of steam heat setting had significant effects on the rate and severity of O_3 damage, even for chemically identical systems. Moore et al. (1984) found that the rate of O_3 fading of acid and disperse dyes on polyamide fibers appeared to be a function of the rate of dye migration to the surface of the fibers. Thus, using dyes that diffuse slowly (high-molecular-weight dyes) improved resistance to O_3 fading. Given this complexity and sensitivity for both dye and fiber type, it is not possible to relate a specific mechanism of damage to a broad class of damage situations.

5.10.2.3 Paint

The mechanisms of architectural paint and coil coating damage caused by O_3 have not been well defined. Damage is probably related to oxidation of the organic binders that hold the pigment and form the protective seal over the surface. Damage is likely to be similar to that of elastomers; that is, embrittlement and cracking as the result of chain scission and cross-linking. The data available on O_3 damage to architectural paints, however, come primarily from studies of surface erosion caused by gaseous pollutants, and the suspected O_3 damage patterns (embrittlement and cracking) are not quantified. Because the polymeric structure of dried paint film is significantly different from that of an elastomer under elongation stress, direct comparisons should be made with great caution.

In a series of experiments (Shaver et al., 1983; Grosjean et al., 1987, 1988a,b, 1989) the direct attack of O_3 on artists' pigments and paints was investigated. Ozone was found to react with alizarin pigments, indigos, curcumin, and triphenylmethane colorants. The exact mechanism and site of the attack (e.g., carbon-carbon unsaturated bonds, aromatic rings, or carbon-nitrogen bonds) and subsequent reactions with the daughter products depended on the initial structure of the pigment. Often the products of these reactions were colorless or of a noticeably different color than the original pigments, resulting in fading or color changes.

5.10.3 Exposure-Response Data

Laboratory exposure-response studies are criticized for their reliance on artificial environments that do not contain all the critical variables encountered under ambient conditions. Scientists realize the limitations of laboratory tests; no model could simulate conditions identical to an ambient environment. Nevertheless, many laboratory tests have represented the outdoor environment to some extent, and the findings from these tests have been used in conjunction with field tests to estimate the nature and amount of damage to materials. Controlled field tests have the advantage of being carried out under real exposure conditions, but, because of the highly variable nature of real exposure conditions, data interpretation is difficult.

5.10.3.1 Elastomer Cracking

Table 5-40 presents an overview of the available laboratory and field studies of the effects of O_3 on elastomers. Hofmann and Miller (1969) demonstrated correlations between laboratory tests and the actual service use of passenger vehicle tires in the Los Angeles area. Basically, three laboratory test methods were used: (1) indoor and outdoor belt flex, (2) indoor and outdoor wheel, and (3) stress relaxation. The investigators found that the behavior of rubber exposed to O_3 under laboratory conditions correlated well with the service behavior of tires in localities where atmospheric O_3 concentrations were high.

Bradley and Haagen-Smit (1951) evaluated a natural rubber (NR) formulation for susceptibility to O_3 cracking. Strips were strained approximately 100% by bending and then exposed in a small chamber to 20,000 ppm of O_3; these specimens cracked almost instantaneously and broke completely within 1 s. When these NR formulations were exposed to lower concentrations of O_3 (approximately 0.02 to 0.46 ppm), time periods of about 5 min to over an hour were required for cracks to develop.

Meyer and Sommer (1957) exposed thin polybutadiene specimens to constant load, ambient room air, and O_3. Specimens exposed in the summer to average O_3 concentrations of about 0.048 ppm broke after 150 to 250 h. In the fall, at average O_3 concentrations of 0.042 ppm, specimens failed after exposures of 400 to 500 h. In the winter, at average O_3 concentrations of 0.024 ppm, failures occurred between 500 and 700 h. These data show the strong dependence of breakage on O_3 dose over the average time of exposure at which failure occurred (average $C \times T$).

Edwards and Storey (1959) presented data demonstrating the O_3 resistance of two styrene-butadiene rubber (SBR) compounds (Polysar S and Polysar Krylene). Both compounds were exposed with and without different levels of antiozonant protection to 0.25 ± 0.05 ppm of O_3 at 120 °F (49 °C) under 100% strain (twice the original sample length). Without antiozonants, a linear relationship was found between O_3 dose (ppm·h) and

Table 5-40. Laboratory and Field Studies on Effects of Ozone on Elastomers[a]

Conditions	Material/ Product	Pollutant	Concentration, (ppm)	Exposure	Environmental Variables	Dose, (ppm-h)	Effects	Comment	Reference
Laboratory/ field	Automotive tires	Ozone	0.25 to 0.5	NA	Tires under stress	—	Cracking of white side wall.	Purpose was to correlate lab and field tests. Exposure time, detailed pollutant measurements, and statistical analyses were not reported.	Hofmann and Miller (1969)
		Ambient air	0.04 (annual average)	>1 year	Los Angeles environment; actual service use	>350	Positive correlation between laboratory and ambient air tests.		
Laboratory	Vulcanized rubber strips	Ozone	0.02 to 0.46, 20,000	3 to 65 min	Physical stress	~0.02 to 0.03	Surface cracking.	Test was designed to establish dose/response curves on O_3-sensitive rubber for use as an analytical method.	Bradley and Haagen-Smit (1951)
Field	Rubber tires and various polymers	Ambient air	0.023 to 0.048	150 to 700 h	Physical stress and ambient environment	9 to 20	Time of cracking.	Cracking occurred over a broad range of values and was related to stress.	Meyer and Sommer (1957)
Laboratory	SBR: Plysar S and Plysar Krylene, with and without antiozonants	Ozone	0.25	19 to 51 h	120 °F, 100% strain	4.75 to 12.75	Percent antiozonant was related to cracking depth rate.	Demonstrated dose/response linear relationship for O_3 on unprotected rubber.	Edwards and Storey (1959)
Laboratory	White sidewall tire specimens	Ozone	0.05 to 0.5	250 to 1,000 h	10 and 20% strain	20 to 500	Mean cracking rates were determined for different stress and O_3 levels.	Detailed data not available to verify author's statement that 2 to 5 years of ambient conditions were required for O_3 cracks to penetrate cord depth.	Haynie et al. (1976)
Laboratory	Polyisoprene	Ozone	0 to 1.8	2 h	22 °C	Up to 3.6	Cracking and stress relaxation.	Rate of attack rapid and proportional to O_3 concentration.	Razumovskii et al. (1988)

Table 5-40 (cont'd). Laboratory and Field Studies on Effects of Ozone on Elastomers[a]

Conditions	Material/ Product	Pollutant	Concentration (ppm)	Exposure	Environmental Variables	Dose (ppm-h)	Effects	Comment	Reference
Laboratory	Ten different NR, SBR, and CR formulations with and without protection	Ozone	0.5	Up to 300 h	30 °C	Up to 50	Time to 10 to 20% relaxation.	Both formulation and protection affected relaxation.	Ganslandt and Svensson (1980)
Laboratory	Natural rubber, epoxidised rubber, and copolymers	Ozone	0.05 to 1,000	To 16 h	−20 to 70 °C, 10 to 100% strain	0 to 240	Time to first cracking.	Temperature dependence of antiozonant protection.	Lake and Mente (1992)
Laboratory	Several NR/SBR blends, with and without protection	Ozone	0.05 to 0.15	~3 to 16 h	Sunlight, humidity	~0.15 to 2.4	Interply adhesion affected at 0.05 ppm and above.	Both waxes and antiozonants needed for protection against sunlight plus O_3.	Davies (1979)
Laboratory	Tire cords (66 nylon; Dacron polyester; Kevlar aramid)	Ozone	0 to 1.5	0 to 48 h	UV light; heat (100 °C); RH (20 to 90%); NO_2	Up to 72	RFL adhesion loss occurred primarily during 6-h exposure to high RH and 0.2 ppm O_3.	Synergism between O_3 and RH; RFL deterioration occurred at surface.	Wenghoefer (1974)

[a]See Appendix A for abbreviations and acronyms.

cracking depth. Increasing the amount of antiozonants significantly reduced the rate of cracking for both rubber compounds in a dose-related manner.

Haynie et al. (1976) conducted a chamber study to evaluate the effects of various pollutants, including O_3, on several materials. In one part of the study, white sidewall specimens from a top-quality, steel-belted radial tire were exposed (strained at 10 and 20%) for 250, 500, and 1,000 h to O_3 concentrations of 0.082 ppm (160 $\mu g/m^3$) and 0.5 ppm (1,000 $\mu g/m^3$). The O_3 level was found to be statistically significant in the rate of cracking of this rubber; however, cracking rates were not directly proportional to O_3 concentrations for these two levels. Using the mean cracking rate calculated after long-term (1,000-h) exposure to conditions representative of the primary air quality standard for O_3 and the annual average standard for NO_2, Haynie et al. (1976) concluded that it would take a minimum of 2.5 years for a crack to penetrate to the cord depth. For this particular premium tire, therefore, sidewall failure from O_3 damage does not appear to be the cause of reduced tire life. Tread wear, rather than sidewall failure, probably determines the life of a typical rubber tire.

Razumovskii et al. (1988) studied the decrease in stress (stress relaxation) of polyisoprene vulcanizates in an exposure chamber at 22 °C at five O_3 concentrations ranging from O_3- free to 3,450 $\mu g/m^3$ (1.76 ppm). Stress relaxation resulting from the growth of surface cracks caused irreversible changes in the dimension of the elastomer and decreased tensile strength. Figure 5-39 presents the rate of change of stress as a function of time for various O_3 concentrations. The rate of stress reduction was proportional to O_3 concentration, with virtually no change for the O_3-free samples and progressively more rapid relaxation as O_3 levels increased. Razumovskii et al. (1988) concluded that O_3 absorption, attack of the C=C bonds, cracking, and the resulting stress relaxation were fast processes for unprotected elastomers.

Ganslandt and Svensson (1980) tested 10 different mixtures of three rubber compounds, NR, SBR, and CR, with the isoelastic force method. The O_3 protection afforded each rubber formulation is summarized in Table 5-41. The samples at 50% elongation were exposed to O_3 concentrations of 0.5 ppm at 30 °C. The time to 10 and 20% relaxation of the isoelastic force in the rubber test samples was used to gauge the O_3 resistance of the formulation. Compounds GL 2073 B, SS 202, and SS 200 C showed greatest resistance to the effects of O_3, and those formulations that were unprotected (GL 2073 D, SS 200 B, SS 202 A, SS 203) and the formulations protected only by paraffin wax (GL 2073 G) demonstrated the least resistance to O_3 attack. The testing showed great variety in the kinds of visible cracking effects as a result of the exposure. The compounds with no protection often showed a large number of small cracks over the entire surface of the material, but those compounds protected by a combination of wax and antiozonant or by wax alone sometimes showed only a single crack, which grew rapidly. These effects are demonstrated in Figure 5-40. Compounds SS 202 B (Figure 5-40A) and SS 200 C (Figure 5-40B), both protected with wax and antiozonant, showed fairly good resistance when gauged by the 10 and 20% stress relaxation tests but failed after approximately 50 and 58 h of exposure, respectively. On the other hand, compounds SS 203 and SS 200 B, both unprotected, exhibited small surface cracking and outlasted some of the protected compounds. Moreover, protection with wax and antiozonant may afford long-term protection, but when one crack appears, it can grow rapidly and cut off the test piece, as shown in Figure 5-40b.

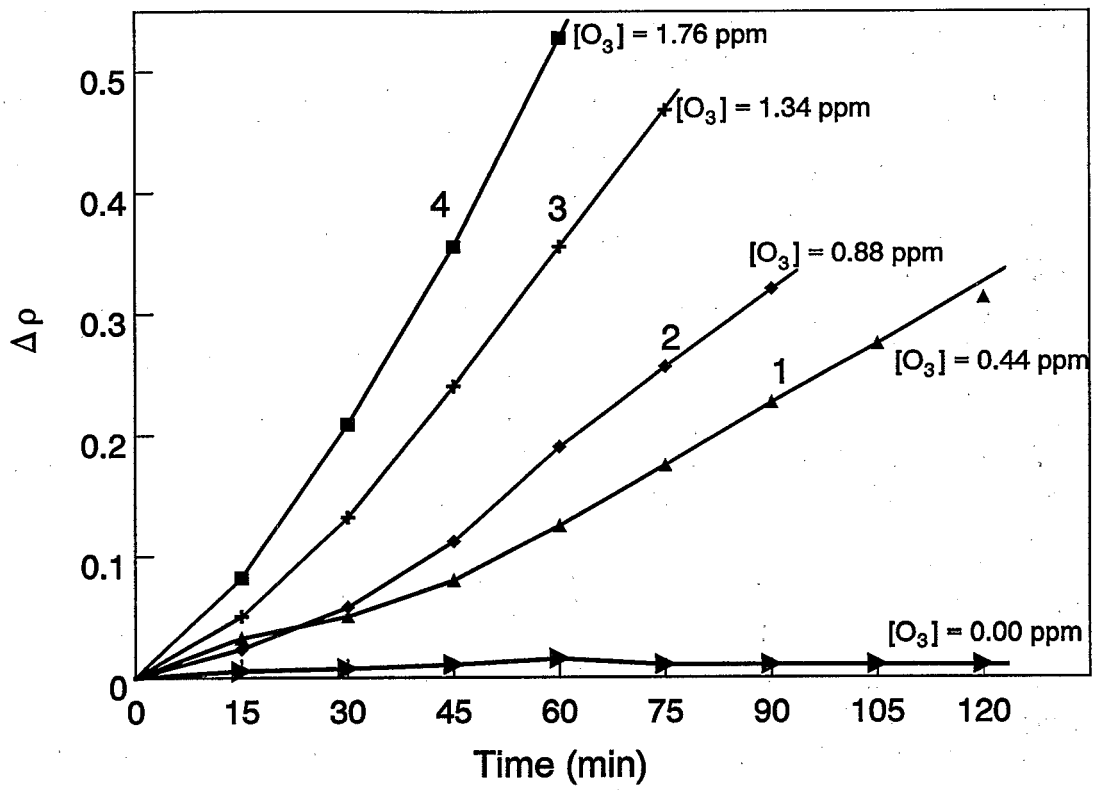

Figure 5-39. Relative decrease in stress (Δρ) with time as a function of ozone concentration for polyisoprene vulcanizate.

Source: Razumovskii et al. (1988).

Table 5-41. Protection of Tested Rubber Materials[a]

			Protected	
Rubber Formulation	Mixtures	Unprotected	Wax	Antiozonant
GL 2073 (NR)	B, C		X	X
	D	X		
	G		X	
SS 200 (NR)	A, C		X	X
	B	X		
SS 202 (SBR)	A	X		
	B		X	X
SS 203 (CR)		X		

[a]See Appendix A for abbreviations and acronyms.

Source: Ganslandt and Svensson (1980).

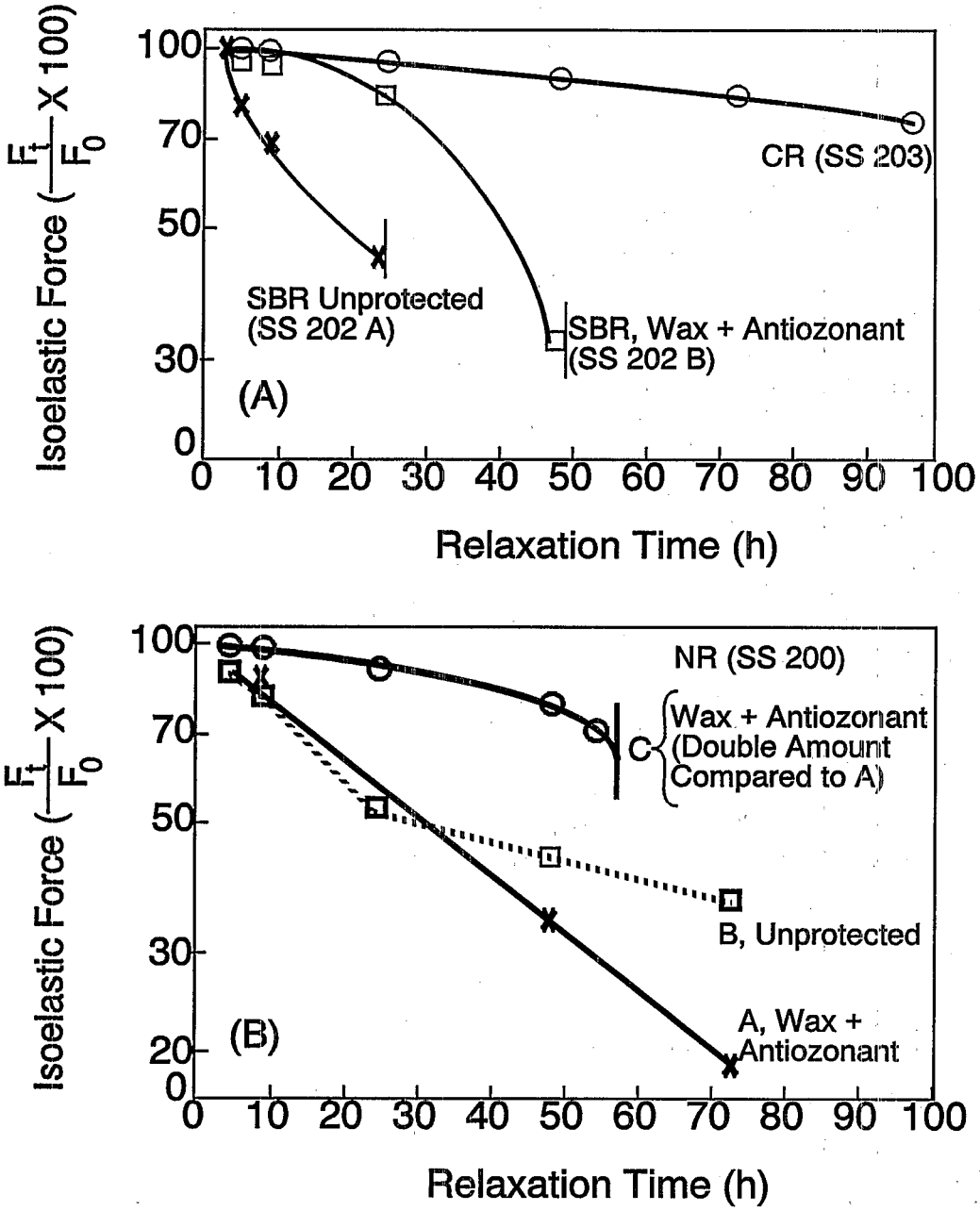

Figure 5-40. ***Relaxation of rubber compounds in ozone (O_3) is affected by the combination of rubber formulation and type of O_3 protection. Compounds SS 202B (A) and SS 200C (B) were tested at an O_3 concentration of 0.5 ppm, a temperature of 30 °C, and elongation of 50%. The vertical lines at the end of curves mean total failure, and vertical axes represents relaxation, where F_O is the initial force, and F_t is the force after time t.***

Source: Ganslandt and Svensson (1980).

Lake and Mente (1992) exposed natural rubber, epoxidised rubber, and two acrylonitrile-butadiene copolymers with chemical antioxidants, waxes, or a combination of antioxidants and waxes to a variety of O_3 concentrations and temperatures in environmental testing enclosures. Ozone concentrations ranged from 0.05 ppm to 1,000 ppm with temperatures from −20 to 70 °C. Samples were kept under constant strain between 10 and 100%. Antiozonant chemicals in the concentration range from 2 to 20 p.h.r. (parts per hundred of rubber by weight) were tested, and wax/antiozonant combinations at 6 p.h.r. wax and 3 p.h.r. antiozonant also were tested. Lake and Mente found that O_3 protection was most effective at higher temperatures, when diffusion of the antiozonant and wax to the surface of the elastomers was most rapid. This relationship is fortunate because ambient O_3 concentrations correlate well with higher temperatures. Antiozonants became generally less effective as temperatures dropped; however, dialkyl paraphenylenediamine provided reasonable protection for natural rubber to −17 °C.

Davies (1979) reported on the effects of O_3 and other environmental factors on interply adhesion of natural and synthetic rubber compounds. Excellent adhesion of plies is essential to the proper manufacturing of tires. The rubber strips must make interlocking contact at the joint boundary, or the strength of the tire will be inadequate. Ozone attack on synthetic poly isoprene and polybutadiene produces a surface layer of ozonides. With NR, the film consists of ozonides and carbonyl groups (Andries and Diem, 1974; Andries et al., 1979). The results of the Davies (1979) tests indicated that, before curing, the adhesion of SBR compounds is unaffected by exposure to O_3 concentrations of 0.15 ppm, but the adhesion of the NR/SBR blend decreases by approximately 30%. Large reductions (on the order of 70%) in adhesion between plies were noted with the NR compounds; even exposure for a few hours at 0.05 ppm reduced adhesion considerably. The adhesion tests on cured NR, SBR, and isoprene rubber (IR) compounds after exposure to various levels of O_3 and humidity are summarized in Table 5-42. The adhesion of the SBR compound is superior to that of the other two compounds, which were affected greatly by increased RH.

Table 5-42. Effect of Ozone and Humidity on Interply Adhesion[a,b]

		Final Adhesion[c]		
Compound	Initial Adhesion	0.15 ppm O_3 (294 $\mu g/m^3$), 30% RH	0.25 ppm O_3 (490 $\mu g/m^3$), 30% RH	0.15 ppm O_3 (294 $\mu g/m^3$), 60% RH
NR	5	2 to 3	1	1
IR	5	4 to 5	2 to 1	1
SBR	5	4 to 5	3 to 4	3 to 4

[a]Adhesion is rated from 1 (bad) to 5 (excellent), based on visual scale standardized by the authors.
[b]See Appendix A for abbreviations and acronyms.
[c]All exposures were 16 h in duration.

Source: Adapted from Davies (1979).

Wenghoefer (1974) studied the effects of O_3 on adhesion of tire cords dipped in resorcinal-formaldehyde latex (RFL). Many fibers and dip formulations were studied to determine their sensitivity to O_3, humidity, NO_2, UV light, and heat. Wenghoefer exposed these materials at a constant temperature of 100 °F (37.8 °C) to O_3 levels that varied between 0 and 1.5 ppm (0 and 2,940 $\mu g/m^3$) and to RH levels ranging from 20 to 90%. Adhesion deteriorated from changes in surface properties of the RFL-dipped cords as a result of exposure to O_3, humidity, UV light, and heat. The adhesion losses from O_3 and the combined effects of O_3 and humidity were most notable in the first 6 h of exposure. The detrimental effects of heat, NO_2, and the synergistic interaction of NO_2 and humidity were much less pronounced.

5.10.3.2 Dye Fading

Color fading of certain textile dyes has been attributed to the effects of ambient O_3. Although NO_2 was originally identified as the pollutant most important to color fading, the effects of O_3 were noted by Salvin and Walker (1955). The primary products affected were permanent-press garments (polyester and cotton) and nylon carpeting. Table 5-43 summarizes studies on the effects of O_3 on dyes. By using a combination of laboratory chamber studies and outdoor exposures, Salvin and Walker (1955) demonstrated that O_3 was responsible for dye fading observed on drapery fabrics. Blue anthraquinone dyes and certain red anthraquinone dyes were markedly bleached after exposure to just 0.1 ppm of O_3. Azo red and yellow dyestuffs and diphenylamine yellow dyes were shown to be resistant to fading at these concentrations, also confirming the results of the field study. The use of known antiozonants, such as diphenyl-ethylenediamine and diallyl phthalate, in combination with disperse blue dyes, was effective against O_3 fading, thus providing additional evidence of the effects of O_3 on dyed fabrics.

Ajax et al. (1967) summarized the results of a study of 69 dye-fabric combinations that were exposed outdoors in light-free cabinets at 11 sites. These sites were Sarasota, FL; Phoenix, AZ; Cincinnati, OH; and four urban-rural combinations: (1) Chicago and Argonne, IL; (2) Washington, DC, and Poolesville, MD; (3) Los Angeles and Santa Paula, CA; and (4) Tacoma and Purdy, WA. Among those fabrics exhibiting a high degree of fading at both urban and rural sites in the first 6 mo, fading was much greater at the urban sites than at the rural sites. The samples exposed in Phoenix, Sarasota, and Purdy showed the lowest amount of fading, which indicated that humidity and temperature are not, by themselves, the primary factors in fading. The highest fading rates occurred in samples exposed in Los Angeles, Chicago, and Washington, DC. In addition, there was a marked seasonal variation in the test results, with greater fading during the spring and summer seasons. Generally, the results correspond with seasonal peaks in O_3 concentrations.

Ajax et al. (1967) also exposed the fabrics to irradiated and nonirradiated auto exhaust, with and without SO_2, for 9 h/day for 6 consecutive days. From the results of this chamber study, the investigators noted that "photochemically produced by-products of automobile exhaust are a prime cause of fading compared to fading caused by nonirradiated auto exhaust or by clean air with sulfur dioxide added." In the presence of SO_2, however, a more than additive effect was seen in the dye-fading tests for both chamber and field studies. Although the conclusions of Ajax and co-workers concerning O_3 itself are easily substantiated in the research literature, the O_3 levels measured in their test chamber are questionable. The daily 9-h average O_3 concentrations (measured by neutral-potassium

Table 5-43. Laboratory and Field Studies of the Effects of Ozone on Dye Fading[a]

Dye	Fabric	Concentration (ppm)	Exposure	Environmental Variables	Effects	Comments	Reference
Blue and red	Drapery	0.1	—	—	Both dyes were markedly bleached. No fading occurred when antioxidants were added.	Insufficient data for dose-response determinations. This study followed a field study showing that oxidants other than NO_x caused fading.	Salvin and Walker (1955)
Disperse Direct, fiber reactive vat, sulfur, azo Disperse Disperse, basic Disperse, acid Direct Premetolized, acid	Cellulose acetate Cotton Polyester Orlon Nylon taffeta Viscose Wool	Laboratory 0.02 to 0.55; field exposure concentrations not reported	54 h 3 mo	Light-proof cabinets, 11 rural and urban sites	Photochemical agents caused more fading than nonradiated samples. Urban locations produced more fading, and temperature and humidity are not the primary causes of fading.	Both laboratory and field measurements. Reported laboratory O_3 concentrations questionable. SO_2 was also present in laboratory exposures.	Ajax et al. (1967)
Direct red 1 Reactive red 2 Sulfur green 2 Azoic[b] red Direct red 1 Acid red 151 Acid yellow 65 Acid violet 1 Basic red 14 Basic yellow 11 Acid orange 45 Disperse blue 3 Disperse blue 3 Disperse blue 3 Disperse blue 27 Disperse blue 27 AATCC O_3 ribbon	Cotton Cotton Cotton Cotton Rayon Wool Wool Wool Acrylic Acrylic Nylon Nylon Cellulose Acetate Acetate Polyester Acetate	0.05 0.5	12 weeks	Temperature = 55 °F, 90 °F RH = 50%, 90%	Induced fading at both levels, but at a nonlinear rate. Both temperature and humidity increased fading rate, and RH was more important. Eight of the tested fabric-dye combinations faded measurably in response to O_3. Only trace amounts of fading occurred in the remaining fabrics.	Insufficient data to show detailed dose-response relationships. Although samples were measured throughout the exposure, only the 12-week data were presented.	Beloin (1973)

Table 5-43 (cont'd). Laboratory and Field Studies of the Effects of Ozone on Dye Fading[a]

Dye	Fabric	Concentration (ppm)	Exposure	Environmental Variables	Effects	Comments	Reference
Olive I and II Disperse blue 3 and 7	Nylon fibers Nylon fibers	0.2 0.9	1 to >6 h	RH = 70 to 90% Temperature = 40 °C	Visible fading in Olive I after 16 h at 70% RH; same effect after 4 h at 90% RH. Linear increase in fading at 0.9 ppm O_3.	Both RH and O_3 concentration affected fading and in a nearly linear fashion. Sleeve form was more susceptible than skein form. Haylock and Rush (1976) found that: (1) increased fiber draw ratio reduced fading; (2) increased heat-setting temperature increased fading; and increased fiber surface area increased fading.	Haylock and Rush (1976)
Disperse blue dye in an avocado green mixture	Nylon 6 yarn	0.5	—	RH = 85% Temperature = 40 °C	Fading was closely correlated with fiber surface area (diameter).	Insufficient data for dose-response relationship determinations.	Huevel et al. (1978)
2 Disperse dyes and 2 acid dyes	Nylon 6 and Nylon 66 carpet		3 mo to 3 years	28 homes in different parts of the country	Geographic and seasonal variation in fading.	Field study.	Nipe (1981)
Disperse blue 3	Nylon 6 yarn	0.2	2 to 120 h	RH = 65%, 85%, and 90% Temperature = 40 °C	Nearly linear increase in fading with time. RH had a major influence on fading rate.	This study focused more on mechanisms of O_3 fading than on dose-response relationships.	Kamath et al. (1983)
Disperse, basic Disperse Direct vat, sulfur, fiber reactive Disperse Disperse, acid Direct Acid, mordant	Acrylic Cellulose acetate Cotton Polyester Nylon Viscose rayon Wool	—	2 years in 3-mo blocks	Light-proof cabinets, eight rural and urban sites	Two-thirds of samples exhibited substantial fading, O_3 was significant for eight fabric/dye combinations.	Field study.	Beloin (1972)

Table 5-43 (cont'd). Laboratory and Field Studies of the Effects of Ozone on Dye Fading[a]

Dye	Fabric	Concentration (ppm)	Exposure	Environmental Variables	Effects	Comments	Reference
Disperse blue 3 Acid blue 25 Acid blue 40 Acid blue 45 Acid blue 80 Acid blue 127	Nylon 6 yarn Nylon 66 yarn	0.2	0 to 96 h	RH = 85% Temperature = 40 °C	Fading proceeded consistent with diffusion of dye to fiber surface.	Study of mechanisms of O_3 fading, follow-on the Kamath et al. (1983).	Moore et al. (1984)
Royal blue Red Plum	Drapery fabric Rayon acetate Rayon acetate Cotton duck	0.5 and 1.0	250 to 1,000 h	50 and 90% RH, NO_2 and SO_2.	O_3 was not a statistically significant cause of fading.	Laboratory study.	Haynie et al. (1976)

[a]See Appendix A for abbreviations and acronyms.
[b]Coupling component 2, azoic diazo component 32.

iodide and a Mast instrument) were identical for UV-irradiated and nonirradiated exhaust (0.02 ppm); irradiated exhaust plus SO_2 produced 0.55 ppm of O_3.

Beloin (1972, 1973) investigated the effects of air pollution on various dyed textiles by conducting field and controlled-environment laboratory studies. For the field study, a wide range of dyed fabric was exposed in light-tight cabinets at the same four urban-rural combined sites used in the Ajax studies. The study was carried out over a 2-year period, in eight consecutive 3-mo, seasonal exposure periods. Color-change data and air pollution and weather measurements were analyzed to identify the factors that caused fading. About two-thirds of the fabrics studied showed appreciable fading. Most of these fabrics faded significantly more at urban sites than at rural sites. The small amount of fading evidenced by the samples exposed at extreme temperatures or humidity indicated that these factors, by themselves, have no effect on fading. The samples also showed some seasonal variations in fading. In areas of high oxidant concentration, maximum fading occurred primarily in summer and fall. Fabrics exposed in Chicago, where SO_2 concentrations are higher in the winter, showed greater fading during this season.

The results of the outdoor fading study were used in a multiple regression analysis. The analysis focused on 25 fabric dye samples, 23 of which showed SO_2 to be a significant variable. Ozone was also a significant contributor to the fading of eight dyed fabrics, as was NO_2 to the fading of seven dyed fabrics. The dominance of SO_2 as a factor in fading may have been complicated by soiling.

Beloin's laboratory study was designed to assess the effects of air pollutants, temperature, and RH on the colorfastness of 30 samples selected from those exposed during the field study. Fabric samples were exposed to two concentrations of O_3: 0.05 and 0.50 ppm. The laboratory studies demonstrated that high O_3 levels produced more significant fading in more fabric samples than did low levels. Visible fading did occur in about one-third of the sensitive fabrics (cellulose acetate, viscose, and cotton muslin with red and blue dyes) exposed to O_3 concentrations of 0.05 ppm. These levels are similar to those frequently found in metropolitan areas. The laboratory study also demonstrated that high RH (90%) is a significant factor in promoting and accelerating O_3-induced fading.

Haynie et al. (1976) and Upham et al. (1976) reported on the degree of fading of three different drapery fabrics exposed in a laboratory chamber to combinations of high and low O_3 concentrations (0.5 and 0.1 ppm, respectively), high and low RH (90 and 50%, respectively), and high and low concentrations of NO_2 and SO_2. The three commercially obtained fabrics selected for this study were royal blue and red rayon-acetates and a plum cotton duck. The samples were exposed in the chamber for periods of 250, 500, and 1,000 h; the degree of fading was measured with a color difference meter. The fading of the plum-colored material was related statistically to RH and the NO_2 concentration. For the red and blue fabrics, only RH appeared to be a significant factor. The effects of concentrations of O_3 on the amount of fading of these dyes were not statistically significant, even after exposure for 1,000 h to 0.5 ppm, levels much higher than typical ambient exposures.

Haylock and Rush (1976, 1978) studied the fading of anthraquinone dyes on nylon fibers. In the first test, nylon carpet yarn dyed with Olive I (0.081% Celliton Pink RF, 0.465% Celliton Yellow GR, 0.069% Celliton Blue FFRN) and Olive II (0.082% Latyl Cerise Y, 0.444% Celliton Yellow GA, 0.143% Cellanthrene Blue CR) was exposed to varying levels of temperature, RH, and O_3. Material dyed with Olive I and exposed at 70% RH, 40 °C (104 °F), and 0.2 ppm of O_3 showed visible fading after 16 h of exposure. At 90% RH, similar fading occurred in less than 4 h. Under the same RH and temperature

conditions, increasing the O_3 concentration from 0.2 to 0.9 ppm resulted in a corresponding increase in fading. Samples in knitted sleeve form demonstrated much greater susceptibility to O_3 attack than samples in skein form.

Using Disperse Blue 3 and 7 dyes exposed to constant conditions of 40 °C (104 °F), 90% RH, and 0.2 ppm of O_3, Haylock and Rush (1976) investigated the effect on fading of changing the fiber cross section, the fiber-draw ratio, and the method of setting the nylon fibers with steam heat. They found that increasing the surface area of the fibers resulted in an increased fading rate. Increasing the fiber draw ratio reduced dye fading, and increasing the heat-setting temperature decreased resistance to fading in disperse dyes.

The effect of high temperature and high humidity for induction of O_3 fading in nylon was confirmed further by the additional work of Haylock and Rush (1978). Their studies showed a good correlation between accelerated O_3 fading in the laboratory and in outdoor, in-service exposure, during which temperature and humidity extremes were common. Control samples exposed indoors, however, where temperatures and humidities were lower, did not exhibit nearly the same magnitude of fading as the laboratory samples.

Huevel et al. (1978) investigated the importance of the physical nature of Nylon 6 yarns on the O_3 fading behavior of a disperse blue dye. Samples of Nylon 6 yarns dyed avocado green with a dye mixture including Disperse Blue 3 were exposed in a laboratory cabinet to 0.5 ppm of O_3 at 40 °C and an RH of 85%. Huevel et al. found that the microfibril diameter and specific surface area of the fiber were the fiber characteristics most closely related to O_3 fading, thus confirming suspicions expressed earlier by Salvin (1969).

Nipe (1981) summarized the results of a 3-year study to establish the relationship between in-service fading of carpets in a home versus O_3 fading as determined by the American Association of Textile Chemists and Colorists (AATCC) Standard Test Method 129, "Colorfastness to Ozone in the Atmosphere Under High Humidities." (Measurements also were taken to compare the fading caused by NO_x.) The test carpets were made of Nylon 6 and 66 dyed with two disperse and two acid dye formulas. Test samples from the homes of 28 participants were returned every 3 mo for the 3-year period. The exposure sites selected for this long-term study represented variations in home heating and cooling, utilities, climate, and geographical locations. The carpet samples were placed in areas as close as possible to the kitchen but away from exposure to sunlight or any traffic. No measurements of O_3 concentrations were collected; however, an O_3-sensitive sample strip was included with each carpet sample. Analysis of the sample strip enabled the researchers to determine the relative O_3 exposure of each carpet sample.

Geographical location appeared to have a significant effect on fading. Test samples from sites in the Southeast and Northeast showed far more O_3 fading than did those in the West and Far West. Test samples in homes with air conditioning exhibited less fading during the summer than those without air conditioning. In all samples, much greater fading was caused by O_3 during July, August, and September than in January, February, and March. Typically, O_3 levels indoors are higher during the summer, when doors and windows are more likely to be open, thus allowing a greater exchange between inside and outside air. The results of the study of in-service interior carpet exposures were compared with the results of AATCC Test 129. In a sample that performs satisfactorily through 1.08 cycles of O_3 exposure in AATCC Test 129, there is a 98% probability against in-service fading over a 1-year period. A sample that performs satisfactorily through only 0.6 cycles of O_3 testing has only a 90% probability of satisfactory performance after 1 year of in-service exposure.

Kamath et al. (1983) studied the effect of atmospheric O_3 dye fading on nylon fibers. Prior studies had postulated that O_3 does not penetrate into the fiber to destroy the dye, but instead attacks the dye at the surface of the fiber. Dye then diffuses outward from the fiber interior because of the concentration gradient set up as the surface dye is destroyed. Using microspectrophotometry to test this postulated mechanism, Kamath et al. (1983) studied the diffusion and destruction of C.I. Disperse Blue Dye 3 on Nylon 6 continuous filament yarn measuring about 45 μm in diameter. With this method, the investigators were able to generate a dye distribution profile across the cross section of the fiber and to determine the diffusion coefficient of a dye in the fiber. The fibers were exposed in a controlled environment to O_3 concentrations of 0.2 ppm for 2 to 120 h at a temperature of 40 °C and RH levels of 90, 85, and 65%. The results of these laboratory studies indicated that RH has a significant positive effect on fading, that destruction of the dye begins near the surface of the fiber in the early stages of exposure, and that O_3 penetration into the fiber may be an important mechanism in O_3 fading. The dependence of fading rates on humidity was substantial. Even slight rises in humidity from 85 to 90% caused a significant increase in the extent of fading. At 65% RH, the fading rate drops dramatically. This effect was attributed to the breakage of hydrogen bonds in the presence of water, which leads to a more open structure with high segmented mobility; this condition is more favorable to diffusion of O_3 and disperse dyes.

A follow-on study by Moore et al. (1984) used the Kamath et al. (1983) approach with a variety of dyes, yarns, and treatments. Moore and coworkers used untreated, phenol-treated, and steam-treated Nylon 6 and Nylon 66 continuous filament yarns, with six disperse blue and acid blue dyes. Molecular weights of the dyes ranged from MW = 296 (Disperse Blue 3) to MW = 872 (Acid Blue 127). Dyed filaments were exposed to 0.2 ppm O_3 at 40 °C and 90% RH for various periods up to 96 h. For Nylon 6, steam-treated fibers faded more quickly than untreated fibers, whereas phenol-treated fibers faded less quickly. In Nylon 66, both treatments increased the rate of dye loss. The authors attributed this effect, at least in part, to the change in morphology of the treated fibers. Faster fading was attributed to higher diffusion rates of the dye in the fiber. They also observed that low-molecular-weight dyes faded faster than high-molecular-weight dyes, again suggesting the dye mobility within the fiber (rate of diffusion of the dye molecules to the surface of fiber) played a significant role in the fading process. Cross-sectional analysis of the exposed fibers showed that most of the dye loss appeared to occur due to reactions at the fiber surface, and that penetration of O_3 into the fiber did not seem to be significant.

Salvin (1969) reported that O_3 and, to a lesser extent, NO_2 caused dye fading of cotton-polyester/permanent-press fabrics. As summarized by Dorset (1972), O_3 was found to be the major fading agent, with NO_x also capable of causing fading, although to a lesser extent. Remedial measures to avoid this problem include selecting dyes more resistant to reaction with O_3 and NO_2, avoiding the use of magnesium chloride ($MgCl_2$) catalyst in the permanent-press process, and using different surfactants and softeners. The use of $MgCl_2$ as a catalyst makes O_3-sensitive dyes more sensitive to O_3 (Dorset, 1972). When the catalyst is zinc nitrate, dyes are more washfast and resistant to O_3 fading. The use of a zinc nitrate catalyst appears generally to have eliminated the problem of the prefading of dyes in permanent-press fabrics from O_3 exposure.

Much of the research reported on dye fading is qualitative in nature. Earlier studies relied on comparisons among various geographical locations and seasonal variations, with little attention given to actual concentration and exposure characterizations. For several

of the initial field investigations reported here, neither O_3 nor oxidant concentrations were given; rather, notations such as high versus low or urban versus rural were the only description of oxidant levels. The few laboratory studies employed, at most, only two concentrations of O_3, making it nearly impossible to derive meaningful exposure-response relationships. Comparisons among studies are difficult owing to the various dye and fabric combinations tested. Also, the importance of RH on O_3 fading rate confounds comparisons among many of the studies that did not use the same RH percentages. Despite these shortcomings, the current body of research clearly demonstrates a strong relationship between dye fading and O_3 exposure. A definitive study to develop exposure-response functions that covers a broad spectrum of fabric/dye combinations, O_3 exposures, humidities, and temperatures has not been undertaken, although the available literature establishes the likely significant variables for such a study.

5.10.3.3 Fiber Damage

Sunlight, heat, alternate wetting and drying, and microorganisms are causative factors in the weathering and deterioration of fabrics exposed outdoors. The influence of O_3 at normal ambient levels is generally small by comparison. Table 5-44 summarizes the experiments of the effects of O_3 on textile fibers.

Bogaty et al. (1952), as part of a program aimed at segregating some of the elements that cause weathering, carried out experiments to study the possible role of O_3 in the deterioration of cotton textiles. These investigators exposed samples of duck and print cloth to air containing 0.02 and 0.06 ppm of O_3. Samples were exposed both dry and wet and tested for 50 days. The wet samples were water-saturated once per week, and moisture was added regularly so that the moisture content of the cloth was never less than 50%. Similar fabric samples were exposed to similar O_3 concentrations with no moisture added, and another control group was wetted similarly but exposed to clean (O_3-free) air. After exposure to O_3, the wetted samples showed a loss in breaking strength of approximately 20%. The wet print control cloth showed a loss in breaking strength of only half this amount. The study showed that low levels of O_3 degrade cotton fabrics if they are sufficiently moist. Bogaty and co-workers surmised that an estimated 500 to 600 days of natural exposure might be required to reach a stage of degradation similar to that caused by a 50-day exposure to O_3 alone. Because unprotected fabrics typically reach a much more advanced state of decay after such long exposures to weathering, Bogaty and co-workers concluded that the effect of O_3 is slighter than that of other agents. Although not noted by Bogaty and co-workers, the O_3 and increased moisture may have caused the formation of H_2O_2, which could account for the loss in breaking strength.

Morris (1966) also studied the effects of O_3 on cotton. Samples were exposed in the absence of light to 0.5 ppm of O_3 (more than four times the NAAQS of 0.12 ppm) for 50 days in a chamber maintained at 70 °F (21 °C) and 72% RH. No appreciable effect on breaking strength was found. Apparently, the moisture content of the cotton was not high enough to produce the degradation that Bogaty et al. (1952) measured in wet cotton samples, even though the concentration of O_3 was considerably higher.

The laboratory study of Kerr et al. (1969) examined the effects of the periodic washing of dyed cotton fabrics exposed to O_3 and the amount of fading and degradation of moist, dyed fabrics exposed to O_3. They exposed samples of print cloth, dyed with C.I. Vat Blue 29, in a chamber to a continuous supply of purified air containing O_3 concentration levels of 1 ± 0.1 ppm. The samples were exposed at room temperature (25 °C) in the

Table 5-44. Laboratory and Field Studies of the Effects of Ozone on Fibers[a]

Fiber	Concentration (ppm)	Exposure	Environmental Variables	Effects	References
Cotton	0.02 and 0.06	50 days	Cloth, both wet and dry	O_3-exposed wetted samples had 20% loss of breaking strength.	Bogaty et al. (1952)
Cotton	0.5	50 days	21 °C, 72% RH	No loss of breaking strength.	Morris (1966)
Cotton	1.0	60 days	25 °C, periodic washing or wetting	Washed O_3-exposed fabrics had 18% loss of breaking strength.	Kerr et al. (1969)
Modacrylic, Acrylic, Nylon 66, Polyester	0.2	7 days	48 °C, 39% RH, atificial sunlight, wetting	No effect on modacrylic and polyester. Slightly reduced breaking strength in acrylic and nylon.	Zeronian et al. (1971)
Nylon	0.03	Up to 445 days	Exposed in industrial warehouse	Loss of dyeability.	Makansi (1986)

[a]See Appendix A for abbreviations and acronyms.

absence of light, and a shallow container of water was kept on the chamber floor to increase the humidity. Samples were withdrawn from the chamber after 12, 24, 36, 48, and 60 days. After an exposure period of 60 days, which included either 20 washing or 20 soaking treatments, the change in strength of control fabrics was not significant. By comparison, the fabrics exposed to O_3 changed significantly; the loss in strength of the washed fabrics was 18%, and that of the soaked fabrics, 9%. Fading was also evident in the fabrics exposed to O_3 but not in the control samples. Differences in the amount of fading between the washed and soaked samples were evident, but the reason for the differences was not. Kerr et al. concluded that washing in hot, soapy water may have affected properties of the dye.

In laboratory studies, Zeronian et al. (1971) simultaneously exposed modacrylic (dynel), acrylic (orlon), Nylon 66, and polyester (dacron) fabrics to artificial sunlight (xenon arc) and CF air contaminated with 0.2 ppm of O_3 at 48 °C (118 °F) and 39% RH. During exposure, the fabric samples were sprayed with water for 18 min every 2 h. Ozone damage was measured by comparing these samples with fabrics exposed to the same environmental conditions without O_3. After exposure for 7 days, Zeronian and co-workers found that O_3 did not affect the modacrylic and polyester fibers. The exposure did seem to affect the acrylic and nylon fibers slightly by reducing breaking strength. The degree of difference, however, in the change of fabric properties between those exposed to light and air and those exposed to light and air containing 0.2 ppm of O_3 was not significant.

Ageing of nylon yarns causes a reduction in the dyeability of the yarn. Ageing is caused by the reaction of amine end groups in the filament skin with O_3 and other pollutants (NO_x, SO_2, etc.). This phenomenon is well known within the textile trade, and procedures such as minimizing time from yarn production to yarn dyeing are in place to reduce problems of ageing. Makansi (1986) investigated the relationship between yarn ageing, as defined by reduction in dyeability, and pollutant levels in yarn storage warehouses. Makansi assessed the yarn dyeability with Acid Blue 45 and Acid Blue 122 dyes of exposed test fiber versus unexposed control samples. Gaseous pollutant concentrations in the warehouse were estimated either using nearby air quality station data or measured twice weekly during the tests with commercial sampling tubes (Draeger™ Tubes). Yarn samples were exposed for up to 1 year of ageing. Makansi found that dyeability decreased proportionally with the O_3 exposure during storage. Dyeability, as weight of dye absorbed for Acid Blue 45, decreased over 75% for Nylon 66 stored in the warehouse at an average concentration of 0.03 ppm O_3. It was not possible to statistically isolate the effects of O_3 exposure from other pollutant exposures for the samples in these tests; thus other factors besides O_3 may have contributed to the loss in dyeability. Makansi suggested that yarns should be dyed as quickly as possible after manufacture or should be stored in airtight wrappings to prevent ageing.

In general, the contribution of O_3 to degradation of fabrics has not been quantified well. Bogaty et al. (1952) concluded that the effects of other factors (sunlight, heat, wetting and drying, and microorganisms) far outweighed the effects of O_3 on cotton duck and print cloth. The work by Morris (1966) and Kerr et al. (1969) does point to the synergistic effect of moisture and O_3 as an important ingredient in material degradation, possibly caused by the formation of a more potent oxidizing agent. Finally, the work of Zeronian et al. (1971) also indicates little if any effect of O_3 on synthetic fibers. Thus, it appears that O_3 has little if any effect on textiles, fibers, and synthetic cloth exposed outdoors. Because most fabrics are used primarily indoors, where they are partially shielded from O_3 exposure, O_3 damage to textile fibers is considered an insignificant problem. This was a finding of Murray et al.

(1986) in a study of material damage and costs in the Los Angeles area, an area with relatively high ambient O_3 concentrations.

5.10.3.4 Paint Damage

A paint surface may suffer several types of damage (including cracking, peeling, erosion, and discoloration) that affect its usefulness. Of these, erosion (i.e., wearing away of the paint surface) is the type of damage most often studied with respect to the impact of gaseous pollutants on architectural and coil-coating finishes. (Coil coatings are industrial, continuous-dip process finishes typically applied to sheet metal.) Studies of paint cracking and peeling have focused on the effects of moisture and have not dealt with the possible influence of ambient pollutants on these types of finishes.

Several damage functions for O_3-induced erosion of paint have been reported in the literature. Such reports are based either on accelerated chamber studies or on long-term outdoor exposure studies. Unfortunately, all studies to date have shortcomings that render their results questionable in regard to actual exposures. Damage to a paint surface is the cumulative effect of the conditions to which the surface is exposed, including various combinations of temperature, moisture, sunlight, and pollution level. No exposure study to date has been able to match all factors exactly to separate the impact of O_3 from the other factors. Table 5-45 summarizes the studies of the effect of O_3 on architectural and industrial paint and coating systems.

In a laboratory chamber exposure study, Haynie et al. (1976) exposed oil-based house paint, latex house paint, vinyl coil coating, and acrylic coil coating to 0.5- and 0.05-ppm concentrations of SO_2, NO_2, and O_3 in various combinations. Statistically significant effects of O_3-caused damage were observed on the vinyl and the acrylic coil coatings: a positive interaction between O_3 and RH on the vinyl coil coating and a positive direct O_3 effect on the erosion rate of the acrylic coil coating. The rate of erosion was low, however, and both vinyl and acrylic coil coatings were shown to be very durable. A linear regression for the acrylic coil coating data gives

$$\text{Erosion rate} = 0.159 + 0.000714\ O_3, \qquad (5\text{-}2)$$

where erosion rate is in micrometers per year and O_3 is in micrograms per cubic meter.

Although the O_3 effect on this coating was found to be statistically significant, it has no practical significance because the erosion rate is so slow; at 0.12 ppm of O_3, the erosion rate is 0.33 μm/year. At an average annual O_3 level of 0.05 ppm, this regression predicts that a 20-μm-thick coating would last over 80 years.

In a comprehensive study by Campbell et al. (1974), panels painted with different exterior paints (automotive refinish, latex coating, coil coating, industrial maintenance coating, and oil-based house paint) were exposed to air pollutants in an environmental chamber under accelerated weathering conditions. The panels were exposed to low (0.1 ppm) and high (1.0 ppm) concentrations of O_3 and SO_2. After exposure, the panels were examined by measuring erosion, gloss, surface roughness, tensile strength, attenuated total reflectance (ATR), and the surface effects that were revealed by scanning electron microscopy and infrared examination. The panels were examined after 0, 400, 700, and 1,000 h of chamber exposure (considered as equivalent to 0, 200, 350, and 500 days of exposure, respectively).

Table 5-45. Laboratory and Field Studies of the Effects of Ozone on Architectural/Industrial Paints and Coatings[a]

Paint/Coating Type	Substrate	Concentration (ppm)	Exposure	Environmental Variables	Effects	Comments	Reference
Latex house paint Oil house paint Vinyl coil coating Acrylic coil coating	Aluminum panels	0.05 and 0.5	To 1,000 h	Chamber exposures with SO_2, NO_2, and O_3; 50 and 90% RH; 13 and 35 °C; and artificial dew and sunlight cycles.	Very slow erosion of coil coatings.		Haynie et al. (1976)
Automotive refinish Latex Coil coating Industrial maintenance coating Oil house paint	Stainless steel panels	0.1 and 1.0	To 1,000 h	Chamber study with SO_2, 70 to 100% RH, 50 to 65 °C, and artificial dew and sunlight cycles.	Although 1 ppm O_3 produced significant changes in finishes, 0.1 ppm O_3 did not produce statistically increased erosion.		Campbell et al. (1974)
			To 24 mo	Field studies in four sites, rural to industrial.	Erosion greater in urban areas.	No environmental measurements conducted.	
Latex house paint Oil house paint	Stainless steel	0.006 to 0.055	3 to 30 mo	Field study.	Effects of O_3 not independently statistically significant.	Nine sites around St. Louis.	Mansfeld (1980)

[a]See Appendix A for abbreviations and acronyms.

In general, exposures to 1 ppm of O_3 produced greater increases in erosion rates than did clean air. Concentrations of this magnitude, however, do not represent typical ambient exposure levels of O_3. At the more representative level of 0.1 ppm, O_3 did not produce statistically significant increases in erosion rates. The various finishes produced a variety of changes for the other measures. Some finishes lost gloss or showed changes in ATR, but O_3 exposure did not produce consistent changes over the suite of finishes examined.

In conjunction with Campbell's chamber studies, field measurements were made of the erosion of paint from test panels exposed to outdoor environments consisting of a clean, rural atmosphere (Leeds, ND); a moderately polluted atmosphere (Valparaiso, IN); a heavily polluted (SO_2) atmosphere (Chicago); and a high-oxidant, moderately polluted atmosphere (Los Angeles). The results of this study showed that paint erosion was much greater in the polluted areas than in relatively clean, rural areas. The highest erosion rates were observed for the coil coating and oil-based house paints at the Chicago and Los Angeles exposure sites. Because meteorology and air quality were not measured at the exposure sites, correlation of film damage with the environmental parameters was not possible. The study does suggest that SO_2 exerts an adverse effect on exterior paints with calcium carbonate as an extender pigment. The coil coating and oil house paints were formulated with calcium carbonate. Oxidants were probably reacting with the organic binder of the coil coating and oil house paints, although no mechanism for this reaction was developed from this exposure study.

In an outdoor exposure test of the effects of air pollutants on materials, Mansfeld (1980) exposed latex and oil-based house paints, as well as galvanized steel, weathering steel, stressed aluminum, silver, marble, and nylon, at nine test sites in St. Louis. In conjunction with the material exposures, measurements of meteorological parameters, O_3, NO_x, total hydrocarbons, total sulfur, SO_2, and hydrogen sulfide were made.

Haynie and Spence (1984) analyzed Mansfeld's (1980) St. Louis data, accounting for covariances among the pollutant and meteorological variables. They analyzed the paint damage data and found significant correlations of O_3 flux with time, temperature, and NO_2 flux for the experimental period. Although Haynie and Spence expected O_3 to attack the binder in latex paint, multiple regression analysis showed little dependence of paint erosion on O_3 flux. They speculate that the effects of O_3 are masked by the covariance of O_3 with temperature and NO_x.

5.10.3.5 Cultural Properties Damage

Ozone-induced degradation of cultural properties (e.g., fine arts paintings) contributes to the deterioration and, ultimately, to the loss of these unique objects. Many cultural properties are expected to last indefinitely, and irreversible damage, even at a slow rate, is considered unacceptable by curators and the art community.

A significant series of tests of the effects of O_3 on a variety of artist's pigments and dyes was reported by Shaver et al. (1983), Grosjean et al. (1987), Whitmore et al. (1987), Grosjean et al. (1988a,b), Whitmore and Cass (1988), Grosjean et al. (1989), Cass et al. (1991), and Grosjean et al. (1993). The experiments are summarized in Table 5-46. The doses of O_3 applied during these tests were the equivalent of less than 10 years exposure in a typical air conditioned indoor environment. Many pigments, notably traditional organic pigments such as indigo, were found to be very sensitive to O_3 exposure. Many of the affected pigments underwent significant color changes on exposure to O_3, and some were

Table 5-46. Laboratory Studies of the Effects of Ozone on Artists' Pigments and Dyes[a]

Pigment Types	Substrate	Concentration	Exposure	Environmental Variables	Effects	Comments	Reference
17 Artists' watercolor pigments	Paper	0.4 ppm	95 days	23 °C 47% RH	Alizarin-based watercolors were very sensitive; other pigments showed lesser degrees of fading.	Also investigated fading on Japanese wood-block print.	Shaver et al. (1983)
Alizarin, Alizarin crimson, anthraquinone	Silica gel cellulose — Teflon	0.4 ppm 0.4 ppm — 10 ppm	95 days 95 days — 18-80 h	22 °C 50% RH — 24 °C ≤40% RH	Each pigment tested faded on all substrates.	Presented possible reaction mechanisms and products.	Grosjean et al. (1987)
16 Traditional organic colorants	Paper	0.4 ppm	12 weeks	23 °C 50% RH	Eleven colorants were reactive with O_3, three were possibly reactive.		Whitmore et al. (1987)
Indigo, dibromoindigo, thioindigo, tetrachlorothio-indigo	Teflon	10 ppm	4 days	24 °C 5% RH	All indigo, dibromoindigo consumed. Thioindigo and tetrachlorothioin-digo were much less reactive and still retained much color.	Presented possible reaction mechanisms.	Grosjean et al. (1988a)
Curcumin	Cellulose, watercolor Paper, silica gel — Teflon	0.4 ppm 0.4 ppm — 10 ppm	95 days 95 days — 4 days	25 °C 50% RH — 24 °C ≤20% RH	Faded rapidly on all substrates, producing colorless products.	Somewhat slower fading on watercolor paper.	Grosjean et al. (1988b)

Table 5-46 (cont'd). Laboratory Studies of the Effects of Ozone on Artists' Pigments and Dyes[a]

Pigment Types	Substrate	Concentration	Exposure	Environmental Variables	Effects	Comments	Reference
Traditional Japanese colorants and dyes	Paper, silk cloth	0.4 ppm	12 weeks	22 °C 50% RH	Several organic and one inorganic pigment faded significantly.	Also investigated fading on ca. 1810 Japanese woodblock print.	Whitmore and Cass (1988)
Triphenylmethane colorants	Teflon	10 ppm	4 days	24 °C ≤20% RH	Found that, although some are not affected, those colorants with unsaturated C-C bonds may fade.	Presented possible reaction mechanisms.	Grosjean et al. (1989)
Alizarin crimson	Watercolor paper	0.4 ppm	7 days	22 °C 50% RH	Severe fading.	Framed sample behind glass exhibited virtually no fading.	Cass et al. (1991)
Various artists' colorants	Watercolor paper Cellulose	Mixture 0.2 ppm O_3 0.01 ppm PAN 0.08 ppm NO_2	12 weeks	16 to 26 °C 46 to 83% RH	11 colorants, negligible changes; 12 colorants, small changes; 3 colorants, modest changes; 9 colorants, substantial changes.		Grosjean et al. (1993)

[a]See Appendix A for abbreviations and acronyms.

virtually completely consumed, producing colorless reaction products. Cass et al. (1991) noted that O_3 damage to artwork is proportional to the O_3 exposure ($C \times T$). Because artworks are intended to have long service lives and their appearance is important, fading is generally considered to be unacceptable, and even low concentrations for long periods of time can lead to noticeable fading. Grosjean et al. (1987) suggest that formulations of substitute pigments be developed with O_3 sensitivity in mind.

Druzik et al. (1990) investigated the indoor/outdoor O_3 concentration ratios at 11 museums, art galleries, and historical houses in the Los Angeles area. They found that the indoor/outdoor ratio of 8-h average O_3 concentrations ranged from 0.10 to 0.87. The ratio was strongly dependent on the type of building ventilation. Buildings with high air-exchange rates (about two to three air changes per hour) had the highest indoor/outdoor ratios. Low exchange rate buildings (ca. less than one air change per hour) and buildings with air conditioning systems had significantly lower indoor/outdoor O_3 concentration ratios.

De Santis et al. (1992) investigated concentrations of SO_2, HNO_3, HNO_2, and O_3 as well as particulate sulfate, nitrate, and ammonium in the Galleria degli Uffizi in Florence for a 5-day period. Although the museum was equipped with an air conditioning system, O_3 concentrations in the galleries correlated strongly with outdoor O_3 concentrations. Indoor hourly average O_3 concentrations ranged from 0.019 to 0.030 ppm. To reduce concentrations in the galleries, they suggested that the Uffizi's air handling system be upgraded to include filtration and modified to include less make-up air. Cass et al. (1991) and Grosjean et al. (1993) suggest that museums design and maintain air conditioning and air filtration systems to control the concentrations of oxidants in order to protect their collections. Cass et al. (1991) note that framing behind glass is an effective means of protecting oxidant sensitive pigments. Grosjean and Parmar (1991) found that activated carbon and Purafil (4% potassium permanganate on neutral activated alumina) could be used to reduce O_3 and oxidant concentrations in museum display cases.

5.10.4 Economics

5.10.4.1 Introduction

Damage to materials from O_3 usually is expressed in terms of one or both of the following two general classes of costs to producers and consumers: (1) O_3-accelerated replacement and repair costs, as when the service life or aesthetics of a material are impaired, and (2) increased avoidance costs, as when certain industries (e.g., tires, plastics, paints, dyes, and fabrics) are obligated to incur expenditures for antiozonant research and development, substitute processes and materials, additives and formulations, product packaging, advertising, etc., in order to offset sales losses that otherwise would occur.

In theory, the approach selected should depend on the observed behavior of the producers and consumers of the materials in question, and the type of damage to which they are reacting. In practice, the empirical estimates of O_3 damage to materials are far from reliable.

5.10.4.2 Methods of Cost Classification and Estimation

Computation of accelerated replacement is probably the most widely applied method of estimating the costs of materials damage to air pollutants. In this approach, a materials damage function is developed to show the increase in physical damage for an increase in the dose of the pollutant. Then a cost schedule is constructed to show how

maintenance or replacement schedules are influenced by the pollutant level. Hershaft et al. (1978) note, however, that this method usually assumes existing inventories and does not take into account substitutions of materials with more (or less) resistance to pollution. As a result, this method tends to overestimate the cost of damage from pollutant increases and to underestimate the net savings realized from pollutant reductions.

A second approach considers avoidance costs. This refers to practices such as adopting alternative production processes and materials. Some industries add antiozonants to their products or change the chemical formulation of their output. All of these measures mitigate the impact of O_3 on the service life or aesthetics of the products in question. Moreover, these measures also require research, development, and implementation expenditures. As such, estimation of these costs is conceptually and empirically difficult, since the opportunity to use different materials changes in response to the level of O_3 concentration.

A number of factors complicate the use of both the replacement and the avoidance methodologies. Data on key variables generally are missing or merely assumed. Lessening the reliability of the final cost estimates are deficiencies in knowledge of the physical damage functions; the quantities and types of materials exposed to O_3 indoors, outdoors, and in respective regions of the country; the actual expenditures incurred for increased replacement, maintenance, and avoidance that can be directly attributed to O_3; the threshold O_3 damage levels that prompt mitigating action; and the range of substitution strategies that can be used to ameliorate degradation. On the last point, few attempts have been made to identify current technology practices and potential innovations. The variety of rubber compounds, paint mixtures, and fabric dyes reflects the number of proprietary formulations, and each formulation presumably has a different response to O_3 exposure.

An additional complication is that repair, replacement, and substitution are frequently dominated by factors unrelated to O_3 concentrations. This can lead to spurious correlations if studies are accepted uncritically. For example, tire replacement may be high in a given region of the country because of high O_3 levels associated with automotive exhaust. Alternatively, tire replacement may be high simply because the total miles of automotive use per year are higher in that region than in the nation as a whole.

5.10.4.3 Aggregate Cost Estimates

The important caveats identified in the preceding discussion qualify the empirical data presented in this and following sections. Table 5-47 summarizes reports of highly aggregated estimates of oxidant damage of all materials. Unfortunately, there are no known recognized studies that are more recent than those reported in the table. For purposes of gross comparison only, where possible, the figures are expressed in 1984 currency equivalents along with 1970 currency equivalents, the base data for most of the reference studies. The figures do *not*, however, represent 1984 supply-demand relationships, production technologies, or O_3 concentrations. It must be emphasized that the costs cited in 1984 currency equivalents therefore cannot be considered true 1984 costs. Because the data in Table 5-47 are reported to four significant figures, the accuracy of this information is exaggerated.

Salmon (1970) was among the first to attempt to estimate the annual cost of air pollution damage to materials. His computation included the dollar value of annual materials production, a weighted average economic life of each material included in his study, a weighted average factor for the percentage of the material exposed to air pollution, and a

Table 5-47. Summary of Damage Costs to Materials by Oxidants (in millions of 1970 and 1984 dollars)[a]

Study	Materials Costs		
	Elastomers/Plastics	Fabric/Dye	All
Barrett and Waddell (1973)	ND	(260)	(3,878)
Mueller and Stickney (1970)	500.0 (1,500)	ND	ND
Salmon (1970)	295.2 (915)	358.4 (1,111)	653.6 (2,026)
Salvin (1970)	ND	83.5 (259)	ND
Waddell (1974)	ND	ND	900.0 (2,790)
Yocom and Grappone (1976)	ND	ND	572.0 (1,773)
Freeman (1979)	ND	ND	505.0 (1,566)

[a]ND = No data; investigator(s) did not develop estimates in this category. 1984 dollars are listed parenthetically.

factor for increased labor to treat damaged materials. Cost was defined as the value of the material multiplied by the difference between the rate of material deterioration in a polluted urban versus an unpolluted rural environment. All data, except for annual production levels of materials, were assumed.

If it is assumed that O_3 affected all of the fibers, plastics, and rubber in the study by Salmon, then annual damage costs attributed to O_3 would have been $2.026 billion (1984$). Salmon did not consider O_3-related damage to paint, since the dominant paint-damaging mechanisms are soiling and gaseous SO_2. His costs refer to maintenance and replacement only, and do not allow for materials protection, substitution, etc.

In discussing other limitations of his study, Salmon cautioned that his estimates were of potential loss, not of actual observed loss. Despite this and other qualifications that lessen the usefulness of the figures derived, the Salmon study has been cited extensively and used quantitatively in a number of the subsequent studies cited here.

For example, the materials estimate by Barrett and Waddell (1973) is based primarily on the work of Salmon (1970). Barrett and Waddell supplemented this by drawing on Mueller and Stickney (1970) for damage costs on elastomers and on Salvin (1970) for damage costs related to dye fading. Combining some of these numbers, Barrett and Waddell stated that materials damage costs attributable to oxidants alone were $3.878 billion (1984$).

Freeman (1979) reviewed earlier studies that categorized the cost of damage to materials. Using the work of Waddell (1974) and Salvin (1970), Freeman calculated that the materials damage costs attributable to oxidants and NO_x were $2.031 billion (1984$). Of this total, roughly 46% was damage to textiles and dyes (from Salvin, 1970), whereas the remaining 54% was damage to elastomers (from Mueller and Stickney, 1970). Freeman then assumed a 20% reduction in oxidant levels since 1970 and concluded that the monetary benefits of controlling oxidants, oxidant precursors, and NO_x were between $170 and $510 million (1984$). Freeman computed that the savings attributable to oxidant controls alone were $128 to $383 million (1984$).

Waddell (1974) likewise depended primarily on existing studies to calculate the national cost of air pollution in 1970. Waddell used Salmon (1970), Salvin (1970), Mueller and Stickney (1970), and Spence and Haynie (1972) to derive an estimate of $6.820 billion (1984$) as the total gross annual damage for materials losses in 1970 resulting from air pollution. The component attributable to O_3 and oxidants alone was $2.790 billion (1984$), within a wide range of $1.550 to $4.030 billion (1984$).

Yocom and Grappone (1976), in work for the Electric Power Research Institute, estimated that the cost of air pollution damage to materials was about $6.820 billion (1984$) in 1970. Of this total, O_3 was estimated to be responsible for $1.773 billion (1984$), or some 26% of the total.

Because of the reliance of the later studies on the questionable data and unverified assumptions contained in the earlier ones, the results compared here are of extremely limited usefulness for cost-benefit purposes. Updated research, using current economic evaluation approaches, should be undertaken to determine the costs of O_3-induced damage.

5.10.5 Summary and Conclusions

More than four decades of research show that O_3 damages certain materials. The materials most studied in O_3 research are elastomers and textile fibers and dyes. The amount of damage to actual in-use materials and the economic consequences of that damage are poorly characterized.

Natural rubber and synthetic polymers of butadiene, isoprene, and styrene, used in products like automobile tires and protective outdoor electrical coverings, account for most of the elastomer production in the United States. The action of O_3 on these compounds is well known, and dose-response relationships have been established and corroborated by several studies. These relationships, however, must be correlated with adequate exposure information based on product use. For these and other economically important materials, protective measures have been formulated to reduce the rate of oxidative damage. When antioxidants and other protective measures are incorporated in elastomer production, the dose rate for cracking is reduced considerably, although the extent of reduction differs widely according to the material and the type and number of protective measures used.

The effects of O_3 on dyes have been known for nearly four decades. In 1955, Salvin and Walker exposed certain red and blue anthraquinone dyes to a 0.1-ppm concentration of O_3 and noted fading, which until that time was thought to be caused by NO_2. Subsequent work confirmed the fading action of O_3 and the importance of RH in the absorption and reaction of O_3 in vulnerable dyes. Both the type of dye and the material in which it is incorporated are important factors in resistance of a fabric to O_3. Researchers found no effects from O_3 on royal blue rayon-acetate, red rayon-acetate, or plum cotton.

On the other hand, anthraquinone dyes on nylon fibers were sensitive to fading from O_3. Field studies and laboratory work showed a positive association between O_3 levels and dye fading of nylon materials. At present, the available research is insufficient to quantify the amount of damaged material attributable to O_3 alone.

The degradation of fibers from exposure to O_3 is poorly characterized. In general, most synthetic fibers like modacrylic and polyester are relatively resistant, whereas cotton, nylon, and acrylic fibers have greater but varying sensitivities to the gas. Ozone reduces the breaking strength of these fibers, and the degree of reduction depends on the amount of moisture present. The limited research in this area indicates that O_3 in ambient air may have a minimal effect on textile fibers, but additional research is needed to verify this conclusion.

The effects of O_3 on paint are small in comparison with those of other factors. Past studies have shown that, of various architectural and commercial paints, only vinyl and acrylic coil coatings are affected, and that this impact has a negligible effect on the useful life of the material coated. Preliminary results of current studies have indicated a statistically significant effect of O_3 and RH on latex house paint, but the final results of those studies are needed before conclusions can be drawn.

A number of artists' pigments and dyes have been found to be sensitive to O_3 and other oxidants. Many organic pigments in particular are subject to fading or other color changes when exposed to O_3. Although most, but not all, modern fine arts paints are O_3 resistant, many older works of art are at risk of permanent damage because of O_3-induced fading. Museums and private collectors should take steps to ensure that susceptible artwork is protected from O_3 exposure.

For a number of important reasons, the estimates of economic damage to materials are problematic. Most of the available studies are outdated in that the O_3 concentrations, technologies, and supply-demand relationships that prevailed when the studies were conducted are no longer relevant. Additionally, little was (and is) known about the physical damage functions, and cost estimates were simplified to the point of not properly recognizing many of the scientific complexities of the impact of O_3. Assumptions about exposure to O_3 generally ignored the difference between outdoor and indoor concentrations. Also, analysts have had difficulty separating O_3 damage from other factors affecting materials maintenance and replacement schedules. For the most part, the studies of economic cost have not had the resources to marshal factual observations on how materials manufacturers have altered their technologies, materials, and methods in response to O_3. Rather, the analysts have had to rely on assumptions in this regard, most of which remain unverified.

It is apparent that a great deal of work remains to be done in developing quantitative estimates of materials damage from photochemical oxidant exposures. This is not meant to deprecate the years of research reported in this document, for much has been gained in refining the initial methodologies used for assessing damage. The current state of knowledge still can be summarized by the following from Yocom et al. (1985):

> "We have learned that some costs may be difficult to quantify either because they are minimal or because they are overshadowed by other factors, such as wear or obsolescence. We have learned that damage functions are complex and are influenced by the presence of other pollutants and by weather. We have learned that more accurate estimates of materials in place may be obtained using selective

sampling and extrapolation. And we have learned that a mere cost-accounting of damage does not present a true estimate of economic cost if it does not account for the welfare effects induced by shifts in the supply-demand relationship."

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Appendix A

Abbreviations and Acronyms

AA	Ambient air
AATCC	American Association of Textile Chemists and Colorists
ABA	Abscisic acid
ADP	Adenosine diphosphate
AH_2	Ascorbic acid
AIRS	Aerometric Information Retrieval System
ATP	Adenosine triphosphate
ATR	Attenuated total reflectance
C	Concentration
CEC	Controlled environmental chamber
CF	Charcoal or carbon filtered
CO_2	Carbon dioxide
CSTR	Continuous stirred tank reactor
cv.	Cultivar
CVM	Contingent valuation method
dbh	Diameter at breast height
DLA	Damaged-leaf area
DNA	Deoxyribonucleic acid
EDU	Ethylene diurea
EPA	U.S. Environmental Protection Agency
GSH	Glutathione
2HDM	Second-highest daily maximum 1-h concentration
HF	Hydrogen fluoride
HNO_2	Nitrous acid
HNO_3	Nitric acid
H_2O_2	Hydrogen peroxide
IR	Isoprene rubber
K	Mass transfer coefficient
ks	Mean diffusive conductance
M7	Seven-hour mean exposure

M12	Twelve-hour mean exposure
M24	Twenty-four-hour mean exposure
$MgCl_2$	Magnesium chloride
n	Number
N_2	Elementary nitrogen
NA	Not applicable
NAAQS	National Ambient Air Quality Standards
NAPAP	National Acid Precipitation Assessment Program
NCLAN	National Crop Loss Assessment Network
NF	Nonfiltered
NH_3	Ammonia
no.	Number
NO	Nitric oxide
NO_2	Nitrogen dioxide
N_2O_5	Nitrogen pentoxide
NO_x	Nitrogen oxides
NR	Natural rubber
O_3	Ozone
OTC	Open-top chamber
p	Probability
PAN	Peroxyacetyl nitrate
PANs	Peroxyacyl nitrates
PAR	Photosynthetically active radiation
pH	Hydrogen ion concentration
p.h.r.	Parts per hundred of rubber, by weight
PIPP	*N'*-phenyl-*N'*-isopropyl-*p*-phenylenediamine
Pn	Net photosynthesis
PRYL	Predicted relative yield loss
PST	Pacific Standard Time
RFL	Resorcinal-formaldehyde latex
RH	Relative humidity
ROPIS	Response of Plants to Interacting Stresses
SAR	Simulated acid rain
SAROAD	Storage and Retrieval of Aerometric Data (U.S. Environmental Protection Agency centralized database; superseded by Aerometric Information Retrieval System)

SD	Standard deviation
SIGMOID	An index using a sigmoidal weighting function
SMD	Soil moisture deficit
SO_2	Sulfur dioxide
SOD	Superoxide dismutase
SUM00	Sum of all hourly average concentrations
SUM06	Seasonal sum of all hourly average concentrations $\geq$0.06 ppm
SUM07	Seasonal sum of all hourly average concentrations $\geq$0.07 ppm
SUM08	Seasonal sum of all hourly average concentrations $\geq$0.08 ppm
SUM10	Seasonal sum of all hourly average concentrations $\geq$0.10 ppm
T	Time
TVMV	Tobacco vein mottling virus
UV	Ultraviolet
UV-B	Ultraviolet radiation of wavelengths from 280 to 320 nm
VPD	Vapor-pressure deficit
WTA	Willingness to accept
WTP	Willingness to pay
WUE	Water-use efficieny
W126	An index using a sigmoidal weighting function
ZAPS	Zonal Air Pollution System

Appendix B

Colloquial and Latin Names

Alder	*Alnus serrulata* (Aiton) Willdenow
Alder, red	*Alnus rubra* Bong.
Alder, speckled	*Alnus incana* (L.) Moench.
Alfalfa	*Medicago sativa* L.
Almond	*Prunus amygdalus* Batsch cv. Nonpariel
Apple	*Malus* spp.
Apricot	*Prunus armeniaca* L.
Ash, green	*Fraxinus pennsylvanica* Marsh.
Ash, white	*Fraxinus americana* L.
Aspen, trembling	*Populus tremuloides* L.
Avocado	*Persea americana* Mill.
Azalea	*Rhododendron* spp.
Barley, spring	*Hordeum vulgare* L.
Basswood (linden)	*Tilia americana* L.
Bean, broad	*Vicia faba* L.
Bean, bush	*Phaseolus vulgaris* L. var. *humulis* Alef.
Bean, kidney, pinto, snap, white	*Phaseolus vulgaris* L.
Beech, European	*Fagus sylvatica* L.
Beet, sugar	*Beta vulgaris* L.
Begonia	*Begonia* sp.
Begonia, bedding	*Begonia semperflorens* Link & Otto
Bentgrass	*Agrostis capillaris* L.
Birch, European white	*Betula pendula* Roth.
Birch, downy	*Betula pubescens* Ehrb.
Birch, paper	*Betula papyrifera* Marsh.
Blackberry, common	*Rubus allegheniensis* Porter
Black-gram	*Vigna mungo* L.
Bluegrass, Kentucky	*Poa praetensis* L.
Buckhorn	*Plantago lanceolata* L.

Cabbage	*Brassica oleracea capitata* L.
Campion, bladder	*Silene cucabalus* Wibel.
Campion, moss	*Silene acaulis* L.
Carnation	*Dianthus caryophyllus* L.
Cedar, incense	*Libocedrus decurrens* Torr. = *Calocedrus decurrens* [Torr.] Florin.
Cedar, western red	*Thuja plicata* Donn ex D. Don
Celery	*Apium graveolens* L. var. *dulce* Pers.
Chestnut, American	*Castenea dentata* (Marsh.) Borkh.
Cherry, black	*Prunus serotina* Ehrh.
Chickpea	*Cicer arietinum* L.
Clover, ladino, white	*Trifolium repens* L.
Clover, red	*Trifolium pratense* L.
Corn	*Zea mays* L.
Cotton	*Gossypium hirsutum* L.
Cottonwood (poplar)	*Populus deltoides* Marsh
Cress, garden	*Lepidium sativum* L.
Cucumber	*Cucumis sativus* L.
Dock	*Rumex obtusifolius* L.
Fenugreek	*Trigonella foenum-graecum* L.
Fescue, tall	*Festuca elatior* L. = *Festuca praetensis* Huds.
Fir, balsam	*Abies balsamea* (L.) Mill.
Fir, Douglas	*Pseudotsuga menziesii* (Mirb.) Franco.
Fir, Douglas, big-cone	*Pseudotsuga macrocarpa* (Vasey) Mayr
Fir, Fraser	*Abies balsamea* (L.) *fraseri* (Pursh) Poir.
Fir, silver	*Abies alba* Mill.
Fir, white	*Abies concolor* Lindl.
Geranium	*Pelargonium* x *hortorum* Bailey
Golden-rain	*Koelreuteria paniculata* Laxm.
Grape	*Vitis labruscana* Bailey
Grape, wild	*Vitis* spp.
Grapefruit, Ruby Red	*Citrus paradisi* L.
Grass, colonial bent	*Agrostis tenuis* Sibthorp.
Grass, orchard	*Dactylis glomerata* L.
Grass, red	*Festuca rubra* Gaud.
Grass, rye	*Lolium perenne* L.

Gum, sweet	*Liquidambar styraciflua* L.
Hemlock, eastern	*Tsuga canadensis* (L.) Carr.
Hemlock, western	*Tsuga heterophylla* (Raf.) Sarg.
Ivy	*Hedera helix* L.
Kenaf	*Hibiscus cannabinus* L.
Juniper, shore	*Juniperus conferta* Parl.
Lemon, Volkamer	*Citrus volkameriana* Ten. & Pasq
Lettuce	*Lactuca sativa* L.
Lichen	*Lobaria* spp.
Lichen, parmelia	*Flavoparmelia caperata*
Lichen, umbilical	*Umbilicaria mammulata*
Lilac	*Syringa vulgaris* L.
Locust, black	*Robinia pseudoacacia* L.
Locust, honey	*Gleditsia triacanthos* L.
Lupine	*Lupinus bicolor* Lindl.
Mangel	*Beta vulgaris* L.
Maple, red	*Acer rubrum* L.
Maple, sugar	*Acer saccharum* Marsh
Milkweed	*Asclepias syriaca* L.
Milkweed	*Asclepias* sp.
Mint	*Mentha piperita* L.
Oak, California black	*Quercus kelloggii* Newb.
Oak, Canyon live	*Quercus chrysolepis* Liebm.
Oak, red	*Quercus rubra* L.
Oak, white	*Quercus alba* L.
Oats	*Avena sativa* L.
Onion	*Allium cepa* L.
Orange	*Citrus sinensis* (L.) Osbeck
Pea	*Pisum sativum* L.
Peach	*Prunus persica* (L.) Batsch cv. Halford
Pepper	*Capsicum annuum* L.
Pear	*Pyrus pyrifolia* Rhd. cv. 20th Century
Petunia	*Petunia hybrida* Vilm.
Pine, eastern white	*Pinus strobus* L.
Pine, Coulter	*Pinus coulteri* D. Don
Pine, Jeffrey	*Pinus jeffreyi* Grev. & Balf.

Pine, loblolly	*Pinus taeda* L.
Pine, pitch	*Pinus rigida* Mill.
Pine, ponderosa	*Pinus ponderosa* Laws.
Pine, Scots	*Pinus sylvestris* L.
Pine, shortleaf	*Pinus echinata* Mill.
Pine, Sierra lodgepole	*Pinus contorta* var. *murrayana* (Grev. & Balf.) Engelm.
Pine, slash	*Pinus elliotti* Englem. ex Vasey
Pine, sugar	*Pinus lambertiana* Dougl.
Pine, Table Mountain	*Pinus pungens* Lamb.
Pine, Virginia	*Pinus virginiana* Mill.
Plane, London	*Platanus* x *acerifolia* (Ait.) Willd.
Plantain, (plantago) common	*Plantago major* L.
Plum	*Prunus domestica* L.
Poinsettia	*Euphorbia pulcherrima* Willd.
Poplar, hybrid	*Populus maximowiczii* x *P. trichocarpa*
Poplar, yellow or tulip	*Liriodendron tulipifera* L.
Potato	*Solanum tuberosum* L.
Radish	*Raphanus sativus* L.
Radish	*Raphanus sativus* L. cv. Cherry Bell
Rape, spring	*Brassica napus* L. var. *napus*
Rhododendron, azalea	*Rhododendron obtusum* (Lindl.) Planch.
Rice, domestic	*Oryza sativa* L.
Sassafras	*Sassafras albidum* [Nutt.] Nees
Sequoia, giant	*Sequoiadendron giganteum* Buchholz
Sorghum, hybrid	*Sorghum bicolor* (L.) Moench x *Sorghum* x *drummondii* (Steudel) Millsp. & Chase
Soybean	*Glycine max* (L.) Merr.
Spinach	*Spinacea oleracea* L.
Spruce, Norway	*Picea abies* (L.) Karst.
Spruce, red	*Picea rubens* Sarg.
Spruce, sitka	*Picea sitchensis* (Bong.) Carr.
Strawberry, cultivated	*Fragaria* x *ananassa* Duch.
Strawberry, wild	*Fragaria virginiana* Duch.
Sunflower	*Helianthus annuus* L.
Skunk bush	*Rhus trilobata* Nutt.

Sycamore	*Platanus occidentalis* L.
Timothy	*Phleum pratense* L.
Tobacco	*Nicotiana tabacum* L.
Tomato	*Lycopericon esculentum* Mill.
Virgin's Bower	*Clematis virginiana* L.
Watermelon	*Citrullus lanatus* (Thunb.) Mastsum & Nakai
Wheat	*Triticum aestivum* L.

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Air Quality Criteria for Ozone and Related Photochemical Oxidants

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Preface

In 1971, the U.S. Environmental Protection Agency (EPA) promulgated National Ambient Air Quality Standards (NAAQS) to protect the public health and welfare from adverse effects of photochemical oxidants. In 1979, the chemical designation of the standards was changed from photochemical oxidants to ozone (O_3). This document focuses primarily on the scientific air quality criteria for O_3 and, to a lesser extent, on those for other photochemical oxidants such as hydrogen peroxide and the peroxyacyl nitrates.

The EPA promulgates the NAAQS on the basis of scientific information contained in air quality criteria issued under Section 108 of the Clean Air Act. The previous O_3 criteria document, *Air Quality Criteria for Ozone and Other Photochemical Oxidants*, was released in August 1986 and a supplement, *Summary of Selected New Information on Effects of Ozone on Health and Vegetation*, was released in January 1992. These documents were the basis for a March 1993 decision by EPA that revision of the existing 1-h NAAQS for O_3 was not appropriate at that time. That decision, however, did not take into account some of the newer scientific data that became available after completion of the 1986 criteria document. The purpose of this revised air quality criteria document for O_3 and related photochemical oxidants is to critically evaluate and assess the latest scientific data associated with exposure to the concentrations of these pollutants found in ambient air. Emphasis is placed on the presentation of health and environmental effects data; however, other scientific data are presented and evaluated in order to provide a better understanding of the nature, sources, distribution, measurement, and concentrations of O_3 and related photochemical oxidants and their precursors in the environment. Although the document is not intended to be an exhaustive literature review, it is intended to cover all pertinent literature available through 1995.

This document was prepared and peer reviewed by experts from various state and Federal governmental offices, academia, and private industry and reviewed in several public meetings by the Clean Air Scientific Advisory Committee. The National Center for Environmental Assessment (formerly the Environmental Criteria and Assessment Office) of EPA's Office of Research and Development acknowledges with appreciation the contributions provided by these authors and reviewers as well as the diligence of its staff and contractors in the preparation of this document at the request of the Office of Air Quality Planning and Standards.

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6

Toxicological Effects of Ozone and Related Photochemical Oxidants

6.1 Introduction

A wide range of effects of ozone (O_3) has been demonstrated in laboratory animals (see reviews by U.S. Environmental Protection Agency [1986], Lippmann [1989, 1993], and Graham et al. [1991]). The major research findings are that environmentally relevant levels of O_3 cause lung inflammation; decreases in host defenses against infectious lung disease; acute changes in lung function, structure, and metabolism; chronic changes in lung structure and lung disease, some elements of which are irreversible; and systemic effects on target organs (e.g., liver, immune system) distant from the lung. The research also has served to expand understanding of the mechanisms of toxicity and relationships between concentration (C) and duration of exposure (time [T]). The framework for presenting the health studies of O_3 in animals begins with a discussion of respiratory tract effects and is followed by a presentation of systemic effects and interaction of O_3 with other common co-occurring pollutants. Respiratory tract effects are often interrelated; however, for purposes of presentation, effects on lung inflammation and permeability, host defenses, morphology, pulmonary function, biochemistry, and mutagenic/carcinogenic potential are discussed separately in the main text, drawing correlations where appropriate. This type of organization enables focus on specific effect categories. In the few cases where one study addresses several different categories of endpoints, cross references are made to the appropriate sections of the chapter. Each major section on a specific effect category is followed by a summary for that section. The summary and conclusions section for the entire chapter (Section 6.5) attempts to draw together findings on related endpoints and to highlight key issues, such as the relative importance of exposure concentrations and durations and the identification of potential risk factors.

A purpose of this criteria document is also to describe any key health effects of photochemical oxidants in addition to O_3. Nitrogen dioxide (NO_2) and nitric oxide are the other two primary photochemical oxidants; they have been evaluated recently in another criteria document (U.S. Environmental Protection Agency, 1993). Formaldehyde (HCHO), which is formed photochemically and can be toxic, also has been reviewed recently by the U.S. Environmental Protection Agency (EPA) (Grindstaff et al., 1991). Literature searches did not reveal any animal toxicology inhalation studies of peroxyacetyl nitrate (PAN) since the last O_3 document (U.S. Environmental Protection Agency, 1986). A myriad of other

individual photochemical oxidants are formed in ambient air (Chapter 3), but they have not been investigated by animal inhalation toxicology. The very few publications on the effects of exposures to a mixture of oxidants are summarized in Section 6.4, which discusses pollutant interactions. Therefore, other than in Section 6.4, this chapter does not address other photochemical oxidants. Even so, considering the limited literature within the aforementioned documents, the available evidence from animal toxicology studies shows that O_3 is the most potent of the oxidants for noncancer effects at environmentally relevant concentrations.

The animal toxicology database for O_3 is extremely large, making it necessary to adopt conventions for presenting succinctly the pertinent findings. Priority was placed on analysis of research published after closure of the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986); however, for the purposes of broader interpretation, the older literature is very briefly summarized. Generally, only the highlights of the key recent studies and their interpretation are provided here. Confirmatory recent studies are mentioned and presented in the tables. Furthermore, studies having O_3 concentrations ≤ 1.0 ppm are highlighted with rare exception (e.g., genotoxicity studies). Genotoxicity studies at O_3 concentrations higher than 1.0 ppm were included to enable coverage of all the specific endpoints, some of which were tested only above 1.0 ppm. In most other cases, however, the 1-ppm cut point allows portrayal of the full array of the effects of O_3 that may occur from ambient air exposure and also avoids the potential for confounding mechanisms that can occur at very high, environmentally unrealistic concentrations. For example, very high levels of O_3 can cause severe pulmonary edema, resulting in types and magnitudes of pulmonary function changes that would not occur in ambient air. In summarizing the literature, changes from control are described if they were statistically significant at $p < 0.05$, rather than citing the probability values for each study. Where appropriate, critique of a statistical procedure is mentioned. A probability value is provided if it aids the understanding of trends observed in a study (e.g., $p < 0.1$).

As stated above, only literature published since the last O_3 criteria document is described in detail here. The earlier findings are summarized to facilitate cross-referencing. For example, in some cases, the older work is presented in overview in the beginning of each main section; in other cases, the overview is at the subsection level. Generally, the newer literature elucidates the influence of different exposure regimens and the mechanisms of several key effects, rather than portrays undiscovered categories of effects. The newer knowledge on molecular and biochemical interactions increases the understanding of mechanisms of effects. For example, it is unlikely that the O_3 molecule itself penetrates the lung and enters the circulation. As another example, the relationship between inflammation measured in tissue and lung lavage assists in the interpretation of lung lavage findings. Information on the immune system suggests that the cell-mediated limb may be more susceptible than the humoral limb. The ability of O_3 to decrease antibacterial host defenses has long been recognized, but only recently have viral defenses been analyzed. Much remains to be learned, but apparently antibacterial defenses are more at risk from O_3 exposure than antiviral defenses. Cellular and interstitial changes in the lungs of O_3-exposed animals were among the very early studies, with newer work adding to a detailed understanding of morphologic lesions in the pulmonary region identified through advanced morphometric procedures. Scientists just recently have begun to study the effects of O_3 on the nose and have discovered epithelial changes, identifying this tissue as a significant target site of O_3. One new body of information concerns the influence of exposure concentration,

duration, and pattern. For several endpoints (e.g., increased lung permeability), under acute exposure conditions, concentration has more impact than exposure duration. The importance of exposure duration is clearly illustrated by the newer chronic studies that show different patterns of effects (compared to acute exposures). The most sensitive indicators appear to be morphological changes (compared to pulmonary function changes) which is consistent with the concept that functional abnormalities follow morphological changes and may not become apparent until a given threshold is achieved. Studies using intermittent exposures (e.g., exposures every day versus every other week or month for equal times) indicate that interrupted exposures can produce equal or, in some cases, enhanced effects compared to uninterrupted exposures, suggesting a cumulative effect. Thus, seasonal "lows" in O_3 do not have benefit in these animal studies. Newer work also has mimicked and extended human clinical studies of repeated exposures. As with humans, the pulmonary function of rats was attenuated with several days of exposure. However, other changes (e.g., cellular) did not attenuate in the rat, illustrating the need for comprehensive evaluations. For the first time, a classical cancer bioassay has been performed with O_3. It helps put some of the earlier genotoxicity and carcinogenicity studies in perspective. This brief identification of the newer additions to the O_3 database is not meant to be a summary of effects; that is the last section of the chapter. Rather, it does show the importance of considering all the literature, not just the newer work in interpreting the effects of O_3. As mentioned, it was not feasible to repeat the 1986 O_3 criteria document herein; this makes it necessary to use both the current and the former document in evaluations.

Animal toxicological studies of O_3 are of major interest because they illustrate a fuller array of effects and exposure conditions than can be investigated in humans. Most experts accept a qualitative animal-to-human extrapolation (i.e., O_3 effects observed in several animal species can occur in humans if causative exposure concentrations, durations, and patterns also occur). However, there is less consensus on an approach to quantitative extrapolation (e.g., the exposures at which effects in animals actually occur in humans). Chapter 8, on extrapolation, provides more information on this topic.

6.2 Respiratory Tract Effects of Ozone

6.2.1 Biochemical Effects

6.2.1.1 Introduction

This section outlines studies designed to identify biochemical targets of O_3, as well as biochemical measurements of antioxidant and microsomal enzyme activities, lipids, and proteins. It should be noted that interpretation of biochemical changes resulting from whole lung measurements is complicated by the heterogeneity in cell type and function present in lung tissue and the changes in cell populations that result from O_3-induced inflammatory cell infiltration and epithelial cell and fibroblast proliferations. The ability to extrapolate from in vitro to in vivo studies and from high to low levels of O_3 is further complicated by an inability to detect biochemical changes in the whole lung when only a small proportion of the lung may be affected by O_3, especially at concentrations of O_3 less than 1 ppm. Interpretation of all biochemical measurements, therefore, needs to take into account the airway sites of O_3 interaction and concomitant changes in cell populations and numbers that take place at times other than the onset of exposure.

6.2.1.2 Cellular Targets of Ozone Interaction

In vitro experiments have indicated that O_3 has the potential to interact with a wide range of different cellular components that include polyunsaturated fatty acids (PUFAs); some protein amino acid residues (cysteine, histidine, methionine, and tryptophan); and some low-molecular-weight compounds that include glutathione (GSH), urate, vitamins C and E, and free amino acids (U.S. Environmental Protection Agency, 1986; Mustafa, 1990; Pryor, 1991, 1992). The mechanisms to explain the initial biochemical and physiological effects of O_3 exposure in vivo are therefore complex. Hypotheses have been developed on the direct action of O_3 with lung macromolecules, the reaction of secondary biochemical products that could result from the generation of free radical-precursor molecules, the release of endogenous mediators of physiological response, and the reactive oxygen intermediates and proteinases associated with the activities of inflammatory cells that subsequently infiltrate into O_3-damaged lungs (see Section 6.2.2). Based on some theoretical calculations, Pryor (1992) hypothesized that, because O_3 is so reactive, it most likely does not penetrate beyond the surface-lining fluids of the lung except in those terminal airway regions having minimal lining thickness where epithelial cells may well be relatively unprotected by either mucus or surfactant. In a review, Pryor (1991) proposed that O_3-induced cell damage more likely results from the reactions of more stable but less reactive ozonide, aldehyde, and hydroperoxide products of O_3 interaction with surface-lining fluid components than from direct interactions of O_3 with intracellular components. Although the alveolar lining fluid is relatively rich in saturated phospholipids, it does contain some lipids with unsaturated fatty acids, cholesterol, a protein A component, and small-molecular-weight compounds (e.g., GSH and uric acid) that have been shown to react with O_3 in both in vitro and in vivo studies (Effros et al., 1990; King and Clements, 1985; Shelley et al., 1984).

Polyunsaturated Fatty Acids

Hitherto, the major products of O_3-lipid interaction that account for cell membrane damage have been assumed to be lipid hydroperoxides. However, evidence for the production of hydrogen peroxide and aldehydes has been demonstrated. It has been proposed that, although Criegee ozonation (Figure 6-1) will ultimately lead to the production of ozonides in a lipophilic environment, in the aqueous environment of lung airways, the carbonyl oxide intermediate can form a hydroxyhydroperoxy compound, which on elimination of hydrogen peroxide yields another aldehyde or, in the presence of iron ions, can form an aldehyde and the very reactive hydroxyl radical (Teige et al., 1974; Pryor, 1991). Ozonation of aqueous emulsions of PUFAs, rat erythrocyte ghost membranes, and rat bronchoalveolar lavage (BAL) fluid has shown hydrogen peroxide and aldehyde generation with a much smaller proportion of ozonides and lipid hydroperoxides (Pryor et al., 1991). A mechanistic study by Santrock et al. (1992) of the ozonation of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine in unilamellar phospholipids confirmed the generation of the hydroxyperoxy compounds, which subsequently result in the generation of hydrogen peroxide and aldehydes with further oxidation to carboxylic acids (Figure 6-1). Similar studies conducted under nonaqueous conditions have demonstrated the production of secondary ozonides that, under physiological conditions, would be expected to decompose rapidly to reactive products (Lai et al., 1990). Madden et al. (1993) have demonstrated recently production of arachidonate-derived aldehydic substances and hydrogen peroxide from in vitro O_3 exposure (0.1 and 1.0 ppm for 1 h) of arachidonate in both a cell-free system and

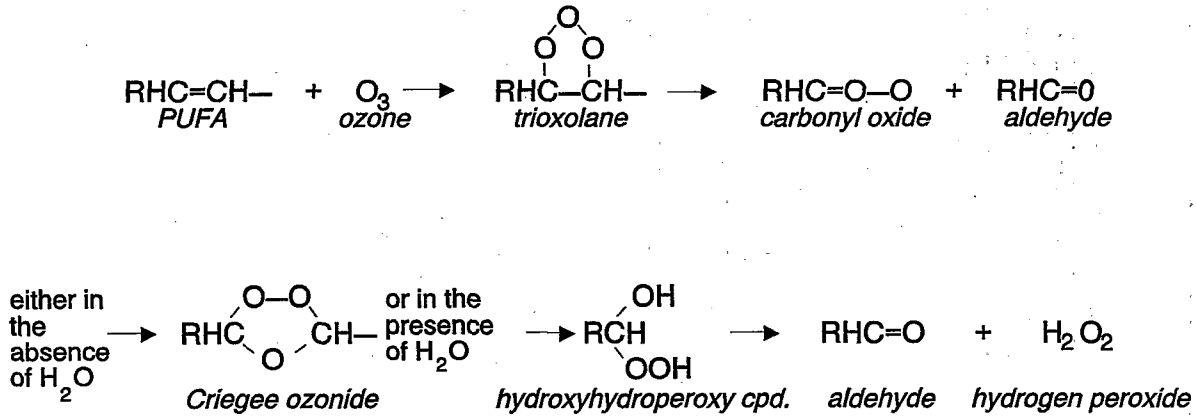

Figure 6-1. Major secondary products of ozone interaction with lung cells.

cultured human bronchial epithelial cells. Ozonides, aldehydes, hydrogen and lipid peroxides, and related reactive oxygen intermediates, together with the phospholipid from which the aldehyde has been removed, represent major products of O_3 interaction with lung cells that all have the potential to cause damage to membranes (see Figure 6-1).

Evidence that interaction of O_3 with PUFAs takes place in vivo has not been so easily obtained. Goheen et al. (1986) investigated the effects of fat-free diets on rats exposed to air or to 0.96 ppm O_3 for 0, 1, 2, and 4 weeks and concluded that O_3 does not oxidize significant levels of the PUFAs linoleate (18:2) and arachidonate (20:4). However, cleavage of lung fatty acid double bonds has been demonstrated in an in vivo study reported by Rabinowitz and Bassett (1988) that involved rat exposures for 4 h to 2 ppm O_3. These authors, by using hydrogen peroxide treatment to convert ozonides and aldehydes to carboxylic acids, were able to demonstrate O_3-induced increases in glutaric and nonanoic acids that are the ozonolysis breakdown products of lung tissue arachidonic and oleic acids, respectively. More recent studies directed towards developing suitable biomarkers and dosimeters for O_3 exposure have analyzed rat BAL lipids after a 12-h exposure to 1.3 ppm and demonstrated the appearance of the aldehydes nonanal and heptanal (Cueto et al., 1992). Pryor et al. (1992) also have been able to identify cholesterol ozonation products extracted from whole lung tissue with the same exposure of rats to 1.3 ppm O_3 for 12 h.

Evidence of the role of hydrogen peroxide in O_3-induced lung damage has been described by Warren et al. (1988), who demonstrated diminished O_3-induced increased BAL protein in rats after 1 day of exposure to 0.64 ppm O_3, when treated with the hydrogen peroxide scavenger dimethylthiourea before exposure. Hitherto, the exhalation of ethane and pentane and tissue measurements of diene-conjugates and thiobarbituric acid reactive substances (TBARS) have been used as evidence for O_3-induced free radical autoxidation of lipids (U.S. Environmental Protection Agency, 1986). However, these measurements have been found to be relatively insensitive for use in inhalation experiments under conditions of low O_3 concentrations (<0.5 ppm). Ichinose and Sagai (1989) were unable to demonstrate any changes in lung TBARS as a result of exposing rats for 2 weeks to 0.4 ppm O_3. As noted by Pryor (1991), malondialdehyde and other thiobarbituric-acid-reacting aldehydes

can be produced by Criegee ozonation of olefinic fatty acids that include arachidonate, as well as by free radical peroxidative processes. In addition, malondialdehyde, being volatile as well as highly reactive, may be lost readily from the lung or during sample preparation. However, measurements of TBARS continue to be used for in vitro experiments designed to demonstrate possible mechanisms by which such agents as taurine (Banks et al., 1991) and uric acid (Meadows and Smith, 1987; Peden et al., 1993) may protect against O_3-induced lipid damage. Rietjens et al. (1987b), by preincubating rat alveolar macrophages (AMs) with either arachidonate (20:4) or phosphatidylcholine to alter PUFA content and membrane fluidity, respectively, demonstrated that PUFA content (not membrane fluidity) determined sensitivity to O_3 damage, measured as decreases in phagocytic activity.

Evidence for free-radical-mediated autoxidation comes indirectly from the demonstration that vitamin E depletion increases O_3 toxicity, as reported previously (U.S. Environmental Protection Agency, 1986) and more recently (Elsayed, 1987; Elsayed et al., 1988). More direct evidence for free radical generation has been obtained using electron spin-trapping technology that correlated increased radical signals in isolated lung lipids from rats exposed to increasing O_3 concentrations (0 to 1.5 ppm, effect beginning at about 0.5 ppm; 2 h) under conditions of carbon dioxide (CO_2)-stimulated respiration (Kennedy et al., 1992). However, the possible contribution of activated inflammatory cell generation of reactive oxygen intermediates to these observed free radical alterations to lung lipids needs to be considered.

Antioxidants

Although vitamin E directly reacts with O_3 at the same rate as PUFAs, vitamin C appears to react more effectively (Pryor, 1991), which, together with intracellular taurine (Banks et al., 1991) and BAL uric acid (Meadows and Smith, 1987; Peden et al., 1993) found in nasal and lung-lining fluids, may act as direct scavengers of O_3. Ozone-induced increases in lung polyamine metabolism in vitamin E-deficient rats suggests their possible role as antioxidants (Elsayed, 1987). Glutathione in its reduced form (GSH) represents another potential direct O_3 scavenger. In addition to being a major intracellular antioxidant, GSH is a component of airway-lining fluids found in BAL. Ozone would have to penetrate the cellular membrane without reaction if it is to directly interact with intracellular GSH, an event considered to be unlikely (Pryor, 1991, 1992). Previously observed oxidation of glutathione and, in some cases, its loss from the lung may more likely reflect its reaction with an O_3-derived oxidant, such as a hydroperoxide or an ozonide, mediated by glutathione peroxidase (GSHPx) and glutathione-S-transferases, respectively (Rietjens et al., 1987a), resulting in the formation of glutathione disulphide or mixed disulphides with sulphydryl (SH)-containing proteins. Although ozone-induced formation of glutathione sulfonate has been reported in vitro, such irreversible oxidation of GSH has not been observed in vivo (U.S. Environmental Protection Agency, 1986; Mustafa, 1990).

Proteins

Early studies reported that nonprotein sulfydryls (NPSHs) and the activities of various cytosolic, microsomal, and mitochondrial enzymes are decreased immediately following short-term exposures to relatively high levels (2 to 4 ppm) of O_3 (U.S. Environmental Protection Agency, 1986; Mustafa, 1990). However, although these early biochemical effects could not be demonstrated after the first day of exposure to the lower O_3 concentration of 0.8 ppm, the methods employed may not have been sensitive enough to

detect coenzyme and enzyme changes in the centriacinar region (CAR), which is a primary target of O_3. However, together with surfactant lipids, the surfactant protein A also has been examined as a potential target of O_3 interaction (Oosting et al., 1991c, 1992). In vitro studies by these authors have suggested that either hydrogen peroxide- or O_3-induced oxidation of methionine and tryptophan residues account for the observed changes in physicochemical properties of canine and human surfactant protein A, measured as an impairment of self-association and a decreased ability to aggregate phospholipid vesicles and to bind mannose (Oosting et al., 1991c). Similar responses were found in vivo; surfactant isolated from rats exposed for 12 h to 0.4 ppm O_3 was less able to stimulate AM superoxide anion generation than surfactant obtained from air-exposed control rats (Oosting et al., 1992). The previously reported presence of giant lamellar bodies in O_3-exposed rat lungs following exposure to 0.3 ppm for 3 h/day for 16 days is also consistent with the hypothesis that O_3 reacts with surfactant protein A (Shimura et al., 1984) and thereby interferes with its homeostatic role in surfactant release from alveolar Type 2 cell lamellar bodies and its subsequent reuptake by Type 2 cells and AMs.

6.2.1.3 Effects of Ozone Exposure on Lung Lipid Metabolism

Arachidonate Metabolites

Ozone-induced damage to airway epithelia (Leikauf et al., 1988) and AMs (Madden et al., 1991) in vitro has been associated with the production of arachidonic acid metabolites by both cyclooxygenase and lipoxygenase pathways. These metabolites have been implicated in a variety of different physiological processes that include changes in airway permeability, infiltration of polymorphonuclear leukocytes (PMNs) and eosinophils, and airway smooth- muscle reactivity, discussed elsewhere in this chapter (Sections 6.2.2 and 6.2.5). Leikauf et al. (1993) have examined the effects of fatty acid O_3-degradation products on human airway epithelial eicosanoid metabolism and concluded that the stimulating effects were increased with product chain length, with the 3-, 6-, 9-hydroxyhydroperoxides being more potent than their corresponding aldehydes. Madden et al. (1993) concluded that aldehydic degradation products of arachidonate, but not hydrogen peroxide, increased in vitro polarization of leukocytes, and decreased peripheral blood T-cell mitogenesis and natural killer (NK) cell cytotoxicity. In vivo experiments on rabbits, guinea pigs, mice, and rats of different ages exposed to ≤ 1.0 ppm O_3 have demonstrated increases in the products of arachidonic acid metabolism (see Section 6.2.2).

Surfactant

Although alveolar surfactant lipids purified from lavage fluids have been shown to be relatively enriched in saturated lipids, a varying percentage of the lipids do contain unsaturated fatty acids depending on the species studied (King and Clements, 1985; Shelley et al., 1984). These unsaturated lipids, together with the apoprotein as possible targets of O_3 interaction, may be expected to have an altered composition as a result of O_3 inhalation. However, surfactant-enriched material isolated by BAL from rats following an 8-h exposure to 0.8 ppm O_3 retained its ability to lower surface tension in spite of an increase in protein content (Nachtman et al., 1986). In long-term exposure studies, monkeys were exposed for 8 h/day to 0.15 and 0.3 ppm O_3 for 21 and 90 days (Rao et al., 1985a,b). In contrast to measurements of total lung lipids that demonstrate a relative decrease in PUFAs after 21 days of exposure (Rao et al., 1985a), there was a relative increase in the proportion of PUFA in the percentage of BAL unsaturated fatty acids (increases from 34% in

air controls to 41, 42, and 45% in BAL lipids recovered from monkeys exposed for 21 days to 0.15 ppm, 90 days to 0.15 ppm, and 90 days to 0.3 ppm O_3, respectively) (Rao et al., 1985b). The major increases were observed in linoleate (18:2) and arachidonate (20:4). Because these PUFAs are potential targets for O_3 interaction, their increase, rather than a decrease, in BAL fluid may best be explained by changes in surfactant lipid production associated with alveolar Type 2 epithelial proliferation (Section 6.2.4). Interestingly, a relative decrease in cholesterol ester with a concomitant increase in phosphatidylcholine was observed, which supports the hypothesis that cholesterol may represent a major target of O_3 interaction (Rao et al., 1985b; Pryor et al., 1992). The observed O_3-induced changes in BAL PUFA composition were consistent with those previously reported for rats by Roehm et al. (1972), but only for BAL lipids isolated from vitamin E-depleted rats following 6 weeks of exposure to 0.5 ppm O_3. Wright et al. (1990) were unable to detect changes in BAL lipid and fatty acids recovered from normally fed rats following 0.12-, 0.25-, and 0.5-ppm O_3 exposures for 20 h/day for 18 mo. Results from these studies are summarized in Table 6-1.

Tissue Lipids

In vivo pulse labeling with carbon-14-labeled acetate was used to estimate phospholipid biosynthesis (Wright et al., 1990). Although found to be diminished at certain time points (3 and 12 mo), no consistent trend could be demonstrated that would suggest that O_3 exposures of less than 0.5 ppm alter lung surfactant homeostasis. Bassett and Rabinowitz (1985), using isolated perfused lungs taken from rats after 3 days of continuous exposure to 0.6 ppm O_3, demonstrated an enhanced incorporation of glucose carbons into both fatty acid and glycerol-glyceride moieties of total lung lipids by 180 and 95%, respectively. The relative increase in carbon incorporation into free fatty acids, phosphatidic acid, phosphatidyl inositol, and sphingosine containing lipids was consistent with the needs of a dividing cell population for increased lipids synthesis associated with alveolar epithelial proliferative repair. It should be noted that, in a separate study, under the same exposure conditions of 0.6 ppm O_3 for 3 days, rat lungs demonstrated increased glycolytic activity and generation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) consistent with the energy and synthetic needs of a lung undergoing repair of O_3-induced damage (Bassett and Bowen-Kelly, 1986). Results from these studies are summarized in Table 6-1.

6.2.1.4 Effects of Ozone on Lung Antioxidant Systems

The O_3-induced increased levels of the antioxidant NPSHs, identified mainly as GSH in the lung, and the enzyme activities involved in GSH utilization, GSHPx and glutathione-*S*-transferase (GST), and for maintaining GSH in a reduced state, glutathione reductase (GR) and the NADPH-linked dehydrogenases of glucose-6-phosphate (G6PD) and 6-phosphogluconate (6PGD), typically have been attributed to concurrent morphological changes rather than to any specific biochemical response (U.S. Environmental Protection Agency, 1986). Numerous studies conducted in mice, rats, and monkeys show increases in many of these enzyme activities at exposures as low as 0.2 ppm O_3 for 1 week (rat) (U.S. Environmental Protection Agency, 1986). The earlier research also included studies of age-dependent responsiveness of rats (Tyson et al., 1982; Lunan et al., 1977; Elsayed et al., 1982). Rats ranging in age from 5 to 90 days old were exposed to 0.8 or 0.9 ppm O_3 for 3 or 4 days or for about 20 days, depending on the experiment. Ozone altered activities of antioxidant enzymes in an age-dependent manner. Generally, prior to weaning, enzyme

Table 6-1. Effects of Ozone Exposure on Lung Lipids[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.12 0.25 0.5	235 490 980	20 h/day, 7 days/week for 18 mo	Rat, M (F344) 28 days old	Age-related increase in BAL and tissue phospholipids generally unaffected by O_3 exposure; at 0.5 ppm, total phospholipid increased at 6 and 12 mo.	Wright et al. (1990)
0.15 0.3	353 588	8 h/day for 90 days	Monkey (Bonnet)	Fraction of total lung lipid fatty acids that were PUFAs decreased from 22 to 9% and 6% following 0.15-ppm and 0.3-ppm exposures, respectively.	Rao et al. (1985a)
0.15 0.3	353 588	8 h/day for 21 and 90 days	Monkey (Bonnet)	BAL PUFAs (linoleate [18:2] and arachidonate [20:4]) increased, with a relative decrease in cholesterol esters.	Rao et al. (1985b)
0.5 0.6	980 1,176	Continuous for 0-4 weeks	Rat, M (S-D) 50 g	No change in lung fatty acid content; no acceleration of essential fatty acid deficiency in rats on fat-free diet.	Goheen et al. (1986)
0.5 1.0 1.5 2.0	980 1,960 2,940 3,920	2 h	Rat, M (CD) 65-85 days old	Extracted lung lipid EPR signal intensity proportional to O_3 concentration following pretreatment with spin-trapping agent and CO_2 stimulation of respiration in vivo.	Kennedy et al. (1992)
0.58	1,137	Continuous for 3 days	Rat, M (Wistar) 220-250 g	Increased lipid synthesis associated with increased glucose catabolism for ATP and NADPH generation.	Bassett and Bowen-Kelly (1986)
0.6	1,176	Continuous for 3 days	Rat, M (Wistar) 220-250 g	Increased synthesis of perfused lung glyceride-glycerol and fatty acid moieties of neutral lipids and phospholipids from glucose carbons, with a greater proportion of shingosine and inositol synthesis.	Bassett and Rabinowitz (1985)
0.8	1,568	18 h	Rat (F344) 260 g	Increase in BAL protein; no alteration in surface-tension-lowering ability of BAL.	Nachtman et al. (1986)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

activities decreased, and, at older ages, they increased. The reasons for these differences are not known, but may be due to differences in (1) dose of O_3 to the lung (due to differences in exposure concentrations in huddled neonates on bedding prior to weaning or to differences in doses delivered to lung target sites), (2) basal levels of antioxidants and antioxidant enzymes, or (3) cellular sensitivity. Increased lung enzyme activities can result from either increased activity within a particular cell population or increased numbers of cells with that activity. Age, nutritional, and species differences in O_3-mediated responses must therefore be interpreted with consideration of the underlying morphological changes (Section 6.2.4). Relevant studies are summarized in Table 6-2.

An increase in lung alveolar Type 2 cells and in infiltrating inflammatory cells adequately explained the observed increases in succinate oxidase, G6PD, and 6PGD activities observed after 3 days of continuous exposure of rats to 0.75 ppm O_3 when represented on a per-milligram-deoxyribonucleic-acid (DNA) basis (Bassett et al., 1988a). These cell types are enriched in mitochondria and in NADPH-generating capacity, needed for both lipid biosynthesis and GSH maintenance. Similarly, no significant changes in these enzyme activities could be detected after 3 days of exposure to the lower O_3 concentration of 0.35 ppm, further illustrating the need to take into account the concomitant changes in cell population and number when interpreting whole-lung enzyme measurements. Increases of 150 and 108%, respectively, were observed in the per-milligram DNA activities of the ornithine carboxylase and *S*-adenosyl-methionine decarboxylase enzymes involved in polyamine synthesis, which, together with enhanced tritiated thymidine incorporation into DNA, have been considered to be more sensitive measures of biochemical changes in lungs of rats exposed continuously for 3 days to 0.45 ppm O_3 (Elsayed et al., 1990).

The potential role of superoxide dismutase (SOD) and catalase in protecting the lung against O_3 toxicity is not clear. Bassett et al. (1989), using a pretreatment with a phenyl-urea compound (*N*[2·(2-oxo-1-imidazolindinyl)ethyl]-*N*′-phenylurea; EDU) that increased rat lung SOD and catalase activities, failed to demonstrate any protection against acute lung injury from a single 3-h exposure to 2.0 ppm O_3. However, Zidenberg-Cherr et al. (1991) have demonstrated that copper (Cu)- and manganese (Mn)-deprived mice may be more susceptible to continuous O_3 exposure of 1.2 ppm for 7 days. Rahman and Massaro (1992) have demonstrated protection against edemagenic exposures to ozone (2.5 ppm for 24 h) in rats pretreated with endotoxin. Endotoxin pretreatment is associated with increases in lung tissue mitochondrial Mn-SOD activity without any concomitant increases in catalase, GSHPx, and the cytosolic Cu,Zn-SOD enzymes. Although it is difficult to conclude that mitochondrial SOD might directly protect against O_3 interactions, these results do suggest a central role of mitochondrial SOD in the protection of the cell against oxidative stress (Rahman and Massaro, 1992).

Rahman et al. (1991) also have demonstrated that lungs from O_3-exposed rats had increased activities of Cu, Zn-SOD, Mn-SOD, catalase, and GSHPx after 5 days of exposure to 0.7 ppm O_3. These increases were attributed to enhanced gene expression, indicated by higher messenger ribonucleic acid (mRNA) concentrations, rather than to the infiltration of cells enriched with these enzyme activities. Chronic exposure of rats to an urban pattern of O_3 for 12 mo did not affect total SOD activity in rats, although GSHPx and GR activities per lung were increased (Grose et al., 1989). Use of microdissection techniques following 90 days and 20 mo of rat exposures to 0, 0.5, and 1.0 ppm O_3 have shown concentration-dependent increases in SOD, GST, and GSHPx per milligram of DNA in the distal bronchioles. In contrast, decreases in GST and GSHPx activities in major bronchi and

Table 6-2. Effects of Ozone Exposure on Lung Antioxidants[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.06 base, 0.25 spike	118 base, 490 spike	Base 13 h/day, 7 days/week; ramped spike 9 h/day, 5 days/week for 12 mo	Rat, M (F344)	Whole lung increase in GSHPx and GSH reductase activities. SOD activity and NPSH content not affected.	Grose et al. (1989)
0.12 0.2 0.64	235 392 1,254	Continuous for 7 days	Rat, M (S-D) 250-300 g	Pretreatment with the H_2O_2 scavenger dimethylurea decreased O_3-induced tissue DNA and protein and BAL protein, acid phosphatase, and *N*-acetyl-β-D-glucosaminidase. No effect of vitamin E or β-carotene.	Warren et al. (1988)
0.12 0.5 1.0	235 980 1,960	6 h/day, 5 days/week for 90 days or 20 mo	Rat, M and F (F344)	Using microdissection techniques and representing data as units/mg DNA, GST, GSHPx, and SOD were increased in distal bronchioles after 90 days and 20 mo in a concentration-dependent fashion. After 90 days, SOD and GST were lower in major daughter bronchi. After 20 mo, SOD was increased in distal trachea; GSHPx was decreased in major bronchi but enhanced in minor bronchi; and GST decreased in major bronchi.	Plopper et al. (1994b)
0.35 0.75	686 1,470	Continuous for 3 days	Rat, M (Wistar) 200-250 g	0.75 ppm O_3-induced whole lung increases in GSHPx and GR not significant when corrected for increases in cell number. Increases in succinate oxidase, G6PD and 6PGD activities per mg DNA were consistent with increased Type 2 and inflammatory cell content. No increases per mg DNA at 0.35 ppm O_3.	Bassett et al. (1988a)
0.4	784	Continuous for 2 weeks	Rat, M (Wistar) 6 weeks old Guinea pig (Hartley) 6 weeks old	Small increases in whole rat lung levels of NPSH, vitamin C, GSHPx. Guinea pig GSHPx and GSH transferase activities decreased.	Ichinose and Sagai (1989)
0.41	800	12 h during day or night for 3 days or continuous for 72 h	Rat, M (Wistar) Guinea pig, M (Hartley) 9 weeks old	Rats: No effect of daytime exposure. Nighttime or continuous exposure increased activities of LDH, G6PD, GR, and GSHPx. Guinea pig: No daytime-only exposure. No effect on GR or GSHPx, G6PD increased after nighttime or continuous exposure; lactate dehydrogenase activity increased only after continuous exposure.	Van Bree et al. (1992)
0.45	882	Continuous for 2 days	Rat, M (S-D) 90 days old	Large increase in ornithine decarboxylase activity and DNA labeling reflecting polyamine metabolism and DNA synthesis and/or repair, respectively.	Elsayed et al. (1990)
0.5	980	Continuous for 5 days	Rat (Long-Evans) 10 weeks old	Ozone increased lung putrescine in both vitamin E-deficient or 1,000 IU/kg groups, but increases in spermidine content and decarboxylase activities of ornithine and *S*-adenosylmethionione only in vitamin E-deficient group.	Elsayed (1987)

Table 6-2 (cont'd). Effects of Ozone Exposure on Lung Antioxidants[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.5	980	Continuous for 5 days	Rat (Long-Evans) 10 weeks old	Ozone increased lung vitamin E level in supplemented rats and remained unchanged in all other tissues measured.	Elsayed et al. (1990)
0.5	980	2.25 h/day for 5 days	Rat, M (F344) 110 days old	Lung GSH initially enhanced, declining to control levels by Day 4. Lung ascorbate levels enhanced on Days 3 and 5 only.	Tepper et al. (1989)
0.64	1,254	Continuous for 7 days	Rat, M (S-D) 3-5 weeks old	The whole lung O_3-induced increase in ascorbate and GSH content unaffected by protein deficient diets.	Dubick et al. (1985)
0.64	1,254	Continuous for 7 days	Rat, M (S-D) 52 and 295 g	Whole adult lung contents of Cu,Zn-SOD and GSHPx increased by O_3 in all diet groups (ad libitum, 4-16% protein diets); GSHPx only increased in weanling rats fed 16% protein diet. Mn-SOD only increased in lungs from 4 and 16% protein-fed adult lungs.	Heng et al. (1987)
0.7	1,373	Continuous for 1-5 days	Rat, M (S-D) 45, 80, and 300 g	By 5 days, increased lung Cu,Zn-SOD, Mn-SOD, catalase, and GSHPx per DNA in all age groups. Adult lungs: Concomitant increases in mRNAs for Cu,Zn-SOD, catalase, and GSHPx without differences in mRNA stability.	Rahman et al. (1991)
0.8	1,568	8 h/day for 2 mo	Rat, M (S-D) 2 mo old	Absence of vitamin E exacerbates O_3-induced damage related to increases in whole lung levels of metabolic enzymes. No additional amelioration by diet supplementation above 50 IU vitamin E.	Elsayed et al. (1988)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

an increase in GSHPx were observed in minor bronchi after 20 mo of O_3 exposure (Plopper et al., 1994b). Rahman et al. (1991) concluded that changes in antioxidant enzyme activities in some cases could be associated with alterations in cellular pathology (see Section 6.2.4), whereas, in other cases, no correlation could be made even though the results were represented on a per-milligram-DNA basis. The observed changes in antioxidant enzyme activities appear to be site-specific and different at different airway locations. The response are concentration dependent and altered by the length of O_3 exposure (Plopper et al., 1994b).

Representing data on a per-gram, wet-lung basis, Ichinose and Sagai (1989) demonstrated increases in lung NPSH, vitamin C, and GSHPx but observed no effect on vitamin E levels, after continuous exposure of rats to 0.4 ppm O_3 for 2 weeks. In contrast, guinea pig lungs exhibited no changes in these antioxidant components when similarly exposed. However, although using the higher concentration of 0.64 ppm for 7 days of continuous O_3 exposure, Dubick et al. (1985) demonstrated that whole lung content of ascorbate and GSH was elevated, these changes were not significantly different when the data were represented on a per-100-g, wet-tissue basis. Rat BAL analysis following a 12-mo exposure to an urban pattern of O_3 demonstrated decreased vitamin E and enhanced ascorbate and protein levels (Grose et al., 1989). Because these antioxidants also have been shown to be targets of ozone interaction, any observed increases in their steady-state level suggest an increase in the ratio of production to degradation that could reflect either enhancement in cellular functions in response to continued O_3 exposure or alteration in the number of cells associated with their production.

In order to demonstrate that dietary vitamin E reduces the effects of O_3 exposure on lung biochemical parameters, comparisons between vitamin E-depleted and -supplemented diets have been used (U.S. Environmental Protection Agency, 1986) and reviewed by Pryor (1991). Elsayed et al. (1988) fed rats a test diet containing 0 or 50 International Units (IU) of vitamin E per kilogram for 2 mo prior to exposure to 0.8 ppm O_3 for 8 h/day for 7 days. Ozone exposure increased the whole-lung activities of mitochondrial, microsomal, and cytosolic enzymes. Vitamin E deficiency alone had no significant effect on these lung enzyme measurements, which were taken on a per-lung basis, but the addition of 50 IU vitamin E per kilogram to the diet prior to O_3 exposure diminished the observed O_3-induced increases in mitochondrial succinate cytochrome *c* reductase and GSHPx, microsomal NADPH cytochrome *c* reductase, and cytosolic GSHPx and SOD observed in vitamin E-deficient rats by up to 50%. Additional experiments using a relatively low range of vitamin E supplementation for short time periods demonstrated that, although absence of vitamin E in the diet exacerbates the effects of O_3 on lung injury, the magnitude of a protective effect does not increase proportionately with increased dietary vitamin E. These data support the conclusion that any supplementation beyond the normal recommended daily allowance for vitamin E may not necessarily provide humans with any additional protection against the effects of ambient O_3 exposure (Pryor, 1991). However, possible failure in these animal experiments to reach a steady-state tissue level of vitamin E may have obscured protective effects.

6.2.1.5 Effects of Ozone on Lung Protein Metabolism

Exposure of rodents to $\geq$0.45 ppm O_3 has been associated with increases in lung collagen, collagen synthesis, and prolyl hydroxylase activity associated with fibrogenesis (U.S. Environmental Protection Agency, 1986). These earlier studies showed an influence of exposure pattern on the responses. When rats were exposed to 0.8 ppm O_3 for 7 days,

prolyl hydroxylase activity continued to increase, but hydroxyproline content plateaued about Day 3 of exposure and remained elevated 28 days after exposure ceased (Hussain et al., 1976a,b). Last et al. (1984b) employed 90-day exposure regimens of rats to 0.96 ppm O_3 that included (1) a continuous 90-day exposure and (2) intermittent periods of 5 days (8 h/day) of O_3 and 9 days of air, repeated seven times with a total of 35 O_3 exposure days over a 90-day period. Both groups had equivalent increases in lung collagen content. When durations were decreased to 3 weeks, the continuous and intermittent (1 week O_3, then 2 weeks air) regimens resulted in equivalent increases in lung collagen. In nonhuman primates receiving 0.25 ppm O_3 daily or seasonally (every other month) for 18 mo, only the seasonal group had an increase in collagen (Section 6.2.4, Tyler et al., 1988). Results from studies of lung protein metabolism are summarized in Table 6-3.

More recently, Choi et al. (1994) examined the earliest time points from the onset of continuous O_3 exposure of rats to 1.0 ppm that caused alterations in extracellular matrix protein gene expression. These authors demonstrated an early increase in lung fibronectin mRNA at 2 days, which preceded an increase in Type I collagen mRNA observed at 4 days; however, increased collagen content indicated by lung hydroxyproline content was not significantly enhanced until after 7 days of exposure. Pickrell et al. (1987a) demonstrated concentration-dependent decreases in antiproteinase activities in serum and lung tissue of rats exposed to 0.5 and 1.0 ppm O_3 for 48 h. Exposure to 1.0 ppm was accompanied by a concomitant increase in inflammatory-cell-derived proteinases. A second study that examined lung collagen metabolism and proteinolysis in rat lungs exposed to 0.57 and 1.1 ppm O_3 for 19 h/day for 11 days suggested that collagen accumulation, in part, may result from decreased collagen degradation (Pickrell et al., 1987b).

Chronic exposures of monkeys to 0.61 ppm 8 h/day for 1 year demonstrated increased lung collagen content, even 6 mo postexposure (Last et al., 1984b). Further analysis also has demonstrated that the collagen isolated from these O_3-exposed lungs exhibited abnormalities, as indicated by increased levels of the difunctional cross-link dehydrodihydroxylysinonorleucine (DHLNL) and of the ratio of DHLNL to hydroxylysinonorleucine (HLNL) (Reiser et al., 1987). Although collagen content remained elevated, difunctional DHLNL and HLNL cross-link levels returned to normal by 6 mo postexposure, whereas trifunctional mature cross-links (hydroxypyridinium) remained elevated. These data suggest that structurally abnormal collagen is actively synthesized during O_3 exposure and that it becomes irreversibly deposited in the lungs.

Because O_3-induced lung effects are multifocal by nature, it is reasonable that changes in collagen content within the lung may not be easily detectable by measuring alterations in whole lung hydroxyproline at earlier time points or in those experiments that have used lower O_3 concentrations. For example, Wright et al. (1988) calculated values for the extent of lung collagen deposition using measured synthesis rates and concluded that 18 mo of exposure (20 h/day) of rats to concentrations up to 0.5 ppm O_3 did not change either synthesis or accumulation of lung collagen. On the other hand, Chang et al. (1992) demonstrated sustained thickening of rat lung extracellular matrix on long-term exposure to a simulated urban pattern of O_3 exposure (baseline of 0.06 ppm, 7 days/week, with a slow rising peak for 9 h/day, 5 days/week to 0.25 ppm) of up to 38 weeks. More recently, Last et al. (1993a, 1994) observed excess stainable collagen in the lung CAR of rats exposed to 0.5 and 1.0 ppm O_3 for 6 h/day, 5 days a week for 20 mo. Biochemical analyses demonstrated slight but significant increases in collagen with relatively more hydroxylysine-derived cross-links in female but not male rats, when compared with age-matched,

Table 6-3. Effects of Ozone Exposure on Lung Proteins[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.12 0.25 0.5	235 490 980	20 h/day for 18 mo	Rat, M (F344) 28 days old	Age-related increases in hydroxyproline content as a measure of collagen were unaffected by O_3 exposure.	Wright et al. (1988)
0.125 0.25 0.5	245 490 980	1 year	Rat (F344) 6 weeks old	No changes in collagen content, increased turnover at ≥0.25 ppm after 3 or more mo of exposure.	Filipowicz and McCauley (1986b)
0.12 0.5 1.0	235 980 1,960	6 h/day 5 days/week 20 mo	Rat, M and F (F344) 4-5 weeks old	Excess stainable collagen in the CAR at ≥0.5 ppm. Biochemical analysis demonstrated slight but significant increases in collagen in female but not male rats exposed to ≥0.5 ppm with increased hydroxylysine-derived cross-links.	Last et al. (1993a, 1994)
0.25	490	8h/day 7 days/week, "daily" for 18 mo or "seasonal" O_3 odd months for 18-mo period (9 mo of O_3)	*Macaca fascicularis* 6 mo old	Increased collagen content in seasonal group only.	Tyler et al. (1988)
0.4	784	12 h	Rat, M (Wistar) 8 weeks old	Surfactant less able to stimulate AM superoxide anion generation, confirming in vitro results suggesting damage to surfactant protein A.	Oosting et al. (1992)
0.5	980	4 h/day for 2 days and 6 weeks	Sheep, F 23-41 kg	At 2 days, increased sulfated glycoproteins secretion; at 6 weeks, diminished tracheal mucosal gland hyperplasia secretion.	Phipps et al. (1986)
0.5 1.0 1.5	980 1,960 2,940	Continuous for 48 h	Rat, F (F344) 12-14 weeks old	Concentration-dependent decrease in antiproteinase activity at 0.5 and 1.0 ppm. Increases in acid proteinase activity 1.0 and 1.5 ppm correlated with increased inflammatory cell content.	Pickrell et al. (1987a)
0.57 1.1	1,117 2,156	19 h/day for 11 days	Rat, F (F344) 120-180 g	After 11 days of 1.1 ppm O_3, inflammatory cell infiltrate and Type 2 cell and fibroblast proliferation, increased cathepsin D and AM elastase activity, decreased rate of intracellular collagen degradation, and increased extracellular matrix collagen turnover (indicated by enhanced BAL hydroxyproline). These changes preceded increased collagen content observed 50 days PE.	Pickrell et al. (1987b)
0.61	1,196	8 h/day for 1 year	Monkey (Cynomolgus) 6-7 mo old	Increased lung collagen content associated with elevated abnormal cross-links that were irreversibly deposited.	Reiser et al. (1987)

Table 6-3 (cont'd). Effects of Ozone Exposure on Lung Proteins[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.8	1,568	Continuous for 3 days	Rat (S-D) 24-365 days old	Increased lung dry weight, protein, and collagen synthesis greatest after 60 days of age.	
0.8	1,568	6 h/night for up to 90 days	Rat, M (S-D) 10-12 weeks old	Increased lung content of collagen. No change in hydroxypyridinium or elastin.	Last et al. (1993b)
1.0	1,960	Continuous for 14 days	Rat, M (Wistar) 200-250 g	Lung mRNAs for c-myc proto-oncogene and fibronectin enhanced on Day 2 and Type I collagen mRNA not increased until Day 4, preceding increases in collagen hydroxyproline observed on Day 7.	Choi et al. (1994)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

air-exposed control animals. It should be noted that no excess of mRNA for Type I procollagen was observed by in situ hybridization in lungs of rats exposed to 1.0 ppm for 20 mo, although increases after a 2-mo exposure under similar conditions did indicate some increase expression of this mRNA in alveolar interstitial cells (Last et al., 1993a).

Ozone exposure also affects airway secretion of mucous glycoproteins. After 2 days of exposure of sheep to 0.5 ppm O_3, with subsequent evaluation of tracheal sulfated glycoprotein and ion fluxes in vitro, there was an increase in basal secretion that was associated with a moderate hypertrophy of lower tracheal submucosal glands (Phipps et al., 1986). Although 7 days of exposure resulted in hypertrophy of upper and lower tracheal submucosal glands, glycoprotein secretion was reduced, but chloride secretion was increased, which can be explained by a relative decrease in gland mucous content.

6.2.1.6 Effects of Ozone Exposure on Lung Xenobiotic Metabolism

Previous studies have demonstrated that exposure to 0.75 to 1.0 ppm O_3 for a few hours diminishes microsomal cytochrome P-450 content and decreases the activities of benzo[*a*]pyrene hydroxylase and benzphetamine *N*-demethylase of lungs isolated from several different experimental animal species (U.S. Environmental Protection Agency, 1986). Because bronchiolar Clara cells and alveolar Type 2 cells are considered to be relatively enriched with microsomal cytochrome P-450 enzyme systems, it is reasonable that damage and subsequent proliferative repair of these cell types would be expected to change the lung's capacity to conduct xenobiotic metabolism. In a series of rat studies, Takahashi et al. (1985) and Takahashi and Miura (1985, 1987, 1989, 1990) have demonstrated that, although intermittent exposure of 0.4 ppm O_3 for 7 h/day for 14 days did not affect microsomal metabolism, increasing the concentration to 0.8 ppm (Takahashi et al., 1985) or exposing the rats continually to 0.2 and 0.4 ppm for 14 days (Takahashi and Miura, 1985) increased cytochrome P-450 content and the activities of cytochrome P-450 reductase, benzo[*a*]pyrene hydroxylase, and 7-ethoxycoumarin *O*-deethylase (see Table 6-4). These increased microsomal activities were sustained in rats exposed continuously for up to 12 weeks to 0.1 to 0.4 ppm O_3, with a greater response being observed in the activity of benzphetamine *N*-demethylase, suggesting preferential increase in the associated P-450 cytochrome isozyme (Takahashi and Miura, 1987). Ozone-induced increases in cytochrome P-450 also have been shown not to result in concomitant increases in microsomal xenobiotic metabolism (Rietjens et al., 1988). Rat lung microsomal benzo[*a*]pyrene oxidation and benzphetamine demethylation were found to be enhanced after a 6-mo continuous exposure to 0.5 ppm O_3 (Filipowicz and McCauley, 1986a). More recent studies have explored O_3-induced changes in cytochrome P-450 isozyme patterns and correlated changes in lung xenobiotic metabolism with Clara cell enlargement and increased numbers during a 14-day exposure of rats to 0.4 ppm O_3 (Takahashi and Miura, 1990; Suzuki et al., 1992). These authors also demonstrated, by immuno-electron microscopy, the presence of cytochrome P-450b (IIB1) in the Clara cell endoplasmic reticulum.

Changes in the extent and pattern of formation of benzo[*a*]pyrene products were investigated by Bassett et al. (1988c) in lungs from rats undergoing epithelial proliferative repair resulting from 3 days of continuous exposure to 0.6 ppm O_3. Although metabolism to all benzo[*a*]pyrene metabolites was enhanced 4.7-fold, the relative proportion of metabolism involving quinone formation was enhanced from 10 to 25%. The toxicity of other inhaled pollutants that undergo lung xenobiotic metabolism may therefore be dependent not only on

Table 6-4. Effects of Ozone Exposure on Lung Xenobiotic Metabolism[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.1 0.2 0.4	196 392 784	Continuous for 4-12 weeks	Rat, M (Wistar) 19-22 weeks old	Concentration-dependent increases in NADPH-cytochrome P-450 reductase activity and cytochrome P-450 content during 4-12 weeks exposure to 0.2 and 0.4 ppm O_3, reaching a maximum at 12 weeks with concomitant increases in benzo[*a*]pyrene hydroxylase and 7-ethoxycoumarin *O*-deethylase activities. NADH-cytochrome b_5 reductase activity unaffected. Four weeks at 0.1 and 0.2 ppm demonstrated a preferential increase in benzphetamine *N*-demethylase activity, with no alterations in coumarin hydroxylase activity.	Takahashi and Miura (1987)
0.2	392	Continuous for 2 weeks	Rat, M (Wistar) 19-22 weeks old	Increases in cytochrome P-450 isozymes ascribed to constitutive types rather than induction of other types.	Takahashi and Miura (1990)
0.2 0.4	392 784	Continuous for 7 and 14 days	Rat, M (Wistar) 22-24 weeks old	By 14 days, NADPH-cytochrome P-450 reductase activity and cytochrome P-450 content enhanced with concomitant increases in benzo[*a*]pyrene hydroxylase and 7-ethoxycoumarin *O*-deethylase activities by Day 7; no change in NADH-cytochrome b_5.	Takahashi and Miura (1985)
0.4 0.8	784 1,568	7 h/day for 14 days	Rat, M (Wistar)	No effect at 0.4 ppm. 0.8 ppm increased NADPH-cytochrome P-450 reductase activity and cytochrome P-450 content, with concomitant increases on Day 7 in benzo[*a*]pyrene hydroxylase and 7-ethoxycoumarin *O*-deethylase activities that further increased by Day 14.	Takahashi et al. (1985)
0.4	784	Continuous for 6 h, 1-14 days	Rat, M (Wistar) 5 weeks old	By 24 h, Clara cell number decreased, but by 14 days had increased. Increase in cytochrome P-450b (IIB1) on Days 7 and 14.	Suzuki et al. (1992)
0.5	980	Continuous for 1 year	Rat, M (F344)	Microsomal benzo[*a*]pyrene oxidation and benzphetamine demethylase activities enhanced after 6 mo and 1 year of exposure.	Filipowicz and McCauley (1986a)
0.6	1,176	Continuous for 3 days	Rat, M (Wistar) 200-220 g	In isolated perfused lung, increase in overall benzo[*a*]pyrene metabolism but with a greater proportion being metabolized to quinones.	Bassett et al. (1988c)
0.8	1,600	Continuous for 7 days	Rat, M (Wistar) 8 weeks old	Cytochrome P-450, cytochrome b_5, and NADPH-cytochrome P-450 reductase enhanced per lung and per gram lung but not per milligram microsomal protein. No concomitant increases in all cytochrome P-450-dependent reactions, suggesting alterations in isozyme patterns.	Rietjens et al. (1988)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

O_3-induced changes in airway protective barrier function and clearance mechanisms, but also on O_3-induced changes in epithelial cell activation and detoxification reactions.

6.2.1.7 Summary

In vitro studies have provided an indication of a wide range of initial biochemical targets of ozone interaction that include lipid PUFAs, SH-containing proteins, and small-molecular-weight electron donors such as GSH and vitamins E and C. Demonstration that these interactions occur in vivo and are responsible for subsequent cytotoxicity has been more difficult to characterize and mainly has required the use of relatively high (>1 ppm) concentrations of O_3. However, because of the high reactivity of ozone and the relatively high abundance of PUFAs in both cell membranes and epithelial lining fluids, PUFAs are considered to be the most likely initial target of interaction of O_3 with the lung. Current understanding of this interaction is that, in the relatively aqueous environment of the lung airways, hydroxyhydroperoxy intermediates are formed that break down to form aldehydes and hydrogen peroxide. Alternatively, it has been proposed that, in more hydrophobic environments (e.g., within a cell membrane), O_3 interaction with PUFAs yields ozonides and their free radical products. Ozonides, aldehydes, hydrogen peroxide, and other lipid breakdown products and oxygen intermediates are therefore considered to be secondary products of the initial O_3 interaction with PUFAs that would account for the observed alterations of cell lipids, SH-containing enzyme proteins, and antioxidants associated with O_3-induced cell damage. As a result of these observations, it has been hypothesized that O_3 most likely does not reach far beyond the surface lining fluids of the upper and lower airways, which are rich in mucopolysaccharides and surfactant lipids, respectively. However, at points where coverage is either discontinuous or thin, epithelial cell components might be expected to directly interact with inhaled O_3.

A wide array of lung biochemical measurements have been made at different times from the onset of O_3-exposure. These measurements have included lung lipids, antioxidants, and enzyme and structural proteins that, in some cases, can be attributed to particular cell populations. However, many of these biochemical determinations cannot be interpreted fully without consideration of the changes in cell population that occur as a result of O_3 exposure. In addition, the sensitivity of some of these measurements has been limited by the relatively small percentage of the whole lung affected by O_3 exposure. The more recent biochemical determinations being made on airway samples isolated by regional microdissection should help overcome some of these limitations.

In vivo experiments have demonstrated cleavage of total lung lipid PUFA double bonds, with arachidonate being a major target of O_3 interaction, the breakdown of cholesterol, and the production of aldehydes and hydrogen peroxide (results that are consistent with ozonation of cell membrane and epithelial lining lipids). The protein A component of the alveolar surfactant system also has been identified as a possible primary target of O_3 interaction. Changes observed in lung lipid biosynthesis during the first few days from the onset of O_3 exposure can be accounted for by concomitant alveolar epithelial proliferative repair. However, lavage-recovered lipids from monkeys following O_3 exposures of 0.12 ppm for 90 days have demonstrated a relative increase in PUFAs and decrease in cholesterol-esters, suggesting some long-term alteration in surfactant lipid composition. However, age-related changes in lavage-recovered lipids and total lung lipid biosynthesis have been shown to be relatively unaffected in rats exposed to 0.5 ppm O_3 for periods of up to 18 mo.

Many studies have utilized whole-lung measurements of antioxidant enzyme changes as indicators of biochemical responses to O_3 exposure. The increased levels of the cytosolic enzymes G6PD, 6PGD, GR, and GSHPx and mitochondrial succinate dehydrogenase observed during the first week from the onset of exposure to O_3 levels of 0.5 to 1.0 ppm are most likely a result of the epithelial proliferation and infiltration of inflammatory cells taking place during this period. Failure to observe similar biochemical changes at lower O_3 concentrations most likely reflects an inability to detect focal changes of altered pathology when using whole-lung tissue samples. Longer-term exposure of rats to an urban pattern of O_3 with daily peaks of 0.25 ppm has demonstrated increases in tissue GSHPx and GR but not SOD. These enzyme changes could reflect changes in either cellular antioxidant capacity in response to chronic O_3 exposure or the steady-state cell population.

Although no long-term changes in collagen content have been observed in rats exposed to <0.5 ppm O_3 for 18 mo, extracellular matrix thickening has been observed in rats exposed to an urban pattern of O_3 with daily peaks of 0.25 ppm for 38 weeks. Exposure of female but not male rats for 20 mo to concentrations of 0.5 and 1.0 ppm O_3 for 6 h/day has demonstrated increased centriacinar stainable collagen and collagen and difunctional cross-links. Similar results were obtained in lungs from monkeys exposed to 0.61 ppm O_3 for 1 year, providing a sensitive indicator that long-term O_3 exposure does cause some fibrogenic alterations to the lung extracellular matrix.

Ozone-induced changes in the extent and pattern of lung microsomal metabolism of xenobiotics have provided consistent results, which may, in part, reflect changes in the numbers and function of bronchiolar epithelial Clara cell and alveolar epithelial Type 2 cells at different durations of O_3 exposure. These cell types are relatively enriched with cytochrome P-450-dependent enzyme systems. Changes in both lung activation and detoxification reactions represent important effects when considering whether or not low-level O_3 exposures alter the ability of the lung to deal adequately with the co-exposure to inhaled xenobiotics found in urban air.

6.2.2 Lung Inflammation and Permeability Changes

6.2.2.1 Introduction

The barrier functions of the airway epithelia have been investigated by isotope tracer techniques for detecting mucosal permeability and by analysis of the BAL for total protein and albumin concentrations. Under normal conditions, the airway epithelia restrict the penetration of exogenous particles and macromolecules from airway lumen into airway interstitium and blood. The integrity of the zonula occludens (tight junctions) is regarded as a major factor in providing barrier properties to the airway epithelia so that only a small amount of intratracheally introduced tracers finds its way across the airway epithelia into the blood. However, disruption of the epithelial barrier creates a leak across the airway mucosa, resulting in increased permeability of serum proteins into the air spaces and of intraluminal exogenous tracers into the blood. Therefore, permeability is generally detected by either the tracer transport from airway spaces to blood or measurement of total protein and albumin in the BAL. Both of these measures are, therefore, taken into account in discussing permeability changes in this section. Although BAL protein measurement offers a good marker for detecting permeability changes, it is important to note that the proteins in the BAL can result from tissue injury and secretory activity, in addition to leakage of the serum proteins across the airway mucosa (Hatch et al., 1989; Hatch, 1992).

Inflammatory cells in the lung constitute an important component of the pulmonary defense system. In their unstimulated state, the inflammatory cells present no danger to other cells or tissues, but, on activation, they are capable of generating proteolytic enzymes such as elastase and reactive oxygen species such as superoxide, hydrogen peroxide (H_2O_2), and the hydroxyl radical. These oxidants can cause substantial injury to cell membranes and intracellular components by their effects on membrane lipids and proteins (biochemical effects of O_3 were described in Section 6.1). Ozone exposure also can cause the epithelial or activated inflammatory cells to liberate arachidonic acid, which is free to enter enzymatic lipoxygenase or cyclooxygenase pathways that lead to the production of leukotrienes (LTs) and prostaglandins (PGs), respectively. Although some of the studies indicate a lack of change in the production and release of cellular mediators following O_3 exposure, other studies demonstrate an elevation in the levels of arachidonic acid and its metabolites in the bronchial washings of rats, as well as humans (see Chapter 7) exposed to O_3 under controlled conditions. The changes in the lung levels of arachidonic acid metabolites generally were observed in animals exposed to O_3 concentrations higher than 0.5 ppm. These cellular mediators can cause a wide range of pathophysiological changes. For example, LTB_4 can cause PMN aggregation and degranulation in vitro and margination of circulating PMNs to capillary endothelium in vivo, whereas LTC_4 and LTD_4 can cause contraction of vascular smooth muscle, PGE_1 has bronchodilator activity, and LTD_4 and $PGF_{2\alpha}$ are regarded as bronchoconstrictors. Because of the toxic potential of the products released by PMNs, AMs, mast cells, and other inflammatory cells, it has been suggested that the recruitment of these cells into the pulmonary interstitium is associated with lung injury and associated edema. An inflammatory response in the lung and an elevation of transmucosal permeability are observed after O_3 exposure, but the interdependence of these two events is a topic of debate. Although AMs are involved in cellular changes during the course of inflammation, AMs are discussed only in terms of their primary function in the section on host defense (Section 6.2.3.4).

The previous O_3 criteria document (U.S. Environmental Protection Agency, 1986) discussed studies available at that time on the inflammatory and permeability effects of O_3. These studies recognized the increased thickness of the alveolar septa, presumably due to increased cellularity after acute exposure to O_3 and excess collagen after chronic exposure to O_3. The inflammatory cell response was reported in rats and monkeys receiving single or repeated exposures to O_3 concentrations ranging from 0.2 to 0.8 ppm (Castleman et al., 1980; Brummer et al., 1977; Moore and Schwartz, 1981; Crapo et al., 1984). Exposures to O_3 also resulted in increased mucosal permeability, as detected by the nonspecific diffusion of phenol red from the lung into circulation (Williams et al., 1980) or the appearance of serum proteins in the air spaces. Increased BAL levels of total protein, albumin, and immunoglobulin (Ig) G were detected in rats, dogs, and guinea pigs exposed acutely to O_3 concentrations ranging from 0.1 to 2.5 ppm (Alpert et al., 1971; Reasor et al., 1979; Hu et al., 1982). For example, Hu et al. (1982) found that a 72-h exposure of guinea pigs to $\geq$0.26 ppm O_3 increased BAL protein immediately after exposure and that, when the exposure duration was decreased to 3 h, protein increased 10 to 15 h postexposure (not immediately after exposure ceased).

6.2.2.2 Permeability Changes

A number of studies have demonstrated an increase in airway mucosal permeability following inhalation exposure to O_3 concentrations of $\leq$1.0 ppm (Table 6-5).

Table 6-5. Lung Inflammation and Permeability Changes Associated with Ozone Exposure[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.1 1.2	196 2,352	2 h/day for 1, 2, 6, and 13 days	Rabbit, M (NZW) 2-4 mo old	Increase in AM number at 7 days following single exposure to 0.1 ppm and increase in number of AMs and PMNs on 1-day after cessation of 6 or 13 days of exposure. Increase in number of PMNs at 24 h after single exposure to 1.2 ppm.	Driscoll et al. (1987)
0.1 0.3 1.0	196 588 1,960	2 h	Rabbit, M (NZW) 15-16 weeks old	Increase in levels of PGE_2 and $PGF_{2\alpha}$ in BAL immediately after exposure to 1.0 ppm O_3 only. No significant effects were observed on the levels of 6-keto-$PGF_{1\alpha}$, TXB_2 or LTB_4	Schlesinger et al. (1990)
0.1 0.3 1.2	196 588 2,352	2 h In vitro and in vivo	Rabbit, M (NZW) 2-4 mo old	In vitro: Increase in PGE_2 after 0.3 ppm and increase in $PGF_{2\alpha}$ after 1.2 ppm by AMs. In vivo: Increase in the release of PGE_2 and $PGF_{2\alpha}$ by AMs after 1.2 ppm, but no effect of 0.1 ppm.	Driscoll et al. (1988)
0.1 0.3 1.0	196 588 1,960	2 h In vitro	Rat (S-D) 12-18 weeks old	Increased production of arachidonic acid metabolites by AMs at 1.0 ppm only.	Madden et al. (1991)
0.1 to 10	196 to 19,600	2 h In vitro	Cow	Increased production of PGE_2 and $PGF_{2\alpha}$ by tracheal epithelial cells after exposure to 0.1 and 0.3 ppm. Increased production of other arachidonic acid metabolites at >1.0 ppm.	Leikauf et al. (1988)
0.1 0.2 0.4 0.8	196 392 784 1,568	2, 4, and 8 h	Rat (F344) 90 days old Guinea pig (Hartley) 60 days old	C × T exposure design; BAL 25 h after exposure started. PMNs measured in rats only; no C and T interaction; effect dependent on C. Exponential and polynomial response surface model used. Similar protein responses at low C × T products; generally, the influence of T increased as C increased. Exponential model explained 86% of the data.	Highfill et al. (1992)
0.1 0.3 1.2	196 588 2,352	2 h In vitro	Rabbit, M (NZW) 2-4 mo old	Exposure of AMs to ≥0.3 ppm O_3 resulted in increased secretion of factors capable of stimulating migration of inflammatory cells.	Driscoll and Schlesinger (1988)
0.1 0.2 1.2 4.0	196 392 2,352 7,840	Continuous for 1 to 12 weeks	Rat, M (Wistar) 16 weeks old for 1 week exposure; 21 weeks old for longer exposures	Number of AMs in BAL increased after exposure for 11 weeks to 0.2 ppm. Infiltration of PMNs did not occur.	Mochitate et al. (1992)
0.12 0.3	235 588	24 h 48 h and 72 h	Mice, M (C57BL/6J[B6]); (C3H/HeJ[C3]) 6-8 weeks old	BAL immediately PE. Comparable increases in BAL protein, AMs, PMNs, and lymphocytes in the two strains after exposure to 0.12 ppm, but greater number of inflammatory cells and protein concentration in B6 than in C3 mice after exposure to 0.3 ppm.	Kleeberger et al. (1993a)

Table 6-5 (cont'd). Lung Inflammation and Permeability Changes Associated with Ozone Exposure[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.12 0.8 1.5	235 1,568 2,940	6 h	Rat, F (F344/N) 12-18 weeks old	Increased number of PMNs in nasal lavage, but not in BAL at 18 h after 0.12 ppm; increased number of PMNs in BAL, but not in nose after 1.5 ppm; number of PMNs decreased with time in nose, with a concomitant increase in BAL PMNs after 0.8 ppm.	Hotchkiss et al. (1989a)
0.12 0.8 1.5	235 1,568 2,940	6 h	Rat, M (F344/N) 12-18 weeks old	AMs and PMNs increased in number in BAL at various times PE at ≥0.8 ppm.	Hotchkiss et al. (1989b)
0.12 to 0.96	255 to 1,882	6 h, 24 h, or 2 days	Rat, M (S-D) 250-300 g	Total protein in BAL increased after exposure to ≥0.4 ppm for 6 h and ≥0.12 ppm for 1 or 2 days. Transport of radiolabeled albumin from blood to the airways increased after 6- or 24-h exposure to ≥0.4 ppm and after 2 days exposure to 0.2 ppm.	Guth et al. (1986)
0.2 0.5 1.0 2.0	392 980 1,960 3,920	4 h	Mouse (Swiss Albino) 19-25 g Guinea pig (Hartley) 314-522 g Rat (S-D) 280-350 g Rabbit (NZW) 1.7-2.5 kg Hamster (Golden Syrian) 94-107 g	Species differences in responsiveness. At 18-20 h PE, total protein in BAL increased in guinea pigs exposed to 0.2 ppm, whereas mice, hamsters, and rats responded to ≥1.0 ppm, and rabbits responded only to 2.0 ppm.	Hatch et al. (1986)
0.2 0.4 0.6 0.8	392 784 1,178 1,568	6, 8, 12, and 24 h/day for 3 days	Rat (S-D) 10-12 weeks old	C and T matched such that all C × T = 14.4 ppm · h. BAL immediately after exposure ceased. Increase in PMNs equivalent in all O_3 groups. Increase in protein equivalent for 6-, 8-, and 12-h exposure groups, all of which are greater than protein in 24-h groups. Equivalent results for BAL PMNs.	Gelzleichter et al. (1992b)
0.2 0.4 0.6 0.8	392 784 1,176 1,568	7 h/day for 1, 2, or 4 days	Rat, M (PVG) 12-16 weeks old	BAL approximately 17 h PE. The proportion of AMs in the BAL decreased, with a concomitant increase in the proportion of PMNs after 1 or 2 days exposure to ≥0.6 ppm O_3. No significant effect on total number of lavageable cells or on the ability of neutrophils to injure epithelial cells.	Donaldson et al. (1991, 1993)
0.25 peak over a bkg of 0.06	490 118	13 h bkg, rose to peak and returned to bkg over 9 h	Rat, M (F344) 60 days old	Interstitial AMs increased in number in proximal alveolar region and TBs at one week of exposure, but the effects had subsided by 3 weeks of exposure.	Chang et al. (1992)

Table 6-5 (cont'd). Lung Inflammation and Permeability Changes Associated with Ozone Exposure[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.3 2.0	588 3,920	24, 48, or 72 h for 0.30 ppm and 3 h for 2.0 ppm	Mice (C57BL/6J[B6]); (C3H/HeJ[C3]) DBA/2J (D2), hybrids, and recombinant inbred strains (R1): BXD and BXH 6-8 weeks old	Inflammatory response was greater in B6 than in C3 or D2 mice. F1 progeny was categorized as resistant; F2 generation segregated into 45:16 for resistant vs. susceptible phenotypes. Among BXD R1 strains, 4 of 10 responded discordantly to the two exposures (0.3 and 2.0 ppm). Among BXD R1, 4 of 16 were discordant.	Kleeberger et al. (1990, 1993b)
0.35 0.5 1.0	686 980 1,960	2.25 h/day for 5 days	Rat, M (F344) 3-4 mo old	Persistent increase in BAL protein and progressive inflammation at ≥0.5 ppm.	Tepper et al. (1989)
0.35 0.5 0.65 0.8	686 980 1,274 1,568	2, 4, and 7 h	Rat, M (F344) 13 weeks old	C × T exposure design. All exposures included 45 min of CO_2 for 1 h to increase ventilation. BAL after pulmonary function tests completed. The quadratic model explained 92% of the variance. The models suggest that C may have a more dominant influence than T.	Tepper et al. (1994, in draft)
0.38 0.76 1.28 2.04	750 1,500 2,500 4,000	1, 2, 4, and 8 h daytime	Rat (Wistar) 7 weeks old	C × T exposure design. BAL protein measured at various times PE. Daytime exposures: At 0.76 ppm, maximal increase 22 h after exposure started; after 4 and 8 h of exposure, protein still elevated at 54 h from start of exposure.	Rombout et al. (1989)
0.13 0.26 0.38	250 500 750	4, 8, and 12 h nighttime		Nighttime exposures: Temporal increase and decrease of protein more gradual, with maximal response at 36 h after exposure started. Protein still elevated 72 h after start of 8- or 12-h exposure to 0.26 or 0.38 ppm. Smallest tested C × T effect was with 0.13 ppm × 4 h. Both: Multivariate regression analysis. Polynomial function shows that T has progressive influence as C increases.	
0.4 0.6	784 1,176	8 h/days for 90 days	Monkey, M (Bonnet) 5.2-8 years old	Inflammatory response in RBs at 0.64 ppm.	Moffatt et al. (1987)
0.4	800	12 h during day or night	Rat, M (Wistar) Guinea pig, M (Hartley) 9 weeks old	Nighttime exposure of rats resulted in greater increase in BAL protein, albumin, and PMNs than the daytime exposure. A similar difference was not observed in guinea pigs.	Van Bree et al. (1992)

Table 6-5 (cont'd). Lung Inflammation and Permeability Changes Associated with Ozone Exposure[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.5	980	2 h	Dog, M (Mongrel) 15 ± 0.9 kg, Baboon, M 25-40 kg	No effect on levels of 6-keto $PGF_{1\alpha}$, PGE_2, TXA_2, TXB_2, or $PGF_{2\alpha}$ in BAL.	Fouke et al. (1990, 1991)
0.5	980	Continuous exposure for 1-14 days	Mouse, F (Swiss) 20-25 g	PGE and total protein levels in BAL increased after the exposure, peaked at 3 days, then declined with time, but remained higher than the controls at 7 days; protein still increased at 14 days. Total cells in BAL decreased on Days 1 to 3 after exposure.	Canning et al. (1991)
0.5 1.0	980 1,960	4 h	Guinea pig, M (Hartley) 300-400 g	Depleting lungs of ascorbic acid enhanced effects of 0.5 but not 1.0 ppm on BAL protein. Depletion of lung nonprotein sulfhydryl had no effect.	Slade et al. (1989)
0.75	1,410	Continuous exposure for 3 days	Rat, M (Wistar) 200-250 g	Increased number of PMNs and AMs and elevated levels of albumin in the BAL. At 4 days PE, no PMNs were detected, but AM numbers and albumin levels were elevated.	Bassett et al. (1988a)
0.8	1,568	2 h	Rat, M (S-D) 300 g	Transient increase in tracheal and bronchoalveolar permeability, as revealed by tracer transport from airways to blood and tracer localization in intercellular spaces.	Bhalla et al. (1986) Bhalla and Crocker (1986)
0.6 0.8	1,176 1,568	2 h exposures during rest or exercise	Rat, M (S-D) 47-52 days old	Airway permeability increased after exposure of resting animals; trends of greater and more persistent effects in exercising group.	Bhalla et al. (1987)
0.8	1,568	2 h	Rat, M (S-D) 50-60 days old	Increased transport of radiolabeled tracers from blood to the air spaces following exposure.	Bhalla and Crocker (1987)
0.8	1,568	3 h	Rat, M (S-D) 250-300 g	The number of PMNs in lung parenchyma increased immediately after exposure, peaked at 8 h PE, and returned to baseline by 16 h PE. Total protein and albumin levels in BAL increased immediately after exposure, peaked at 8 h PE, and then declined with time, but the albumin levels were higher than the controls at 24 h PE.	Bhalla and Young (1992)
0.8	1,568	3 h	Rat, M (S-D) 250-300 g	Time-related changes in tracheal permeability, detected by tracer transport, and PMN influx in tracheal wall following exposure. Increase in permeability prior to increase in PMNs.	Young and Bhalla (1992)
0.8	1,568	2 h	Rat, M (F344) 11-12 weeks old	Increased DTPA transport across the tracheal mucosa and elevated levels of protein and albumin in BAL. Effects attenuated in leukopenic rats or rats pretreated with indomethacin or FPL 55712.	Bhalla et al. (1992)

Table 6-5 (cont'd). Lung Inflammation and Permeability Changes Associated with Ozone Exposure[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.8	1,568	2 h	Rat, M (F344) 250-275 g	PMNs isolated from blood of O_3-exposed rats displayed deformation of shape, indicative of motility and greater cell adhesion than the PMNs from air-exposed rats.	Bhalla et al. (1993)
0.8	1,568	3 h	Mouse, F (CD-1) 5 and 9 weeks old	Increase in PGE_2 in BAL in 5-week-old mice only; effect blunted by indomethacin pretreatment.	Gilmour et al. (1993b)
0.96	1,882	8 h	Monkey, M (Rhesus) 2-8.5 years old	Number of labeled PMNs into lung tissue and BAL increased immediately after exposure, peaked at 12 h PE, and returned to baseline by 24 h PE. Total labeled and unlabeled PMNs in BAL remained elevated at 24 h, but returned to control levels by 72 h PE. Total protein in BAL was elevated only at 24 h PE.	Hyde et al. (1992)
1.0	1,960	4 to 24 h	Rat (S-D) 63-70 days old	Total protein and PMNs in BAL and PMNs in the CAR of the lung increased with exposure duration, but the number of AMs in BAL decreased. Treatment with anti-rat-PMN serum resulted in elimination of PMNs in BAL, but it did not affect the O_3-induced increase in BAL protein.	Pino et al. (1992a,b)
1.0	1,960	5 min O_3 delivered to a localized area of lung via a Teflon catheter fitted to bronchoscope	Dog, M (Mongrel) 21.2 ± 0.5 kg	Number of PMNs in the subepithelial tissue increased at 1-3 h PE. Number of BAL PMNs increased at 24, but not at 1-3 h PE.	Kleeberger et al. (1989)
1.0	1,960	6 h	Rat, F (S-D) 8-9 and 13-17 weeks old	BAL 16 h PE. Enhanced responsiveness to O_3-induced inflammation and elevated protein levels in BAL developed during pregnancy, was maintained during lactation, and disappeared following lactation.	Gunnison et al. (1992b)
1.0	1,960	2, 4, or 6 h	Rat, M (S-D) 13, 18 days and 8 and 16 weeks old	BAL immediately after exposure. PGE_2 concentrations in BAL greatest in 13-day-old rats after 2 h of exposure, but in older rats the response was seen after 6 h of exposure. In 13-day-old rats, 50% of leukocytes in BAL were dead after 6 h of exposure; no such effect on 16-week-old adults. No age dependence for BAL protein increase or PMN increase.	Gunnison et al. (1992a)
1.0	1,960	2 h	Rat (S-D) 18 days or 14 weeks old Rabbit (NZW) 6, 11, 16, or 30 weeks old	BAL immediately after exposure. In youngest animals, greater amounts of PGE_2 and $PGF_{2\alpha}$. In youngest rabbits, 6-keto $PGF_{1\alpha}$ and TXB_2 increased. No effect on LTB_4. No age dependent effects on BAL protein or cell number.	Gunnison et al. (1990)

Table 6-5 (cont'd). Lung Inflammation and Permeability Changes Associated with Ozone Exposure[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
1.0	1,960	1 h	Guinea pig, M (Hartley) 300-400 g	Appearance of horseradish peroxidase in plasma, following its intratracheal administration, was accelerated at 2 and 8 h PE, but not at 24 h PE.	Miller et al. (1986)
1.0	1,960	1 h	Guinea pig, M (Hartley) 250-300 g	The concentrations of PGE_1, 6-keto $PGF_{1\alpha}$, and TXB_2 in BAL increased at various times following exposure.	Miller et al. (1987)
1.0	1,960	3 h isolated perfused lung	Rat (S-D) 350 ± 42 g	No effect on BAL protein.	Joad et al. (1993)
1.8	3,528	2 or 4 h	Rat, M (Wistar) 200-250 g	A decrease in number of AMs in BAL immediately after exposure. PMNs and albumin content of BAL increased at 1 day PE. Increased albumin levels, but not PMNs, persisted on Day 3 PE.	Bassett et al. (1988b)
2.0	3,920	4 h	Guinea pig, M (Hartley) 300-350 g	Interstitial PMNs increased in number immediately after exposure, but declined by 24 h PE. BAL PMNs were maximal by 3-6 h and remained elevated by 3 days PE.	Schultheis and Bassett (1991)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

In rats exposed for 2 h to 0.8 ppm O_3, labeled tracers, such as diethylenetriaminepentaacetate (DTPA) and bovine serum albumin, introduced into the airway lumen were transferred to blood to a greater extent than in the air-exposed rats (Bhalla et al., 1986; Bhalla and Crocker, 1986; Crocker and Bhalla, 1986). The rapidly rising concentration of the tracers in the blood during the initial period of instillation of the tracers into the airways reflected both the accumulation, due to slow instillation over a 5-min period, of the tracers in the airway lumen and subsequent transfer across the respiratory epithelium. The changes in permeability observed in this study were transient in nature, returning to the baseline value by 24 h postexposure in the trachea and by 48 h in the distal airways. Reversible increases in airway epithelial permeability also were observed in guinea pigs acutely exposed to 1 ppm O_3 (Miller et al., 1986). The rate of appearance of intratracheally administered horseradish peroxidase increased in blood at 2 and 8 h after O_3 exposure, as compared to rats at 24 h postexposure to O_3 and air-exposed controls. When rats were exercised at a level that increased the minute ventilation ($\dot{V}_E$) twofold, the effect of 0.8 ppm O_3 was not only greater than in rats exposed at rest, but the increased permeability persisted longer (Bhalla et al., 1987).

Guth et al. (1986) analyzed the permeability effects of O_3 by injecting radiolabeled albumin into the blood and measuring it in the BAL, as well as by measuring the total protein concentration in the BAL. This study revealed a concentration-dependent increase in permeability following a 6-h exposure of rats to $\geq$0.4 ppm or following 1 or 2 days of exposure to $\geq$0.12 ppm O_3. For example, after a 2-day exposure to 0.12 ppm, there was a 71% increase in BAL protein. Tracer transport also was increased in rats exposed for 2 h to 0.8 or 2.0 ppm O_3 (Crocker and Bhalla, 1986; Bhalla and Crocker, 1987).

The relative influence of concentration and duration of O_3 exposure was evaluated by three laboratories using BAL protein as an indicator of effects. In the first study, Rombout et al. (1989), exposed rats for 1, 2, 4, or 8 h to 0.38, 0.76, 1.28, or 2.04 ppm O_3 during the daytime (16 C $\times$ T products). A similar nighttime exposure study was conducted using 0.13 to 0.38 ppm O_3 and 4, 8, or 12 h of exposure (nine C $\times$ T products). The smallest C $\times$ T product causing an increase in protein was 0.52 ppm $\cdot$ h (0.13 ppm $\times$ 4 h). A multivariate regression analysis accounted for 88.6% of the variance in the daytime data and 73.2% in the nighttime data. Animals exposed during the night were more responsive. A quadratic polynomial function showed that the influence of T increased with increasing C and that the influence of T was still important at the lowest O_3 concentration tested (0.13 ppm). The second study employed rats and guinea pigs, each having 12 C $\times$ T products (0.1, 0.2, 0.4, and 0.8 ppm O_3; 2, 4, and 8 h) (Highfill et al., 1992). Using additional modeling approaches, they obtained similar results to those of Rombout et al. (1989) . For example, the exponential response surface model explained 86% of the variance in the data and showed that the influence of T increased as C increased. However, at low C $\times$ T products, similar BAL protein increases were observed. Further modeling of these data (Highfill and Costa, 1995) again showed that C and T had interdependent influences. Tepper et al. (1994) performed a similar C $\times$ T study with 12 C $\times$ T products (0.35 to 0.8 ppm O_3, 2 to 7 h). However, rats were exposed to 8% CO_2 for 45 min of each hour to increase ventilation, and BAL was conducted on lungs that had been measured for pulmonary function. The response surface predicted by the modeling again indicated that the influence of T increased as C increased. Histopathological observations in the rats support the findings that C had more influence than T. Tepper et al. (1994) compared their analysis of BAL protein to that of Highfill et al. (1992) and found very good agreement, even though

there were experimental differences. However, in Tepper et al. (1994), there were larger constants for C terms, indicating that C had a greater influence than in the Highfill et al. (1992) study, probably because Tepper and co-workers increased ventilation (and hence O_3 dose) by using concurrent CO_2 exposures.

Gelzleichter et al. (1992b) exposed rats to a single O_3 C × T (14.4 ppm · h) composed of 16 products (0.2 to 0.8 ppm O_3, 6 to 24 h/day for 3 days). They found that the 24 h/day exposure groups had significantly fewer responses than the other groups, which were all equivalent. Thus, in this study, C and T had equivalent influences on the response, except when T was 24 h/day. This study was well conducted, but had some basic differences from the Rombout et al. (1989) and Highfill et al. (1992) studies in that the longer exposure durations (i.e., 24 h/day) involved a mixture of daytime and nighttime exposure that likely altered the dose-rate of O_3. Also, Gelzleichter et al. (1992b) used one C × T product, whereas the other studies used several C × T products.

6.2.2.3 Concomitant Changes in Permeability and Inflammatory Cell Populations in the Lung

Polymorphonuclear leukocyte infiltration in the lung following O_3 exposure has been investigated in a number of studies (Table 6-1), either by analyzing the cellular content of the BAL or by counting PMNs in lung sections. Bassett et al. (1988a) found an increase in the number of inflammatory cells in the BAL of rats continuously exposed for 3 days to 0.75 ppm O_3. The inflammatory response was accompanied by elevated levels of albumin and lactate dehydrogenase, suggesting increased permeability and cellular injury. Comparable changes were also observed in rats acutely exposed to a higher O_3 concentration (Bassett et al., 1988b). In another study, a random count of PMNs in the lung sections at 4-h intervals, following a 3-h exposure of rats to 0.8 ppm O_3, revealed a gradual increase in the number of PMNs, with a peak at 8 h postexposure and a return to the baseline value by 16 h postexposure (Bhalla and Young, 1992). The total protein and albumin concentrations in the BAL also increased after the exposure, peaking at 8 h postexposure. Although the protein concentrations returned to baseline by 16 h postexposure, the albumin levels remained above the controls after 24 h. Alveolar changes, consisting of thickened septa, parenchymal cellularity, and increased numbers of free cells, began to increase between 12 and 16 h postexposure and were still increasing at 24 h postexposure.

In trachea of rats exposed for 3 h to 0.8 ppm O_3, a peak of PMN infiltration at 12 h postexposure was preceded by a decline in the number of PMNs in pulmonary capillaries, suggesting exit of PMNs from the blood vessels and their migration across the endothelial cells into the tracheal wall (Young and Bhalla, 1992). Although a significant change in the tracheal population of PMNs did not occur until 12 h after the end of exposure, tracheal permeability, as detected by DTPA transport, increased immediately following O_3 exposure. The results of this study suggest that the initial changes in tracheal permeability may be independent of an inflammatory response, but the recruited PMNs may serve to sustain the increased permeability and to amplify O_3 effects at later stages. This conclusion was based on the observed shift of PMNs from the vascular compartment into the tracheal wall and a concurrent peak of increased permeability. In comparable studies, Pino et al. (1992a) exposed rats to 1.0 ppm O_3 for periods ranging from 4 to 24 h. Total protein and the number of PMNs in the BAL increased with time, with the maximum increase at the end of 24 h of continuous exposure. The number of AMs was lower in the exposed animals than in the controls. By morphometry, the peak PMN response in the terminal bronchioles

(TBs) and alveolar ducts (ADs) occurred at 4 h after an 8-h exposure. In dogs, local exposure of peripheral airways to 1 ppm O_3 for a short period (5 min) produced a recognizable inflammatory response (Kleeberger et al., 1989). An increase in the number of PMNs was detected in the subepithelial tissue within 3 h after 5 min of exposure of the dogs, but the response had subsided 24 h later. In BAL, on the other hand, an increase in the number of PMNs was not observed at 3 h postexposure; the number of PMNs increased at 24 h.

Hotchkiss et al. (1989a,b) have investigated the effects on AMs and PMNs of a 6-h O_3 exposure of rats to 0.12, 0.8, or 1.5 ppm O_3 and compared the inflammatory responses by nasal lavage and BAL, as well as by morphometry in the nose and the CAR of the lung, a site at which abnormal cellular changes generally occur following O_3 exposure. Animals were examined 3, 18, 42, or 66 h after exposure ceased. From lavage data, 0.12 ppm O_3 had no effect. At 0.8 ppm, there was an increase in the number of nasal PMNs lavaged immediately after exposure, which tapered off (no significant change at 42 h postexposure). In contrast, BAL PMNs increased later, beginning at 18 h postexposure and peaking at 42 h postexposure. From morphometric data, 0.12 ppm O_3 caused an increase in nasal PMNs 66 h postexposure. At 0.8 ppm, nasal PMNs increased to their greatest extent immediately after exposure and still were increased at later time periods. However, PMNs in the lung increased only at 18 and 66 h postexposure. The interpretation of these results was based on the presence of potential competing mechanisms in the nose and lungs. Therefore, the attenuation of the nasal effects are matched by simultaneous enhancement of the inflammatory response in the lung. Whether such a balance between nasal and alveolar PMNs represents a specialization restricted to rats or is a more general phenomenon remains to be investigated. A similar balance was not observed in humans exposed to O_3 (see Chapter 7). Subtle differences in species, O_3 concentrations, and exposure durations, however, need to be considered when making interspecies comparisons.

Hyde et al. (1992) investigated the inflammatory response in monkeys exposed to 0.96 ppm O_3 for 8 h. Polymorphonuclear leukocytes were isolated from peripheral blood, labeled with indium-111-labeled tropolonate and infused into the cephalic vein of monkeys 4 h before necropsy. Labeled PMNs in the lung tissue and the BAL peaked at 12 h and returned to control values by 24 h postexposure. The total number of labeled and unlabeled PMNs in the BAL, however, remained elevated at 24 h postexposure, but returned to baseline by 72 h. Furthermore, the PMN peak at 24 h postexposure coincided with the maximum increase in BAL protein at this time point. These studies suggest a strong correlation between BAL protein concentration, epithelial necrosis, and inflammatory cells (especially eosinophils) in bronchi, but not in the trachea or bronchioles. This observation may represent a species-specific response. In rats, the inflammatory response in the terminal airways involved an increase in the number of migratory cells, including PMNs, but not eosinophils (Pino et al., 1992a). The available literature suggests that the precise time point at which the maximum change in the number of inflammatory cells occurs is variable and may be dependent on several factors, including animal species, concentration, duration of exposure, and mode of analysis (i.e., BAL versus morphometry of lung parenchyma). Because the PMNs sampled by BAL represent only a small fraction of the cells shown to be present in the air spaces by morphometry (Downey et al., 1993), the inflammatory response detected by analyzing BAL may or may not match the response obtained by microscopic analysis of tissue sections. Even when the PMN response detected by the BAL analysis accurately reflects the tissue PMNs (Hotchkiss et al., 1989a), the times at which the PMN

response peaks do not necessarily coincide when the analyses are made by the two procedures. Therefore, the mode of analysis (BAL versus morphometry) and the time at which this analysis is made need to be taken into account when analyzing the inflammatory response. This recommendation is consistent with the conclusion of Schultheis and Bassett (1991) that BAL does not necessarily reflect cellular changes in the lung interstitium.

Another approach to studying the inflammatory impact of O_3 and its effects on airway permeability is based on exposure of rats to drugs that destroy leukocytes or block the activity of chemical mediators released by these cells. To determine whether the PMNs play a role in O_3-induced increased permeability, Pino et al. (1992b) studied O_3 effects in PMN-depleted rats. Although ip injection of anti-PMN serum resulted in a nearly complete depletion of PMNs in rats, it did not affect the increase in BAL protein following an 8-h exposure to 1.0 ppm O_3. In comparable studies, rats were rendered leukopenic by ip injection of cyclophosphamide (Bhalla et al., 1992). A 2-h exposure of untreated rats to 0.8 ppm O_3 caused a significant increase in the tracheal mucosal permeability, as measured by enhanced trachea-to-blood transport of ^{99m}Tc-radiolabeled DTPA immediately postexposure and accumulation of protein and albumin in BAL at 12 h postexposure. Pretreatment with cyclophosphamide, a potent immunosuppressive agent, which can be toxic to the lung, did not change baseline values but did eliminate the O_3 response. The reasons for the discrepancy between these results and the results of the anti-PMN serum treatment study of Pino et al. (1992b) are not entirely clear, but it is likely that the O_3 effects are dependent, in part at least, on an interaction between different inflammatory cell types. Therefore, it is not unreasonable to assume that, in the absence of PMNs, their role is taken up by another cell type. The attenuation of O_3 effects also was observed in rats pretreated with indomethacin, an inhibitor of cyclooxygenase products, and FPL55712, which blocks LTD_4 activity by preventing its binding to the receptors (Bhalla et al., 1992). Based on these results, it was proposed that, although O_3 is capable of producing direct injury to cells, inflammatory cells and their products may contribute to the injury process (Bhalla et al., 1992). This conclusion is supported by the recent studies of Joad et al. (1993). In the isolated perfused rat lung, PMNs (but not O_3) increased BAL protein concentration. However, PMNs acted synergistically with O_3 in the induction of epithelial injury in the bronchioles. The recent demonstration of the effects of O_3 (0.8 ppm, 2 h) on some of the cellular activities of vascular PMNs (Bhalla et al., 1993) further suggests potential mechanisms involved in the stimulation of PMNs and the induction of inflammatory response. Polymorphonuclear leukocytes isolated from the blood of rats exposed to O_3 displayed shape changes, indicative of cell motility, and greater adhesion to epithelial cells in culture than did the PMNs from rats exposed to purified air.

6.2.2.4 Sensitive Populations

In addition to investigating the inflammatory response and permeability changes in healthy adult animals, studies in recent years have analyzed the effects of O_3 on lung inflammation and airway permeability in different animal species, in potentially susceptible subpopulations, and under special conditions (Table 6-1). Hatch et al. (1986) performed an interspecies comparison to determine their relative responsiveness to O_3. Although the baseline BAL protein concentration of all the species was nearly the same, there were noticeable differences in changes in BAL protein concentration among different species following their exposure to O_3. Significant changes were observed in guinea pigs exposed to 0.2 ppm O_3. Mice, hamsters, and rats responded at O_3 concentrations of 1 ppm and above,

but rabbits responded only to 2 ppm O_3. In the case of rats, no differences were observed in the sensitivity between males and females. When Slade et al. (1989) depleted guinea pigs of lung ascorbic acid, they were more susceptible to an O_3-induced increase in BAL protein when the 4-h exposure was to 0.5 but not 1.0 ppm. Depletion of lung nonprotein sulfhydryls did not enhance susceptibility. Although Hu et al. (1982) report no elevation in BAL protein in O_3-exposed, vitamin C-deficient guinea pigs, when their data were statistically reanalyzed by Slade et al. (1989), vitamin C deficiency enhanced the effects of 0.5 but not 1.0 ppm O_3.

Kleeberger et al. (1990) found interstrain differences in inbred mice with regards to inflammation and permeability changes following high-concentration (2-ppm) O_3 exposure for 3 h that led the investigators to propose that the PMN response to O_3 may be controlled by a single autosomal recessive gene at a chromosomal location designated as "Inf" (inflammation) locus. In the follow-up studies, Kleeberger et al. (1993a) exposed the "susceptible" (C57BL/6J) and the "resistant" (C3H/HeJ) strains of mice to lower concentrations of O_3. Although changes in inflammatory response and BAL protein were observed after exposure for 24 to 72 h to 0.12 as well as 0.3 ppm O_3, the elevation in response in the susceptible strain over that in the resistant strain was observed only at 0.3 ppm O_3. Further studies with recombinant inbred strains of mice suggested that genes at different loci may be responsible for responses to 24-h (Inf locus) and 48-h (Inf-2 locus) O_3 exposures (Kleeberger et al., 1993b).

Gunnison et al. (1992a) exposed rats aged 13 and 18 days and 8 and 16 weeks old to 1 ppm O_3 for 2, 4, or 6 h. In the experiments to be discussed here, BAL was performed immediately after exposure. Ozone exposure resulted in an increase in protein in the BAL and a decrease in the number of leukocytes, but this decrease was not specific for a certain age group. A weak relationship was observed between age and the number of lavageable PMNs; a slightly greater influx of PMNs was observed in the younger rats. A strong inverse relationship was, however, observed between age and leukocyte viability. Approximately 50% of the total leukocytes recovered in the BAL from 13-day-old rats exposed for 6 h were dead, as compared to about 10% dead in the 16-week-old rats. Furthermore, 13-day-old animals were more responsive to a 2-h O_3 exposure than the other age groups of rats in terms of PGE_2 levels in BAL; PGE levels were enhanced more in older animals with the longer exposure durations. The authors attribute the increase in PGE_2 to an increased release of arachidonic acid, rather than an effect on metabolism or formation on PGE_2. Gunnison et al. (1990) also have shown that levels of several eicosanoids in rabbits show a similar pattern of age-responsiveness.

Factors such as physical activity and pregnancy, in addition to age, can modify the airway sensitivity of rats to O_3. Van Bree et al. (1992) have reported circadian variation in response to O_3. In rats exposed to 0.4 ppm O_3 for 12 h, about 70% more PMNs were recovered in the BAL after nighttime exposure than after daytime exposure. This increase was attributed to greater physical activity and increased ventilation in the nocturnal animals. In guinea pigs, a similar difference between daytime and nighttime exposures was not observed; instead, the variations appeared to be related to random physical activity. The nighttime exposures also caused a greater increase in BAL protein and albumin in rats but not in guinea pigs. Gunnison et al. (1992b) have found that pregnant rats are more responsive to O_3 (1 ppm for 6 h) than virgin females, as measured by an enhanced inflammatory response and as detected by the analysis of protein, PMNs, leukocytes, and enzyme activities in BAL at 18 h postexposure. When O_3 exposure occurred on Day 17 of pregnancy or Days 3, 13, and 20 of lactation, the magnitude of the increase in BAL protein

and number of PMNs was greater than the magnitude of increase in virgin rats. No such increased responsiveness was observed in rats at Day 10 to 12 of pregnancy or 14 days after lactation ceased. Enzyme changes followed a similar pattern.

6.2.2.5 Repeated Exposures

The magnitudes of some of the effects of O_3 observed after an acute exposure were smaller following repeated exposure. This phenomenon has been referred to as tolerance, adaptation, or attenuation. Most of the older literature is on tolerance, which classically is defined as the phenomenon in which a previous exposure to a nonlethal concentration of O_3 provides protection against an otherwise lethal level. These studies and others at high O_3 levels are discussed in the last O_3 criteria document (U.S. Environmental Protection Agency, 1986).

Tepper et al. (1989) observed attenuation of pulmonary function changes in rats exposed for 2.25 h/day for 5 days, but a corresponding attenuation of lung inflammation did not occur. Histologic examination of the lung sections revealed substantially more inflammatory cells in alveoli after 5 days of exposure to 0.5 ppm O_3 than after a single exposure to the same O_3 concentration. Increased protein concentration in the BAL observed after a single exposure also persisted after 5 days of repeated exposures. The morphologic studies of Moffatt et al. (1987) identified an inflammatory response in the respiratory bronchioles (RBs) of bonnet monkeys exposed for 8 h/day for 90 days to 0.64 ppm O_3. Significantly greater numbers of AMs, mast cells, and PMNs reflected persistence of inflammation following repeated exposures. Chang et al. (1992) exposed rats to an ambient pattern of O_3. In this morphometric study, the responses (epithelial inflammation in the proximal alveolar region and the TBs, interstitial edema, and infiltration of AMs) to 1 week of O_3 exposure had subsided after 3 and 13 weeks of exposure. Donaldson et al. (1993) did not find a change in the total number of cells in BAL of rats exposed for 7 h/day for 4 days to O_3 concentrations ranging from 0.2 to 0.8 ppm. The number of PMNs, however, increased after exposure to 0.6 and 0.8 ppm O_3. This increase was greatest after the first day of exposure, but it was resolved by Day 4. In the studies of Mochitate et al. (1992), the number of BAL AMs of rats exposed to 0.2 ppm O_3 for 11 weeks was about 60% greater than in the air-exposed controls. The preferential increase in the number of small AMs was not dependent on an enhancement of DNA synthesis. It was concluded that AMs adapt to long-term exposures as a result of recruitment of immature AMs from an influx of monocytes. No increase in the number of PMNs was observed in the BAL of exposed rats.

When analyzing the PMN data from different studies like the ones discussed above, it is important to make a distinction between the PMN response in the lung interstitium versus that observed in the BAL. It is possible that, although the inflammatory response may persist after repeated exposures, the PMNs do not necessarily continue to migrate from pulmonary interstitium into the air spaces. As a result, the inflammatory response is detected in the histological sections of the lung but not in the BAL.

6.2.2.6 Mediators of Inflammation and Permeability

Although the presence of PMNs in the lung in large numbers is regarded as evidence of a morphological response to O_3, the release of chemical mediators by inflammatory cells indicates a state of activation and represents the functional modification as a consequence of O_3 exposure. Mediators with biological and chemotactic properties have been shown to be released as a result of stimulation or injury of AMs, epithelial cells, and

PMNs. Arachidonic acid metabolites play an important role in a variety of processes, including inflammatory response and permeability changes. Driscoll and Schlesinger (1988) found that, although AMs isolated from rabbit lungs continually released chemoattractant factors for monocytes and PMNs, an in vitro exposure of AMs to 0.3 and 1.3 ppm O_3 resulted in the increased secretion of factors that stimulated the migration of PMNs. Driscoll et al. (1988) also found increased eicosanoid biosynthesis following O_3 exposure. In the latter studies, elevated levels of PGE_2 and $PGF_{2\alpha}$ were detected in the supernatant following in vitro exposure of rabbit AMs to 0.3 and 1.2 ppm O_3 for 2 h. In a parallel in vivo study, an effect was seen only at the higher O_3 concentration. In vitro exposure in a roller-bottle system of rat AMs to 1 ppm (but not 0.1 ppm) O_3 causes stimulation of both cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism, as shown by substantial increases in the levels of 6-keto-$PGF_{1\alpha}$, thromboxane B_2 (TXB_2), PGE_2, LTB_4, LTD_4, and 15-hydroxy-eicosatetraenoic acid (15-HETE) in the supernatant of the AM culture (Madden et al., 1991). The authors attribute these effects to both an increase in the availability of arachidonic acid and a stimulation of cycloxygenase and lipoxygenase activities. Another in vitro study also demonstrated effects on arachidonic acid metabolism (Leikauf et al., 1988) . Interpretation of these in vitro studies is difficult. When Gunnison et al. (1990) compared the effect of in vitro and in vivo exposures of AM to O_3 on eicosanoid metabolism of AMs in culture, a disparity was found. Cultured AMs from O_3-exposed rabbits had a decrease in the elaboration of $PGF_{2\alpha}$; in vitro exposure caused an increase.

Changes in the levels of eicosanoids also have been observed in in vivo studies. Schlesinger et al. (1990) found elevation of PGE_2 and $PGF_{1\alpha}$ in BAL of rabbits immediately following a 2-h exposure to 1 ppm O_3. Age may play a role. Five-week-old (but not 9-week-old) mice had increased levels of PGE_2 in BAL (Gilmour et al., 1993b). Lower O_3 concentrations did not affect the levels of BAL eicosanoids. Hyde et al. (1992) found an increase in BAL concentrations of $PGF_{2\alpha}$, PGD_2, and PGE_2 following an 8-h exposure of monkeys to 0.96 ppm O_3. Prostaglandin concentrations in the BAL, detected using an antibody that did not distinguish PGE_1 from PGE_2, also increased with time following a continuous exposure of mice to 0.5 ppm O_3 (Canning et al., 1991). The peak levels of PGE at 3 days were followed by a decline with time, but the levels remained higher than the controls after 14 days of exposure. The time course of changes in the PGE levels was matched by the time sequence of changes in BAL protein following exposure to 0.5 ppm O_3. Plasma concentrations of 6-keto-$PGF_{1\alpha}$ and PGE_1 also were elevated in guinea pigs exposed for 1 h to 1 ppm O_3 (Miller et al., 1987). Kleeberger et al. (1989) delivered 1 ppm O_3 for 5 min to a lobar bronchus of dogs using a wedged bronchoscope. An analysis of the lavage fluid collected at 1 min postexposure revealed significant increases in the concentrations of PGD_2 and histamine. Although, in this study, the concentration of TXB_2 did not change after O_3 exposure, significant increases in concentrations of TXB_2 in the plasma and BAL were observed following acute exposure of guinea pigs to 1 ppm O_3 (Miller et al., 1987) and humans to lower levels of O_3 (see Chapter 7). In addition, increased plasma concentrations of $PGF_{1\alpha}$ and PGE_1 were observed in the guinea pigs exposed to O_3. Fouke et al. (1990, 1991) were unable to detect changes in the BAL concentrations of 6-keto-$PGF_{1\alpha}$, PGE_2, TXB_2, and $PGF_{2\alpha}$ in baboons and mongrel dogs exposed to 0.5 ppm O_3 for 2 h. The reasons for the lack of this response in the baboon are not entirely clear, but the lower O_3 concentration used in this study, as compared to the exposure concentrations in dogs and guinea pigs, and species differences offer possible explanations for the discrepancy.

Prostaglandin E_1 and E_2 have been shown to influence the inflammatory processes in the lung. Intrabronchial or iv administration of PGE_2 was accompanied by increased accumulation of the inflammatory cells in the lung and elevation of BAL protein (Downey et al., 1988). A possible mechanism involved in the proinflammatory effects of the PGEs included arteriolar vasodilation without venodilation, resulting in increased transfer of proteins and cells from blood into the lung by hydrostatic pressure.

6.2.2.7 Summary

The airway epithelial lining serves as an efficient barrier against penetration of exogenous particles and macromolecules into the lung tissue and circulation and against entry of endogenous fluids, cells, and mediators into the air spaces. Disruption of this barrier following O_3 exposure represents a state of compromised epithelial defenses, leading to increased transepithelial permeability. Inflammatory cells represent another important component of pulmonary defenses. The recruitment of these cells into the lung following O_3 exposure could result in the release of mediators capable of damaging other cells in the lung.

Toxicological studies from several laboratories demonstrate alterations in epithelial permeability and inflammatory responses in animals exposed to O_3 concentrations of 1.0 ppm and below. In these studies, an inflammatory response, as detected by an increase in the number of PMNs in the BAL or in lung parenchyma, was accompanied by either an increased tracer transport across the airway mucosa or an elevation in the levels of total protein or albumin in the BAL. These changes were observed in animals exposed to O_3 concentrations as low as 0.1 ppm in rabbits (2 h/day for 6 days of exposure [Driscoll et al., 1987]; 0.12 ppm in mice (24 h-exposure [Kleeberger et al., 1993a]) and rats (6-h exposure [Hotchkiss et al., 1989a] and 24-h exposure [Guth et al., 1986]); and 0.2 ppm in guinea pigs (4-h exposure [Hatch et al., 1986]). Although monkeys also exhibit inflammatory responses, concentrations this low have not been tested in this species. The magnitude of response and the time at which it peaked appeared to vary with O_3 concentration, exposure duration, and the mode of analysis. Investigations of $C \times T$ relationships for BAL protein in both rats and guinea pigs showed that T had increasing influence as C increased (Rombout et al., 1989; Highfill et al., 1992; Highfill and Costa, 1995; Tepper et al., 1994). However, at low $C \times T$ products, similar increases were observed (Highfill et al., 1992). The responsiveness to O_3 also depended on the animal species tested (Hatch et al., 1986) and increased under certain conditions, such as physical activity (Van Bree et al., 1992) and pregnancy and lactation (Gunnison et al., 1992b).

To determine the impact of inflammatory cells on O_3-induced airway permeability, rats were exposed to drugs that either destroyed the inflammatory cells or blocked the activity of their products. Treatment of rats with anti-PMN serum resulted in the depletion of PMNs but did not affect the increase in BAL protein produced by O_3 exposure (Pino et al., 1992b). Depletion of all the leukocytes by cyclophosphamide or treatment of rats with PG and LT antagonists resulted in an attenuation of the O_3 effects on permeability (Bhalla et al., 1992).

Inflammatory cells, when activated, are capable of releasing mediators with pathophysiologic and a variety of modulating activities. An increase in the release of arachidonic acid metabolites following O_3 exposure has been shown after both in vitro (Driscoll and Schlesinger, 1988; Driscoll et al., 1988; Madden et al., 1991; Leikauf et al.,

1988) and in vivo exposures (Schlesinger et al., 1990; Miller et al., 1987; Canning et al., 1991).

Some of the effects seen after an acute exposure to O_3 are modified on repeated exposure. The responses following repeated exposures included persistence or an increase in the number of PMNs or AMs on exposure of rats to 0.5 ppm (2.25 h/day) for 5 days (Tepper et al., 1989) or exposure of monkeys to 0.64 ppm for 90 days (Moffatt et al., 1987). Other studies report a reduced inflammatory response following repeated exposures (Chang et al., 1992; Donaldson et al., 1993). The section on morphometry (Section 6.2.4) has an extended discussion of microscopically evaluated inflammatory responses.

In brief, these studies show that acute exposures to O_3, at concentrations of 0.12 ppm and above, are capable of producing inflammatory and permeability changes in laboratory animals. It is clear that an assessment of the effects of O_3 and interpretation of the results requires that several factors be taken into consideration; these include O_3 concentration, duration of exposure, exposure conditions (e.g., repeated versus continuous exposure, daytime versus nighttime exposure, rest versus exercise during exposure), animal species, method of evaluation, sensitive populations, and time of analysis postexposure.

6.2.3 Effects on Host Defense Mechanisms

6.2.3.1 Introduction

The mammalian respiratory tract has a number of closely integrated defense mechanisms that, when functioning normally, provide protection from the adverse effects of a wide variety of inhaled particles and microbes (Green et al., 1977; Kelley, 1990; Schlesinger, 1989; Sibille and Reynolds, 1990). For simplicity, these interrelated defenses can be divided into two major parts: (1) nonspecific (transport and phagocytosis) and (2) specific (immunologic) defense mechanisms. A variety of sensitive and reliable methods have been used to assess the effects of O_3 on these components of the lung's defense system to provide a better understanding of the health effects associated with the inhalation of this pollutant.

The previous *Air Quality Criteria for Ozone and Other Photochemical Oxidants* (U.S. Environmental Protection Agency, 1986) provided a review and evaluation of the scientific literature published up to 1986 regarding the effects of O_3 on host defenses. This section briefly summarizes the existing database through 1986; describes the data generated since 1986; and, where appropriate, provides interpretations of the data. This section also discusses the various components of host defenses, such as the mucociliary escalator, the phagocytic and regulatory role of the AMs, the immune system, and integrated mechanisms that are studied by investigating the host's response to experimental pulmonary infections.

6.2.3.2 Mucociliary Clearance

This nonspecific defense mechanism removes particles deposited on the mucous layer of the conducting airways by ciliary action. Ciliary movement directs particles trapped on the overlying mucous layer toward the pharynx, where it is swallowed or expectorated. The effectiveness of the mucociliary transport system can be measured by the rate of transport of deposited particles, the frequency of ciliary beating, and the structural integrity of the cells that line the conducting airways. Impaired mucociliary clearance can result in an unwanted accumulation of cellular secretions, increased infections, chronic bronchitis, and complications associated with chronic obstructive pulmonary disease.

Studies cited in the previous criteria document (U.S. Environmental Protection Agency, 1986) provided evidence on the effect of O_3 on the morphologic integrity of the mucociliary escalator and its ability to transport deposited particles from the respiratory tract. For example, a number of studies with various animal species reported morphologic damage to the cells of the tracheobronchial tree from exposures to O_3 (see Section 6.2.4). The cilia had become noticeably shorter or were completely absent. Based on such morphologic observations, related effects such as ciliostasis, increased mucous secretion, and a slowing of mucociliary transport rates might be expected. Functional studies on mucociliary transport of deposited particles from the respiratory tract have, in general, observed a delay in particle clearance in early time periods following acute exposure. For example, a 4-h exposure of rats to 0.8 ppm O_3 slowed early clearance of inhaled latex spheres (Phalen et al., 1980).

Since the publication of the previous criteria document (U.S. Environmental Protection Agency, 1986), several studies have been performed on the effects of acute O_3 exposure on the mucociliary transport apparatus (Table 6-6). Retarded mucociliary particle clearance was observed following a 2-h exposure of rabbits to 0.6 ppm O_3; extended exposures (up to 14 days) caused no effects (Schlesinger and Driscoll, 1987). Acute exposure of adult sheep for 4 h/day for 2 days to 0.5 ppm O_3 increased basal secretion of glycoproteins in sheep trachea, whereas a longer exposure (4 h/day, 5 days/week for 6 weeks) to 0.5 ppm O_3 reduced tracheal glycoprotein secretions (Phipps et al., 1986). In a similar manner, continuous exposure of ferrets to 1.0 ppm O_3 for 3 days increased tracheal gland secretion of glycoproteins, which remained elevated following 7 days of exposure (McBride et al., 1991). Because the integrity of the periciliary space is vital for efficient mucociliary action, O_3-induced hyper- or hyposecretion by the mucous glands along the conducting airways can alter the effectiveness of the mucociliary escalator.

Mariassy et al. (1990) exposed sheep during the first week of life to 1.0 ppm O_3 for 4 h/day for 5 days and observed retardation of normal morphologic development of the tracheal epithelium and a decrease in the tracheal mucous velocity. In a similar manner, exposure of sheep during the first week of life for 4 h/day for 5 days to 1.0 ppm O_3 decreased epithelial mucosa density and retarded the developmental decrease of tracheal mucous cells and their carbohydrate composition (Mariassy et al., 1989). Finally, exposure of adult sheep for either 2 h or for 5 h/day for 4 days to 1.0 ppm O_3 decreased tracheal mucous velocity (Allegra et al., 1991).

6.2.3.3 Alveolobronchiolar Transport Mechanism

In addition to the transporting of particles deposited on the mucous surface layer of the conducting airways, particles deposited in the deep lung may be removed either up the respiratory tract or through interstitial pathways to the lymphatic system (Green, 1973). The pivotal mechanism of alveolobronchiolar transport involves the movement of AMs with phagocytized particles to the bottom of the mucociliary escalator. Failure of the AMs to phagocytize and sequester the deposited particles from the vulnerable respiratory membrane can lead to particle entry into the interstitial spaces. Once lodged in the interstitium, particle removal is more difficult and, depending on the toxic or infectious nature of the particle, its interstitial location may allow the particle to set up a focus for pathologic processes. Although Phalen et al. (1980) and Kenoyer et al. (1981) observed decreases in early (tracheobronchial) clearance after acute O_3 exposure of rats; late (alveolar) clearance was accelerated.

Table 6-6. Effects of Ozone on Host Defense Mechanisms: Physical Clearance[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.06 base, spike rising to 0.25	118 base, spike rising to 490	13 h/day, 7 days/week base; ramped spike 9 h/day, 5 days/week; 6 weeks	Rat, M (F344) 35 days old	Increased retention of asbestos fibers in the lung parenchyma.	Pinkerton et al. (1989)
0.1 0.6 1.2	196 1,176 2,352	2 h/day for 1 or 13 days	Rabbit, M (NZW) 2.5-3.0 kg	Acceleration of early alveolar particle clearance at 0.1 and 0.6 ppm for 13 days. After single exposure, increased clearance at 0.1 ppm and decrease at 1.2 ppm.	Driscoll et al. (1986)
0.1 0.6 1.2	196 1,176 2,352	2 h/day for 1 or 13 days	Rabbit, M (NZW) 2.5-3.0 kg	Acceleration of early alveolar particle clearance at 0.1 and 0.6 ppm for 13 days. After single exposure, increased clearance at 0.1 ppm and decrease at 1.2 ppm	Driscoll et al. (1986)
0.1 0.25 0.6	196 490 1,176	2 h/day for 14 days	Rabbit, M (NZW) 2.5-2.7 kg	Retarded mucociliary particle clearance at 0.6 ppm only following a single 2-h exposure; no effect of 14-day exposure.	Schlesinger and Driscoll (1987)
0.5	980	4 h/day, 5 days/week for 6 weeks	Sheep, F 23-41 kg	Increase of tracheal glycoprotein secretion following acute exposure (4 h/day for 2 days) with a decrease following longer term exposure.	Phipps et al. (1986)
1.0	1,960	24 h/day for 7 days	Ferret	Increased secretion of glycoconjugates by tracheal glands.	McBride et al. (1991)
1.0	1,960	4 h/day for 5 days	Sheep 1st week of life	Retardation of normal morphologic development of the tracheal epithelium. Decreased tracheal mucous velocity. Decreased tracheal mucosa epithelial density. Retardation of developmental decrease of tracheal mucous cells and their carbohydrate composition.	Mariassy et al. (1989, 1990)
1.0	1,960	2 h and 5 h/day for 4 days	Sheep 26-41 kg	Decreased tracheal mucous velocity.	Allegra et al. (1991)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

Exposure of rabbits for 2 h/day for 13 days to 0.1 and 0.6 ppm O_3 resulted in acceleration of early alveolar clearance of polystyrene latex particles (Driscoll et al., 1986). After a single exposure to 0.1 ppm, the greatest acceleration occurred over the period shortly after exposure ceased (1 to 4 days), although the effect was still observed at Day 14 postexposure. A single exposure to 0.6 ppm caused no effect, whereas a higher concentration (1.2 ppm) retarded clearance. To investigate the effects of longer term O_3 exposure on alveolobronchiolar clearance, rats were exposed to an urban pattern of O_3 (continuous 0.06 ppm, 7 days/week with a slow rise to a peak of 0.25 ppm and subsequent decrease to 0.06 ppm over a 9-h period for 5 days/week) for 6 weeks and were exposed 3 days later to chrysotile asbestos, which can cause pulmonary fibrosis and neoplasia (Pinkerton et al., 1989). Ozone did not affect the deposition of asbestos at the first AD bifurcation, the site of maximal asbestos and O_3 deposition. However, 30 days later, the lungs of the O_3-exposed animals had twice the number and mass of asbestos fibers as the air-exposed rats.

6.2.3.4 Alveolar Macrophages

Within the gaseous exchange region of the lung, the first line of defense against microorganisms and nonviable particles that reach the alveolar surface is the AM. This resident phagocyte is responsible for a variety of activities, including the detoxification and removal of inhaled particles, maintenance of pulmonary sterility, and interaction with lymphocytes for immunologic protection. Under normal conditions, AMs seek out particles deposited on the alveolar surface and ingest them, thereby sequestering the particles from the vulnerable respiratory membrane. If the particle is insoluble, the AMs serve as a repository for the transport of the particle from the alveolus to the bottom of the mucociliary escalator located at the far distal portion of the conducting airways. Degradable particles are detoxified by powerful lysosomal enzymes, whereas microorganisms are killed by biochemical mechanisms, such as superoxide anion radicals and lysosomal enzymes. To adequately fulfill their defense function, the AMs must maintain active mobility, a high degree of phagocytic activity, and an optimally functioning biochemical and enzyme system.

As discussed in the previous criteria document (U.S. Environmental Protection Agency, 1986), short periods of O_3 exposure can cause a reduction in the number of free AMs available for pulmonary defense, and these AMs are more fragile, less phagocytic, and have decreased lysosomal enzyme activities. The lowest O_3 concentration showing AM effects in this early work was 0.25 ppm; a 3-h exposure of rabbits decreased lysosomal enzyme activities (Hurst et al., 1970).

Since the publication of the previous criteria document, the studies performed have been, in general, confirmatory of previous observations (Table 6-7). Morphologic observations showed that continuous exposure for 7 days to 0.13, 0.25, 0.5, and 0.77 ppm O_3 resulted in concentration-related increases in the number of rat AMs at 5 days postexposure, as well as increased AM size and morphologic changes consisting of surface microvilli and bleb formation (Dormans et al., 1990). Other morphological studies discussed in Section 6.2.4 also show increased numbers of AMs. A 2-h exposure to 0.1 ppm O_3 did not affect the AM number in the BAL when the analyses were made immediately postexposure, but, 7 days later, the total number of AMs increased by about 70% (Driscoll et al., 1987). On repeated exposure for 6 or 13 days, the number of AMs increased on the day after exposure. A single exposure to a higher concentration (1.2 ppm O_3) did not affect the number of AMs when assessed immediately after exposure. It is assumed that, although

Table 6-7. Effects of Ozone on Host Defense Mechanisms: Macrophage Alterations[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.05 0.1 0.2 0.4	98 196 392 784	16 h	Rat, M (Wistar) 210 ± 10 (SD)g	Increased adherence to nylon fibers at 0.05 and 0.1 ppm, but not at 0.2 and 0.4 ppm.	Veninga and Evelyn (1986)
0.1 0.2	196 392	Continuous for 11 weeks	Rat, M (Wistar) 16-21 weeks old	Increases in enzyme activity.	Mochitate et al. (1992)
0.1 1.2	196 2,352	2 h/day for 1, 2, 6, and 13 days	Rabbit, M (NZW) 2-4 mo old	Single exposure: Decreased phagocytosis at 0.1 and 1.2 ppm immediately and 1 day after exposure; recovery by 7 days after exposure in the 0.1-ppm group. Multiple exposure to 0.1 ppm only: decreased phagocytosis 1 day after 2 and 6 days of exposure.	Driscoll et al. (1987)
0.1 1.2	196 2,352	2 h	Rabbit (NZW)	Increased release of PGE_2 and $PGF_{2\alpha}$ at 1.2 ppm; no effect at 0.1 ppm.	Driscoll et al. (1988)
0.11 to 3.6	216 to 7,056	3 h	Mouse, M (Swiss) Rat, F (S-D)	Concentration-dependent decrease in superoxide anion radical production; mouse more sensitive. No effect on murine AM phagocytosis at 0.42, 0.95, 1.0, and 1.2 ppm.	Ryer-Powder et al. (1988) Amoruso et al. (1989)
0.12 0.8 1.5	235 1,568 2,940	6 h	Rat, M (F344) 12-18 weeks old	At 42 and 66 h PE, concentration-dependent increase in mitotic index beginning at 0.8 ppm; increased size at 1.5 ppm at 18 and 42 h PE.	Hotchkiss et al. (1989b)
0.12 0.27 0.8	235 529 1,568	6 h	Rat, F (F344) 12-14 weeks old	Increase in mitotic index and chromosome damage at 0.27 and 0.8 ppm, no effect at 0.12 ppm.	Rithidech et al. (1990)
0.12 0.25 0.5	235 490 980	20 h/day for 1, 2, 3, 7, and 14 days	Rat, M (F433)	Increase in AM DNA synthesis at 2 and 3 days at 0.25 ppm O_3 and at 1, 2, and 3 days at 0.50 ppm O_3.	Wright et al. (1987)
0.13 to 0.77	250 to 1,500	Continuous for 7 days	Rat, M (Wistar) 8 weeks old	Concentration-related effects on number, size, and surface morphology.	Dormans et al. (1990)
0.13 0.26 0.51 0.77	250 500 1,000 1,500	Continuous for 7 days	Rat, M (Wistar) 8 weeks old	Decreased phagocytic ingestion (at all concentrations) and intracellular killing (at ≤0.26 ppm) of *Listeria monocytogenes*.	Van Loveren et al. (1988)
0.2	392	Continuous for 14 days	Rat, M (Wistar) 10 weeks old	Increases in enzyme activity at ≥3 days. Increased number of AMs by Day 3.	Mochitate and Miura (1989)

Table 6-7 (cont'd). Effects of Ozone on Host Defense Mechanisms: Macrophage Alterations[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.4	784	3, 6, or 12 h; 12 h/day for 1, 3, or 7 days	Mouse, M (NIH) 23-28 g Rat, M (Wistar) 180-200 g	Decreased Fc-receptor mediated phagocytosis of AMs from mice at Days 1 and 7. Decreased phagocytosis at 6 h, increased phagocytosis of AMs from rats on Day 1. Decrease in superoxide anion production at some exposures.	Oosting et al. (1991a)
0.4	784	6 or 12 h, or 12 h/day for 3 or 7 days	Mouse, M (NIH) 25-30 g Rat, M (Wistar) 200-250 g	Increased ATP levels in mouse AMs only after 7 days of exposure.	Oosting et al. (1991b)
0.4 0.8	784 1,568	3 h	Mouse, F (C3H/HeJ C57Bl/6) 30 days old	Decreased phagocytosis of *Streptococcus zooepidemicus* (in vivo assay) and latex beads (in vitro assay). C57B1 mice more susceptible than C3H at 0.4 ppm only for bacteria phagocytosis, reverse susceptibility for latex beads.	Gilmour et al. (1993a)
0.4 0.8	784 1,568	3 h	Mouse, F (CD-1) 5 and 9 weeks old	Decreased in vivo phagocytosis of *Streptococcus zooepidemicus* independent of age. Decrease in number of phagocytic cells and number of bacteria/AM.	Gilmour et al. (1993b)
0.4 0.8	784 1,568	3 h	Mouse, F (C3H/HeJ C57Bl/6) 30 days old Rat, F (F344)	Decreased phagocytosis of latex beads.	Gilmour and Selgrade (1993)
0.5	980	24 h/day for 14 days	Mouse, F (Swiss) 20-25 g	Decreased Fc-receptor mediated phagocytosis of AMs at Days 1-14, with trend towards maximal decrease on Day 3. Effect correlated with increases in PGE. Decreased phagocytosis of peritoneal AMs.	Canning et al. (1991)
0.5	980	24 h/day for 14 days	Mouse, F (Swiss) 20-25 g	Decreased Fc-receptor mediated phagocytosis on Days 1, 3, and 7, but not at Day 14.	Gilmour et al. (1991)
0.64	1,254	23 h/day for 27 days	Rat, M (S-D) 130-150 g	Decreased lysozyme enzyme content during chronic *Pseudomonas aeruginosa* bacterial infection.	Sherwood et al. (1986)
1.0	1,960	2 h/day for 3 days	Rabbit, M (NZW) 14 weeks old	Decreased cytotoxicity vs. xenogeneic tumor cells. No effect on TNF-α and H_2O_2 production. Depression of superoxide anion production immediately after exposure, with an increase at 24 h.	Zelikoff et al. (1991)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

the lower O_3 concentrations are stimulatory for AMs activity, the higher concentrations are inhibitory because of their ability to produce substantial cellular injury. The number of AMs in BAL is reduced after a single exposure to 0.8 or 1.8 ppm O_3 (Bassett et al., 1988b; Bhalla and Young, 1992; Donaldson et al., 1993). Hotchkiss et al. (1989b) also observed a slight decrease in the number of AMs in the BAL immediately after a 6-h exposure to 0.8 and 1.5 ppm O_3, but the number of AMs increased at 42 and 66 h after the exposure. The results of the studies on AMs in general indicate that, following O_3 exposure, there is a short-term reduction in the number of lavageable AMs, but these cells increase in number over several days following exposure.

Exposure of rats to 0.12, 0.8, and 1.5 ppm O_3 for 6 h also resulted in a concentration-dependent increase in mitotic index at 0.8 ppm at 92 h after postexposure; AM size was only increased at the highest concentration (18 and 48 h postexposure) (Hotchkiss et al., 1989b). In a similar study, exposure of rats for 6 h to 0.12, 0.27, and 0.8 ppm O_3 resulted in AM chromosome damage at the two higher concentrations 28 h after exposure (Rithidech et al., 1990).

Several studies have investigated the effect of O_3 exposure on AM phagocytosis. Exposure of C3H/HeJ and C57Bl/6 mice for 3 h to 0.4 and 0.8 ppm O_3 decreased AM phagocytosis of *Streptococcus zooepidemicus* and latex beads (Gilmour et al., 1993a). In a similar study, a 3-h exposure of 5- and 9-week-old CD-1 mice decreased AM phagocytosis of *S. zooepidemicus*, but there was no effect of age (Gilmour et al., 1993b). Decreased phagocytic ingestion of *Listeria monocytogenes* also was observed following continuous exposure of rats to 0.13, 0.26, 0.51, and 0.77 ppm for 7 days; only the two lower concentrations inhibited intracellular killing (Van Loveren et al., 1988). Although lower O_3 concentrations were not tested, rats exposed to 1.02 ppm O_3 were unable to clear *Listeria* from their lungs. Exposure of rabbits to 0.1 ppm O_3 for 2 h/day resulted in decreased AM phagocytosis of latex microspheres after 2 or 6 (but not 13) days of exposure (Driscoll et al., 1987). In the same study, a single exposure to 0.1 or 1.2 ppm decreased AM phagocytosis immediately after exposure; recovery occurred by 7 days postexposure in the 0.1-ppm group but not in the 1.2-ppm group. That repeated exposures to O_3 results in an initial suppression of AM phagocytosis, which is followed by recovery of phagocytic potential while exposure continues (Driscoll et al., 1987), was confirmed by the studies of Gilmour et al. (1991) and Canning et al. (1991). Using identical exposure systems, it was observed that continuous exposure of mice to 0.5 ppm O_3 decreased AM Fc-receptor-mediated phagocytosis of sheep erythrocytes on Days 1, 3, 5, 7, and 8 of exposure, with return to control phagocytic levels by Day 14. This temporal trend paralleled the pattern of O_3-induced reduction of lung bactericidal activity against *Staphylococcus aureus*.

Interspecies comparisons of AM phagocytic potential were made by Gilmour and Selgrade (1993), who exposed C3H/HeJ and C57Bl/6 mice and Fischer 344 rats to 0.4 and 0.8 ppm O_3 for 3 h. Alveolar macrophage phagocytosis of latex beads was suppressed in all animals immediately after 0.4 ppm O_3 exposure, with the percent suppression greater in both strains of mice as compared to similarly treated rats. No differences in phagocytic suppression were observed between 0.4- and 0.8-ppm-O_3-exposed rats or the C57Bl/6 mice, but phagocytosis by AMs from 0.8-ppm-O_3-exposed C3H/HeJ mice was more suppressed as compared to the 0.4-ppm-O_3-exposed group. In a similar comparative study, Oosting et al. (1991a) exposed mice and rats to 0.4 ppm O_3 for single (3, 6, and 12 h) and repeated (12 h/day for 7 days) regimens. A decrease was observed in rat and mouse AM Fc-receptor mediated phagocytosis following the single O_3 exposure protocol. With the repeated

O_3 exposure protocol, rat AM phagocytosis was increased a day after exposure with no significant changes on Days 3 and 7. In contrast, phagocytosis by mouse AMs was suppressed at Day 1 of exposure and still did not recover at Day 7. In the same study, when mice were allowed to recover for 4 days following 3 days of O_3 exposure, phagocytosis by AMs was increased. These interspecies comparisons on the effect of O_3 exposure on AM phagocytic potential indicate that mice may be more susceptible than rats.

A species comparison of superoxide anion radical production between mouse AMs and rat AMs following a single 3-h exposure to O_3 concentrations ranging from 0.11 to 3.6 ppm showed the O_3 concentration that inhibits superoxide anion radical production by 50% to be 0.41 ppm for mouse AMs and 3.0 ppm for rat AMs (Ryer-Powder et al., 1988). Oosting et al. (1991a) also found a species difference in superoxide anion production using a more varied exposure-duration protocol; mice appeared to be more responsive than rats. This oxygen radical is important in antibacterial activity, and both sets of authors suggest that O_3-induced impairment of pulmonary antibacterial defenses may be related to decreases in superoxide anion radical production. Decreased lysozyme enzyme levels in rat AMs also were observed during chronic *Pseudomonas aeruginosa* bacterial infection following exposure to 0.64 ppm O_3 for 23 h/day for 27 days (Sherwood et al., 1986).

Exposure of rats for 16 h to 0.05, 0.1, 0.2, and 0.4 ppm O_3 increased AM adherence to nylon fibers at 0.05 and 0.1 ppm, but had no effect at 0.2 and 0.4 ppm (Veninga and Evelyn, 1986). Increased metabolic activity of AMs retrieved from rats following continuous O_3 exposure for 14 weeks to 0.1 and 0.2 ppm was observed by Mochitate et al. (1992). In a similar study, long-term O_3 exposure of rats (continuous 0.2 ppm for 11 weeks) continued to increase AM metabolic activity (Mochitate and Miura, 1989). Exposure of mice and rats for 14 h/day for 7 days to 0.4 ppm O_3 also increased adenosine triphosphate (ATP) levels in the mouse AMs, but had no effect on ATP levels in rat AMs (Oosting et al., 1991b).

In addition to their phagocytic function and particle removal, AMs also play several other roles in host defense that include (1) a regulatory role through their release of mediators (soluble substances secreted by the AMs that produce biologic effects on other cells) such as tumor necrosis factor, interleukin-1, and PGs; (2) activities associated with tumor surveillance; and (3) accessory cell function in antigen presentation to lymphocytes in the initiation of the immune response. Investigating the effect of a single 2-h exposure of rabbits to 0.1 and 1.2 ppm O_3, Driscoll et al. (1988) observed an increased release of PGE_2 and $PGF_{2\alpha}$ by AMs following exposure to 1.2 but not 0.1 ppm. Prostaglandin E_2 can depress AM and natural killer cell cytotoxicity to tumor cells. Perhaps this is a mechanism involved in the depression of AM-mediated cytotoxicity toward xenogeneic tumor cells following exposure of rabbits for 2 h/day for 3 days to 1.0 ppm O_3 (Zelikoff et al., 1991). No studies were found on the effects of O_3 on antigen presentation.

6.2.3.5 Immunology

In addition to the above nonspecific defense mechanisms, the respiratory system also has specific immunologic mechanisms that can be initiated by inhaled antigens. There are two types of immune mechanisms: antibody (humoral)-mediated and cell-mediated. In general, 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. Cell-mediated mechanisms enhance the microbiocidal capacity of AMs in defense to intracellular bacteria such as *Mycobacterium tuberculosis* and

Listeria monocytogenes, whereas another arm of the cellular immune response generates a class of lymphocytes that are cytotoxic for virus-infected cells. Both the humoral and cell-mediated responses protect the respiratory tract against infectious agents and operate in three major temporal waves: (1) natural killer cells (nonspecific lymphocytes that can destroy bacteria, viruses, and tumor cells), (2) cytotoxic T lymphocytes (lymphocytes that lyse specifically recognized targets), and (3) antigen-specific antibodies.

Little information was available in the previous criteria document (U.S. Environmental Protection Agency, 1986) on the effects of O_3 on immunologic defenses. However, the data base indicated an immunotoxic effect of O_3 exposure, especially on T-cell populations. For example, Aranyi et al. (1983) found that a 90-day (5 h/day, 5 days/week) exposure of mice to 0.1 ppm O_3 suppressed blastogenesis of splenic lymphocytes to T-cell, but not B-cell mitogens; the ability of these cells to produce antibodies was not affected either. As can be seen from Table 6-8, this database has greatly expanded and also has been recently reviewed (Jakab et al., 1995). Many of the studies include both the pulmonary and systemic immune system, which, to a degree, are compartmentalized; both systems are discussed here.

Studies on the effect of O_3 exposure on the immune system can be divided into three broad categories. These are (1) measurement of lymphoid organ weights and cellular composition, (2) determination of the functional capacity of lymphocytes in the absence of antigenic stimulation, and (3) measurement of the immune response following antigenic stimulation.

Dziedzic and White (1986a) observed that exposure of mice to 0.3, 0.5, and 0.7 ppm O_3 for 20 h/day for 28 days resulted in a concentration-dependent initial depletion of cells in the mediastinal lymph nodes (MLNs) (Days 1 and 2); this was followed by a T-cell hyperplasia peaking about Days 3 and 4. There was an enhanced blastogenic response to the T-cell mitogen concanavalin A (ConA) at 0.7 ppm O_3 (only level tested). There was no effect of O_3 on cell division morphology of B cells. In a similar study, exposure of mice to 0.7 ppm O_3 for 20 h/day for 28 days resulted in an initial thymic atrophy, with return to normal thymus weights by Day 14 of exposure (Dziedzic and White, 1986b). Exposure of rats to 0.5 ppm O_3 for 20 h/day for 14 days also increased bronchus-associated lymph node and MLN cell proliferation at 2 and 3 days of exposure, but not at 1, 7, and 14 days of exposure (Dziedzic et al., 1990). Bleavins and Dziedzic (1990) observed that exposure of BALB/c mice to 0.7 ppm O_3 for 20 h/day for 14 days resulted in decreased spleen and thymus weights at Day 4, with recovery at Day 14. The absolute number of thymocytes decreased following exposure of mice to 0.7 ppm O_3 for 24 h/day for 7 days (Li and Richters, 1991a) and to 0.3 ppm O_3 for 24 h/day for 3 weeks (Li and Richters, 1991b). Although the latter exposure protocol (0.3 ppm for 24 h/day for 3 weeks) decreased the absolute number of thymocytes, an increase in the percentage of thymocytes was observed in the absence of any changes in splenic T cells (Li and Richters, 1991b). Continuous exposure of rats to $\geq$0.26 ppm O_3 for 7 days increased MLN T:B lymphocyte ratios immediately and 5 days postexposure (Van Loveren et al., 1988). Bleavins and Dziedzic (1990) observed an increased infiltration of Thy-1.2$^+$ lymphocytes and IgM$^+$ cells into the O_3-induced pulmonary lesion following exposure of mice to 0.7 ppm O_3 for 20 h/day for 14 days. The lesion was defined by quantitative histomorphometric analysis as any lung area with inflammatory cell infiltration, cellular proliferation, consolidation, or edema. Dziedzic and White (1987a) further investigated these T-cell effects by exposing normal and athymic nude

Table 6-8. Effects of Ozone on Host Defense Mechanisms: Immunology[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.06 base, spike rising to 0.25	118 base, spike rising to 490	13 h/day, 7 days/week base; ramped spike 9 h/day, 5 days/week	Rat, M (F344) 60 days old	No effect on splenic NK cell activity or splenic lymphocyte blastogenic response to T-cell mitogens (PHA and ConA) or B-cell mitogen (*S. salmonella* glycoprotein).	Selgrade et al. (1990)
0.1 0.5 1.0	196 980 1,960	23.5 h/day for 10 days	Rat, M (F344) 8-12 weeks old	Decreased lung NK cell activity at 1.0 ppm on Days 1, 5, and 7, with recovery at Day 10; decreased NK cell activity at 0.5 ppm on Day 1 (only day tested).	Burleson et al. (1989)
0.13 0.26 0.51 0.77 1.02	250 500 1,000 1,500 2,000	Continuous for 7 days	Rat, M (Wistar) 8 weeks old	At ≥0.26 ppm, increased T:B cell ratios in MLN. Decreased T:B cell ratios in MLN and delayed-type hypersensitivity response following *Listeria monocytogenes* immunization.	Van Loveren et al. (1988)
0.2 0.4 0.82	400 800 1,600	24 h/day for 7 days	Rat, M (Wistar) NS	Decreased lung NK cell activity at 0.82 ppm; increased lung NK cell activity at 0.2 and 0.4 ppm.	Van Loveren et al. (1990)
0.3	588	24 h/day for 3 weeks	Mouse, F (BALB/c) 3 weeks old	Increase in percentage of thymocytes with lower absolute numbers. No changes in splenic T-lymphocytes.	Li and Richters (1991b)
0.3 0.5 0.7	588 980 1,372	20 h/day for 28 days	Mouse, F (CD-1)	Concentration- dependent MLN cell depletions (Days 1 and 2) followed by T-cell hyperplasia (Days 4, 7, 14, and 28). Enhanced blastogenic response to T-cell mitogen ConA at 4 and 7 days at 0.7 ppm (other levels not tested).	Dziedzic and White (1986a)
0.5	980	24 h/day for 14 days	Mouse, F (Swiss)	Decreased antiviral serum antibody following influenza virus infection and decreased T-cells in lung tissue.	Jakab and Hmieleski (1988)
0.5	980	20 h/day for 14 days	Rat, M (F344) 11 weeks old	BALT and MLN cell proliferation at 3 days, but not at Days 1, 2, 7, and 14.	Dziedzic et al. (1990)
0.7	1,372	20 h/day for 28 days	Mouse, F (CD-1)	MLN hyperplasia. Thymic atrophy through Day 7 with return to control level at Day 14.	Dziedzic and White (1986b)
0.7	1,372	24 h/day for 7 days	Mouse, F (BALB/c) 5-12 weeks old	Decrease in the absolute number of thymocytes.	Li and Richters (1991a)
0.7	1,372	20 h/day for 7 and 14 days	Mouse, F (Athymic and euthymic)	Compared to euthymic (nu/+) mice, athymic nude (nu/nu) mice have increased lung inflammation and lesion volume, but no MLN hyperplasia.	Dziedzic and White (1987a)

Table 6-8 (cont'd). Effects of Ozone on Host Defense Mechanisms: Immunology[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.7	1,372	20 h/day for 14 days	Mouse, F (BALB/c) 10-12 weeks old	Decreased spleen and thymus weight at Day 4 with recovery at Day 14. Increased infiltration of Thy-1.2$^+$ lymphocytes and IgM$^+$ cells into the O_3-induced pulmonary lesion.	Bleavins and Dziedzic (1990)
0.8	1,568	23 h/day for 14 days	Mouse, F ($B6C3F_1$) 6-8 weeks old	Decreased blastogenesis of MLN and splenic lymphocytes to PHA mitogen on Day 1; no effect on Days 3, 7, and 14. Decreased splenic NK cell activity on Days 1 and 3; no effect on Days 7 and 14. Decreased pulmonary IgG and IgA response to ovalbumin immunization.	Gilmour and Jakab (1991)
0.8	1,568	24 h/day for 14 days	Mouse, M (BALB/c) 2-3 mo old	Suppression of delayed-type hypersensitivity response to SRBCs on Day 7; no effect on Days 1, 3, and 14. Thymic atrophy on Day 7 with decreased Thy-1.2$^+$ cells in thymus.	Fujimaki et al. (1987)
0.8	1,568	24 h/day for 4 weeks	Mouse, M (BALB/c) 7 weeks old	Suppression of serum IgG following ovalbumin immunization.	Ozawa (1986)
1.0	1,960	24 h/day for 4 weeks	Mouse, M (C57B/6 × DBA/2 F1) 11-14 weeks old	Decreased ability of spleen cells to generate a primary antibody response to SRBCs in vitro.	Wright et al. (1989)
1.0	1,960	8 h/day for 7 days	Rat, M (Long-Evans) 7-8 weeks old	No effect on lung and splenic ADCC activity. Enhanced blastogenesis of splenic lymphocytes to PHA, ConA, and lipopolysaccharide mitogens at 1.0 ppm.	Eskew et al. (1986)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

mice to 0.7 ppm O_3 for 7 or 14 days (20 h/day). After 7 days of exposure, the athymic nude mice did not have the MLN hyperplasia seen in the euthymic mice. However, the athymic nude mice had a greater inflammatory response and an increase in lung lesion volumes compared to the euthymic mice. Thus, it appears that T cells have some involvement in protecting the lungs from the morphological effects of O_3.

The above longitudinal studies on the effects of O_3 exposure on lymphoid organ cell numbers provide information on cellular traffic and cell numbers but provide few insights into the functional capacity of the lymphocytes. A number of studies have investigated the effect of O_3 exposure on the blastogenic response of lymphocytes to nonspecific mitogens. These assays measure nonspecific clonal expansion of the lymphocyte population, a critical step during the amplification of the immune response. Exposure of mice to 0.7 ppm O_3 for 20 h/day for 28 days enhanced the MLN cell blastogenic response to the T-cell mitogen ConA at 4 and 7 days of exposure, with return to control levels by Day 14 (Dziedzic and White, 1986a). In a similar manner, Gilmour and Jakab (1991) observed that continuous exposure of mice to 0.8 ppm O_3 decreased the MLN and splenic lymphocyte blastogenic response to the T-cell mitogen phytohemagglutinin (PHA) on Day 1 of exposure, with the effect abrogated after prolonged exposure. An enhanced blastogenic response of splenic lymphocytes to PHA and Con A and a B-cell mitogen (*Escherichia coli* lipopolysaccharide) was observed following exposure of rats to 1.0 ppm O_3 for 8 h/day for 7 days (Eskew et al., 1986).

Natural killer cell activity also has been studied. One such study in rats observed a decreased lung NK cell activity following 1, 5, and 7 days of exposure to 1.0 ppm for 23.5 h/day, with recovery by Day 10 (Burleson et al., 1989). In a similar experiment, Van Loveren et al. (1990) observed decreased lung NK cell activity following 7 days of continuous exposure of rats to 0.82 ppm O_3; however, exposure to 0.2 and 0.4 ppm enhanced lung NK cell activity. Exposure of mice for 23 h/day for 14 days to 0.8 ppm O_3 also decreased splenic NK cell activity on Days 1 and 3, with a return to control values on Days 7 and 14 (Gilmour and Jakab, 1991). Finally, Selgrade et al. (1990) used an experimental protocol designed to mimic diurnal urban O_3 exposure patterns. Rats were exposed to a background level of 0.06 ppm for 13 h (7 days/week), followed by a broad exposure spike (5 days/week) rising from 0.06 to 0.25 ppm and returning to 0.06 ppm over 9 h, and then followed by 2-h downtime. After 1, 3, 13, 52, or 78 weeks of exposure, spleen cells were assessed for NK cell activity and responses to T-cell mitogens (PHA and ConA) and a B-cell mitogen (*Salmonella typhimurium* glycoprotein). Ozone exposure had no effect on NK cell activity, nor were there any O_3-related changes in mitogen responses in splenic or blood leukocytes. There were also no effects of a single 3-h exposure to 1.0 ppm O_3 on spleen cell responses to the mitogens immediately after exposure or at 24, 48, and 72 h thereafter.

Several studies also have investigated the effect of O_3 exposure on the immune response following antigenic stimulation. Fujimaki (1989) observed that exposure of mice to 0.8 ppm O_3 for 24 h/day for 56 days suppressed the primary splenic antibody response to sheep red blood cells (SRBCs; T-cell-dependent antigen) but not to DNP-Ficoll (T-cell-independent antigen). In a similar study, exposure of mice to 0.8 ppm O_3 for 24 h/day for 14 days suppressed the delayed type hypersensitivity response to SRBCs on Day 7, but not on Days 1, 3, and 14 (Fujimaki et al., 1987). Suppression of serum IgG levels on ovalbumin immunization was observed following exposure of mice to 0.8 ppm O_3 for 24 h/day for 4 weeks (Ozawa, 1986). Decreased pulmonary IgG and IgA responses on

ovalbumin immunization were also observed in mice during a 2-week O_3 exposure for 23 h/day to 0.8 ppm (Gilmour and Jakab, 1991). Exposure of mice to 0.5 ppm O_3 continuously for 14 days during the course of influenza virus infection also decreased the serum hemagglutinin antiviral antibody response (Jakab and Hmieleski, 1988).

Van Loveren et al. (1988) investigated antigen-specific responses following pulmonary *Listeria* infection and observed no significant changes in the delayed-type hypersensitivity response when O_3 exposure (continuous, 0.77 ppm) was for 7 days prior to infection. However, if the O_3 exposure took place when an infection with *Listeria* was also present (from Days 0 to 7 or from Days 7 to 14), the delayed-type hypersensitivity response was significantly decreased. In a similar manner, no significant changes were observed in the splenic lymphoproliferative response to *Listeria* antigen when the 0.77-ppm O_3 exposure preceded the infection, whereas the response was suppressed when the 0.77-ppm O_3 exposure occurred immediately after infection or from Days 7 to 14 after infection. In the same series of experiments, Van Loveren et al. (1988) observed that continuous exposure to 0.26 ppm O_3 impaired the increase in T:B lymphocyte ratios that occurred in response to the *Listeria* infection.

6.2.3.6 Interaction with Infectious Agents

Because respiratory infections remain one of the most common public health problems, it is important to determine whether or not exposure to air pollutants reduce susceptibility to infectious agents. Measurement of the competence of the host's antimicrobial mechanisms can best be tested by challenging air-pollutant-exposed animals and the clean-air-exposed control animals to an aerosol of viable organisms. If the test substance, such as O_3, decreases the efficiency of the host's integrated protective mechanisms (i.e., physical clearance via the mucociliary escalator, microbicidal activity of the AMs, and associated humoral and cellular immunologic events), the microorganisms are less efficiently killed in the lungs, or the organisms may even multiply, resulting in the demise of the host. The defensive function of the lung is remarkably similar across animals species, and available human data suggests that qualitative findings obtained on functional resistance mechanisms using appropriate animal models may be extrapolated to humans (Green, 1984).

The studies detailed in the previous criteria document (U.S. Environmental Protection Agency, 1986) primarily used the mouse "infectivity model" (Gardner, 1982). Briefly, animals are randomly selected to be exposed to either clean air or O_3. After exposure, the animals from both groups are combined and exposed to an aerosol of microorganisms. The vast majority of these studies have been conducted with streptococcus species. At the termination of the infectious exposure period, the animals are housed in clean air and the mortality rate in the two groups is determined during a 15-day holding period. In this system, the concentrations of O_3 used do not cause any mortality. The mortality in the control group (clean air plus exposure to the microorganism) ranges from approximately 10 to 20% and reflects the natural resistance of the host to the infectious agents. The difference in mortality between O_3-exposure groups and the controls is concentration-related (Gardner, 1982). These studies showed that, depending on the O_3 exposure protocol, a 3-h exposure to concentrations as low as 0.08 ppm O_3 can enhance the increased mortality of CD-1 mice from streptococcus infection (Coffin et al., 1967; Coffin and Gardner, 1972; Miller et al., 1978). However, although a prolonged intermittent exposure (103 days) to 0.1 ppm O_3 increased mortality in this model system, the magnitude of the effect was not substantially greater than that after acute exposure (Aranyi et al., 1983).

Another approach to assess the effect of air pollutants on host defenses is to quantitate rates of pulmonary bacterial inactivation following aerosol infection with microorganisms. In this system, the animals are exposed either to clean air or to the air pollutant and then are exposed to an aerosol of microorganisms in a manner similar to the method used for the infectivity model. However, instead of assessing enhancement of mortality, viable bacteria are quantitated in lung homogenates at various times after inhalation of the microorganisms (Goldstein et al., 1971a,b). In air-exposed control animals, there is a rapid inactivation of the inhaled microorganisms that have been deposited in the respiratory tract. However, O_3 exposure alters the ability of the microbicidal mechanisms of the lungs to function normally and bacterial inactivation proceeds at a slower rate, indicating impairment of host defenses. For example, Goldstein et al. (1971b) showed that a 4-h exposure of mice to $\geq$0.6 ppm O_3 after infection with *S. aureus* decreased lung bactericidal activity. Studies appearing in the literature since publication of the previous criteria document (U.S. Environmental Protection Agency, 1986) are described below (also see Table 6-9).

Gilmour et al. (1993a) observed that exposure of C3H/HeJ and C57Bl/6 mice for 3 h to 0.4 and 0.8 ppm O_3 resulted in decreased intrapulmonary killing of *S. zooepidemicus* in both strains of mice. Although both strains were affected, the C3H/HeJ mice appeared to be more susceptible because bactericidal activity was decreased sooner and mortality was enhanced more. Gilmour et al. (1993b) expanded these studies to CD-1 mice of different ages (5 and 9 weeks old) exposed for 3 h to 0.4 and 0.8 ppm O_3. The higher concentration decreased intrapulmonary killing 4 h after infection with *S. zooepidemicus*; there was no effect of age. However, the 5-week-old mice were more susceptible to the infection because mortalities were 9, 41, and 61% in the air, 0.4-ppm, and 0.8-ppm exposure groups, respectively; whereas only 4, 15, and 28% of the older animals died with analogous exposure. Pretreatment of the mice with indomethacin reduced the O_3-induced enhancement of PGE_2 levels in BAL as well as the enhanced mortality in the 5-week-old mice, suggesting an involvement of arachidonic acid metabolites in antibacterial defenses.

Gilmour and Selgrade (1993) studied the interspecies response to experimental *S. zooepidemicus* infection of rats and C3H/HeJ and C57Bl/5 mice following a 3-h exposure to 0.4 and 0.8 ppm O_3. Exposure of rats to O_3 suppressed intrapulmonary bacterial killing, with no differences observed between the 0.4- and 0.8-ppm O_3 exposure groups. Exposure of C57Bl/6 mice to 0.4 ppm O_3 also resulted in a suppression of bactericidal activity, and exposure to 0.8 ppm O_3 led to bacterial proliferation in the lungs, resulting in 60% mortality at Day 4. Exposure of C3H/HeJ mice to both 0.4 and 0.8 ppm O_3 resulted in bacterial proliferation with, respectively, 60 and 80% mortality at Day 4 after exposure. Increased mortality from *S. zooepidemicus* infection following 24 h/day exposure for 5 days/week for 3 weeks also was observed following 0.3 and 0.5 but not 0.1 ppm O_3 exposure (Graham et al., 1987).

To investigate the effect of longer exposures and challenges with bacteria, Gilmour et al. (1991) exposed mice continuously to 0.5 ppm O_3 for 14 days. At 1, 3, 7, and 14 days, intrapulmonary killing was assessed by inhalation challenge with *S. aureus* and *Proteus mirabilis*. Ozone exposure impaired the intrapulmonary killing of *S. aureus* at 1 and 3 days. However, with prolonged exposure, the bactericidal capacity of the lungs returned to normal. In contrast to *S. aureus*, when *P. mirabilis* was the challenge organism, O_3 exposure had no suppressive effect on pulmonary bactericidal activity. The authors attribute this difference to the defense mechanisms involved. Alveolar macrophages are

Table 6-9. Effects of Ozone on Host Defense Mechanisms: Interactions with Infectious Agents[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.1 0.3 0.5	196 588 980	24 h/day, 5 days/week for 3 weeks	Mouse, F (CD-1) 4-6 weeks old	Increased mortality from *Streptococcus zooepidemicus* infection at 0.3 and 0.5 ppm.	Graham et al. (1987)
0.13 0.26 0.51 0.77 1.02	250 500 1,000 1,500 2,000	Continuous for 7 days	Rat, M (Wistar) 8 weeks old	Decreased bactericidal activity vs. *Listeria monocytogenes* at 0.77 and 1.02 ppm. Increased mortality at 1.02 ppm.	Van Loveren et al. (1988)
0.25 0.5 1.0	490 980 1,960	3 h/day for 5 days	Mouse, F (CD-1) 3-4 weeks old	Increased mortality from influenza virus infection and increased pulmonary virus titers at 1.0 ppm when infection followed 2 days of exposure, but not at other time points. Histopathologic and pulmonary function changes more severe with this regimen. At ≥0.5 ppm, increased lung wet weight when virus given after 2 days of O_3 exposure.	Selgrade et al. (1988)
0.4 0.8	784 1,568	3 h	Mouse, F (C3H/HeJ C57Bl/6) 30 days old	Decreased bactericidal activity vs. *Streptococcus zooepidemicus*. Increased mortality in both strains, with greater mortality in the C3H/HeJ strain.	Gilmour et al. (1993a)
0.4 0.8	784 1,568	3 h	Mouse, F (CD-1) 5 and 9 weeks old	Decreased bactericidal activity vs. *Streptococcus zooepidemicus*. Increased mortality in both age groups, with greater mortality in the 5-week-old mice.	Gilmour et al. (1993b)
0.4 0.8	784 1,568	3 h	Mouse, F (C3H/HeJ C57Bl/6) Rat (F344) 30 days old	Decreased intrapulmonary killing of *Streptococcus zooepidemicus*.	Gilmour and Selgrade (1993)
0.5	980	24 h/day for 14 days	Mouse, F (Swiss) 20-23 g	Decreased bactericidal activity vs. *Staphylococcus aureus* on Days 1 and 3; no effect on Days 7 and 14. Enhanced bactericidal activity vs. *Proteus mirabilis* on Days 3, 7, and 14; no effect on Day 1.	Gilmour et al. (1991)
0.5	980	24 h/day for 15 days	Mouse, F (Swiss) 20-23 g	No effect on pulmonary virus titers during influenza virus infection. O_3 decreased lung morphological injury due to virus (Day 9).	Jakab and Hmieleski (1988)
0.5	980	24 h/day for 120 days	Mouse, F (Swiss) 20-23 g	Ozone decreased acute lung influenzal injury, but increased pulmonary fibrosis during the course of and period after influenza virus infection.	Jakab and Bassett (1990)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

active against the gram-positive *S. aureus*; AMs and PMNs defend against the gram-negative *P. mirabilis*. The effects of O_3 on bactericidal activity against *S. aureus* paralleled the effects on AM phagocytosis (early decrease, then no change). With *P. mirabilis*, there was more than a 1,000-fold increase in PMNs in the lung that was not altered by O_3, enabling bactericidal activity to occur. In a similar manner, exposure of rats for 24 h/day for 7 days to 0.13, 0.26, 0.51, 0.77, and 1.02 ppm O_3 decreased pulmonary bactericidal activity against *Listeria* at 0.77 and 1.02 ppm, with increased mortality at 1.02 ppm (Van Loveren et al., 1988). These effects were associated with increased pathologic lesions, characterized by multifocal infiltrates of histiocyctic and lymphoid cells, found in the lungs and liver of O_3-exposed and *Listeria* infected animals as compared to *Listeria* infection alone.

Fewer studies of viral infectivity have been conducted. Exposure for 15 days to 0.5 ppm O_3 during the course of murine influenza virus infection had no effect on pulmonary virus titers (Jakab and Hmieleski, 1988). A 5-day exposure for 3 h/day to 1.0 ppm O_3 with influenza virus infection on the second day of exposure had no effect on pulmonary virus titers, but did show increased mortality, increased lung wet weight, and more severe nonsuppurative pneumonitis and epithelial metaplasia and hyperplasia, with changes in lung function consistent with that effect (Selgrade et al., 1988). Lung wet weight also was increased when the mice were infected after the second day of exposure to 0.5 but not 0.25 ppm. When infection occurred on other days during the 5-day O_3 exposure, no such effects were found.

Typically, influenza virus infection causes pneumonitis characterized by severe acute lung damage that eventually resolves to persistent alveolitis and changes in the parenchyma (focal interstitial pneumonia and collagen deposition). Jakab and Bassett (1990) investigated the effect of long-term O_3 exposure (24 h/day for 120 days to 0.5 ppm) on mice administered influenza virus immediately before O_3 exposure started. The authors observed an increase in pulmonary fibrosis with the virus infection, as compared to O_3 exposure alone. During the course of the viral infection, O_3 exposure had no effect on pulmonary virus titers and reduced the virus-induced acute lung injury. However, from Day 30 after infection, increased numbers of AMs, lymphocytes, and PMNs were recovered from animals exposed to virus plus O_3, as compared to virus infection alone or O_3 exposure alone. This increased alveolitis correlated with increases in morphometrically determined lung damage and lung hydroxyproline content, a biochemical marker indicative of pulmonary fibrosis. Ozone exposure administered 10 days after viral infection enhanced lung hydroxyproline content at Day 30, as compared to either virus infection or O_3 exposure alone. Thus, O_3 enhanced postinfluenzal aveolitis and parenchymal changes. From these data, the authors speculated that the mechanism for the postinfluenza lung damage may be related to O_3 impairing the repair process of the viral-induced acute lung injury.

In the studies reported to date, it is clear that the temporal relationships between O_3 exposure and influenzal infection are important. This is not surprising because there are several waves of different antiviral defense mechanisms that might be affected differently by O_3. However, they have not been studied adequately for susceptibility to O_3. Apparently, O_3 does not alter defenses responsible for clearing virus from the lungs, as evidenced by the lack of effect of O_3 on viral titers (Selgrade et al., 1988; Jakab and Hmieleski, 1988). The interaction between virus and O_3 on histological changes in lung tissue can be damaging (Selgrade et al., 1988; Jakab and Bassett, 1990) or beneficial (Jakab and Bassett, 1990), possibly depending on the time of observation relative to the stage of the infectious process. The exact reasons are not known, but perhaps the induction of interferon production by the

virus plays a role. In noninfectious studies, Dziedzic and White (1987b) observed that interferon induction mitigates O_3-induced lung lesions, defined as areas with inflammatory cell infiltration, cellular proliferation, consolidation, or edema, and that anti-interferon treatment exacerbates those lesions.

6.2.3.7 Summary

Exposure to O_3 can result in alterations of all the defense mechanisms of the respiratory tract, including mucociliary and alveolobronchiolar clearance, functional and biochemical activities of AMs, immunologic competence, and susceptibility to respiratory infections. Structural (see Section 6.2.4), functional, and biochemical alterations in the mucociliary escalator occur after O_3 exposure. Mucociliary clearance is slowed in rabbits after a single 2-h exposure to 0.6 ppm, but repeated (up to 14-day) exposures have no such impact (Schlesinger and Driscoll, 1987). Secretions of mucous components are affected by repeated exposure (Phipps et al., 1986; McBride et al., 1991). When lambs were exposed (1.0 ppm O_3, 4 h/day, 5 days) shortly after birth, tracheal mucous components did not develop normally (Mariassy et al., 1989, 1990). In contrast, alveolar clearance of rabbits after acute O_3 exposure (0.1 ppm, 2 h/day, 1 to 4 days) is accelerated (Driscoll et al., 1986). In the same study, a 14-day exposure caused no effects, and a higher concentration (1.2 ppm) slowed alveolar clearance. A similar pattern of slowed tracheobronchial clearance and accelerated alveolar clearance occurs in rats (Phalen et al., 1980; Kenoyer et al., 1981). A subchronic (6-week) exposure of rats to an urban pattern of O_3 increased the retention of asbestos fibers (Pinkerton et al., 1989).

Although AMs have numerous functions, one primary role is to clear the lung of infections and noninfectious particles. Phagocytosis of bacteria, inert particles, and antibody-coated red blood cells (RBCs) is inhibited by acute exposure to O_3. The lowest effective concentration tested was 0.1 ppm O_3 (2 h) in rabbits (Driscoll et al., 1987). If exposures are repeated for several days, phagocytosis returns to control levels (Driscoll et al., 1987; Gilmour et al., 1991; Canning et al., 1991). The ability of AMs to produce superoxide anion radicals (important to bactericidal activity) is inhibited by acute exposure to O_3, especially in mice as compared to rats (Ryer-Powder et al., 1988; Oosting et al., 1991a). The effect is clearly evident after exposure for 3 h to 0.4 ppm, as observed by dysfunction in AM phagocytosis and enhanced susceptibility to experimental respiratory infection (Gilmour et al., 1993a, 1993b). Thus, the evidence indicates that the AM-dependent alveolobronchiolar transport mechanisms are impaired, as are their phagocytic and microbicidal activities, leading to decreased resistance to respiratory infections.

The experimental database also shows that the effects of O_3 on the immune system are complex. These effects are not yet fully evaluated, and the reported effects on immune parameters are dependent on the exposure regimen and the observation period. It appears that the T-cell-dependent functions of the immune system are more affected than B-cell-dependent functions (U.S. Environmental Protection Agency, 1986; Fujimaki, 1989). Generally, there is an early immunosuppressive effect that, with continued O_3 exposure, results in either return to normal responses or immunoenhancement. For example, in mice exposed for 28 days (20 h/day) to 0.3 to 0.7 ppm O_3, there was an early (Days 1 and 2) depletion of cells in the MLN, followed by MLN T-cell hyperplasia and increased blastogenic response to a T-cell mitogen (Dziedzic and White, 1986a). Several investigations have found an initial (Days 1 to 4) decrease in blastogenic response to T-cell mitogens in the MLN and spleen of mice exposed for a few weeks to 0.7 or 0.8 ppm O_3 that returned to

control levels by the end of the exposure (Dziedzic and White, 1986a; Gilmour and Jakab, 1991). There also are changes in cell populations in lymphatic tissues. For example, T:B-cell ratios in the MLN increase when rats are exposed for 7 days to $\geq$0.26 ppm O_3 (Van Loveren et al., 1988). Natural killer cells in the lung are affected under some circumstances. Van Loveren et al. (1990) showed that a 1-week exposure to 0.2 or 0.4 ppm O_3 increased NK cell activity, but a higher concentration (0.82 ppm) decreased it. Ozone also alters response to antigenic stimulation. For example, antibody responses to a T-cell-dependent antigen were suppressed after a 56-day exposure of mice to 0.8 ppm O_3, and a 14-day exposure to 0.5 ppm O_3 decreased the antiviral antibody response following influenza virus infection (Jakab and Hmieleski, 1988). The temporal relationship between O_3 exposure and antigenic stimulation is important. When O_3 exposure preceded *Listeria* infection, there were no effects on delayed-type hypersensitivity or splenic lymphoproliferative responses; when O_3 exposure occurred during or after *Listeria* infection was initiated, these immune responses were suppressed (Van Loveren et al., 1988). With experimental viral infections, O_3 exposure decreases the T-lymphocyte responses and the antiviral antibody response (Jakab and Hmieleski, 1988); the latter impairment may pave the way for lowered resistance to reinfection.

The significance of O_3-induced suppression of immune parameters in relation to risk of infectious disease is an example of a generic problem that remains to be clarified. For example, correlative studies between immune parameters and acquired immune deficiency syndrome have provided clear insights on the magnitude of the immune dysfunction and the progression of the disease. However, there is a paucity of such correlative studies on alterations of immune parameters and function resistance mechanisms or disease endpoints. Because the major functions of the immune apparatus are to protect against infectious agents and conduct tumor surveillance, suppression of immune parameters is considered a signal of increased susceptibility to infections and acquisition of tumors. One of the few studies that has addressed this issue (Selgrade, 1995) illustrates how the temporal relationships between exposure to a compound and exposure to infectious agents or tumor cells will have an impact on the risk associated with immune suppression in experimental animal models. The types of immune responses affected by a chemical and their importance to defense against any particular infectious agent, the recovery time of the immune response, the length of exposure, and the time required for mobilization of alternative defenses are among the factors that can impact the risk of enhanced infectious disease that might be associated with an immunosuppressive event.

In addition to a suppressive effect on pulmonary immunity, O_3 exposure also can affect systemic immunity. Although these depressive effects on the systemic compartment occur at approximately twice the O_3 exposure concentrations observed for pulmonary immunosuppression (with the exception of a study by Aranyi et al. [1983] at 0.1 ppm O_3), the observations are important because they show that the effects of O_3 exposure on host resistance are not limited to the lung alone, but may increase susceptibility to systemic infections as well as pulmonary infections. However, Selgrade et al. (1990) found no effects on selected systemic immune functions in rats exposed for up to 78 weeks to an urban pattern of O_3.

Numerous studies have confirmed that acute or short-term exposure to O_3 decreases lung bactericidal activity and increases susceptibility to respiratory bacterial infections. The lowest exposure showing such effects was 0.08 ppm (3 h) in the mouse streptococcal model (Coffin et al., 1967; Coffin and Gardner; 1972; Miller et al., 1978).

Further research has indicated that changes in antibacterial defenses are dependent not only on exposure regimens, but also on species and strain of animal, species of bacteria, and age of animal (e.g., young mice are more susceptible) (Gilmour et al., 1991, 1993a,b; Gilmour and Selgrade, 1993). Furthermore, increasing the duration of an exposure to 0.1 ppm O_3 from a few hours to 3 weeks either causes no effect or does not enhance the streptococcal-induced mortality observed after acute exposure (Graham et al., 1987; Aranyi et al., 1983). In general, the effect of O_3 exposure on antibacterial host defenses appears to be concentration- and time-dependent. Acute exposures result in an impairment of host defenses, whereas the defense parameters become reestablished with more prolonged exposures.

Effects of O_3 on the course of viral infections are more complex and highly dependent on the temporal relationship between O_3 exposure and viral infection. For example, Selgrade et al. (1988) found increases in mortality and lung wet weight in mice infected with influenza only after the second day of O_3 exposure (1 ppm, 3h/day). Jakab and Bassett (1990) found no detrimental effect of a 120-day exposure to 0.5 ppm O_3 on acute lung injury from influenza virus administered immediately before O_3 exposure started. However, O_3 enhanced postinfluenzal alveolitis and lung parenchymal changes. Because O_3 did not affect lung influenza viral titers in any of these studies, it is unlikely that O_3 has an impact on antiviral clearance mechanisms.

Ozone-induced susceptibility to experimental respiratory infections has been correlated with the immunotoxic effects of O_3 by the observation that O_3 exposure increases the severity of *Listeria* infection while concurrently suppressing the antigen-specific immune responses (Van Loveren et al., 1988).

6.2.4 Morphological Effects

6.2.4.1 Introduction

All mammalian species studied react to inhaled concentrations of <1.0 ppm O_3 in a generally similar manner, with species variation in morphological responses depending on the distribution of sensitive cells and the type of junction between gas conducting and exchange areas of the lung (U.S. Environmental Protection Agency, 1986). The cells most damaged by O_3 are the ciliated epithelial cells in airways and Type 1 cells in gas-exchange areas. Both of these cell types have very large surface areas (relative to volume) exposed to inhaled gases. The many factors that influence the distribution of inhaled O_3 within the respiratory system (see Chapter 8) result in some of the largest effective doses to the epithelial cells lining the nose and to epithelial cells located at the junction of the conducting and exchange areas, the CAR, in lungs. The 1986 criteria document did not contain studies of morphological effects of O_3 on the nose. There are species differences both in the basic structure of the CAR and in the epithelial cells that line the CAR (Tyler, 1983; Plopper, 1983). The CAR of humans, other primates, dogs, cats, and a few other domesticated species consists of the last conducting airway, the TB, several generations of RBs, alveoli that open directly into RB lumens, and ADs that branch from RBs (Figure 6-2). In lungs from many other mammals, including those most commonly used for inhalation toxicology (i.e., rats, mice, guinea pigs, and rabbits), RBs are poorly developed or absent and the CAR consists of TBs that open directly into ADs.

Epithelial degenerative changes in TBs and alveoli occur early, 2 to 4 h, in an O_3 exposure (Stephens et al., 1974a; Castleman et al., 1980). Depending on the dose to

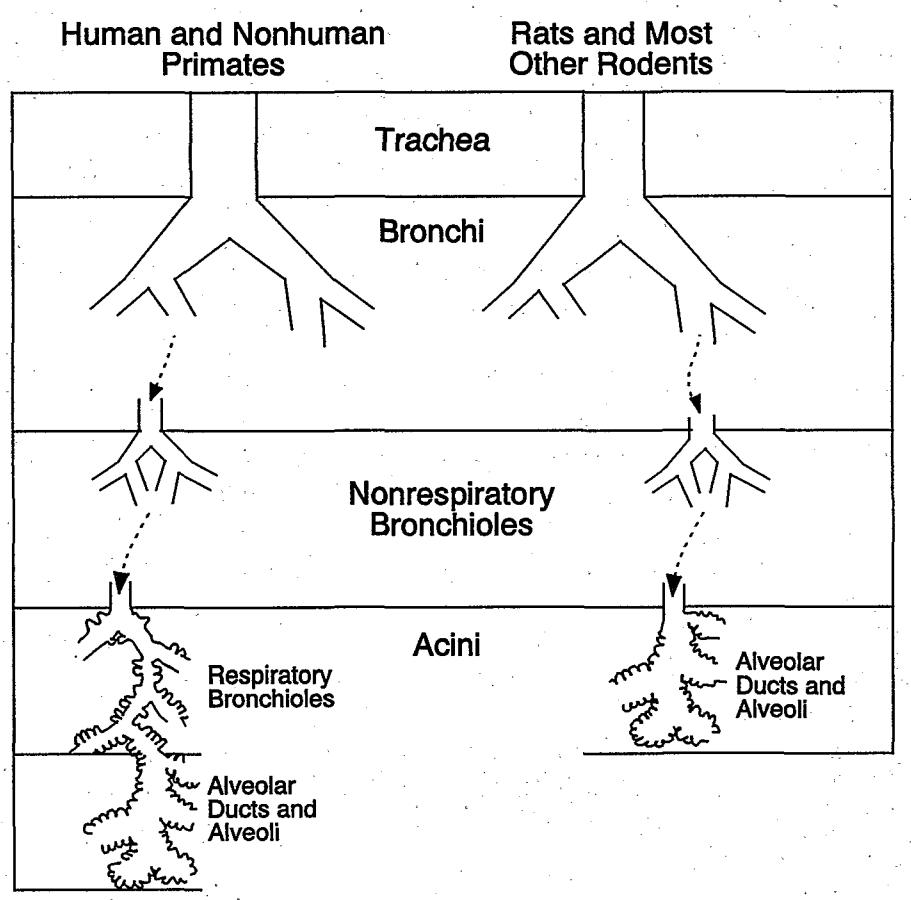

Figure 6-2. Schematic representation of intrapulmonary conducting airways and acini from animals with respiratory bronchioles (RBs) (human and nonhuman primates) and without RBs (rats and most rodents). For simplicity, several generations of bronchi and nonrespiratory bronchioles are not depicted.

Source: Redrawn from Weibel (1963) using information from Tyler and Julian (1991).

individual ciliated cells, they may lose cilia; undergo degenerative changes; or become necrotic and be sloughed into the lumen, leaving bare basement membrane until other cells replace them (Stephens et al., 1974a; Castleman et al., 1980). In the TB of the CAR, sloughed ciliated cells are replaced by nonciliated bronchiolar cells, which may become hyperplastic following longer exposures. Although these changes in TB epithelial cells can be studied readily by light microscopy (LM) or transmission electron microscopy (TEM), the surface views provided by scanning electron microscopy (SEM) provide a more comprehensive understanding of the three-dimensional aspects of CAR changes (Schwartz et al., 1976; Castleman et al., 1980).

Changes in mucus-secreting cells of conducting airways were considered minor in the 1986 criteria document (U.S. Environmental Protection Agency, 1986). Schwartz et al.

(1976) did not find changes in mucous cells suggesting damage to cell organelles in rats exposed to 0.2, 0.5, or 0.8 ppm O_3 for 7 days continuously or 8 h/day intermittently. Mellick et al. (1977) reported similar negative findings in mucous cells of monkeys following exposure to 0.5 or 0.8 ppm 8 h/day for 7 days. Wilson et al. (1984) reported more prominent small-mucous-granule (SMG) cells in the tracheas from monkeys exposed to 0.64 ppm O_3 continuously for 3 or 7 days. They speculated that these SMG cells may be related to repair processes.

Following O_3 exposure, Type 1 cells lining alveoli in the CAR, especially those opening into either RBs or ADs, may undergo vacuolization, fragmentation, or necrosis and be sloughed from the surface, leaving bare basement membrane (Stephens et al., 1974a). Although these degenerative changes, called lesions for purposes of this document, can be seen by careful LM of thin plastic sections, they are more reliably identified and the amount of damage can be estimated morphometrically using TEM (Barry et al., 1983; Crapo et al., 1984; Fujinaka et al., 1985). In alveoli, the bare basement membrane that follows O_3 exposure is recovered by Type 2 alveolar epithelial cells. Some Type 2 cells differentiate into Type 1 cells (Evans et al., 1975), but the epithelium remains thickened (Barry et al., 1983; Crapo et al., 1984).

Epithelial replacement in both TBs and alveoli can be followed and the amount can be estimated using radiolabeled thymidine and autoradiography (Evans et al., 1976a,b). Although bare basement membrane in alveoli is usually recovered by multiplication of Type 2 cells, in chronic exposures, bronchiolar cells, especially nonciliated bronchiolar cells, may cover part of the basement membrane formerly occupied by Type 1 or 2 cells. This process, termed bronchiolization (Nettesheim and Szakal, 1972), results in remodeling of CAR airways with the formation of new RBs. New RBs are identified readily by SEM or by LM in lungs from species in which RBs are normally absent or poorly developed (Boorman et al., 1980; Moore and Schwartz, 1981). In animals whose lungs normally have well-developed RBs, the extent of remodeling can be estimated using LM morphometry (Fujinaka et al., 1985).

The above epithelial changes are accompanied by an inflammatory response in the CAR characterized by increased numbers of PMNs in early stages, by increased numbers of AMs in lumens and in tissue at later stages, by hyperemia and interstitial edema, and by a fibrinous exudate (Stephens et al., 1974a; Schwartz et al., 1976; Boorman et al., 1980; Castleman et al., 1980; Fujinaka et al., 1985). As exposure continues, alveolar septa in the CAR thicken due to increased matrix, basement membrane, collagen, and fibroblasts and other cells, as well as by thickened alveolar epithelium (Boorman et al., 1980; Barry et al., 1983; Crapo et al., 1984; Fujinaka et al., 1985).

Two morphometric studies cited in the 1986 criteria document reported thickened walls in pulmonary arteries (P'an et al., 1972) and arterioles (Fujinaka et al., 1985).

Relative susceptibility to morphological change due to nutritional factors, age at start of exposure, and pneumonectomy also were considered in the previous criteria document (U.S. Environmental Protection Agency, 1986). Some investigators reported that rats on diets that were deficient or had basal levels of vitamin E tended to develop more severe lesions at lower O_3 concentrations (Plopper et al., 1979; Chow et al., 1981). Other studies concluded that the extent of CAR lesions was independent of the level of vitamin E in lung tissue (Stephens et al., 1983). The effects of age at start of exposure also were studied. Stephens et al. (1978) reported that rats were not susceptible until weaning at 21 days and that CAR lesions increased until they reached a plateau at 35 days. Barry et al. (1983) used

TEM morphometry to study the CAR lesions in lungs from 1-day-old rats exposed 12 h/day for 6 weeks to 0.25 ppm O_3. They reported CAR alveoli had more Type 1 and 2 epithelial cells and more AMs. The Type 1 cells were smaller in volume, covered less surface, and were thicker. They were aware of the results of Stephens et al. (1978) and speculated that the changes they described may have occurred primarily during the last 3 weeks of exposure. Boatman et al. (1983) did not find effects of O_3 on lung growth following pneumonectomy.

Several studies included both an exposure and a postexposure period during which the animals breathed air without O_3. Plopper et al. (1978) reported that CAR epithelial cells returned to normal appearance 6 days after a 72-h exposure to O_3. Incomplete resolution was reported 7 days after a 50-h O_3 exposure of monkeys (Castleman et al., 1980), 10 days after a 20-day O_3 exposure of mice (Ibrahim et al., 1980), and 62 days after a 180-day O_3 exposure of rats (Moore and Schwartz, 1981).

The previous criteria document (U.S. Environmental Protection Agency, 1986) comprehensively evaluated several citations reporting emphysema following O_3 exposure using current definitions of human emphysema (Snider et al., 1985). The morphological changes described in those earlier publications did not meet the current criteria for emphysema of the type seen in human lungs.

6.2.4.2 Sites Affected

The sites in the respiratory system that are affected will be discussed in anatomical sequence, beginning with the upper respiratory tract and proceeding downward. The upper or extrathoracic conducting airways (also referred to as the nasopharyngeal region) include the nasal cavity, pharynx, and larynx. Lower conducting airways begin with the trachea and include the bronchi and nonterminal bronchioles (also referred to as the tracheobronchial region). A summary of available information on these sites in the respiratory system is in Table 6-10, Effects of Ozone on Conducting Airways. Summaries of the information available concerning effects on the CAR, the gas exchange region (also referred to as the pulmonary region), and other pulmonary structures are divided into effects of short-term (<2-week) exposures in Table 6-11 and effects of long-term exposures in Table 6-12.

Nasal Cavity and Nasopharynx

The nasal cavity "conditions" inhaled air and in that process "scrubs" some reactive pollutants from the inhaled air, thereby reducing the concentration to which other portions of the respiratory system are exposed (Yokoyama and Frank, 1972; Miller et al., 1979). Although this scrubbing process is protective of other portions of the respiratory system, it results in a large dose of pollutant to the cells and tissues that line the nasal cavity.

There is a large range of variation in the structure of the nasal cavity among the animals used for inhalation toxicology and between those animals and humans (Schreider and Raabe, 1981). Schreider and Raabe (1981) found a striking similarity between the nasopharyngeal cavities of monkey and humans. They proposed that, with appropriate scaling, the monkey could serve as a model for aerosol and gas deposition in the nasopharyngeal region of humans. Thus, the studies on monkeys by Harkema et al. (1987) provide useful information for extrapolation to humans, as well as information concerning cellular responses in monkeys.

Table 6-10. Effects of Ozone on Conducting Airways[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.05 0.1 0.5 1.0 2.0 4.0 6.0 8.0	98 196 980 1,960 3,920 7,840 11,760 15,680	30 min (In vitro monolayer cultures)	Rabbit (NZW)	Phase contrast LM and trypan blue exclusion of tracheal epithelium. No consistent changes at 0.05 or 0.1 ppm, but 0.5 to 2.0 ppm resulted in cytoplasmic vacuolization without decreased viability. More evidence of cell damage, including gaps in previously confluent cultures and loss of cell viability at 4.0 and 8.0 ppm. Lipid peroxidation also was studied in these cultures.	Alpert et al. (1990)
0.12 0.8 1.5	235 1,568 2,940	6 h	Rat, F (F344/N) 280-400 g	LM pathology and LM morphometry of PMNs in lung and nasal epithelia. Also nasal and bronchoalveolar lavage. See Section 6.2.2. Nasal epithelium: No necrosis, loss or attenuation of cilia, or hyperplasia at any exposure. Increases in pavemented PMNs at most concentrations and times. Lung: No lesions due to 0.12 ppm. CAR lesions not obvious at 0.8 or 1.5 ppm immediately or 3 h PE, but a progressive increase in CAR lesions at all other times. CAR TBs and proximal alveolar septa thickened and increased inflammatory cells. Increased CAR tissue PMNs in 0.8- and 1.5-ppm groups at 18 and 66 h PE and 1.5-ppm group at exposure end.	Hotchkiss et al. (1989a)
0.12 0.8	235 1,568	6 h/day for 7 days	Rat, F (F344/N) 12-14 weeks old	LM pathology and morphometry of LM histochemistry of nasal epithelia. No LM pathology at 0.12 ppm, but 0.8 ppm caused hyperplasia of transitional nonciliated epithelium. In 0.8-ppm group, shortened cilia in respiratory epithelium of nasopharynx, but not of nasal septum. Increased intraepithelial mucosubstances in all areas at 7 days PE to 0.8 ppm.	Harkema et al. (1989)
0.12 0.25 0.5	235 490 980	20 h/day, 7 days/week for 2 years (4, 12, 26, 52, 78, and 104 weeks)	Rat, M (F344, CrlBR) 42 days old	Smaller body weights after 7 weeks exposure to 0.5 ppm. LM histopathology of nasal epithelia. Nose: At ≥0.25 ppm, mucous cell and respiratory epithelial hyperplasia. No lesions in mainstem or large bronchi. CAR: Described in Table 6-12.	Smiler et al. (1988)
0.12 0.27 0.8	235 529 1,568	6 h/day for 3 or 7 days	Rat, F (F344/N) 8-12 weeks old	LM pathology and LM BrdU for DNA synthesis by nasal epithelia. No LM pathology in squamous epithelium or in ciliated respiratory epithelium. Hyperplasia of nonciliated cuboidal/transitional epithelium at 0.8 ppm. BrdU uptake (DNA synthesis) increased at end of 3- and 7-day exposure to 0.8 ppm. BrdU uptake decreased in squamous epithelium only after 7 days exposure to 0.8 ppm and 7 days PE.	Johnson et al. (1990)
0.12 0.5 1.0	235 980 1,960	6 h/day, 5 days/week for 20 mo	Rat, M (F344) 6-8 weeks old	Nasal changes limited to nasal transitional nonciliated epithelium at 0.5 and 1.0 but not 0.12 ppm. LM histochemistry of intraepithelial mucosubstances in the nose and bronchi. Epithelial cell hyperplasia; mild to moderate inflammatory cell influx into the mucosa; and increased mucosubstances. Mucous flow rates decreased.	Harkema et al. (1994)

Table 6-10 (cont'd). Effects of Ozone on Conducting Airways[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.12 0.5 1.0	235 980 1,960	6 h/day, 5 days/week for 20 mo	Rat, M (F344) 6-8 weeks old	LM morphometry and histochemistry of "short-" and "long-path" conducting airways and CAR. *Trachea*: No changes in epithelial thickness, cell populations or stored glycoconjugate, but a dose-dependent loss of stored glycoconjugate was found. *Bronchi*: No changes in epithelial thickness or cell populations. Rats exposed to 1.0 ppm had increased stored gylcoconjugates in cranial (short-path) and caudal (long-path) bronchi, but not in central (short-path) bronchi. *Bronchioles*: TB of rats exposed to 1.0 ppm had thicker epithelium with increased V_v of nonciliated bronchiolar cells. V_v also increased in caudal (long-path) TB of 0.5-ppm exposed group. Mass ($\mu m^3/\mu m^2$) (V_s) of nonciliated cells increased in caudal (long-path) TBs of all exposed rats, but not in short-path TBs. *CAR*: Increased V_s of bronchiolar epithelium in former ADs in cranial and caudal CARs of rats exposed to 1.0 ppm and in cranial CAR of rats exposed to 0.5 ppm.	Plopper et al. (1994a)
0.12 0.24 0.48	235 470 940	3, 6, 12, or 24 h	Rat, F (F344/N) 11-13 weeks old	LM pathology and semiautomatic image analysis system for DNA synthesis (BrdU uptake) of epithelium in nasal maxilloturbinates. C × T design. No effects at 0.12 or ≤1.44 ppm·h. For a given C × T, increased DNA synthesis equal at different Cs and Ts. Nonlinear increase in DNA synthesis as C × T increased.	Henderson et al. (1993)
0.15 0.3	294 588	8 h/day for 6 days to 0.15 ppm or for 90 days to 0.15 or 0.3 ppm	*Macaca radiata*, M, F 2-6 years old, 2.3-9.7 kg	LM, SEM, and TEM morphometry of nasal epithelia. Respiratory epithelium: Ciliated cell necrosis, shortened cilia, and increased small mucous granule cells at all exposures, even at 0.15 ppm for 6 days. Transitional epithelium: Decreased nonciliated cells without granules, increased nonciliated cells with granules, and increased small mucous granule cells. Increased intraepithelial leukocytes only at 6 days in both types of epithelium.	Harkema et al. (1987)
0.15 0.25	294 490	8 h/day for 6 days to 0.15 ppm or for 90 days to 0.15 or 0.3 ppm	*Macaca radiata*, M, F 2-6 years old, 2.3-9.7 kg	LM and TEM histochemistry for intraepithelial mucosubstances in nasal mucosa. More mucous cells that had dilated granular endoplasmic reticulum. Also changes in mucosubstances.	Dimitriadis (1992)
0.15 0.25	294 490	8 h/day for 7 days	*Macaca radiata* NS	The O_3 concentration is not clear—the abstract states 0.64 ppm, the text mentions only 0.25 ppm. LM morphometry of vocal fold mucosa. Disruption and hyperplasia of stratified squamous epithelium. Epithelium thickened at 12 h and 7 days PE. Connective tissue of lamina propria thickened at 7 days PE. Basement membrane is undulating rather than smooth. Even though thickened, epithelium appeared normal at 7 days PE.	Leonard et al. (1991)
0.2 0.4 0.8	392 784 1,568	22 h/day for 3 days	Rat, M (Wistar RIVM [TOX]) 150-190 g	LM pathology and tritiated thymidine uptake by nasal epithelia. Observations on mixtures are in Section 6.4. Respiratory epithelium: No changes at 0.2 ppm O_3. Loss of cilia and disarrangement at 0.4 and 0.8 ppm. Some epithelia were hyperplastic or metaplastic or both. Thymidine uptake increased in rostral (anterior) portions.	Reuzel et al. (1990)

Table 6-10 (cont'd). Effects of Ozone on Conducting Airways[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.8	1,568	6 h/day for 3 or 7 days	Rat, F (F344/N) 12-14 weeks old	LM morphometry and histochemistry of nasal nonciliated cuboidal epithelium. Other types of nasal epithelia not examined. After 3 days O_3 and 18 h PE, no changes in cell density or in intraepithelial mucus. After 7 days exposure or after 3 days exposure and 4 days PE, hyperplasia and increased intraepithelial mucosubstances with no change in the ratio of acidic to neutral mucosubstances.	Hotchkiss et al. (1991)
0.8	1,568	6 h	Rat, F (F344/N) 12-16 weeks old	LM pathology and DNA synthesis by BrdU uptake by nasal nonciliated transitional epithelium. Ozone did not result in necrosis, exfoliation, or inflammation, but did increase DNA synthesis.	Hotchkiss and Harkema (1992)
0.96	1,882	8 h/night, 7 nights/week for 3 or 60 nights	Rat, M (S-D) 234-263 g	LM morphometry, histochemistry, autoradiography, and SEM and TEM morphometry of tracheal epithelium. Neither 3 nor 60 days exposure altered the cell density of ciliated, serous, basal, brush, migratory, or unidentified cells in tracheal epithelium. 3 days: Damage to cilia and ciliated cells, including necrosis. Thymidine labeling index increased. Serous cell histochemistry unchanged. 60 days: Less evidence of injury than at 3 days, but more damaged ciliated cells than in controls. Complete recovery of the epithelial changes by 42 days PE.	Nikula et al. (1988a)
0.96	1,882	8 h	*Macaca mulata*, M 2.0-8.5 years old 2.1-6.3 kg	LM and TEM morphometry of trachea, bronchi, and RBs. Increased necrotic cells in trachea and RBs at 1 h PE and in bronchi at 12 and 24 h PE. Decreased ciliated and basal cells in bronchi at 1, 12, and 24 h PE. Basal cells in bronchi also decreased at 72 and 168 h PE. Nonciliated bronchiolar cells in RBs increased only at 24 h PE. In bronchi, smooth muscle increased and amorphous matrix decreased at 24, 72, and 168 h PE. In RBs, smooth muscle increased at 24 h, fibroblasts increased at 24 and 72 h, and amorphous matrix increased at 12 h PE.	Hyde et al. (1992)
1.0	1,960	4 h/day for 5 days (examined at 2 weeks)	Sheep New-born	LM morphometry of tracheal epithelium. Also see Section 6.2.3. Percentage of ciliated and mucous cells remained at newborn levels, rather than ciliated cell percent increasing and mucous cell percent decreasing as in control lambs.	Mariassy et al. (1990)
1.0	1,960	4 h/day for 5 days (examined at 2 weeks)	Sheep New-born	LM morphometry of mucosubstances in tracheal epithelium. No evidence of damage or inflammatory changes. Decreased epithelial cell density, decreased ciliated and basal cells. Lectin-detectable intraepithelial mucosubstances did not undergo the maturation changes seen in control lambs.	Mariassy et al. (1989)
1.0	1,960	96 h (in vitro tracheal explants)	Rat, M (S-D) 250-270 g	Tracheal organ cultures exposed in vitro. Filtered air + O_3 resulted in extensive damage to cilia, and intermediate cells were seen. Cultures exposed to 95% O_2 + O_3 had stratified thickened epithelium with metaplastic cells in a middle zone and no ciliated cells at the surface.	Nikula and Wilson (1990)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

Table 6-11. Effects of Ozone on Lung Structure: Short-Term Exposures (<2 Weeks)[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.12 0.8 1.5	235 1,568 2,940	Continuous for 6 h	Rat, M (F344/N) 12-18 weeks old	LM histopathology of lungs and LM morphometry of lavaged AMs: No LM histologic effect detected at 0.12 ppm. No LM histologic effect at 0.8 and 1.5 ppm immediately or 3 h PE. At later PE times, there was mild, patchy CAR bronchiolitis and alveolitis. Increase in AMs and PMNs from 18-66 h PE. Progressive thickening of TB walls and CAR AD septa at 18, 42, and 66 h PE.	Hotchkiss et al. (1989b)
0.12 0.8 1.5	235 1,568 2,940	6 h	Rat, M (F344/N) 12-18 weeks old	Observations of nose and CAR. Same rats as in Hotchkiss et al. (1989a). LM histopathology of CAR is the same; new morphometry of PMNs in CAR and nasal mucosa. Emphasis on PMNs in nasal mucosa and nasal lavage compared with PMNs in CAR tissues and BAL at exposure end and at 3-66 h PE.	Hotchkiss et al. (1989a)
0.12 0.5 0.75	250 1,000 1,500	Continuous for 1-7 days	Rat, M (Wistar RIV:TOX) 8 weeks old	LM: Increased AMs in CAR and parenchyma. CAR increase persisted 5 days PE. TEM and SEM: BAL AMs had microvilli and blebs in addition to ruffles characteristic of AMs from controls. Also see Section 6.2.3.	Dormans et al. (1990)
0.15 0.5		Continuous for 3 or 7 days	Rat, M (Wistar RIV:TOX) 8 weeks old	Elastase-induced emphysema and saline control rats. LM histopathology and morphometry for alveolar size. Also see Section 6.2.5. The incidence and severity of CAR LM lesions was the same in elastase- or saline-treated rats exposed to O_3. No change in alveolar size due to O_3.	Dormans et al. (1989)
0.2 0.4	393 784	4 h	Rat, M (S-D) 7 weeks old	LM histopathology. Necropsy 24 h PE. Lung lesions from 0.2 ppm not reported, but 0.4 ppm resulted in increased AMs and increased cellularity of alveolar septa with focal thickening. No increase in DNA synthesis by nasal epithelium. See mixture effects in Section 6.4.	Mautz et al. (1991)
0.2 0.38	393 745	3.75 h, Rest or exercise	Rat (S-D)	LM histopathology and morphometry. No effect from 0.2 ppm at rest. Increased free cells in airspaces at 0.38 ppm at rest. Exposure during exercise resulted in larger areas with free cells and in areas of septa thickened by infiltrating cells.	Mautz et al. (1985b)
0.25 0.8	490 1,568	29 min/day for 2 days, Strenuous exercise	Horse (Thoroughbred) 5-6 years old Gelding	LM and TEM histopathology (see also Section 6.2.5). 0.25 ppm: Lesions limited to vacuoles seen only by TEM in TB ciliated cells. 0.8 ppm: Gross hemorrhage and edema in two of three horses. CAR lesions, visible only by TEM, included edema, necrosis and sloughing of Type 1 cells, slight increase in AMs, shortened cilia, and vacuoles in ciliated and nonciliated bronchiolar cells.	Tyler et al. (1991c)
0.35 0.6	686 1,176	4 h at rest, 3 h with exercise	Rat, M (S-D) 7 weeks old	Examination 48 h PE. Lung: LM morphometry of lesions as a percent of parenchyma section area. No statistical evaluation of groups exposed to O_3 at rest and exercise. The lesion percent of parenchyma appears concentration-dependent and increased by exercise similar to Mautz et al. (1985b). Nasal epithelium: Evaluated percent thymidine labeled cells in respiratory epithelium. No change due to O_3 at rest. Effects of mixtures in Section 6.4.	Mautz et al. (1988)
0.35 0.8	686 1,568	72 h	Rat, F (S-D) 60 or 444 days old	LM morphometry and SEM. V_v of CAR lesions. Exposed adults lost body weight. Adults exposed to 0.8 ppm had smaller fixed lung volumes. Lesion V_v larger in young than adult rats at both concentrations. Free cell (AM) V_v increased in young rats at 0.35 ppm compared to adults. The CAR lesions were similar by SEM, but younger rats had more AMs in the CAR. Younger rats had larger CAR lesions and more AMs, but older rats had greater changes in body weight and fixed lung volume.	Stiles and Tyler (1988)

Table 6-11 (cont'd). Effects of Ozone on Lung Structure: Short-Term Exposures (<2 Weeks)[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.35 0.5 1.0	686 980 1,960	2.25 h/day for 5 days	Rat, M (F344) 110-120 days old	LM histopathology and morphometry for parenchymal density and alveolar size. No significant changes in these morphometric parameters. Histopathology: Reported only for 0.5 ppm O_3 + CO_2 challenge group, which had maximal CAR tissue damage on Days 4 and 5. Increased AMs on Days 2 and 3 and foci of necrotic TB epithelium. By Day 5, hyperplasia of TB epithelium and increased AMs and other inflammatory cells, which completely filled some CAR alveoli. Morphologic damage continued, whereas pulmonary functional changes attenuated. Also see Section 6.2.5.	Tepper et al. (1989)
0.4	784	Continuous up to 14 days	Rat, M (Wistar Jcl) 5 weeks old	SEM morphometry, immunocytochemistry. See Section 6.2.1 for biochemistry. Number of Clara cells/μm^2 increased at 14 days, but not earlier. The length of the Clara cell apical projection was increased after 6 h, decreased at 1 day, and not different at other periods. Cytochrome P-450 was localized to agranular endoplasmic reticulum of Clara cells.	Suzuki et al. (1992)
0.5	980	20 h/day for 1-14 days	Rat, M (F344) 13 weeks old	LM morphometry of thymidine-labeled cells in bronchus-associated lymph node and MLN. Other lung changes not described. Also see Section 6.2.3.	Dziedzic et al. (1990)
0.64	1,254	Continuous, 7 days	Rat, M (S-D) 250-300 g	LM morphometry of CAR (proximal alveolar) lesions. Increased centriacinar lesions. Rats treated with dimethylthioruea (a H_2O_2 scavenger) had smaller lesion volumes.	Warren et al. (1988)
0.7 0.9	1,372 1,764	20 h/day for 4 days	Mouse, F (CD-1 Crl:CD1/ (CR)BR) 20-22 g	LM morphometry for areal density (e.g., volume density) of lesions. No comparison of O_3 and air exposures. Also see Section 6.2.3.	Dziedzic and White (1987b)
0.75	1,470	Continuous for 3 days	Rat, M (Wistar) 200-250 g	LM histopathology. Ozone-exposed rats gained less body weight. Increased cells in TB and CAR AD septa. Number of cells diminished by Day 4 PE, but foci of AMs remained.	Bassett et al. (1988a)
0.8 0.4	1,568 784	Continuous for 7 days	Rat, M (Wistar) 8 weeks old	LM: Clara cell numbers/mm of TB basement membrane unchanged. Also see Section 6.2.3. Cell isolation: Although the number of isolated Clara cells/10^6 cells isolated/lung was increased, the percent Clara cells in the isolate was not changed. The percent Type 2 cells in the isolate was increased. No morphologic observations at 0.4 ppm.	Van Bree et al. (1989)
0.8	1,568	Continuous for 3 h	Rat, M (S-D) 250-300 g	By LM, PMNs in alveolar septa increased three times at 4 h PE. Number of septal PMNs peaked at 8 h PE and then rapidly declined. Free cells, septal thickening, and cellularity increased with increasing time PE. Also see Section 6.2.2.	Bhalla and Young (1992)
0.82	1,600	Continuous for 7 days	Rat, M (Wistar RIV:TOX) 8 weeks old	LM morphometry of histochemically identified Type 2 cells. Increased number of Type 2 cells than in controls.	Dormans (1989)

Table 6-11 (cont'd). Effects of Ozone on Lung Structure: Short-Term Exposures (<2 Weeks)[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.97	1,901	Continuous for 7 days	Rat, M 225-275 g	Smaller body weights. No other statistical comparison of controls and O_3 alone (see Sections 6.2.6 and 6.4).	Last et al. (1986)
1.0 2.0	1,960 3,920	4 h/day for 5 days	Dog, M, F (Beagle) 6 weeks old	LM morphometry for alveolar size. Mean linear intercepts larger (indicating larger alveoli) in 1.0- but not 2.0-ppm group.	Phalen et al. (1986)
1.0 2.0	1,960 3,920	Continuous for 3 h (Isolated perfused lungs)	Rat, M (S-D) 300-380 g	LM and TEM morphology. Necrosis and sloughing of airway epithelial cells of bronchi and larger bronchioles. TB had less severe lesions, including fewer necrotic cells and less damage to cilia. Fragmentation of some Type 1 cells with some areas of bare basal lamina.	Pino et al. (1992a)
1.0	1,960	Continuous for 8 h	Rat, M (S-D) 10 weeks old	LM and TEM morphometry. Also see Section 6.2.2. Rats received either normal rat serum or rabbit anti-rat PMN serum before the exposure. At exposure end, both exposed groups had a smaller volume of ciliated cells per unit area of epithelial basal lamina (V_s) compared with filtered air controls with similar serum. Ciliated cell V_s was also smaller at 4 and 16 h PE.	Pino et al. (1992b)
1.0	1,960	4, 6, 8, and 24 h	Rat, M (S-D) 63 days old	TEM morphometry. Also see Section 6.2.2. Volume of necrotic cells per area basal lamina (V_s) in the TB larger than controls at the end of 4- and 24-h exposure, but not at other exposure or PE times. With increasing exposure time, there was a shift from necrotic cells on the basal lamina to necrotic cells free in the TB lumen. The V_s of necrotic alveolar cells was increased after 4, 6, and 24 h of exposure. Viable undifferentiated cell V_s in TBs was increased after 6-h exposure followed by 18 h PE, 8-h exposure followed by 16 h PE, and after a 24-h exposure. In alveoli, viable Type 1 cell V_s was increased after a 24-h exposure. Total connective tissue V_s changes only increased in TBs after 8-h exposure followed by 4 h PE and in alveoli at the end of 8-h exposure. The V_s of migratory cells in TB interstitium was only increased 4 h after a 6-h exposure. In alveoli, the V_s of capillaries was increased after 8-h exposure.	Pino et al. (1992c)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

Table 6-12. Effects of Ozone on Lung Structure: Long-Term Exposures (>2 Weeks)[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
Base 0.06; spike to 0.25	Base 118; spike to 490	Base 13 h/day, 7 days/week; Ramped spike 9 h/day, 5 days/week (1, 3, 13, and 78 weeks)	Rat, M (F344) 60 days old	TEM morphometry, data expressed as volume per unit epithelial basement membrane (V_s). Acute response in the CAR (proximal alveolar) region included increased AMs, interstitial edema, and interstitial cell hypertrophy. These changes subsided by 3-weeks exposure. Changes at 13 and 78 weeks include increased V_s, total alveolar tissue, and epithelium. Type 1 cell increases include V_s at 13 weeks; number at 13 weeks, 13 + 6 weeks PE, and 78 weeks; and decreased surface area at 78 weeks. Type 2 cell V_s increased at 78 weeks and after 78 + 17 weeks PE, and number increased at 78 weeks. Total interstitium was increased at 78 weeks, and noncellular interstitium (collagen and basement membrane) was increased at 13 and 78 weeks. Thickened basement membrane had crystalline deposits. No bronchiolization or centriacinar airway remodeling. Changes in terminal bronchiolar cells include a decrease in surface area of ciliated and nonciliated at 78 weeks. No changes in bronchiolar cell numbers. All changes diminished, except the increased volume of Type 2 cells and the thickened basement membrane, 17 weeks PE.	Chang et al. (1992)
A: 0.25 B: Base 0.06; spike to 0.25	490; 118 to 490	6 weeks 3 weeks 13 weeks 12 h/day 22 h/day	Rat (F344) 7 weeks old	Compared effects of A and B exposure regimens. Cumulative doses for A (Barry et al., 1985) were 60.5 and 126.0 ppm-h and, for B, were 45.3 ppm-h at 3 weeks and 196.0 ppm-h at 13 weeks. The B regimen alone (Chang et al., 1992) is described earlier in this table. TEM morphometry of CAR. The pattern of exposure did not affect the degree of injury.	Chang et al. (1991)
0.1	196	2 h/day, 5 days/week for 1 year	Rabbit, M (NZW) 3-3.5 kg	LM morphology and morphometry of intrapulmonary conducting airways. (Also see Section 6.4). No difference in number of airways/area or in distribution of airway size. ESCs in the smallest conducting airways (< 0.30 mm) increased at 4, 6, and 12 mo of exposure to O_3 and decreased at 6 mo PE. ESCs in the next larger airways (0.31-0.49 mm) only increased after 4 mo of exposure and decreased at 6 mo PE. No effect on airways >0.50 mm.	Schlesinger et al. (1992a)
0.12 0.25	235 490	12 h/day for 6 weeks	Rat, M (F344) 1 day or 6 weeks old	TEM morphometry of proximal alveolar region (CAR). Type 1 epithelial cells increased in number and thickness, but decreased in luminal and basal lamina surface area. Some bare basement membrane where Type 1 cells sloughed, but not significantly increased. At 0.25 ppm, Type 2 epithelium increased in number, but not in volume, thickness, or surface area. Interstitium increased in thickness in adults at 0.25 ppm, but not at 0.12 ppm or in juveniles. AMs increased by 0.25 ppm at both ages, but only older rats had increased interstitial AMs. No differences due to age.	Barry et al. (1985)
0.12 0.5 1.0	235 980 1,960	6 h/day, 5 days/week for 20 mo	Rat, M (F344) 6-8 weeks old	LM morphometry of CAR remodeling. Thickened tips of alveolar septa lining ADs (alveolar entrance rings) 0.2 mm from TB in rats exposed to 0.12 ppm and to 0.6 mm in rats exposed to 1.0 ppm. Interstitial changes accompanied these epithelial changes.	Pinkerton et al. (1995)
0.12 0.5 1.0	235 980 1,960	6 h/day, 5 days/week for 20 mo	Rat, M (F344) 6-8 weeks old	Laser scanning confocal LM immunohistochemistry for CC10 in nonciliated bronchiolar (Clara) cells. Clara cells from rats exposed to 1.0 ppm, but not to 0.12 ppm, had increased cell volume of granule-based CC10, increased CC10 concentration within the granules, and increased number of granules per Clara cell profile.	Dodge et al. (1994)
0.12 0.25 0.5	235 490 980	20 h/week, 7 days/week for 2 years (Examined at 4, 12, 26, 52, 78, and 104 weeks)	Rat, M (F344 CrlBR) 42 days old	Rats exposed to 0.5 ppm had smaller BW after 7-weeks exposure. LM histopathology. Nose: At ≥0.25 ppm, mucous cell respiratory epithelium hyperplasia; no lesions in mainstem or large bronchi. CAR: ≥0.25 ppm, TB epithelium hyperplastic and hypertrophic; bronchiolarization and airway remodeling. No changes in 0.12-ppm group after 26 weeks of exposure. Peribronchiolar tissue and AD walls thickened by eosinophilic material after 12 weeks at 0.5 ppm and after 26-weeks at 0.25 ppm. Collagen found in these areas using special stains. Increased AMs at ≥0.25 ppm.	Smiler et al. (1988) Wright et al. (1989, 1990)

Table 6-12 (cont'd). Effects of Ozone on Lung Structure: Long-Term Exposures (>2 Weeks)[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.15 0.3	294 588	8 h/day for 6 or 90 days	*Macaca radiata*, F, M 2-6 years old	TEM, SEM, and LM morphometry. First generation RBs had epithelial hyperplasia, and alveoli opening into these RBs had increased AMs. RB epithelium thickened, but no difference due to either exposure time or concentration. RB interstitium was thickened in all exposed monkeys, but both cellular and acellular compartments were individually thickened only after 90-days exposure to 0.3 ppm. No differences due to age or gender. No evidence of epithelial cell necrosis nor of inflammatory cell infiltration other than the increased AMs.	Harkema et al. (1993)
0.25	490	12 h/day for 6 weeks	Rat, M (F344) 1 day or 6 weeks old	TEM morphometry of the TBs. Luminal surface area covered by cilia decreased, as did the luminal surface of Clara (nonciliated bronchiolar) cells. Number of brush cells decreased. No differences due to age.	Barry et al. (1988)
0.25	490	8 h/day, 7 days/week, "daily" for 18 mo or "seasonal" O_3 odd months, filtered air even months for 18 mo (9 mo of O_3)	*Macaca fascicularis*, M 6 mo old	LM morphometry. Also see Section 6.2.5. Low-grade respiratory bronchiolitis in both exposed groups. Compared with controls, both groups of exposed monkeys had increased V_v of tissue other than parenchyma and V_v of RBs and their lumens. Both V_v and V of RB wall increased in the "daily" group but not in the "seasonal" group. The only significant morphometric difference between the two exposed groups was the V_v of cells, mostly AMs, free in airspace lumens. This difference and the difference in significance of the RB wall thickness was presumed due to the difference in time after the last O_3 exposure and necropsy. Daily group necropsied the day after the last exposure, whereas seasonal group necropsied after a month of filtered air. Seasonal group had an amount of morphological changes similar to the daily group.	Tyler et al. (1988)
0.25	490	8 h/night, 7 nights/week, "daily" for 18 mo or "seasonal" O_3 odd months, filtered air even months for 18 mo	Rat, M (S-D) 22 days old	LM morphology and morphometry. Monkey data from Tyler et al. 1988 (above) compared with rats exposed to a similar regimen. Rats: Estimated the extent of centriacinar remodeling by counting the number of junctions of bronchioles with ADs per area of lung section (B/A J/cm^2). At the end of the exposure, both exposed groups had more B/A J/cm^2 than filtered air controls. Recovery by 30 days PE. No difference between the two exposed groups, even though the daily group was exposed twice as many days as the seasonal group.	Tyler et al. (1991a)
0.3	588	7 h/day, 5 days/week for 6 weeks	Mouse, M (Swiss-Webster) Newborn	LM morphometry of histochemically identified Type 2 cells. Type 2 cells tended to be larger (longer linear intercepts), and the number per microscope field tended to be greater, but the p values were >0.05 in final data in which the images were edited electronically. However, these values were significant ($p < 0.05$) in unedited data. Exposed mice had larger ($p < 0.05$) body weights at both 3 and 6 weeks.	Sherwin and Richters (1985)

Table 6-12 (cont'd). Effects of Ozone on Lung Structure: Long-Term Exposures (>2 Weeks)[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.35	686	4.5 h/day, 5 days/week for 4 weeks, 380 mmHg (5,400 m) or sea level	Mouse, M (Swiss-Webster) 32 g	Automated LM morphometry of stainable elastin in alveolar walls. Simulated high altitude (5,400 m) with O_3 (SHA-X) or without O_3 (SHA-C) resulted in larger lung volumes than sea-level controls (SL-C), but not different from each other. Unlike most studies, sea-level, O_3-exposed mice had the smallest lung volumes. Alveolar wall areas, after adjustment to SL-C lung volumes, were increased only in the SHA-X group. Alveolar wall elastin area, adjusted to the SL-C lung volumes, increased in both high-altitude groups compared to SL-C and also were different from each other with the largest amount of elastin area in the SHA-X group. However, if the elastin areas were not adjusted for differences in lung volumes, there were no differences between the groups.	Damji and Sherwin (1989)
0.4 0.64	784 1,254	8 h/day, 7 days/week for 90 days	*Macaca radiata*, M 5-8 years old	LM and TEM morphometry with emphasis on RBs. Respiratory bronchiolitis and peribronchiolar inflammation. RB walls thicker with smaller lumens. Increased wall thickness due both to thicker epithelium (significant only at 0.64 ppm) and interstitial components. Epithelial changes in both O_3 groups include increased nonciliated bronchiolar epithelial cells and decreased Type 1 cells. Interstitial changes in both O_3 groups included increased smooth muscle cells, mast cells, and fibers. Components increased at 0.64 ppm, but not at 0.4 ppm, included interstitial AMs, PMNs, and amorphous ground substance.	Moffatt et al. (1987)
0.5	980	6 h/day, 6 days/week for 2, 3, 5, and 12 mo	Rat, M (Wistar) 100 g	LM, TEM, and LM morphometry of collagen fibers. Bronchitis, peribronchitis, CAR remodeling, and increased stainable collagen in bronchioles. Rats apparently had intercurrent respiratory disease, as 10 of 44 exposed rats died of pneumonia or pulmonary edema. In addition, it appears that only one set of 12 rats maintained in room air served as controls for all exposed groups, even though controls and exposed rats would be of significantly different age and size.	Hiroshima et al. (1989)
0.5	980	20 h/day, 7 days/week for 52 weeks	Rat (F344)	LM histopathology. 6-mo exposure: Inflammation, mononuclear cells, and fibroblasts in AD walls and walls of adjacent CAR alveoli. TB not involved. 12-mo exposure: Similar to 6 mo with possibly a slight increase in AMs, some increased thickening of centriacinar AD and alveolar walls, and a few foci of bronchiolization (CAR remodeling). 12-mo exposure + 6 mo PE: Slight dilation of ADs, minimal inflammatory reaction, slight thickening of AD and CAR alveolar walls, and a few foci of bronchiolization.	Gross and White (1987)
0.5	980	Continuous for 120 days	Mouse, F (Swiss) 20-23 g	LM morphometry and histopathology. Also see Section 6.2.3. More tissue, primarily inflammatory cells, at Day 9. Little change from Days 10 to 120. Thickened airway walls with increased collagen. Increased collagen in alveolar walls along the ADs.	Jakab and Bassett (1990)
0.64 0.96	1,254 1,881	8 h/night, 7 nights/week for 42 nights, "pair" fed	Rat, M (S-D) 28 days old	LM morphometry and SEM. Also see Section 6.2.5. End of 42-night exposure: No difference in BW, hemoglobin, or total serum proteins. At 0.96 but not 0.64 ppm, larger fixed and saline-filled lung volumes, lung volume/BW ratios, and volumes of parenchyma. At both concentrations, increased V_v and V of RB and RB walls and their ratios to BW. By SEM, remodeling of CAR airways with the formation of RBs and thickened CAR septa at both ppm. After 42 days PE: Fixed lung volume at 0.96 ppm increased. V_v and V of RB walls and ratio to BW increased at 0.96 ppm, as did ratios of volumes to BW for parenchyma, alveoli, total RB, and RB wall. SEM revealed persistence of CAR remodeling and thickened septa.	Tyler et al. (1987)

Table 6-12 (cont'd). Effects of Ozone on Lung Structure: Long-Term Exposures (>2 Weeks)[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.64	1,254	8 h/day for 12 mo	*Macaca faciscularis*, M 6-7 mo old	LM morphometry of CAR airway remodeling. Both V_v and V of RBs, and their walls and lumens, increased at end of exposure and 6 mo PE. RB internal diameters were smaller at exposure end, but not at 6 mo PE. V_v free cells, mostly AMs, increased only at exposure end. No differences in BW or fixed lung volumes.	Tyler et al. (1991b)
0.7	1,372	20 h/day for 4 weeks	Rat, M (F344) 14 weeks old	LM histopathology. Also see Section 6.2.5. Exposure end: CAR inflammation of TB, AD, and CAR (proximal) alveoli characterized by edema with mononuclear and leukocyte infiltration. 4 weeks PE: Few inflammatory foci, edema decreased, and interstitial mast cells. Slight thickening of ADs and septa. 9 weeks PE: Inflammation cleared, TB walls slightly thickened by amorphous matrix.	Gross and White (1986)
0.95	1,862	8 h/day for 90 days	Rat, M (S-D) 61 days old	LM and TEM morphology. RB: Increased volume of total RB and of RB wall and lumen. RB walls thickened by interstitial inflammation with edema, hyperemia, fibrosis, and hypertrophied smooth muscle and by interstitial mononuclear cells, granulocytes, and plasma cells. Epithelial and vascular basal lamina fused. TB: Smaller internal diameter and smaller luminal volume, but no change in total TB volume or in wall volume. Proximal AD: Most severe cell damage and inflammation at alveolar septal tips (alveolar entrance rings). Epithelium at these tips was frequently necrotic or missing, leaving bare basement membrane. Duct walls thicker due mainly to increased interstitial edema, fibrosis, and cellular infiltrates. Basal lamina thickened, split or duplicated, and had granular deposits. Site of most severe injury shifted progressively distally as new segments of RB were formed.	Barr et al. (1988)
0.95	1,862	8 h/day for 90 days or 5-day episodes followed by 9 days PE	Rat, M (S-D) 52 days old	LM and TEM morphometry. Both groups examined at exposure end. The lesions were as previously described by Barr et al. (1988). Both groups had CAR airway remodeling with the formation of new RBs. The only morphometric difference in RBs between the groups was the volume of RB wall, which was greater in the daily group, but both groups were greater than controls. The volume of the total RB and of RB lumen was increased in the daily group. RB epithelium of the daily group was more differentiated. TB interstitium was increased in the episodic group. Alveolar duct/sac lumen volume was increased in both groups with the increase in the episodic group significantly greater than the daily group. Alveolar volume was decreased in the episodic group. The total amount of CAR damage was not different for both episodic (35 exposures) and daily (90 exposures) groups.	Barr et al. (1990)
0.96	1,882	8 h/night, 7 days/week for 3 or 60 nights	Rat, M (S-D) 234-263 g	LM morphometry, histochemistry, autoradiography, and SEM, and TEM morphometry. Neither 3 nor 60 days of exposure altered the cell density of ciliated, serous, basal, brush, migratory, or unidentified cells in tracheal epithelium. 3 days: Damage to cilia and ciliated cells, including necrosis. Thymidine labeling index increased. Serous cell histochemistry unchanged. 60 days: Less evidence of injury than at 3 days, but more damaged ciliated cells than in controls. Complete recovery of the epithelial changes by 42 days PE.	Nikula et al. (1988a)
0.98	1,921	8 h/day, 7 days/week for 90 days	Rat, M (S-D) 65 days old	LM morphometry and SEM of CAR. Remodeling of CAR. Increased thickness of septal edge (tips) of alveoli, which form the walls of ADs (alveolar entrance rings) up to 0.6 mm from TB. Alveolar septa thickened by replacement of Type 1 cells by Type 2 and bronchiolar cells to 0.6 mm from TB.	Pinkerton et al. (1992)

Table 6-12 (cont'd). Effects of Ozone on Lung Structure: Long-term Exposures (>2 Weeks)[a]

Ozone Concentration		Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
ppm	$\mu g/m^3$				
1.0	1,960	6 h/day, 5 days/week for 20 mo	Rat, M (F344) 6-7 weeks old	LM morphometry, SEM, confocal microscopy, immunocytochemistry, and conventional histochemistry. Remodeling of CAR. Former ADs were converted to RBs. Bronchiolar epithelium in these former ADs consisted of well-differentiated ciliated and nonciliated bronchiolar (Clara) cells.	Pinkerton et al. (1993)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

Harkema et al. (1987) exposed bonnet monkeys to 0.15 or 0.30 ppm O_3, 8 h/day for 6 or 90 days. They sampled four regions of the nasal cavity and nasopharynx. Changes were limited to the respiratory and transitional epithelium in the two most rostral (anterior) of the four sections. No changes were reported in the caudal (posterior) two sections, the last of which included the nasopharynx. The respiratory epithelium of the rostral nasal cavity had both qualitative and quantitative changes. Quantitative changes included decreased density of ciliated cells characterized qualitatively by multifocal loss of cilia, necrotic ciliated cells, ciliated cells with attenuated cilia, and others with only microvillar surface. The respiratory epithelium also had an increased density of SMG cells, presumably related to repair processes. Monkeys exposed to 0.30 ppm O_3 for 90 days also had increased abnormal cells with intracytoplasmic lumens containing both cilia and microvilli. Qualitative changes were also seen in mucous (goblet) cells, which appeared to have fewer secretory granules and dilated endoplasmic cisternae. Ozone exposure resulted in more nonciliated cells with secretory granules and with dilated cisternae of the endoplasmic reticulum. Like the respiratory epithelium, the transitional epithelium had an increased density of SMG cells. In both epithelia, inflammatory cells were increased only in the monkeys exposed to 0.15 ppm O_3 for 6 days. Most of the morphometric changes in the respiratory but not the transitional epithelium were as large after 6 days of exposure to 0.15 ppm O_3 as after 90 days of exposure to either 0.15 or 0.30 ppm. The histochemistry and cytochemistry of the nasal epithelia from these monkeys were studied by Dimitriadis (1992). This investigator reported changes in the intraepithelial mucosubstances and the presence of mucous cells with dilated cisternae in the granular endoplasmic reticulum.

Acute changes in nasal epithelia from rats exposed to O_3 concentrations of 0.12 to 1.0 ppm for 6 h to 7 days have been studied extensively (Table 6-10). In general, short-term exposure to $\leq$0.2 ppm O_3 results in either no changes detectable by LM or in mild hyperplasia. Higher concentrations for up to 7 days can result in damaged cilia, hyperplasia, and increased stored intraepithelial mucosubstances. Several studies document the hyperplasia using morphometry or DNA synthesis and document the stored mucosubstance by histochemistry and morphometry. In one study, the increased stored intraepithelial mucosubstances reached their largest quantity 7 days postexposure (Harkema et al., 1989). Details of individual studies follow.

Exposure to 0.12, 0.8, or 1.5 ppm O_3 for 6 h followed by postexposure periods up to 66 h resulted in inflammatory changes characterized by increased PMNs, but without LM evidence of necrosis, ciliary loss, or hyperplasia (Hotchkiss et al., 1989a). Hotchkiss and Harkema (1992) reported similar LM findings in rats exposed to 0.8 ppm O_3 for 6 h. They also reported increased DNA synthesis by bromodeoxyuridine (BrdU) uptake in nasal nonciliated transitional epithelium. Exposure to 0.8 ppm O_3 6 h/day for 3 or 7 days, or for 3 days with 4 days postexposure, resulted in hyperplasia of the nasal nonciliated cuboidal (transitional) epithelium with increased intraepithelial mucosubstances without significant changes in histochemical staining characteristics (Hotchkiss et al., 1991). In that study, no changes were reported for rats exposed for 3 days and examined 18 h postexposure.

Reuzel et al. (1990) exposed rats to 0.2, 0.4, or 0.8 ppm O_3, 22 h/day for 3 days. They did not report changes in rats exposed to 0.2 ppm, but those exposed to 0.4 or 0.8 ppm had loss of cilia and disarrangement of the epithelium with hyperplasia and metaplasia. Cell proliferation, as measured by radiolabeled thymidine, was increased at the two higher concentrations. The influence of O_3 C $\times$ T on epithelial cell proliferation in the nasal anterior maxilloturbinates was measured by BrdU uptake (Henderson et al., 1993). Rats

were exposed to 0.12, 0.24, and 0.48 ppm O_3 for 3, 6, 12, and 24 h, resulting in six C × T products. Exposure to 0.12 ppm or C × Ts of 0.72 or 1.44 ppm·h did not cause effects. For a given C × T between 2.88 and 11.52 ppm·h, the increased DNA synthesis was similar; the response did not increase linearly with increasing C × Ts. Generally, above 0.12 ppm O_3 there was a linear increase with increasing C but not T. Thus, exposure duration apparently was responsible for the lack of C × T linearity. Johnson et al. (1990) also used BrdU to study DNA synthesis in rats exposed to 0.12, 0.27, or 0.8 ppm O_3, 6 h/day for 3 or 7 days, and examined 3 or 7 days postexposure. Rats exposed to 0.8 ppm O_3, but not to the lower concentrations, had increased DNA synthesis in the nonciliated cuboidal (transitional) epithelium at 3 and 7 days and increased numbers of labeled cells in the ciliated respiratory epithelium and the olfactory epithelium only at 3 days. No changes were found in squamous epithelia except a decrease in labeled cells 7 days postexposure to 0.8 ppm O_3. Johnson and co-workers reported no LM changes in the ciliated respiratory, olfactory, or squamous epithelia, but hyperplasia occurred in the cuboidal transitional epithelium.

Epithelial mucosubstances were studied in rats exposed to 0.12 or 0.8 ppm O_3, 6 h/day for 7 days or 7 days postexposure (Harkema et al., 1989). They reported no LM pathology in the nasal or nasopharyngeal airways from rats exposed to 0.12 ppm, with the exception of an increase in secretory cells in ciliated epithelium. Rats exposed to 0.8 ppm had attenuation of cilia in the lateral walls of the nasopharynx; 7 days postexposure, an increase in stored intraepithelial mucosubstances was observed. The 0.8-ppm group also had hyperplasia of the nonciliated transitional epithelium accompanied by an increase in PMNs in the lamina propria. Seven days postexposure, rats in the 0.8-ppm exposure group had more stored intraepithelial mucosubstances in some areas of ciliated respiratory and nonciliated transitional epithelia.

In rats exposed to 0.12, 0.25, or 0.5 ppm O_3, 20 h/day for 2 years, Smiler et al. (1988) reported hyperplasia, especially of mucous cells, in the respiratory epithelium over the rostral portion of the nasoturbinate of rats in the 0.25- and 0.5-ppm groups. The respiratory epithelium lining other parts of the nasal cavity were less affected, and no changes were found in the squamous and olfactory epithelia.

Harkema et al. (1994) reported no changes in the amount of mucosubstances in conducting airways, including the nasal cavity, of rats exposed to 0.12 ppm O_3, 6 h/day, 5 days/week, for up to 20 mo. After exposure to 0.5 and 1.0 ppm O_3, however, mucous flow rates were slower and mucous cell metaplasia was evident over the lateral wall and turbinates of the proximal third of the nasal airways. Exposure to 0.5 and 1.0 ppm O_3 also caused epithelial hyperplasia in nasal transitional epithelium, an increase in eosinophilic globules in the surface epithelium lining the distal nasal airways, and mild-to-moderate inflammatory cell influx in the nasal mucosa of the proximal and middle nasal passages.

Larynx

Leonard et al. (1991) reported disruption and thickening of the stratified squamous epithelium over the vocal folds of bonnet monkeys (*Macaca radiata*) exposed to O_3, 8 h/day for 7 days. The basement membrane appeared undulating rather than smooth. At 7 days postexposure, the epithelium appeared thickened, but otherwise normal. The O_3 concentration to which the monkeys were exposed is not clear because different concentrations appear in the summary and text sections of the publication. However, these

larynges were from bonnet monkeys that also were studied by Harkema et al. (1987) and Dimitriadis (1992) and, therefore, most likely were exposed to 0.15 ppm.

Trachea and Bronchi

Several investigators studied effects of 0.96 or 1.0 ppm O_3 on the tracheas of monkeys, rats, and sheep during and after short-term (very brief) or long-term exposures. Hyde et al. (1992) studied the trachea, bronchi, and RBs of rhesus monkeys exposed to 0.96 ppm O_3 for 8 h and examined them at 1, 12, 24, 72, and 168 h postexposure. Although the primary objective of the study concerned inflammation (see Section 6.2.2), the study also provided much new morphometric information concerning reactions to O_3 of tracheal, bronchial, and RB epithelia and their interstitium. Both epithelial and interstitial data were determined as volume per surface area of epithelial basal lamina (V_s). At 1 h postexposure, the major change in the tracheal and RB epithelia was an increase in necrotic cells, whereas in the bronchial epithelium, there were fewer ciliated and basal cells. There were no other changes in tracheal epithelial cell V_s at any of the postexposure times examined. At 12 and 24 h postexposure, the V_s of necrotic cells was increased in bronchi but not in the trachea or RBs. The V_s of ciliated and basal cells was smaller in the bronchial epithelium but not in the trachea. Basal cells in bronchi also were increased at 72 and 168 h postexposure. Respiratory bronchioles had smaller V_s of Type 1 alveolar epithelial cells at all times except 1 h postexposure. In RBs, nonciliated bronchiolar cells were increased only at 24 h postexposure. Epithelial cell DNA synthesis was studied in the filtered air controls and at 1 and 12 h postexposure by radiolabeled thymidine incorporation. The only increase was observed in the bronchial epithelium at 12 h postexposure. Changes in the interstitial components of the trachea were minimal, with a decrease in the amorphous matrix at 24 h postexposure. Bronchi had increased V_s of smooth muscle and decreased amorphous matrix at 24, 72, and 168 h postexposure. Collagen fibers in the bronchial interstitium were decreased at 168 h. In RBs, the arithmetic mean thickness was increased at 12 and 24 h, but not at other times. In RBs, smooth muscle V_s was increased at 24 h, V_s of fibroblasts was increased at 24 and 72 h, and V_s of the amorphous matrix was increased at 12 h postexposure.

Nikula et al. (1988a) exposed rats to 0.96 ppm O_3, 8 h/night for 3 or 60 nights or for 60 nights followed by 7 or 42 days postexposure, and examined the tracheas using LM, TEM morphometry, SEM, LM mucosubstance histochemistry, and DNA synthesis by radiolabeled thymidine incorporation. Ciliated cells with short or damaged cilia were increased after 3 and 60 nights of exposure; cells with short cilia were increased after 60 nights of exposure and 7 days postexposure. Intermediate cells, presumed to be immature ciliated cells, were increased only after 3 nights of exposure. However, the numeric density of total ciliated cells, basal cells, total serous cells, brush cells, and total migratory cells was not different from controls. There were no changes in LM histochemistry for mucosubstances at any time. The only increase in thymidine labeling occurred after 3 days of exposure. Recovery was complete 42 days after 60 nights of exposure.

Mariassy et al. (1989, 1990) exposed newborn lambs to 1.0 ppm O_3, 4 h/day for 5 days, and studied controls at birth and controls and exposed lambs at 2 weeks of age. Tracheal mucous velocity was decreased at 2 weeks and at several additional postexposure times (see Section 6.2.3). In control lambs, the percent of ciliated cells increased and mucous cells decreased in the tracheas from birth to 2 weeks of age. This normal change in cell populations did not occur in the exposed lamb tracheas. In the more detailed

morphological study (Mariassy et al., 1989), epithelial cell density (cells per millimeter), rather than differential cell counts, was reported. In tracheas from control lambs, the density of mucous cells decreased from birth to 2 weeks of age. Ozone exposure resulted in decreased total epithelial cell density, with decreased densities of ciliated and basal cells. Mucous cell density remained at newborn levels. Ozone exposure also prevented the normal maturational changes of lectin-detectable mucosubstances but not of tinctorially stained mucosubstances.

The most comprehensive study of the effects of long-term O_3 exposure on conducting airways of rats is that by Plopper et al. (1994a). They used LM morphometry and tinctorial histochemistry to study conducting airways from the trachea to centriacinar alveoli following two "short" and one "long" pathway by airway dissection of fixed lungs. In rats exposed to 0.12, 0.5, or 1.0 ppm O_3 for 6 h/day, 5 days/week for 20 mo, the investigators did not find differences due to O_3 exposure in tracheal or bronchial epithelial thickness, cell populations, or stored glycoconjugates. However, they did find a concentration-dependent loss of stored glycoconjugates in the tracheas and in the caudal long-path bronchi but not in the cranial or central short-path bronchi. Although not significantly different from controls, there was a concentration-dependent thinning of the epithelium in caudal long-path bronchi. Terminal bronchioles from rats exposed to 0.5 and 1.0 ppm O_3 had increased volume fraction (V_v) of nonciliated bronchiolar (Clara) cells, and the epithelium was thicker in TBs from rats exposed to 1.0 ppm. In all exposed rats, the mass (V_s) of nonciliated bronchiolar cells was increased in TBs that had long pathways (caudal) but not in TBs with short pathways (cranial and central).

Centriacinar Region

As described in the previous criteria document (U.S. Environmental Protection Agency, 1986) and in the summary of it above, the CAR varies with the species. By common usage (Weibel, 1963; Schreider and Raabe, 1981; Weibel, 1983; Rodriguez et al., 1987; Haefeli-Bleuer and Weibel, 1988), the acinus consists of a TB, RBs when present, and the ADs and alveoli supplied by that TB. In some species (e.g., humans, monkeys, dogs, and cats), several generations of RBs are found between the TB and ADs. In other species (e.g., rats, mice, guinea pigs, and rabbits), RBs either are absent or very poorly developed and limited to a single, very short generation (Tyler, 1983; Tyler and Julian, 1991). The CAR consists of the TB, RBs if present and alveoli that open directly into RBs, and the initial portions of ADs. Acini that do not have RBs have a smaller volume than those that do. Rodriguez et al. (1987) estimated the acinar volume in rat lungs to be 1.86 mm^3, and Haefeli-Bleuer and Weibel (1988), using the same methods, estimated the acinar volume in the human lungs at 187.0 mm^3. Mercer and Crapo (1989) and Mercer et al. (1991) found that variation in acinar size within an individual lung is an important determinant of the intensity of lesions due to inhaled reactive gases. Thus, the intensity of CAR lesions may vary when animals with differing size acini are compared (Plopper et al., 1991).

The CAR lesion, both in animals with small acini (e.g., rats) and animals with large acini (e.g., monkeys) has been well described, both in the original reports and in the 1986 document (U.S. Environmental Protection Agency, 1986). Some of the reports published since that document contain additional details concerning cellular and interstitial responses in the CAR to short- or long-term O_3 exposure and are presented in this section (Chang et al., 1992; Pino et al., 1992c; Harkema et al., 1993). Most of these studies need

TEM levels of resolution and magnification and employ morphometric methods. In other reports, morphometric estimates of the volume of the CAR lesion were used to study factors that might alter the intensity of the lesion or evaluate the intensity of reaction to specific exposure regimens (Mautz et al., 1988; Warren et al., 1988; Stiles and Tyler, 1988). Due to the size and definition of the CAR lesion, this approach can use LM morphometry to estimate lesion volume. In other studies, the cumulative effect of O_3 on the CAR is estimated by LM morphometry of one of the components, distal airway remodeling, which results in the formation of new RBs (Barr et al., 1988; Tyler et al., 1988; Pinkerton et al., 1993). Examples of each type of study will be presented.

Pino et al. (1992c) exposed rats to 1.0 ppm O_3 for 4, 6, 8, or 24 h, followed by postexposure periods in filtered air for up to 20 h, so that the total exposure and postexposure period did not exceed 24 h. Some of the rats were used for BAL (see Section 6.2.2), others for TEM morphometry. The morphometric data are expressed as V_s values. After a 4-h exposure, necrosis was the dominant morphologic feature, with increases in V_s of necrotic cells in the TB epithelium (ciliated cells) and in CAR alveoli (Type 1 cells). With increasing time of exposure or postexposure, the volume of necrotic cells in TBs shifted from the epithelium to the lumen, with this change being significant at 24 h. In CAR alveoli, increased V_s of total necrotic cells occurred at 4, 6, and 24 h of exposure and at 24 h in the epithelium. Healing in the TBs, evidenced by increased V_s of undifferentiated cells, was underway 18 h after a 6-h exposure, 16 h after an 8-h exposure, and immediately after 24 h of exposure. The only significant change in viable alveolar cells was an increase in V_s of Type 1 cells after 24 h of exposure. This increase appeared predominantly due to swelling of individual Type 1 cells. Increased V_s of total TB interstitium occurred 4 h after an 8-h exposure. In CAR alveoli, total interstitium was increased after 8 h of exposure, with much of the increase due to an increase in capillary volume.

Chang et al. (1992) used TEM morphometry to evaluate cellular and interstitial responses in the CAR, TB, and alveoli (proximal alveoli) of rats exposed to a 9-h peak slowly rising to 0.25 ppm O_3 superimposed on a 13-h background level of 0.06 ppm (the background was 7 days/week, the peak was 5 days/week). Chang and co-workers examined rats after 1, 3, 13, and 78 weeks of exposure; 6 weeks after a 13-week exposure; and 17 weeks after a 78-week exposure. Centriacinar region alveoli had a larger volume of total tissue and total epithelium per area of basement membrane (V_s) only after 13 or 78 weeks of exposure, and these values were not different after postexposure periods. Type 1 cells had a larger volume only at 13 weeks of exposure, increased numbers at 78 weeks, and increased numbers after 13 weeks of exposure plus 6 weeks postexposure. Type 2 cell V_s was increased only after 78 weeks of exposure and 78 weeks of exposure plus 17 weeks postexposure. Macrophages in the CAR alveoli were increased only after 1 week of exposure. In CAR alveoli, both interstitial cells and matrix were increased after 1 week of exposure, and the matrix increased again after 13 and 78 weeks of exposure. This difference was no longer significant after either postexposure period. Although the data are not in the tables or figures in the article, the text indicates that both epithelial and endothelial basement membranes were thickened after 13 and 78 weeks of exposure and after the 17-week postexposure period. Crystalline deposits in the basement membrane are demonstrated in Figure 6-3. In TBs, the luminal surface area of Clara cells was reduced at 1 week of exposure, and both ciliated and Clara cells had smaller luminal surface areas after 78 weeks of exposure; these returned to control values during the postexposure period. However, increased V_s of Type 2 cells persisted for the 17-week postexposure period that followed the

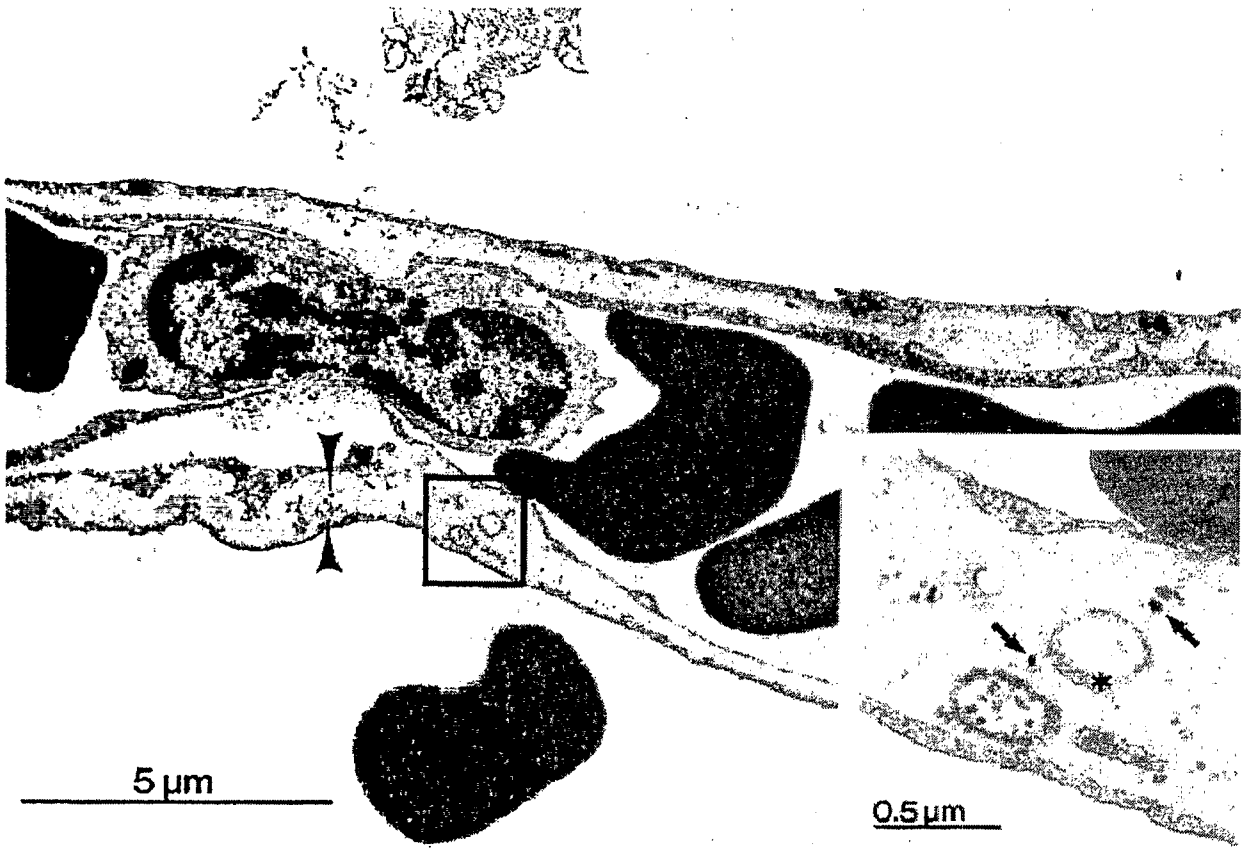

Figure 6-3. Electron micrograph of alveolar septa in the centriacinar region of the lungs from laboratory rats exposed to a simulated pattern of ambient O_3 for 78 weeks, showing thickened basement membrane (arrow heads). Inset micrograph shows dense crystaline deposits (arrows) and cellular extensions (*).

Source: Chang et al. (1992).

78 weeks of exposure. Chang et al. did not find significant bronchiolization of alveoli (i.e., distal airway remodeling). This observation may be due to the way the investigators sampled the CAR proximal alveoli and the strict orientation of the airways to obtain exact cross sections. This study complements those of Barry et al. (1985, 1988), who used similar TEM morphometric methods, by extending the exposure period and adding postexposure periods.

Cellular and interstitial changes were studied in nonhuman primates exposed 8 h/day for 90 days by both Moffatt et al. (1987) and Harkema et al. (1993), using TEM and morphometry. The study by Harkema et al. (1993) is reported here because the concentrations used, 0.15 and 0.3 ppm O_3, were lower than those used by used Moffatt et al. (1987), which were 0.4 and 0.64 ppm. Harkema and co-workers also studied reactions after only 6 days of exposure to 0.15 ppm. There were no major differences among the three

exposed groups (i.e., 6 days to 0.15 ppm, 90 days to 0.15 ppm, and 90 days to 0.3 ppm O_3). All exposed monkeys had thicker RB epithelium and thicker RB interstitium. There were more nonciliated cuboidal epithelial cells per millimeter of basement membrane in all exposed groups and increased squamous cells only in the 6-day group. The thickened total interstitium was due to increases in both acellular (matrix) and cellular components, but both compartments were increased individually only in the 90-day O_3 group. There were no differences in RB smooth muscle. Transmission electron microscopy and SEM observations, which were not studied quantitatively, include increased AMs in alveoli opening into RBs and increased "dome"-shaped nonciliated bronchiolar cells, which had more apical cytoplasm, more agranular and granular endoplasmic reticulum, more mitochondria, and more Golgi with secretory granules. With the exception of increased AMs, there was no evidence of necrosis nor of inflammatory cells. No differences due to age or gender were detected.

Harkema et al. (1993) speculate that their finding of a larger percent increase in RB cuboidal cells in monkeys exposed to a lower concentration of O_3 for the same time than that reported by Moffatt et al. (1987) might be due to the difference in sampling methods. Harkema et al. studied only the first generation RBs, whereas Moffatt et al. (1987) studied a random sample of all generations of RBs. The first generation tends to be more damaged than succeeding generations (Mellick et al., 1977; Eustis et al., 1981). The Harkema et al. (1993) sampling procedure also prevented examination for the increased volume density of RBs and decreased RB diameter reported by Fujinaka et al. (1985) and Moffatt et al. (1987). Remodeling of Centriacinar Region Airways. This is a less well-known sequela of long-term O_3 exposure. Using SEM, Boorman et al. (1980) and, later, Moore and Schwartz (1981) reported the development in rats of an airway with the appearance of RBs between the TB and ADs. This new segment was longer than those occasionally seen in control rats. Respiratory bronchioles in rats either are absent or developed to only a single, very short segment (Tyler, 1983; Tyler and Julian, 1991).

Barr et al. (1988) examined the development of this new segment using LM and TEM morphometry on lungs from rats exposed to 0.95 ppm O_3, 8 h/day for 90 days. They reported a significant increase in the total volume of RB and of RB lumen and wall. The new RBs reached a maximum length of four alveolar opening rings. They also noted that, in some of these RB segments, the capillary and epithelial basal laminae were fused as they are in TBs, rather than separate as in alveoli. Most Type 1 cell necrosis was found at the tips of alveolar septa immediately adjacent to the RB/AD junction. Thus, the most severe epithelial damage did not occur at the most proximal alveolus in the CAR, but rather in the alveolus immediately distal to the newly formed RB.

Recently, Pinkerton et al. (1993) developed a new LM morphometric method to evaluate remodeling of CAR ADs. In rats exposed to 1.0 ppm O_3 intermittently for 20 mo, the investigators reported well-differentiated ciliated and nonciliated bronchiolar epithelium lining CAR airways that would otherwise be ADs. Some of this epithelium extended five alveoli from the TB. Thus, the Type 1 and 2 cells characteristic of ADs were replaced by both types of bronchiolar cells characteristic of RBs when RBs are present in control rats. Pinkerton et al. (1995) used their new morphometric method to study rats exposed to 0.12, 0.5, or 1.0 ppm O_3 for 6 h/day, 5 days/week for 20 mo. They reported significant thickening of alveolar septal tips 200 mm from the TB in rats exposed to 0.12 ppm, which increased with O_3 concentration to 600 μm in rats exposed to 1.0 ppm, but they did not describe the type of epithelium covering these thickened tips. Several studies that did not

find CAR remodeling also used a slightly different procedure (Barry et al., 1985; Chang et al., 1992).

Plopper et al. (1994a) examined CARs from rats exposed to the same regimen, but studied CARs from one cranial short pathway and a caudal long pathway. They found nonciliated bronchiolar epithelial cells in remodeled former ADs in short- and long-pathway CARs from rats exposed to 1.0 ppm O_3, but only in short-pathway CARs from rats exposed to 0.5 ppm. Central, short-pathway CARs were not examined. Using the airway dissection method of selecting CARs to be studied, nonciliated bronchiolar cells were not found in ADs from rats exposed to 0.12 ppm O_3.

The same phenomena apparently occurs in animals with several generations of RBs as increases in V_v and volume (V) of RBs have been reported in all O_3-exposed monkeys examined using morphometric methods to estimate V_v or V of RBs (Fujinaka et al., 1985; Moffatt et al., 1987; Tyler et al., 1988, 1991b). Inflammatory changes and CAR remodeling occur concomitantly, and inflammatory changes in an airway may indicate future remodeling. Mellick et al. (1977) noted that, in monkeys exposed to 0.8 ppm, 8 h/day for 7 days, the inflammatory process extended throughout the RBs and into ADs. Eustis et al. (1981) reported that, in monkeys exposed to 0.8 ppm, 8 h/day for 90 days, all generations of RBs contained aggregates of inflammatory cells. Monkeys exposed to lower concentrations for the same or longer time have increased V_v and V of RBs (Moffatt et al., 1987).

6.2.4.3 Considerations of Exposure Regimens and Methods

Recovery During Postexposure Periods

Evidence of healing occurs soon after short-term O_3 exposures cease. In the studies of Pino et al. (1992c), evidence of healing is provided by the increased V_s of viable undifferentiated cells in TBs detected 16 h after the end of an 8-h exposure to 1.0 ppm O_3.

Chang et al. (1992) reported an increased V_s of Type 2 cells 17 weeks after a 78-week exposure to a simulated urban exposure regimen with a peak O_3 concentration of 0.25 ppm; there were no changes detected 6 weeks after a 13-week exposure. Gross and White (1987) examined rats 3 and 6 mo after a 52-week exposure (20 h/day, 7 days/week) to 0.5 ppm O_3. Using LM pathology, the only changes visible 6 mo after a 12-mo exposure were a few areas of bronchiolization, slight dilation of ADs, and slight thickening of AD walls and adjacent alveolar septa. In an earlier study, less complete healing was reported by Gross and White (1986), who used LM pathology to study rats 4 and 9 weeks after a 4-week exposure (20 h/day, 7 days/week) to 0.7 ppm O_3. Four weeks postexposure, Gross and White reported a slight, unevenly distributed inflammatory reaction with condensed eosinophilic material, presumed to be collagen, in the interstitium. Nine weeks postexposure, some AD walls and TBs were thickened. Rats exposed to 0.96 ppm, 8 h/night for 42 nights and examined 42 days later using LM morphometry had increased V_v and V of the RB wall and SEM evidence of CAR remodeling (Tyler et al., 1987). Collagen content of these lungs increased during the postexposure period (Last et al., 1984b).

Centriacinar region remodeling was more persistent in monkeys exposed to 0.64 ppm O_3, 8 h/day for 12 mo followed by 6 mo postexposure (Tyler et al., 1991b). By LM morphometry, the V_v and V of total RB, RB lumen, and RB walls were increased both at exposure end and at 6 mo postexposure. At exposure end, but not at 6 mo postexposure, RB internal diameters were smaller, and AMs in the CAR increased.

One study concerned postexposure recovery of the trachea (Nikula et al., 1988a). Complete recovery of the trachea (as evaluated by LM morphometry, SEM, and TEM) of

rats exposed to 60 nights (8 h/night, 7 days/week) to 0.96 ppm O_3 occurred following a 42-day postexposure period.

Effects of Episodic and Seasonal Exposure Regimens

Many investigators have noted that lesions due to O_3 reach a maximum intensity in a very few days and that, with continued exposure, the intensity of the lesion decreases. Eustis et al. (1981) reported half the number of inflammatory cells in the CAR of monkeys exposed to 0.8 ppm for 90 days as found in monkeys exposed to the same concentration for 7 days. Chang et al. (1992) noted that the acute reactions to the 0.06-ppm background O_3 (7 days/week), with a 9-h peak (5 days/week) slowly rising to 0.25 ppm, that they reported at 1 week of exposure had subsided at 3 weeks of exposure. Harkema et al. (1993) reported no difference in first generation RB epithelial thickness or cell numbers among monkeys exposed to 0.15 ppm O_3 for 6 days or to 0.15 or 0.3 ppm for 90 days.

These and other similar observations prompted Chang et al. (1991) to compare effects of two exposure regimens, which were evaluated using the same TEM morphometric approach. The first regimen was a "square wave", 12-h/day, 7-day/week exposure to 0.12 or 0.25 ppm O_3. The second regimen simulated urban O_3 exposures by exposing rats 7 days/week for 13 h to 0.06-ppm background, with a peak slowly rising to 0.25 ppm over a 9-h period (5 days/week). They calculated cumulative O_3 concentration ($C \times T$) for each exposure regimen and concluded that increases in volume of Type 1 and 2 alveolar epithelial cells were linearly related to increasing $C \times T$. The relationship for Type 1 cells was more robust.

Barr et al. (1990) used TEM and LM morphometry to compare effects of 90 days of daily exposure of rats for 8 h/day to 0.95 ppm O_3 with a regimen that modeled 5-day episodes of O_3 exposure. Each 5-day episode was followed by 9 postexposure days of filtered air. The cycle was repeated seven times so that the "episodic" group was exposed a total of 35 days over an 89-day period, and the "daily" group was exposed for 90 days to the same O_3 concentration. Both groups had CAR remodeling with the formation of RBs. The volume of RBs formed was not different when the two exposure groups were compared. The absolute volume of parenchymal lesion was the same in both groups. The RB epithelial thickness was increased in the daily group but not in the episodic group; conversely, the interstitium of both TBs and ADs was thickened in the episodic group but not in the daily group. Thus, rats exposed to the same concentration of O_3 for 35 days over an 89-day period in an episodic regimen had lesions as severe as those rats exposed daily for 90 days.

Effects of "seasonal" and "daily" exposure of young monkeys to 0.25 ppm O_3 were reported by Tyler et al. (1988). The daily group was exposed every day (8 h/day) for 18 mo, whereas the seasonal group was exposed only during odd months for the 18 mo. Thus, the daily group was exposed twice as many days to the same concentration as the seasonal group. By LM morphometry, both groups had increased V_v of total RB and RB lumen, but RB wall thickness was increased only in the daily group. The only significant morphometric difference between the two groups was an increase in CAR AMs in the daily group. This difference, and the difference in significance of the RB wall thickness in the seasonal group, was presumed due to the daily group being exposed to O_3 the day before necropsy, whereas the seasonal group breathed filtered air for 30 days preceding necropsy. This final 30 days of filtered air apparently allowed the more acute inflammatory changes in the seasonal group to regress. The seasonal group, but not the daily group, had increased lung collagen (Section 6.2.1) and increased chest wall compliance (Section 6.2.5). Exposure

to the same concentration of O_3 for half as many days in a seasonal regimen resulted in morphometric effects similar to daily exposure and in physiological and lung collagen changes not found in the daily group.

Tyler et al. (1991a) exposed rats to seasonal and daily regimens similar to those used for the monkeys described above. The concentration used in both studies was 0.25 ppm O_3 and the total length of exposure was 18 mo. Rats were exposed nights, during their natural period of activity. Both groups of rats were studied at the end of the 18-mo exposure cycle and 30 days postexposure. The lungs were evaluated using a simplified LM morphometric method for CAR airway remodeling, estimating the number of junctions of bronchioles (TB and RB) with ADs per surface area of section. At exposure end, the number of junctions of both exposure groups was increased compared to filtered-air controls; the O_3 groups were not different from each other. Neither group was different from the controls at 30 days postexposure.

Ex Vivo and In Vitro Exposures

Results obtained from studies of isolated perfused lungs and organ culture explants were consistent with some of the findings from in vivo studies (Pino et al., 1992a; Nikula et al., 1988b; Nikula and Wilson, 1990).

6.2.4.4 Considerations of Degree of Susceptibility to Morphological Changes

Species Differences in Degree of Response

Plopper et al. (1991) reviewed data from nonhuman primates and rats that had been exposed to O_3 and evaluated using TEM morphometry. The data were generated in several laboratories and the exposure and evaluation methods were somewhat different, but the data were expressed in similar terms. In the CAR, the results were expressed as total epithelial thickness or numbers of cells per square millimeter of basal lamina. Exposure of rats to 0.25 ppm O_3, 8 h/day for 42 days, resulted in an increase of less than 100% in either parameter compared to controls (Barry et al., 1985, 1988). Exposure of monkeys to 0.15 ppm O_3, 8 h/day for 6 days, resulted in a 230% increase in thickness and a 700% increase in cell number compared to controls (Harkema et al., 1993). As noted earlier (Section 6.2.4.2), the CARs of rats and monkeys are structurally different (Tyler, 1983), and the CAR cells are also different (Plopper, 1983).

There was also a difference when Plopper et al. (1991) compared stored secretory product per square millimeter of basal lamina in the nasal septum and lateral wall of the nasal cavity of O_3-exposed rats and monkeys. Data from the exposure of rats to 0.12 ppm, 6 h/day for 7 days, resulted in a $<10\%$ increase in the nasal septum and a $<100\%$ increase in the lateral wall. Exposure of monkeys to 0.15 ppm, 8 h/day for 6 days, resulted in a 300% increase in the nasal septum and a 125% increase in lateral wall. As in the CAR, there are major morphological differences in the nasal cavities of these two species (Schreider and Raabe, 1981).

Plopper et al. (1991) also compared collagen metabolism in rats and monkeys exposed to 1.5 ppm O_3, 23 h/day for 7 days, using the uptake of tritium-labeled proline. In rats, there was an increase of 200% above controls, whereas the increase was 800% in monkeys.

From these data, it appears that the respiratory system of monkeys is much more responsive than that of rats to near-ambient concentrations of O_3. The mechanisms responsible for these species differences in response to O_3 remain to be elucidated.

Effects of Age

Several studies published since the previous criteria document (U.S. Environmental Protection Agency, 1986) have addressed the effects of age on the intensity of O_3 morphological changes. The study by Stephens et al. (1978) and the initial report by Barry et al. (1983) were cited. Briefly, Stephens et al. exposed rats ranging in age from 1 to 40 days old to 0.85 ppm O_3 for 24, 48, or 72 h and examined their lungs by LM and TEM. Stephens and co-workers reported that, prior to 20 days of age, they did not find damage to TB-ciliated cells or to CAR Type 1 cells and that the amount of injury increased from 21 to 35 days when a plateau in response was reached.

Barry et al. (1985, 1988) exposed 1-day-old and 6-week-old rats to 0.12 or 0.25 ppm O_3, 12 h/day for 6 weeks. The 1985 study emphasized TEM morphometry of CAR (proximal) alveoli. The investigators did not find differences in response due to age. In both age groups, they found Type 1 cells increased in number and thickness, but decreased in both luminal and basement membrane surface area. They found bare basement membrane where Type 1 cells had been sloughed, but the amount was not increased in exposed groups. In the 0.25-ppm groups, but not in the 0.12-ppm groups, Type 2 cells were increased in density per square millimeter basement membrane but not in volume. Alveolar interstitium was increased only in adults exposed to 0.25 ppm. Macrophages in alveoli were increased in both age groups exposed to 0.25 ppm, but not in adults exposed to 0.12 ppm. Interstitial AMs were increased only in adults exposed to 0.25 ppm. The TEM morphometry of TBs from these rats did not include adults exposed to 0.12 ppm (Barry et al., 1988). There were no differences due to age at start of exposure. In both juvenile and adult rats exposed to 0.25 ppm, Barry and co-workers found that the luminal surface covered by cilia and by nonciliated bronchiolar (Clara) cells was reduced. The number of brush cells was also decreased.

Stiles and Tyler (1988) studied effects in a wider range of ages using LM morphometry and SEM. They exposed 60- and 444-day-old female rats to 0.35 or 0.8 ppm O_3 continuously for 72 h. Body weights of the 444-day-old rats, but not of those 60 days old, decreased during exposure. Fixed lung volumes of 444-day-old rats exposed to 0.8 but not 0.35 ppm were smaller than same-age controls. The V_v of CAR lesions was larger in 60-day-old rats than in the 444-day-old rats exposed to either concentration. The V_v of cells free in lumens (AMs) was increased in young rats exposed to 0.35 ppm compared to the older rats, but was not different for rats exposed to 0.8 ppm. Young rats exposed to either concentration had larger CAR lesions than the older rats, and young rats exposed to the lower concentration had more AMs. Older rats had greater changes in body weight and, in those exposed to the higher concentration, in fixed lung volume.

Effects of Exercise

Exercise increases the dose of inhaled toxicants delivered to sensitive cells (see Chapter 8). Mautz et al. (1985b) studied the effects of 0.2 and 0.38 ppm O_3 on rats at rest and during several treadmill exercise protocols. They found increased percent of lung parenchymal area containing free cells (AMs) in exercised rats exposed to both concentrations compared to rats exposed at rest. At the higher concentration, there was also an increase in the percent of parenchymal area with thickened ADs and alveolar septa.

Tyler et al. (1991c) exposed thoroughbred horses (trained to a treadmill) to 0.25 or 0.8 ppm O_3 for 29 min on 2 consecutive days using a protocol that included 9 min of graded exercise (3 min at maximum speed) and 20 min of "cool out". During maximal

exercise, horses increase their rate of oxygen consumption more than other species. Two of three horses exposed to 0.8 ppm O_3 had significant areas of hemorrhage and edema, and one of them refused the second day's exercise and exposure. By TEM, all horses exposed to 0.8 ppm had CAR lesions including necrosis of Type 1 cells. Lesions in those exposed to 0.25 were limited to CAR ciliated cells. No horses were exposed at rest for comparison.

Elastase-Induced Emphysema

Rats with elastase-induced emphysema and saline-instilled controls were exposed to 0.15 or 0.5 ppm for 3 or 7 days (Dormans et al., 1989). Mean linear intercepts, a measure of alveolar size, were determined using LM. The incidence and severity of CAR inflammatory changes were the same in O_3-exposed elastase-treated and saline-control rats. There were no changes in mean linear intercepts due to the O_3 exposure.

6.2.4.5 Summary

Research since the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986) continues to support the concept that all mammalian species respond to O_3 concentrations <1.0 ppm in a similar manner, but with significant differences in intensity of reactions among the species studied (Plopper et al., 1991). Dungworth (1989) provided a schematic overview of morphological reactions of the CAR from mammalian lungs to continuous exposure to low concentrations of O_3 as a series of time-response profiles (Figure 6-4). Bronchoalveolar exudative processes are the predominate early response, but the magnitude decreases rapidly with increasing duration of exposure and continues to decline during postexposure periods. Epithelial hyperplasia also starts early and increases in magnitude for several weeks, after which a plateau is reached until the exposure ends. Epithelial hyperplasia declines slowly during postexposure periods. Interstitial fibrosis has a later onset and may not be apparent for a month or more. The magnitude of this response, however, continues to increase throughout the exposure and, at least in some cases (Last et al., 1984b), continues to increase after exposure ends.

Nonhuman primates appear to respond more than rats to O_3 at concentrations <1.0 ppm. However, the mechanisms responsible for these differences in response have not been elucidated. Differences in cell, tissue, and circulating levels of several antioxidants are being studied, as are differences in in vitro responses to O_3 by cultures of cells from the various species. Basic morphological differences in the structure of the most injured portion of the lung, the CAR, and the size (volume) of the basic structural unit, the acinus, may also be factors in the greater response of monkeys to O_3. Both human lungs and lungs from nonhuman primates have CARs characterized by several generations of RBs, whereas rats have no RBs, or only a single poorly developed generation (Tyler and Julian, 1991). Within an individual lung, acinar volume is directly related to the intensity of CAR lesions (Mercer and Crapo, 1989; Mercer et al., 1991). The volume of individual acini in human lungs is 100 times larger than individual acini in rat lungs (Rodriguez et al., 1987; Haefeli-Bleuer and Weibel, 1988). Acinar volume of the monkeys used in O_3 studies is not known but, on the basis of the CAR structure, is assumed to be more like that of human lungs than rat lungs.

Another morphological factor that may be responsible in part for the greater response to O_3 of nonhuman primates than of rats may be differences in the complexity of the nasal cavity. Schreider and Raabe (1981) studied the cross-sectional morphology of the nasal-pharynx in rats, beagle dogs, and a rhesus monkey. They concluded that the

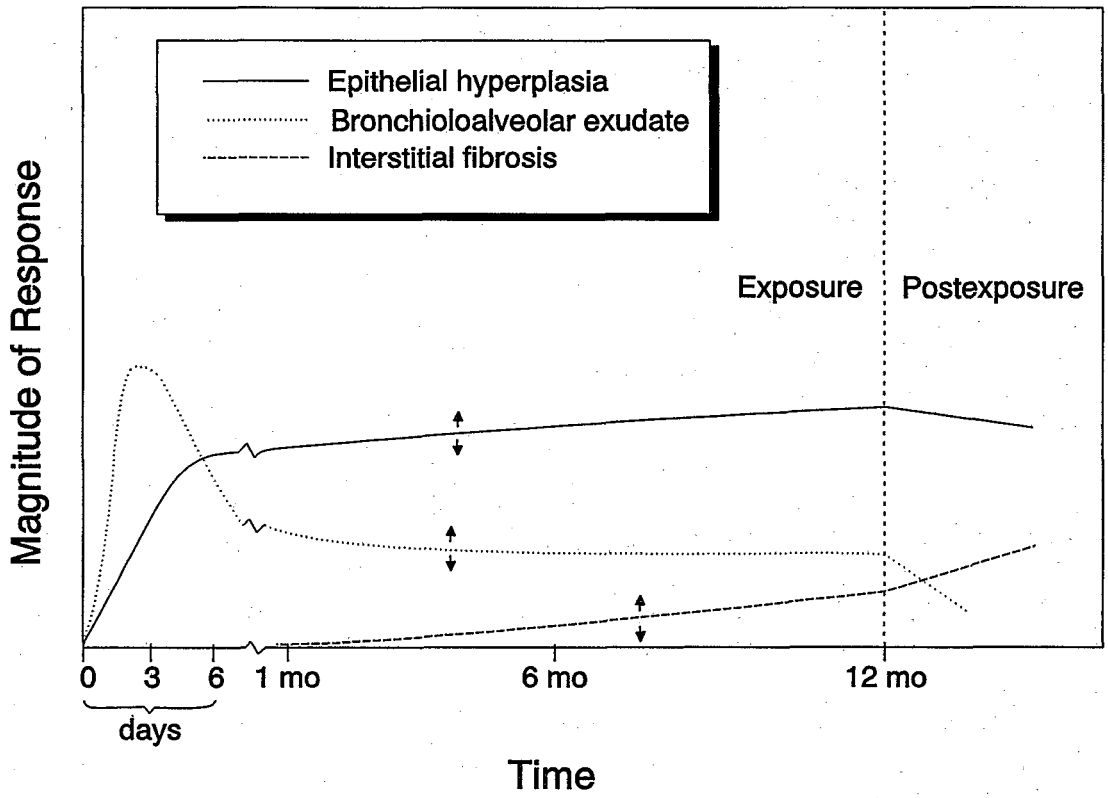

Figure 6-4. Schematic comparison of the duration-response profiles for epithelial hyperplasia, bronchoalveolar exudation, and interstitial fibrosis in the centriacinar region of lung exposed to a constant low concentration of ozone.

Source: Dungworth (1989).

complexity of the nasal cavity, and therefore the "scrubbing" effect (Yokoyama and Frank, 1972; Miller et al., 1979), which reduces the concentration of inhaled O_3 delivered to the lower respiratory tract, would be greater in rats than in monkeys. Schreider and Raabe (1981) proposed that, with appropriate scaling, the monkey could serve as a model for aerosol and gas deposition in the nasopharyngeal region of humans. However, the sensitivity of the nasopharyngeal epithelium may be different because changes in the nasal epithelium that follow O_3 inhalation, like those in the CAR, are more severe in monkeys than in rats (Plopper et al., 1991).

The effect of age at start of exposure on O_3-induced lung injury has not been resolved. Barry et al. (1985, 1988) reported no differences in TEM morphometry of CAR and TB lesions due to age at start of exposure. However, they studied a narrow range of ages, 1 and 42 days old at the start of a 42-day exposure. Barry et al. (1985, 1988) speculate that much of the CAR response in the rats that began exposure at 1 day of age might have occurred during exposure Days 21 to 42 because, as the earlier studies of Stephens et al. (1978) found, rats are not sensitive to morphological effects of O_3 until weaning at 21 days

of age. Thus, the rats that were 1 day old at the beginning of exposure may have developed the same intensity of lesions during the last 21 days of exposure as the older rats did in 42 days of exposure. In studies using a wider range of ages (60- and 444-day-old rats), Stiles and Tyler (1988) reported larger CAR lesions in the younger rats but greater changes in body weight and fixed lung volume in the older rats following a 3-day exposure to O_3.

The effects of exposure regimen and duration were evaluated in more recently published studies. Exposure of young monkeys to 0.25 ppm O_3 in a "seasonal" regimen (i.e., exposure in odd months and postexposure in even months) for 18 mo resulted in the same quantity of CAR lesions as daily exposure to the same concentration for 18 mo (Tyler et al., 1988). A similar quantity of CAR lesions was reported in rats exposed to 0.95 ppm O_3 in a 35 day, "episodic" regimen (units of 5-day exposures and 9 days without exposure) for a total of 89 days, as those exposed to the same concentration each day for 90 days (Barr et al., 1990). Chang et al. (1991) calculated the cumulative O_3 concentration for a "square wave" exposure to 0.12 or 0.25 ppm for 12 h/day, 7 days/week, with a simulated "urban" exposure regimen of 0.06 ppm for 13 h/day, 7 days/week and then raising that background 5 days/week to a peak of 0.25 ppm over a 9-h period. Using TEM morphometry of the CAR, they found no difference due to the pattern of exposure. Thus, it appears that the pattern of daily exposure does not influence the intensity of CAR lesions, but that episodic and seasonal patterns of exposure, with multiple days of clean air between days of exposure, are equivalent to daily exposure.

It has become clear that remodeling of centriacinar airways is cumulative. Using a stereological approach, Barr et al. (1988) reported an increase in the total volume of RB wall and lumen in rats exposed to 0.95 ppm O_3, 8 h/day for 90 days. Barr and co-workers also reported continuing Type 1 cell necrosis at the tips of alveolar septa (alveolar opening rings) immediately distal to the newly formed RB/AD junction (rather than in the TB/AD junction). It appears that some of the necrotic Type 1 cells were replaced by bronchiolar epithelium, rather than by Type 2 cells as previous studies indicated. This was confirmed by Pinkerton et al. (1993), who reported fully differentiated ciliated and nonciliated bronchiolar epithelium lining alveolar tips along a former AD up to 1 mm from the TB in lungs from rats exposed to 1.0 ppm O_3 for 6 h/day, 5 days/week for 20 mo. Remodeling of centriacinar airways appears to be a general phenomena, as increases in the V_v and V have been reported in lungs from all exposed rats and monkeys examined using stereological or morphometric methods that could detect this change (Fujinaka et al., 1985; Moffatt et al., 1987; Tyler et al., 1987; Tyler et al., 1988; Barr et al., 1990; Pinkerton et al., 1992, 1993). Centriacinar region remodeling has been demonstrated to persist in monkeys 6 mo after a 12-mo exposure to 0.64 ppm O_3 (Tyler et al., 1991b) and in rats 42 days after a 42-night exposure to 0.96 ppm O_3 (Tyler et al., 1987).

Several studies have confirmed and extended the earlier reports of epithelial degenerative changes followed by sloughing (i.e., leaving bare basement membrane that is recovered by other cell types), thus altering epithelial cell populations and increasing cell density (hyperplasia) in TB and centriacinar alveoli (Barry et al., 1985, 1988; Moffatt et al., 1987; Chang et al., 1988, 1992; Harkema et al., 1993). Epithelial replacement, a reparative process, occurs very early (Pino et al., 1992c), even though degeneration and necrosis continues (Barr et al., 1988). In specific airways, these processes appear to reach a maximum early in the exposure, as reported by Harkema et al. (1993), who found no difference in the intensity of lesions in first generation RBs, as measured by RB epithelial cell thickness and numbers, among monkeys exposed to 0.15 ppm O_3 for 6 days or to

0.15 or 0.3 ppm for 90 days. However, it is important to note that there may have been differences in response if more distal generations of RBs or random generation RBs had been selected for study. The interstitium in the CAR also thickens by the addition of cells and matrix. Thickening of the basement membrane and the presence of granular material in it were reported by Barr et al. (1988) and Chang et al. (1992). Chang et al. (1992), using TEM morphometry, reported that some changes in epithelial cell populations persist in rats for 17 weeks after a 78-week exposure to a model urban profile with a peak of 0.25 ppm O_3. At the LM level, Gross and White (1987) reported that 6 mo following a 12-mo exposure to 0.5 ppm O_3, 20 h/day, CAR inflammation had all but disappeared, and only a slight dilation and thickening of some ADs and adjacent alveoli remained.

The epithelia of the nasal cavity respond rapidly to O_3. In ciliated regions, cilia are attenuated, and intraepithelial mucosubstances increase. Hyperplasia and increased intraepithelial mucosubstances are reported in areas of nonciliated transitional epithelium (Harkema et al., 1989). These effects persisted throughout a 20-mo intermittent exposure of rats to 0.5 or 1.0 ppm O_3, but were not seen in rats exposed to 0.12 ppm for that period (Harkema et al., 1994). After acute exposure, DNA synthesis of the epithelium of the anterior maxilloturbinates of rats increases according to a given $C \times T$ product at ≥ 2.88 ppm·h, but the increase is not linear with increasing $C \times T$ (Henderson et al., 1993). Changes have not been reported in the olfactory epithelium or in the squamous epithelium of the nasal cavity.

Respiratory epithelia in other conducting airways, especially the trachea, appear to react in a manner similar to early necrosis of ciliated cells (Hyde et al., 1992). Cell replacement starts early (Hyde et al., 1992), and, after 60 nights of exposure of rats to 0.96 ppm O_3, numeric density of specific cell types was not different from controls (Nikula et al., 1988a). In newborn lambs exposed to 1.0 ppm O_3, 4 h/day for 5 days and examined 9 days later, the normal change in epithelial cell population that occurs by 2 weeks of age did not occur (Mariassy et al., 1990).

6.2.5 Effects on Pulmonary Function

6.2.5.1 Introduction

Numerous studies have been published on the effects of O_3 exposure on pulmonary function in animal models. This work has been reviewed by the U.S. Environmental Protection Agency (1986) and Tepper et al. (1995). The evaluation of pulmonary function after exposure may help provide a more integrated assessment of the severity of health effects by indicating the magnitude, location, and duration of functional disability. In an attempt to summarize the literature here, only key studies employing multiple concentrations or studies demonstrating a particular functional effect, testing a different species or strain, or showing the relationship with a unique variable such as age or sex will be discussed. Because purely descriptive pulmonary function studies now are rarely reported, newer studies will be discussed within the context of the study hypothesis. To enable discussion of the full range of studies, some exposures greater than 1 ppm O_3 will be discussed. For example, many of the airway reactivity mechanism studies were conducted at higher concentrations.

This section is organized by duration of exposure (brief, acute, repeated, and long-term). Within each of these sections, there are subsections on different types of pulmonary function measures. These subsections include a discussion of ventilatory patterns,

breathing mechanics, airway reactivity, and more extended characterizations of lung function, whenever such data are available.

Ventilation

Evaluation of the sinusoidal breathing pattern includes the measurement of tidal volume (V_T) and frequency of breathing (f) and their product, $\dot{V}_E$. Such measurements have proven to be sensitive indicators of O_3 effects. Numerous animal and human studies have shown that O_3 exposure increases f and decreases V_T (tachypnea) (U.S. Environmental Protection Agency, 1986). Although there is evidence indicating that tachypnea may serve to protect the deep lung from exposure, other evidence indicates that this sign of pulmonary irritation represents deep lung toxicity and is of greater concern than breathing pattern changes indicative of upper airway irritation.

Breathing Mechanics

Measurement of breathing mechanics (dynamic compliance [Cdyn] and total pulmonary resistance [R_L]) in animals has an advantage over simple measures of ventilation in that these parameters can assess the mechanical effort required to breathe and can help localize the site of dysfunction to the airways (resistance) or the parenchyma (compliance). With sufficient O_3 exposure, increases in R_L and decreases in Cdyn have been observed (U.S. Environmental Protection Agency, 1986). Changes in R_L and Cdyn typically reverse rapidly after high ambient O_3 exposures; however, these alterations can signal underlying inflammatory or lung permeability changes.

Airway Reactivity

Increased airway reactivity, an exaggerated response of the lung to an exogenously administered bronchoconstrictor, has been observed with O_3 (U.S. Environmental Protection Agency, 1986). Typically in humans, heightened airway responsiveness is determined using progressively increasing concentrations of aerosolized bronchoconstrictors, such as methacholine or histamine (see Chapter 7). Although bronchoprovocation protocols employing doubling doses of inhaled bronchoconstrictors have been relatively standardized for human experiments, no such standardization exists for animal studies, making comparisons between animal and human studies difficult. Increased airway reactivity is a hallmark of asthma and occurs in many other lung diseases, yet the long-term pathological consequences of hyperreactive airways are unknown.

Extended Functional Characterizations

A more complete assessment of the nature and magnitude of functional changes related to O_3 exposure includes an extended characterization of the lung using a battery of human clinical pulmonary function test analogs. Such tests include measurement of static lung volumes, volume-pressure and flow-volume relationships, as well as evaluation of inhomogeneity of ventilation and problems associated with oxygen diffusion across the epithelial barrier. Although these latter measurements are technically complex, they may contribute to a more in-depth understanding of the nature and severity of the physiological impairment and may provide in vivo evidence to suggest the anatomical localization of the functional abnormality.

6.2.5.2 Brief Ozone Exposures (Less Than 30 Minutes)

Few experiments have evaluated the effects of brief exposures (<30 min) to O_3. Most of these brief exposure studies have examined changes in regional breathing mechanics through exposures to the lower respiratory tract via a tracheal tube, thus eliminating any scrubbing by the nasal or oropharynx and thereby increasing the effective dose of O_3 delivered to that region of the lung. The relevance of this method of delivering the exposure, as compared to the typical inhalation route, is uncertain. However, positive effects have been observed, indicating that very rapid reflex responses occur with brief, direct O_3 exposure.

Ventilation and Breathing Mechanics

No studies have evaluated the effects of brief O_3 exposure on ventilatory pattern; however, breathing mechanics (Cdyn and R_L) have been evaluated. The previous criteria document (U.S. Environmental Protection Agency, 1986) described two experiments by Gertner et al. (1983a,b,c), which demonstrated increased collateral resistance within 2 min of exposure to 0.1 ppm O_3 in anesthetized dogs exposed via a fiber-optic bronchoscope wedged into a segmental airway. The response rapidly attenuated with exposure to 0.1 but not 1.0 ppm. Atropine or vagotomy blocked the increase in collateral flow resistance to 0.1 ppm, indicating that vagal postganglionic stimulation was involved, but the response to the 1.0-ppm O_3 exposure was blocked only partially.

More recently, Kleeberger et al. (1988), using a technique similar to Gertner et al. (1983a), exposed the segmental airways of mongrel dogs to 1.0 ppm O_3 for 5 min through a wedged bronchoscope (Table 6-13). As previously described, collateral resistance increased, and this increase was reproducible even when four 5-min exposures over a 3-h period were performed. Thus, no immediate tolerance was observed. Furthermore, this response could be blocked partially by administration of a cyclooxygenase inhibitor (indomethacin) and a H_1-receptor blocker (chlorpheniramine), whereas a thromboxane synthetase inhibitor was ineffective. This study suggests that histamine or cyclooxygenase products released from resident cells directly or via the parasympathetic nervous system may mediate the increase in collateral resistance. However, because collateral resistance probably makes up only a small proportion of pulmonary resistance, these results may not be generalizable to more prolonged exposures and to larger airway responses.

Airway Reactivity

Baboons were exposed via an endotracheal tube to 0.5 ppm O_3 for 5 min after a baseline methacholine inhalation challenge test (Fouke et al., 1988) (Table 6-14). Lung resistance increased with O_3 exposure, and the baboons showed an enhanced response to methacholine. This enhanced methacholine response was due almost exclusively to the post-O_3 increase in R_L and, thus, resulted in no change in the provocative dose that increased R_L by 50%. The experiment was repeated 5 to 14 days later, except that before O_3 exposure, cromolyn sodium was administered. In the presence of cromolyn, baseline R_L after O_3 exposure was less (not significant), but the response to methacholine challenge was significantly lower. In a follow-up study (Fouke et al., 1990) using a similar O_3 exposure protocol (no methacholine challenge), cromolyn partially blocked the O_3-induced increase in R_L; however, post-O_3 exposure analysis of BAL indicated that cromolyn did not affect the level of several measured prostanoids (6-keto $PGF_{1\alpha}$, PGE_2, TXB_2, or $PGF_{2\alpha}$), suggesting that these mediators were not related to the change in R_L.

Table 6-13. Effects of Ozone on Pulmonary Function[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Drugs	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
Base 0.06 spike 0.25	Base 118 spike 490	Base 13 h/day, 7 days/week; ramped spike 9-h/day, 5 days/week, for 1 week, 1, 3, 12, and 18 mo		Rat, M (F344) 60 days old	Increased expiratory resistance observed at all time points, but mostly at 78 weeks.	Tepper et al. (1991)
0.13 0.22 0.45	255 431 882	3 h Through tracheal tube	Pentobarbital Gallamine	Dog (Foxhounds) 16-20 kg	Positron camera indicated nonuniform distribution of ventilation in small airways; no change in R_L, Cdyn, or forced expiratory flow.	Morgan et al. (1986)
0.2-0.8	392-1,568	3 h		Rat, M (S-D) 7 weeks old	Maximum O_2 consumption decreased at 0.2 ppm, tachypnea observed at 0.4 ppm, and ventilation and core temperature decreased at 0.6 ppm.	Mautz and Bufalino (1989)
0.25 0.5 1.0	490 980 1,960	2 h; 2, 4, 6, and 8% CO_2 alternating 15 min		Rat, M (F344) 90 days old	Concentration response-related increase in f and flow at zero pleural pressure, decrease in V_T, no change in $\dot{V}_E$, R_L, or Cdyn.	Tepper et al. (1990)
0.25 0.5 1.0	490 980 1,960	3 h for 5 days	Pentobarbital	Mouse, F (CD-1) 3-4 weeks old	O_3 alone had no effect, but, in combination with virus, a decreased DL_{CO}, N_2, and lung volumes were observed more often than in virus alone at 6, 9, and 14 days PE.	Selgrade et al. (1988)
0.5	980	2 h	Chloralose	Dog (Mongrel) 15 ± 0.9 kg	Increased R_L, decreased Cdyn. No change in BAL prostanoids.	Fouke et al. (1991)
0.35 0.5 1.0	686 980 1,960	2 h/day for 5 days, 8% CO_2 alternating 15 min		Rat, M (F344) 110 days old	Attenuation of tachypnea with consecutive exposures; BAL antioxidants and protein did not adapt with exposure, histopathology increased in severity.	Tepper et al. (1989)
0.5 0.8	980 1,568	2 or 7 h, 8% CO_2 alternating 15 or 45 min/h	Halothane	Rat, M (F344) 90 days old	FVC, DL_{CO}, and N_2 slope all decreased with increasing C × T products. The magnitude of the decrement depended on the both the duration and concentration of O_3 exposure and the measured parameter.	Costa et al. (1989)
0.6	1,176	2 h exercise, muzzle		Dog, F (Beagle) 2-7 years old	Tachypnea, $\dot{V}_E$, O_2 consumption, CO_2 output, and R_L increased; Cdyn decreased.	Mautz et al. (1985b)
0.7	1,372	20 h/day for 28 days	Halothane	Rat, M (F344) 14 weeks old	Decreased forced expiratory flow and DL_{CO} and increased FRC immediately PE, no effect at 4 weeks PE, decrease in forced expiratory flow at 9 weeks PE.	Gross and White (1986)

Table 6-13 (cont'd). Effects of Ozone on Pulmonary Function[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Drugs	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
1.0	1,960	5 min Through tracheal tube	Pentobarbital	Dog, M (Mongrel) 20.2 ± 0.8 kg	Ozone-induced increase in collateral resistance blocked by indomethacin and histamine antagonist, not by thromboxane synthetase inhibitor.	Kleeberger et al. (1988)
1.0	1,960	1 h	Ketamine Xylazine	Guinea pig, M (Hartley) 250-300 g	Decreased TLC, VC, FRC, RV, and R_L. Indomethacin and cromolyn blocked change in FRC and RV at 2 and 24 h PE. DL_{CO} increased, blocked by cromolyn.	Miller et al. (1988)
1.0	1,960	1 h	Ketamine Xylazine	Guinea pig, M (Hartley) 250-300 g	R_L increased at 2 but not 8 h. Lung volumes, DL_{CO}, and alveolar ventilation increased at 8 and 24 h PE.	Miller et al. (1987)
1.0	1,960	2 h Through tracheal tube		Dog (Mongrel) 22-25 kg	Tachypnea with inspiratory time and expiratory time equally shortened. No increase in ventilatory drive 1 and 24 h PE.	Sasaki et al. (1987)
1.0	1,960	6 h/day, 5 days/week for 12 weeks	Ketamine Xylazine	Monkey, M (Cynomolgus) 4.5 ± 0.1 kg	No R_L, Cdyn, or forced expiratory flow changes associated with exposure.	Biagini et al. (1986)
1.0 2.0	1,960 3,920	3 h	In vitro	Rat, M (S-D) 300-380 g	R_L increased and Cdyn decreased in rat isolated perfused lung preparation.	Pino et al. (1992a)
1.0 2.0	1,960 3,920	6 h/day for 7 days (1 ppm) or 3 days (2 ppm)	Pentobarbital	Rabbit, M (Albino) 2.5 kg	At 17 h PE, 1 ppm increased R_L; 2 ppm trapped air, decreased Cdyn and forced expiratory flows, and increased R_L.	Yokoyama et al. (1989a)
1.0	1,960	24 h	Pentobarbital	Rat, M (Wistar) 6 weeks old	Decreased Cdyn, Cst, $\dot{V}_{max50\%TLC}$; increased FRC, RV; no additional effect of elastase pretreatment in O_3 exposed rats.	Yokoyama et al. (1987)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

Table 6-14. Effects of Ozone on Airway Reactivity[a]

Ozone Concentration[b]		Exposure Duration	Challenge[c]		Drugs	Species, Sex (Strain) Age[d]	Observed Effect(s)	Reference
ppm	$\mu g/m^3$		Agent	Route				
0.5	980	2-7 h Intermittent 8% CO_2	Ach	IV	Urethane	Rat, M (F344) 70 days old	No increase in airway reactivity despite use of CO_2 during O_3 exposure.	Tepper et al. (1995)
1.0	1,960	4 h	Mch	INH IV		Rat (S-D)	Reactivity in INH- but not IV-challenged rats.	Uchida et al. (1992)
1.0	1,960	2 h	Mch Ach	INH IV	Urethane	Rat, M (F344) 70 days old	Reactivity in INH- but not IV-challenged rats.	Tepper et al. (1995)
1.0	1,960	8 h	Mch	IV	Urethane	Rat, M (F344, S-D, Wistar)	No reactivity in any of three strains.	Tepper et al. (1995)
1.0	1,960	31 h	Ach	IV	Urethane	Rat, M	Increased reactivity.	Tepper et al. (1995)
4.0	7,840	2 h	Ach	INH	Methohexital Pancuronium	Rat, F (Long-Evans) 190-210 g	Reactivity increased immediately, but not 24 h PE, but not accompanied by PMN influx or vascular protein leakage.	Evans et al. (1988)
0.15	294	4 h/day, 5 days/week for 18 weeks	Hist	INH		Guinea pig, M (Hartley) 4-5 weeks old	Increase in sensitivity, but no change in reactivity.	Kagawa et al. (1989)
0.5	980	2 h	Ach	IV	Urethane	Guinea pig, M (Hartley)	Increased reactivity before PMN influx.	Tepper et al. (1990)
0.8	1,568	2 h	Sulfuric acid	INH		Guinea pig, F, M (Hartley) 2 mo old	No increased reactivity to acid; O_3 alone increased gas trapping.	Silbaugh and Mauderly (1986)
1.0 3.0	1,960 5,880	0.5-2 h	Mch	INH	Propranolol	Guinea pig, F (Hartley) 300-400 g	Increased reactivity with 90 min at 1 ppm and with 30 min at 3 ppm. At 2 h, 3 ppm reactivity occurred at 0 and 5 but not 24 h PE.	Nishikawa et al. (1990)
1.0	1,960	2 h	Hist Ach	INH IV	Urethane	Guinea pig, M (Hartley)	Reactivity in INH Hist, but not IV Ach.	Tepper et al. (1990)
3.0	5,880	0.25 h	Ach	IV	Propranolol	Guinea pig, M (English) 550-700 g	Reactivity blocked by lipoxygenase inhibitors but not by indomethacin.	Lee and Murlas (1985)

Table 6-14 (cont'd). Effects of Ozone on Airway Reactivity[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration	Challenge[c] Agent	Challenge[c] Route	Drugs	Species, Sex (Strain) Age[d]	Observed Effect(s)	Reference
3.0	5,880	0.25-2 h	Mch Hist 5-HT SP	IV INH	Pentobarbitone	Guinea pig, M (Dunkin Hartley) 450-550 g	Mch, Hist, 5-HT, and SP caused increase in reactivity INH, but not IV. Ascorbic acid blocked reactivity to Hist and SP. NEP inhibitors increased control response without changing O_3 response (i.e., O_3 inhibits NEP). Atropine and vagotomy did not or only partially reduced reactivity. Neither cyclooxygenase or 5-lipoxygenase inhibitors affected reactivity.	Yeadon et al. (1992)
3.0	5,880	2 h	Mch	INH	Ketamine Xylazine	Guinea pig, M (Dunkin Hartley) 494 ± 39 g	Platelet activating factor antagonist did not inhibit reactivity or eosinophils.	Tan and Bethel (1992)
3.0	5,880	2 h	Ach	IV		Guinea pig, M (Hartley) 550-750 g	Reactivity occurs with leukocyte depletion using cyclophosphamide.	Murlas and Roum (1985b)
3.0	5,880	2 h	Ach	INH		Guinea pig, M (Hartley) 600-700 g	With increased reactivity, increased lysosomal hydrolase observed in BAL.	Lew et al. (1990)
3.0	5,880	2 h	Ach	INH	Propranolol	Guinea pig, M (Hartley) 600-750 g	In vitro reactivity to SP and Ach, but not KCl. Phosphoramidon blocked SP effect, but not Ach reactivity; no increased reactivity was observed when mucosa was removed.	Murlas et al. (1990)
0.5	980	2 h Trach Tube	Mch	INH	Chloralose	Dog (Mongrel) 15 ± 0.9 kg	Increased reactivity, no change in BAL prostanoids.	Fouke et al. (1991)
1.0	1,960	5 min Bronchoscope	*Ascaris suum*	INH	Pentobarbital Succinylcholine	Dog, M (Mongrel) 21.2 ± 0.5 kg	Increased resistance to flow through the collateral system after antigen challenge attenuated with O_3 exposure 1-3 h and 24 h PE; effect independent of PMNs.	Kleeberger et al. (1989)
3.0	5,880	2 h	Ach	INH	Thiopental Chloralose	Dog (Mongrel) 18-23 kg	Cyclo- and lipoxygenase inhibitor BW755C blocked reactivity.	Fabbri et al. (1985)
3.0	5,880	2 h	Ach FS	INH	Thiopental Chloralose	Dog (Mongrel) 16-25 kg	In vivo but no in vitro reactivity; in vitro, trachea showed reactivity to FS.	Walters et al. (1986)
3.0	5,880	1 h	Ach	INH	Pentothal Chloralose	Dog (Mongrel) 24 ± 9 kg	Reactivity blocked with Ambroxyl, but PMN increased; ambroxyl inhibits arachidonic acid products from PMN.	Chitano et al. (1989)
1.0	1,960	2 h Trach tube	Ach	INH	Pentobarbital	Dog (Mongrel) 15-17 kg	Collateral resistance of small airways showed persistent reactivity 15-h PE; no effect on PMN, monocyte, or mast cell numbers at PE time.	Beckett et al. (1988)

Table 6-14 (cont'd). Effects of Ozone on Airway Reactivity[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration	Challenge[c] Agent	Challenge[c] Route	Drugs	Species, Sex (Strain) Age[d]	Observed Effect(s)	Reference
3.0	5,880	0.5 h Trach tube	FS Cch		Pentobarbital	Dog (Mongrel)	In vitro increases in reactivity suggested pre- and postjunctional inhibition (PGE_2) and postjunctional excitation (TXA_2).	Janssen et al. (1991)
3.0	5,880	0.5 h Trach tube	Ach	INH	Pentobarbital	Dog (Mongrel)	Reactivity was not blocked by TX antagonists.	Jones et al. (1990)
3.0	5,880	0.5 h Trach tube	Ach Hist 5-HT	INH	Pentobarbital	Dog (Mongrel) 18-30 kg	In vitro reactivity not altered by epithelial removal, indicating O_3 did not effect epithelial-derived relaxing factor.	Jones et al. (1988b)
3.0	5,880	0.5 h Trach tube	Ach Hist	INH	Pentobarbital	Dog (Mongrel)	Ganglionic blocker hexamethonium did not alter reactivity.	Jones et al. (1987)
3.0	5,880	0.5 h Trach tube	Cch TX	INH	Pentobarbital	Dog (Mongrel)	Airways not responsive to TX mimetic (U46619) but were to carbachol.	Jones et al. (1992)
3.0	5,880	0.5 h Trach tube	Ach FS KCl	INH	Pentobarbital	Dog (Mongrel) 18-30 kg	In vitro hyperresponsiveness of airway smooth muscle was observed with FS and Ach, but not KCl. FS not associated with increased excitatory junction potentials.	Jones et al. (1988a)
3.0	5,880	0.5 h Trach tube	Ach	INH	Pentobarbital	Dog (Mongrel) 21-27 kg	CD11b/CD18 monoclonal antibody prevented PMN influx, but not reactivity.	Li et al. (1992)
3.0	5,880	20 min Trach tube	Ach	INH	Pentobarbital	Dog (Mongrel) 18-32 kg	Allopurinol and desferoxamine inhibited reactivity without inhibiting PMN influx.	Matsui et al. (1991)
3.0	5,880	0.5-2 h Trach tube	Ach	INH	Thiopental Chloralose	Dog (Mongrel) 26-32 kg	Thromboxane synthase inhibitor blocked reactivity without influencing PMN influx.	Aizawa et al. (1985)
0.5 0.6	980 1,176	5 min	Mch	INH	Ketamine Fluorodiazepam	Baboon, M 25-40 kg	Brief exposure caused increased reactivity that was blocked by cromolyn.	Fouke et al. (1988)
0.5 0.6	980 1,176	5 min	Mch	INH	Ketamine Fluorodiazepam	Baboon, M 25-40 kg	Reactivity partially blocked by cromolyn, but no effect on stable prostanoids.	Fouke et al. (1990)
1.0	1,960	6 h/day, 5 days/week for 12 weeks	Ach/Pt	INH	Ketamine Xylazine	Cynomolgus, M 4.5 ± 0.1 kg	No increased reactivity in O_3-only group, but increase with platinum mixture.	Biagini et al. (1986)
1.0	1,960	2 h, 1/week for 19 weeks	Mch	INH	Pentobarbital	*Rhesus Macaca*, F 5-7 kg	5-lipoxygenase inhibitor blocked the development of reactivity.	Johnson et al. (1988)

[a]See Appendix A for abbreviations and acronyms.
[b]Table ordered according to animal species.
[c]Mch = methylcholine, Ach = acetylcholine, Hist = histamine, 5-HT = 5-hydroxytryptamine, SP = substance P, FS = field stimulation, Cch = carbachol, TX = thromboxane, KCl = potassium chloride, Pt = platinum; Route: IV = intravenous, INH = inhalation.
[d]Age or body weight at start of exposure.

The response to antigen-induced bronchoconstriction, an animal model of allergy, also has been evaluated recently. After a 5-min exposure to 1.0 ppm O_3 via a wedged bronchoscope, collateral resistance in dogs increased for 1 to 3 h (Kleeberger et al., 1989). After the O_3-induced resistance returned to baseline, the typical increase in collateral resistance observed in dogs challenged with *Ascaris suum* antigen, to which the dogs were natively sensitive, was attenuated both 1 to 3 h and 24 h post-O_3 exposure. The attenuated antigen response appeared to be independent of PMNs in the airways. In a follow-up study, the late-phase response to antigen (bronchoconstriction 2 to 12 h postantigen challenge) also was blocked in allergic dogs when O_3 exposure (1.0 ppm, 5 min, via a bronchoscope) preceded antigen challenge (Turner et al., 1989). These studies suggest that, at least in the dog, brief local administration of O_3 to the airways may inhibit allergic responses.

6.2.5.3 Acute Ozone Exposures (Less Than One Day)

Ventilation

Alteration of the ventilatory pattern has long been established as a hallmark of acute O_3 exposure. Several animal studies evaluated tidal breathing changes during and after O_3 exposure (U.S. Environmental Protection Agency, 1986). For most species, a tachypneic response (rapid and shallow breathing) has been observed. For example, Murphy et al. (1964) studied unanesthetized guinea pigs exposed for 2-h to 0.34, 0.68, 1.08, or 1.34 ppm O_3 via nose cones, and measured tidal breathing using a constant volume plethysmograph. A similar experimental preparation was used by Amdur et al. (1978) to evaluate the respiratory response of guinea pigs to 0.2, 0.4, and 0.8 ppm O_3. In both experiments, a monotonic increase in f was observed. In the Amdur et al. (1978) study, decreases in V_T were not observed concomitantly.

Lee et al. (1979, 1980) showed that the tachypneic pattern observed in conscious dogs exposed to 0.56 to 0.85 ppm was not altered by bronchodilator pretreatment or atropine administration. These manipulations would suggest that the rapid, shallow breathing was not caused by bronchoconstriction. The response, however, was blocked by vagal cooling, which was interpreted by the authors to suggest that vagal sensory afferent transmission had been blocked. Thus, the authors suggested that increased vagal afferent impulses produced tachypnea and that the response was independent of vagal efferents (increased smooth muscle tone).

Several new studies evaluating ventilation after acute O_3 exposure have appeared in the literature (Table 6-13). Mautz and Bufalino (1989) measured ventilation ($\dot{V}_E$) as well as oxygen consumption and rectal temperature in awake rats exposed for 3 h to 0.2, 0.4, 0.6, and 0.8 ppm O_3. Concentration-related increases in f were significantly different from controls beginning at 0.4 ppm, with a maximal response observed up to 0.6 ppm. Tidal volume was similarly reduced, whereas $\dot{V}_E$ and rectal temperature were less sensitive to O_3 exposure, showing decreases at 0.6 and 0.8 ppm. Oxygen consumption was decreased at all concentrations tested. The authors concluded that the O_3-induced change in breathing pattern did not cause a decrease in metabolic rate or impose a condition of hypoxia. The changes in ventilation and O_2 consumption appeared coincident or possibly preceded the irritant reflex change in breathing pattern.

Tepper et al. (1990) exposed awake rats to 0.12, 0.25, 0.5, and 1.0 ppm O_3 for 2.25 h in head-out pressure plethysmographs. During exposure, CO_2-stimulated breathing was incorporated to augment ventilation, similar to the use of exercise in human studies. Frequency increased and V_T decreased monotonically between 0.25 and 1.0 ppm during a

2.25-h exposure. No decrease in $\dot{V}_E$ was observed. This difference from the Mautz and Bufalino (1989) study could be due to their restraining rats in a tightly fitting plastic flow plethysmograph with the face sealed by an aluminum nose cone; in the Tepper et al. (1990) study, the rats were exposed in oversized, steel, head-out plethysmographs and were intermittently challenged with CO_2, which may have overridden the metabolic depressant effect.

Mautz et al. (1985b), using exercising dogs exposed to 0.6 ppm O_3 for 140 min, showed tachypnea, increased $\dot{V}_E$, and elevated ventilation equivalents for O_2 and CO_2 compared to dogs exposed to air while exercising. Pulmonary resistance fell in air-exposed, exercising dogs, but climbed toward the end of the exposure in the O_3-exposed dogs.

In a follow-up study to Lee et al. (1979, 1980), Sasaki et al. (1987) performed similar experiments on two awake dogs that were trained to run on a treadmill. Dogs were exposed to 1.0 ppm O_3 for 2 h and evaluated before O_3 exposure and at either 1 or 24 h postexposure. In all studies with or without exercise, O_3 increased f and decreased V_T, without affecting $\dot{V}_E$. Vagal blockade diminished, but did not abolish, the tachypneic response, indicating that both vagal and nonvagal mechanisms were important. The O_3-induced change in f was due to equal reductions in inspiratory and expiratory times with no, or small, diminution (only during CO_2 rebreathing experiments) of ventilatory drive (V_T/inspiratory time). Additionally, O_3 did not affect functional residual capacity (FRC) or core temperature in resting, exercising, or vagally blockaded dogs. The authors speculate that the change in f is due to a vagally mediated lowering of the volume threshold of the pulmonary stretch receptor for inspiration and expiration with a concomitant increase in flow rate, thus leaving the FRC constant. Furthermore, the authors speculated that increased sensitization of rapidly adapting receptors, C-fiber nerve endings, and nonvagal mechanisms also may be contributors to the tachypneic response.

Two recent studies provide further insight into the mechanism of O_3-induced changes in ventilatory patterns. In the first study, Schelegle et al. (1993) showed that O_3-induced (3 ppm O_3 for 40 to 70 min) tachypnea in anesthetized, spontaneously breathing dogs largely could be abolished by cooling the cervical vagus to 0 but not 7 °C. This would indicate that large myelinated fibers were not involved in this reflex response, but nonmyelinated C fibers, whose activity is decreased only at the lower temperature, are important. In a companion study, Coleridge et al. (1993) measured the responses of five types of single vagal nerve fibers: (1) bronchial C-fibers, (2) pulmonary C-fibers, (3) rapidly adapting receptors, (4) slowly adapting pulmonary stretch receptors, and (5) unclassified fibers. During exposure to O_3, bronchial C-fibers were most affected. Because discharge of these fibers was not immediate with the onset of exposure, but took time to develop, the authors suggested that O_3 may not directly stimulate these receptors and that autacoid mediators released in the lung, which previously have been shown to stimulate these fibers, were probably responsible for fiber activation. Rapidly adapting receptors were shown to play a small part in this reflex response; although, surprisingly, pulmonary C-fibers and slowly adapting receptors were found to be unimportant. Ozone also stimulated several unidentified vagal fibers that may be responsible for residual effects not abolished by 0 °C cooling of the vagus. Thus, early inflammatory changes, as well as direct stimulation of bronchial C-fibers, may be responsible for the tachypnea seen with O_3 exposure in animal experiments. Results from these studies, together with the amelioration of spirometric changes found after indomethacin in humans (see Chapter 7), suggest that tachypnea,

inspiratory pain, and the reduction in forced vital capacity (FVC) could reflect early inflammatory lesions as well as neurogenic stimulation.

Breathing Mechanics

Although changes in breathing mechanics have been observed in laboratory animals, these changes are not observed consistently and tend to be reported more frequently at higher exposure concentrations (U.S. Environmental Protection Agency, 1986; Table 6-13).

The previously discussed studies by Murphy et al. (1964) and Amdur et al. (1978) evaluated breathing mechanics in unanesthetized guinea pigs. The Murphy et al. (1964) study showed an increase in flow resistance only at concentrations >1 ppm O_3. Pulmonary compliance was not measured. Amdur et al. (1978) observed a decrease in Cdyn after exposure to 0.4 and 0.8 ppm O_3, but no significant change in R_L was noted.

In an attempt to expose unanesthetized rats using regimens analogous to human clinical studies, Tepper et al. (1990) observed no significant changes in R_L or Cdyn after a 2.25-h exposure to 0.12, 0.25, 0.5, or 1.0 ppm O_3, in spite of intermittent 15-min periods of exercise-like hyperventilation induced by CO_2. Similarly, no changes in breathing mechanics were observed by Yokoyama et al. (1987) when they evaluated anesthetized rats exposed to 1.0 ppm O_3 for 24 h. However, when Cdyn was normalized for differences in FRC, the resulting specific compliance was decreased compared to air-exposed controls. Furthermore, when the animals were paralyzed and ventilated between 40 and 200 breaths/min, O_3-treated animals showed a frequency-dependent decrease in Cdyn as f increased above 120 breaths/min. The authors conclude that because R_L was not affected, the effect of O_3 was to obstruct peripheral airways.

Pulmonary mechanics were evaluated in anesthetized, paralyzed dogs acutely exposed to 0.12, 0.22, and 0.45 ppm O_3 for 3 h via a stainless steel tracheal tube. No changes in R_L or Cdyn were observed at any concentration (Morgan et al., 1986).

In papers by Miller et al. (1987, 1988), the effect of a 1-h exposure to 1.0 ppm O_3 was evaluated. Two hours after exposure, anesthetized, tracheostomized guinea pigs showed a significant increase in R_L that resolved by 8 h postexposure. Both indomethacin and cromolyn sodium partially blocked the increase in R_L at 2 h postexposure (Miller et al., 1988). These results suggest that eicosanoids produced from an inflammatory response in the lung may be responsible for the increase in R_L. However, plasma levels of $PGF_{2\alpha}$ and 6-keto PGF_{α} were not affected by O_3 or drug treatment, and PGE_1 was not affected by O_3. In an attempt to understand the involvement of eicosanoids in the increase in R_L observed with O_3 exposure, Fouke et al. (1991) showed that exposure to 0.5 ppm O_3 for 2 h caused an increase in R_L and a decrease in Cdyn in anesthetized dogs. Bronchoalveolar lavage fluid from these dogs did not have any increase in 6-keto $PGF_{1\alpha}$, PGE_2, TXB_2, or $PGF_{2\alpha}$, suggesting that these cyclooxygenase products were not involved in the changes in breathing mechanics. Similar findings were observed by these authors after brief exposures to baboons (see Section 6.2.5.2).

Gas trapping in the excised guinea pig lung was evaluated by water displacement in guinea pigs challenged with acid aerosol exposure after a 2 h, 0.8-ppm O_3 exposure (Silbaugh and Mauderly, 1986). Ozone exposure followed by air exposure increased gas trapping to roughly the same extent as O_3 followed by a sulfuric acid challenge (1 h, 12 mg/m^3) when compared to the air-only control response. These data indicate that

O_3 causes an acute peripheral airway obstruction, but no additive or synergistic effect of sulfuric acid aerosol was observed.

Airway Reactivity

Probably, the most extensive amount of laboratory animal research has been conducted on the role of O_3 in producing acute airway injury resulting in an increase in airway reactivity (U.S. Environmental Protection Agency, 1986; Table 6-14). Much of this research has used O_3 exposures that are never encountered in the ambient environment ($\geq$3 ppm for $\geq$30 min); thus, the relevance of these studies may be questioned. However, the studies are the most thorough mechanistic account of such O_3 effects and have shown some agreement with human O_3 exposure studies (Chapter 7); therefore, these studies are summarized briefly here. The literature is focused around five primary issues that in recent years have been more thoroughly evaluated.

Concentration and Peak Response Time. Easton and Murphy (1967) were the first to demonstrate an increased responsiveness in unanesthetized guinea pigs post-O_3 exposure (2 h, 0.5 to 7 ppm). In their study, responsiveness was assessed by increased mortality due to severe histamine-induced bronchoconstriction, as well as by increased R_L and decreased Cdyn. Lee et al. (1977) examined anesthetized dogs exposed to O_3 (0.7 to 1.2 ppm, 2 h) via a tracheal tube and determined that increased airway reactivity to inhaled histamine occurred at 24 but not 1 h postexposure. A similar experiment, done in unanesthetized sheep by Abraham et al. (1980), indicated that airway responsiveness was increased at 24 h, but not immediately after a 2-h exposure to 0.5 ppm O_3. When the exposure was increased to 1 ppm O_3, an increase in baseline R_L was reported, and reactivity increased immediately and at 24 h postexposure. In apparent contradiction, Holtzman et al. (1983a) showed that airway reactivity increased markedly 1 h after dogs were exposed to 2.2 ppm O_3 for 2 h and was less evident at 24 h postexposure. Gordon and Amdur (1980) also reported that airway reactivity in guinea pigs was maximal 2 h after a 1-h exposure to 0.1, 0.2, 0.4, or 0.8 ppm O_3, as defined by a significant increase in R_L or decrease in Cdyn after a single subcutaneous challenge of histamine. The effect on R_L was concentration dependent, but was significant only at 0.8 ppm. For Cdyn, there was no concentration-related response, but all O_3 exposures exacerbated the decrease in Cdyn after histamine relative to the air-exposed group. The site of bronchoconstriction was suggested to be the conducting airways, rather than the parenchyma, because dynamic compliance was affected and static compliance was not (Gordon et al., 1984).

To examine the role of duration of exposure on experimental outcome, Nishikawa et al. (1990) exposed guinea pigs to C $\times$ T products of 30 (1 ppm $\times$ 30 min), 90 (1 ppm $\times$ 90 min), 90 (3 ppm $\times$ 30 min), and 360 (3 ppm $\times$ 120 min) ppm $\cdot$ min O_3. After exposure, specific airway resistance (SR_{aw}) during an inhaled methacholine challenge was measured in unanesthetized animals at 5 min, 5 h, and 24 h. In all but the 1-ppm, 90-min exposure group, there was an increase in baseline SR_{aw} at 5 min, but the response was neither concentration nor C $\times$ T dependent. At 5 min postexposure, no increase in airway responsiveness was observed at 30 ppm $\cdot$ min. Airway hyperresponsiveness was observed at 90 ppm $\cdot$ min, using either exposure scenario (1 ppm for 90 min or 3 ppm for 30 min), and the response to the 360 ppm $\cdot$ min was greater than that observed with 90 ppm $\cdot$ min exposure. Significant increases in airway responsiveness at both 5 and 24 h postexposure

were observed only in the 360 ppm · min group. The authors concluded that exposure duration was an important determinant of O_3-induced airway hyperresponsiveness.

Uchida et al. (1992) reported increased airway reactivity in rats to inhaled methacholine after a 1.0-ppm (2-h) O_3 exposure. These results conflict with other published studies in rats also using inhaled methacholine, which reported the inability to produce consistent increases in airway reactivity after exposure to less than 4 ppm O_3 (Evans et al., 1988). Tepper et al. (1995) reported that airway hyperresponsiveness in rats challenged with iv acetylcholine occurred only at 1 ppm O_3 or higher. In these latter studies, exposure durations ranged from 2 to 7 h, and, in some tests, CO_2 was added to the exposure to increase ventilation. Although guinea pigs are more responsive than rats, they are not as responsive as humans to O_3-induced increased airway reactivity, even under optimal conditions (Tepper et al., 1995).

Inhaled Versus Intravenous Challenge. In a follow-up study using a similar exposure protocol as described above, Abraham et al. (1984) observed increased responsiveness to iv carbachol in unanesthetized sheep 24 h after a 2-h, 0.5-ppm O_3 exposure; inhaled carbachol did not produce a similar response. The authors interpreted this result to indicate a decreased penetration of the carbachol aerosol in O_3-exposed animals compared with the direct stimulation of smooth muscle by the iv route. Roum and Murlas (1984) observed that O_3-induced hyperresponsiveness was similar for inhaled versus iv acetylcholine or methacholine challenge through 14 h postexposure, but after that time, only iv administration revealed a persistent O_3-related response. In contrast, Yeadon et al. (1992) reported that guinea pigs exposed to 3 ppm for 30 min were hyperresponsive to inhaled histamine, serotonin, acetylcholine, and substance P, but were not hyperresponsive to O_3 after iv administration of the same agonists. Tepper et al. (1995) and Uchida et al. (1992) also showed that rats were more sensitive to inhaled methacholine than to iv administration of the agonist.

Neurogenic Mediation. Lee et al. (1977) reported increased airway responsiveness to histamine in dogs exposed to 0.7 or 1.2 ppm O_3 for 2 h. Atropine and vagal blockade were effective in reducing the O_3-induced hyperresponsiveness to histamine, suggesting that heightened vagal activity was responsible. Katsumata et al. (1990) also showed that in the cat, airway hyperresponsiveness to histamine could be attributed to cholinergic reflex. This is in apparent contrast to the increased O_3-induced (1.0 to 1.2 ppm, 2 h) responsiveness to histamine (subcutaneous) that was not blocked by atropine or vagotomy, indicating minimal vagal involvement in guinea pigs (Gordon et al., 1984). In agreement, Jones et al. (1987) found that hexamethonium, a ganglionic blocker, did not prevent O_3-induced hyperresponsiveness in dogs exposed to 3 ppm O_3 for 0.5 h via an endotracheal tube. Similarly, Yeadon et al. (1992) showed that atropine or bilateral vagotomy only partially reduced the hyperresponsivenss in guinea pigs exposed to 3 ppm for 120 min but did not block the response in animals exposed for only 30 min.

A role for prejunctional muscarinic receptors has been demonstrated by Schulteis et al. (1994). The M_2 receptor, which is inhibitory for acetylcholine release, was shown to be defective immediately after a 4-h, 2-ppm O_3 exposure in guinea pigs. Fourteen days after exposure, M_2 receptor function and vagally stimulated responsiveness were normal. Thus, the role of the cholinergic system in O_3-induced airway hyperresponsiveness has yet to be firmly established.

Peptidergic mediators also have recently been suggested as important modulators of this response. Murlas et al. (1992) demonstrated that phosphoramidon, an inhibitor of neutral endopeptidase (NEP), increased the responsiveness to substance P in air-exposed (but not O_3-exposed [3 ppm, 2 h] guinea pigs). Substance P-induced bronchoconstriction in air-exposed animals was increased after phosphoramidon because NEP degrades substance P. The finding was associated with a decrease in tracheal NEP in O_3-exposed animals. Additionally, the increased airway responsiveness in O_3-exposed animals was reversed by inhalation of partially purified NEP. Taken together, these results suggest that O_3 inactivates NEP, thus increasing the response to endogenous tachykinin release. A similar result was obtained by Yeadon et al. (1992) in guinea pigs exposed to 3 ppm O_3 (30 or 120 min) and challenged with aerosolized substance P after pretreatment with the NEP inhibitors phosphoramidon, thiorphan, and bestatin. Tepper et al. (1995) depleted guinea pigs of substance P, using multiple doses of capsaicin, and found that airway reactivity, after a 2-h exposure to 1 ppm O_3, was partially blocked. However, although tracheal vascular permeability also was blocked by capsaicin pretreatment, protein influx into the BAL and tachypnea were not blocked. On the other hand, Evans et al. (1989) did not find increased tracheal vascular permeability in rats exposed to 4 ppm O_3 for 2 h. These studies suggest, at least for the guinea pig, that enhancement of the substance P response, by inhibition of NEP, may be important in O_3-induced hyperresponsiveness.

Inflammation. Holtzman et al. (1983b) found a strong association between increased airway responsiveness and increased PMNs present in the tracheal biopsy of dogs 1 h after a 2-h O_3 exposure to 2.1 ppm. Fabbri et al. (1984) extended these findings, showing an association between increased airway reactivity and increased lavageable inflammatory cells from the distal airways of dogs. Further support for this hypothesis was engendered by the demonstration that in PMN-depleted dogs (produced by administration of hydroxyurea), O_3-induced airway hyperresponsiveness was blocked. This is in contrast to Murlas and Roum's (1985a) findings in guinea pigs exposed to 3 ppm O_3 for 2 h, which indicate that increased airway reactivity, mucosal injury, and mast cell infiltration occur before PMN influx. The authors speculate that PMN influx is a response to the damage, not a cause of the increased airway reactivity. Furthermore, Murlas and Roum (1985b) showed that PMN depletion in the guinea pig with cyclophosphamide did not prevent O_3-induced airway hyperresponsiveness. Similar results were obtained by Evans et al. (1988), who reported that airway hyperresponsiveness was not accompanied by airway PMN influx in rats, and by Joad et al. (1993), who showed that adding human PMNs to the pulmonary circulation of the rat lung during a 3-h, 1.0-ppm exposure to O_3 did not further enhance O_3-induced airway reactivity. Beckett et al. (1988) evaluated dogs exposed for 2 h to 1 ppm O_3 directly to the peripheral airways via a wedged bronchoscope. Fifteen hours postexposure, the exposed peripheral airway segments were hyperresponsive to aerosolized acetylcholine. However, at the site of increased responsiveness, there was no association with increased PMNs, mast cells, or mononuclear cells. Such studies agree with perhaps the most definitive study of this hypothesis (Li et al., 1992), which used monoclonal antibodies (CD11b/CD18) to prevent PMN influx into the airways. When PMNs were present in the circulation, but prevented from entering the lung, the dogs were still hyperresponsive after a 30-min exposure to 3 ppm O_3. Thus, in three species, it appears that PMN influx may be associated with O_3 exposure but is not necessary for producing airway hyperresponsiveness.

Several studies have suggested that arachidonic acid metabolites may be important in O_3-induced airway hyperresponsiveness. Although the primary source of arachidonic acid metabolites is suspected to be inflammatory cells in the lung, cells other than PMNs could be responsible for the liberation of arachidonic acid metabolites. Only one study in dogs indicates that blockage of cyclooxygenase products with indomethacin can protect animals from developing airway hyperresponsiveness (O'Byrne et al., 1984). However, several, more recent studies have found that cyclooxygenase inhibitors were ineffective in blocking this response (Lee and Murlas, 1985; Holroyde and Norris, 1988; Yeadon et al., 1992). Two papers indicated the importance of LTs, as demonstrated by the inhibition of hyperresponsiveness with prior administration of 5-lipoxygenase inhibitors to guinea pigs (Lee and Murlas, 1985; Murlas and Lee, 1985). In contrast, Yeadon et al. (1992) found that a specific 5-lipoxygenase inhibitor did not block the response in guinea pigs. One study with dogs showed that TX generation may be important in this phenomenon (Aizawa et al., 1985), but, more recently, two papers from the same investigators have dispelled that notion (Jones et al., 1990, 1992). Furthermore, exposure to 0.5 ppm O_3 for 2 h caused a decrease in the provocative dose of methacholine necessary to cause a 50% increase in R_L in anesthetized dogs (Fouke et al., 1991). Bronchoalveolar lavage on these dogs did not show any increase in 6-keto $PGF_{1\alpha}$, PGE_2, TXB_2, or $PGF_{2\alpha}$, suggesting that these cyclooxygenase mediators of inflammation were not involved in the changes in airway reactivity. In summary, the initial hypothesis of the role of PMNs or PMN-derived products in O_3-induced airway hyperresponsiveness is questionable because most newer studies, using more specific inhibitors of PMNs, cyclooxygenase, and 5-lipoxygenase, and studies blocking TX receptors indicate the lack of a protective effect.

Interactions with Antigen and Virus. In mice, Osebold et al. (1980) showed that an increased number of animals became sensitized to ovalbumin after 3 to 5 days of continuous exposures to 0.5 and 0.8 ppm O_3. Matsumura (1970) and Yanai et al. (1990) made similar findings in guinea pigs and dogs exposed acutely to higher O_3 concentrations, suggesting that O_3 may enhance either sensitization or response to antigen. These results appear to agree with recent findings in humans (see Chapter 7).

Ozone (1 ppm, 2 h) also may increase hyperreactivity associated with virus exposure. Tepper et al. (1995) exposed rats to O_3 either before or during an influenza virus infection. Rats exposed to O_3 before virus infection were more hyperresponsive to inhaled methacholine 3 days later (at a time when there was no hyperresponsiveness to O_3 alone) than were rats exposed to only the virus. An additive effect was observed in virus-infected rats when O_3 exposure was immediately before methacholine challenge.

Extended Functional Characterizations

Extended characterizations of pulmonary function in laboratory animals indicate that the general pattern of functional impairment reported in human studies also is observed in animal studies of acute O_3 exposure. Anesthetized and ventilated cats showed a general decline in vital capacity (VC), static lung compliance, or diffusing capacity for carbon monoxide (DL_{CO}) with exposures up to 6.5 h of 0.26 to 1.0 ppm O_3 (Watanabe et al., 1973). Inoue et al. (1979) observed functional evidence of premature airway closure, as indicated by increases in closing capacity, residual volume (RV), and closing volume, after rabbits were exposed to 0.24 or 1.1 ppm O_3 for 12 h. The volume-pressure curve indicated increased lung volume at low distending pressures; additionally, nonuniform distribution of

ventilation was observed. The effects were most prominent 1 day following exposure and had mostly subsided by 7 days postexposure.

Most studies of O_3 in experimental animals make little effort to mimic human study designs, thereby further confounding the extrapolation of their results to humans. Recently, however, rat studies involving periods of intermittent CO_2-induced hyperventilation to enhance the delivered dose of O_3 have attempted to capitalize on the qualitative similarity of the rat and human maximum expiratory flow-volume curves as a potentially sensitive endpoint of toxicity (Costa et al., 1988b; Tepper et al., 1989). In the rat, FVC decreases acutely with O_3 exposure, and this response has been mathematically modeled for O_3 concentrations between 0.35 and 0.8 ppm with exposure durations between 2 and 7 h (Tepper et al., 1989). The magnitude of response is apparently less than that observed in humans (Tepper et al., 1995), although the extent to which anesthesia mitigates the rat response or that there are inherent species differences in dosimetry or sensitivity is not clear from these studies (see Chapter 8).

In addition to changes in the flow-volume curve, changes in lung diffusion also are observed. In a study that examined concentration, duration, and ventilation factors, rats were exposed for 2 or 7 h to 0.5 or 0.8 ppm O_3 with intermittent 8% CO_2 to hyperventilate ($\approx$2 to 3 times resting $\dot{V}_E$) the animals as an exercise analogue to human exposures (Costa et al., 1988a). The DL_{CO} values were reduced by 10% at both 0.5-ppm time points and by 12% with a 2-h exposure to 0.8 ppm. Exposure to 0.8 ppm for 7 h, however, greatly exacerbated the alveolar effect, with a resultant 40% reduction in the DL_{CO}. Static compliance was affected only at this latter exposure concentration and duration. This O_3-induced reduction in DL_{CO} appeared to correlate with the degree of lung edema in affected animals. Yokoyama et al. (1987) found decreases in rat lung volumes (FRC and RV), static compliance (from the volume-pressure curve), and maximal flow at 50% of VC after a 24-h exposure to 1 ppm O_3.

Flow-volume curves and measurements of regional distribution of ventilation, using a positron camera, were evaluated in anesthetized, paralyzed dogs acutely exposed via a stainless steel tracheal tube to 0.12, 0.22, and 0.45 ppm O_3 for 3 h (Morgan et al., 1986). No changes in the flow-volume curve were observed at any concentration, but a less uniform distribution of ventilation was noticed, with the greatest difference occurring between the central and more peripheral regions. The authors conclude that the initial effect of O_3 appears to be obstruction of the small airways.

Miller et al. (1987, 1988) evaluated the effect of a 1-h exposure to 1.0 ppm O_3 on changes in the lung function of anesthetized, tracheostomized guinea pigs. Decreases in lung volumes were noted at 2 h postexposure and were maximum between 8 and 24 h postexposure, after which time they began to resolve. Alveolar ventilation ($\dot{V}_A$) and DL_{CO} also were decreased by exposure. The initial (2 h postexposure) reduction in DL_{CO} may have been caused by a bronchoconstriction-related decrease in $\dot{V}_A$. After this time, disproportionate ratios of DL_{CO} and $\dot{V}_A$ suggest that different mechanisms were responsible for the decreased DL_{CO}. The authors speculate that this latter response probably involves the development of a peripheral inflammatory response (8 to 24 h postexposure) because plasma concentrations of 6-keto $PGF_{1\alpha}$ and PGE_1 also were elevated in guinea pigs exposed for 1 h to 1 ppm O_3 (Miller et al., 1987). Significant increases in the plasma and BAL concentrations of TXB_2 also were observed following acute exposure of guinea pigs to 1 ppm O_3 (Miller et al., 1987) and humans to 0.4 or 0.6 ppm O_3 (see Chapter 7). Both indomethacin and cromolyn sodium partially blocked the reduction in lung volumes at 2 and

24 h postexposure (Miller et al., 1988). Indomethacin was ineffective in blocking the O_3-induced decrease in DL_{CO} at either 2 or 24 h postexposure, but cromolyn sodium blocked this O_3 response. Both drugs were effective in blocking the O_3-induced decrease in $\dot{V}_A$ at the same examination periods. These results suggest that eicosanoids produced from an inflammatory response in the lung may be responsible for the observed changes in lung function in guinea pigs. However, as noted above the role of eicosanoid mediators in O_3-induced lung injury is controversial.

6.2.5.4 Repeated Acute Exposure Experiments (More Than Three Days)

To date, few physiological studies have examined the attenuation that occurs in humans repeatedly (3 to 7 days) exposed to O_3 (U.S. Environmental Protection Agency, 1986; Table 6-13), despite the fact that this exposure scenario most closely mimics a high oxidant pollution episode.

Ventilation

In the only laboratory animal study using a similar exposure protocol and an experimental design analogous to human repeated-exposure studies, Tepper et al. (1989) showed that rats displayed an initial pulmonary irritant response (tachypnea) that attenuated after 5 consecutive days of exposure in a manner quite similar to the response pattern of humans (see Section 7.2). Exposures were for 2.25 h and included challenge with CO_2 during alternate 15-min periods to augment ventilation (2 to 3 times $\dot{V}_E$—equivalent to light exercise in humans). The functional changes were largest on Day 1 or 2, depending on the parameter and the O_3 concentration (0.35, 0.5, and 1.0 ppm were evaluated). Additionally, lung biochemical and structural consequences were examined at 0.5 ppm O_3 and indicated that several indices of lung damage increased (histopathology) or did not adapt (lavageable protein), despite the loss of the functional response over the 5-day exposure period. Functional attenuation, however, did not occur in the 1.0-ppm O_3 group; such a nonreversing effect has not been observed in humans. It is likely that this lack of reversal was attributable to the high concentration of O_3 and, thus, may be predictive of the human response under similar conditions.

Breathing Mechanics

In rats exposed to 1.0 ppm O_3 for 6 h/day for 7 days, the only change in breathing mechanics was an increase in R_L (Yokoyama et al., 1989a). Whether attenuation occurred cannot be ascertained because functional measurements were obtained only after the end of exposure.

Extended Characterizations

Selgrade et al. (1988) evaluated mice exposed to 1.0 ppm O_3 for 5 days (3 h/day), with and without the inoculation of influenza virus on Day 2 of exposure. Ozone alone did not cause an untoward effect on lung volumes, volume-pressure, and flow-volume relationships when mice were evaluated 1, 4, and 9 days postexposure. Mice exposed to the combination of virus and O_3 showed a decrease in DL_{CO} that persisted for 9 days.

A portion of the Tepper et al. (1989) study, discussed above, was conducted using groups of animals that were exposed between 1 to 5 days to 0.5 ppm O_3. Changes in the shape of the flow-volume curve (as indicated by the change in forced expiratory flow [FEF]

at 25% of VC) were maximal on Day 2 but gradually returned to baseline with further repeated exposures.

6.2.5.5 Longer Term Exposure Studies

The question of degenerative or irreversible lung damage when O_3 exposure is extended over periods of days to years remains paramount to the assessment of health risk. Several new studies since the previous criteria document (U.S. Environmental Protection Agency, 1986) have been published using more integrated approaches (structure, function, and biochemical techniques) for understanding this problem. This is especially true for studies evaluating near-lifetime O_3 exposures in rodent species.

Ventilation and Breathing Mechanics

Tepper et al. (1991) evaluated ventilation and breathing mechanics in rats exposed for 1, 3, 13, 52, and 78 weeks to a simulated urban profile of O_3 (Table 6-13). The exposure consisted of a 5-day/week, 9-h "ramped spike" exposure that had an integrated average of 0.19 ppm O_3 and a maximum concentration of 0.25 ppm. During other periods (13 h/day, 7 days/week), the exposure remained at a 0.06-ppm O_3 background level. Pulmonary function measurements were evaluated after 1, 3, 13, 52, and 78 weeks of O_3 exposure in response to a postexposure challenge with 0, 4, and 8% CO_2. Overall, there was a significant increase in expiratory resistance, but only at 78 weeks was resistance significantly different than the time-matched filtered-air control. At all evaluation times, V_T was reduced compared to control rats; this was especially true during challenge with CO_2. Frequency of breathing was significantly decreased when the analysis included all evaluation times, but at no single evaluation time was the reduction significant.

Other evidence of peripheral airflow abnormalities from extended exposures to O_3 is limited. Costa et al. (1983) exposed rats to 0.2 or 0.8 ppm O_3 for 6 h/day, 5 days/week for 12 weeks and did not find a concentration-related increase in pulmonary resistance measured immediately after exposure. Yokoyama et al. (1984) measured increased central resistance in rats exposed for 30 days to 1.0 ppm, but found increased peripheral airway resistance when exposure was for 60 days to 0.5 ppm. Pulmonary resistance was measured at different elastic recoil pressures. Increased R_L at low distending pressures was interpreted as of peripheral origin, whereas uniform increases across all distending pressures were described as originating from the central airways. These changes were consistent with morphological findings of mucus in the large bronchi of rats exposed to 1.0 ppm compared to the rats exposed to 0.5 ppm. These data also agree with a study by Wegner (1982) that suggests the occurrence of airflow obstruction, revealed in terms of small increases in peripheral airways resistance (as measured by oscillation harmonics) that were observed in monkeys after 1 year of exposure to 0.64 ppm O_3 (8 h/day, 7 days/week).

Airway Reactivity

No studies of airway reactivity after long-term exposures were reported before 1985. Since then, several studies have reported no increase in reactivity with daily O_3 exposure (Table 6-14). Biagini et al. (1986) observed no changes in breathing mechanics, FEF parameters, or methacholine and platinum airway responsiveness in a group of monkeys (cynomolgus) exposed to 1 ppm O_3 for 6 h/day, 5 days/week for 12 weeks in a study designed to examine the effects of combining O_3 exposure with the respiratory sensitizer platinum. Kagawa et al. (1989) exposed guinea pigs 4 h/day, 5 days/week for 4 mo to

0.15 ppm O_3. Baseline total respiratory resistance and response to increasing concentrations of inhaled histamine were assessed every 3 weeks, but did not change in response to O_3. The only exception is the Johnson et al. (1988) study that evaluated airway responsiveness in female rhesus monkeys just before a 2-h single weekly exposure to 1 ppm O_3 delivered via an endotracheal tube. After 19 weeks of exposure, increased responsiveness to inhaled methacholine was observed compared to the animal's historic control. The hyperresponsiveness persisted approximately 15 weeks after exposures were discontinued. Hyperresponsiveness to O_3 was reinstated after a similar 7-week exposure to the same animals. After this exposure regimen, animals recovered in approximately 9 weeks, but hyperresponsiveness was again reinstated with four, once-per-week exposures. The investigators described the effect of a 5-lipoxygenase inhibitor on certain portions of this sequence; however, the descriptions of methods and results were insufficient for evaluation of the effect of treatment on exposure.

Extended Functional Characterizations

The previous criteria document (U.S. Environmental Protection Agency, 1986) cataloged several investigators that reported marginal increases in total lung capacity (TLC) or its component volumes in rats after intermittent or continuous exposures to $\geq$0.25 ppm O_3 for 4 to 12 weeks (Bartlett et al., 1974; Costa et al., 1983; Raub et al., 1983). In contrast to these significant results, Yokoyama and Ichikawa (1974) previously had reported no effects on rat static volume-pressure curves after a 6-week exposure to 0.45 ppm (6 h/day, 6 days/week). More recent studies are summarized in Table 6-13.

Exposures of rats to 0.7 ppm O_3 for 28 days (20 h/day) showed an obstructive-type lung function abnormality characterized by a significant reduction in FEFs, lung volumes, and DL_{CO}, and a significant increase in FRC (Gross and White, 1986). These effects largely reversed after an additional 9 weeks of clean air, but some airflow abnormalities persisted.

Tyler et al. (1988) exposed young monkeys to 0.25 ppm O_3 for 8 h/day, 7 days/week for 18 mo or for alternate months of the 18-mo period and observed increased chest wall compliance (C_w) and inspiratory capacity. Because C_w did not decrease with age, as expected, the authors speculated that perhaps O_3 interfered with respiratory system maturation. This effect was greater in monkeys exposed during alternate months than in animals exposed every month of the 18-mo period.

To address the issue of cumulative exposure over a near-lifetime, several rodent studies have been performed using various exposure concentrations. With exposure of rats to 0.5 ppm O_3 for 52 weeks (20 h/day, 7 days/week), increases in RV and FRC were apparent, as was a fall in DL_{CO} (Gross and White, 1987), suggesting substantial end-airway damage and gas-trapping. After a 3-mo period in clean filtered air, these measurements were not different than similarly treated, but air-exposed control rats. In partial contrast, 12 or 18 mo of exposure to a daily urban profile of O_3 (9-h time-weighted average of 0.19 ppm, 5 days/week; a background of 0.06 ppm for 13 h/day, 7 days/week) resulted in small reduction in lung volumes (RV and VC) and an enhanced nitrogen (N_2) washout pattern consistent with a stiffer, restricted lung (Costa et al., 1995). Interestingly, in spite of mural remodeling of small airways (which was concentration dependent), no evidence of airflow obstruction was apparent in this study. However, in a cohort group of animals exposed at the same time, R_L was increased at all time points in unanesthetized animals, as described previously (Tepper et al., 1991). Harkema and Mauderly (1994) exposed rats for 6 h/day,

5 days/week for 20 mo to either filtered air or 0.12, 0.5, or 1.0 ppm O_3. Within 3 days of the end of exposure, an extended functional evaluation was performed; O_3 caused little impact on respiratory function. However, RV was decreased (between 21 and 36%), a finding similar, but of greater magnitude, to the Costa et al. (1995) study. The existing morphological data in monkeys at the higher concentrations noted above appear consistent with end-airway remodeling, but no clear functional evidence of obstruction has been described (Eustis et al., 1981; Wegner, 1982).

6.2.5.6 Summary

Alterations in the pulmonary function of laboratory animals after exposure to O_3 have been reported by numerous investigators. These changes appear to be homologous with the changes in pulmonary function observed in humans exposed acutely to O_3 (see Chapter 7). Although there are apparent differences in sensitivity among species, it is not clear whether these differences are due to the use of anesthesia or restraint or variances in tissue sensitivity or dosimetry.

Brief exposures to O_3 of less than 30 min have been shown to produce reflex responses (increased collateral resistance) and airway hyperresponsiveness. In the dog, these changes appear to be related, in part, to parasympathetic stimulation and release of inflammatory mediators. However, the relevance of these studies must be questioned because O_3 was delivered to a specific lung region via a bronchoscope and the contribution of collateral resistance to total lung resistance was small.

With exposures lasting greater than an hour, a wide variety of effects has been observed. Most notably, tachypnea (increased frequency of breathing and decreased tidal volume) has been noted in several species at exposures as low as 0.25 to 0.4 ppm (Tepper et al., 1990; Mautz and Bufalino, 1989). In addition to changes in breathing pattern, changes in breathing mechanics (compliance and resistance) and increased airway reactivity have been observed, but, generally, these effects have been reported at concentrations of 1 ppm or greater. In dogs, R_L increased and Cdyn decreased after a 2-h exposure to 0.5 or 0.6 ppm O_3 (Fouke et al., 1991; Mautz et al., 1985b). Enhanced reactivity to bronchoconstrictors has been reported in guinea pigs at 0.5 ppm O_3 (Tepper et al., 1990). The mechanisms that may be responsible for the O_3-induced increase in airway reactivity have been investigated extensively; however, no firm conclusion can be drawn. The most consistent evidence suggests a role for sensory afferent fibers, their associated mediators (tachykinins), and the enzyme responsible for tachykinin degradation (NEP). However, many studies suggest that the parasympathetic nervous system and inflammatory cells and mediators also may play a role in O_3-induced increase in airway reactivity.

Extended characterizations of pulmonary function indicate that the general pattern of functional impairment seen in humans acutely exposed to high concentrations of O_3 (decreased lung volumes, diffusional disturbances, and inhomogeneity of ventilation) also is observed in animals exposed to high ambient O_3 (0.5 to 2.0 ppm). For example, FVC, DL_{CO}, and N_2 slope decreased with increasing C $\times$ T products (0.5 and 0.8 ppm O_3, 2 and 7 h) in rats (Costa et al., 1989).

With daily repeated exposure to O_3, Tepper et al. (1989) showed attenuation of lung function changes (tachypnea and flow volume curve) over 5 days (2 h/day to 0.35 to 1.0 ppm, with CO_2 stimulation of breathing) similar to what is observed in repeatedly exposed humans (Chapter 7). It is of interest that, in this study, morphological changes showed a progressive increase in severity, and other biochemical indicators of lung injury

(lavageable protein and antioxidants) did not show attenuation of the response over the same time period. The findings from long-term exposures of O_3 to laboratory animals are even more difficult to summarize. Various findings in rats ranged from no or minimal effects (Biagini et al., 1986; Kagawa et al., 1989; Chang et al., 1992; Harkema and Mauderly, 1994) to obstructive (Gross and White, 1986; Tepper et al., 1991) or restrictive (Costa et al., 1995) lung function abnormalities. However, in all cases where recovery was evaluated, no severe lung injury was detected, and the physiological alterations that were observed resolved several months after termination of exposure.

6.2.6 Genotoxicity and Carcinogenicity of Ozone

6.2.6.1 Introduction

Ozone is a very reactive molecule and a strong oxidizing agent that can dissolve in aqueous solutions and generate superoxide, hydrogen peroxide, and hydroxyl radicals and can oxidize and peroxidize cellular macromolecules (reviewed in Menzel, 1970; Hoigne and Bader, 1975; U.S. Environmental Protection Agency, 1986; Mustafa, 1990; Victorin, 1992; Pryor, 1993). Early studies of the effects of O_3 on purines, pyrimidines, nucleosides, nucleotides, and nucleic acids showed that O_3 rapidly degraded these compounds in vitro (Christensen and Giese, 1954; reviewed in Menzel, 1984). Ozone-generated hydroxyl radicals can abstract hydrogen from organic molecules, leading to further complex free-radical reactions (reviewed in Menzel, 1970, 1984; Mustafa, 1990; Victorin, 1992). In addition, O_3 initiates radical reactions, resulting in ozonolysis of alkenes to form ozonides, which decompose on reaction with water to form peroxyl radicals, peroxides, and aldehydes. Ozone also can oxidize amines to amine oxides and react with PUFA to form products of lipid peroxidation (reviewed in Menzel, 1970, 1984, 1992; Mustafa, 1990; Pryor, 1978, 1991, 1993). Ozone also has been shown to cause a reduction in plaque formation by bacteriophage f2, to release RNA from phage particles, to inactivate RNA, and to degrade protein (Kim et al., 1980). Hence, because O_3 generates hydroxyl radicals in aqueous solution and degrades DNA, RNA, protein, and fatty acids in vitro, it poses a potential genotoxic hazard by virtue of its ability to generate reactive intermediates that can oxidize nucleic acid bases (reviewed in Victorin, 1992). However, the precise reactions that occur in living cells exposed to O_3 have not yet been defined completely. As the ensuing discussion shows, the genotoxic potential of O_3 is, at most, weak.

This section reviews the information available on the genotoxicity of O_3 since the last air quality criteria document (U.S. Environmental Protection Agency, 1986) was published, although earlier reports are cited to create a historical and scientific perspective for the reader. The areas covered in this review are the ability of O_3 to induce DNA damage, mutagenesis, cell transformation, carcinogenesis, co-carcinogenesis, and tumor promotion. Although modulation of the tumorigenic response by indirect effects of O_3 on the immune system is theoretically possible, no evidence for such modulation has been reported (see Section 6.2.3). Unfortunately, experimental data to evaluate whether O_3 is genotoxic are very limited. Hence, relevant data on genotoxic effects of O_3 above 1 ppm also have been included to ensure discussion of the full array of effects as they currently are understood. Although data at points far above 1 ppm of O_3 are not directly relevant to human health, such high-concentration data serve to address (1) whether ozone is genotoxic at all, (2) whether concentration-response relationships exist for the specific genotoxicity

endpoint studied, and (3) what maximum sensitivity is required for discovering genotoxic effects.

A further caveat is that in many experiments utilizing in vitro systems, O_3 was added to bacteria or to mammalian cells covered by cell culture medium. In all such experiments, the reactivity of O_3 makes it highly likely that the reaction products of O_3 with culture fluid, not O_3 itself, actually reach and interact with cells. This complicates interpretation of the results, making it extremely difficult to extrapolate in vitro results to in vivo results and making it extremely difficult, if not impossible, to extrapolate in vitro results to potential genotoxicity in humans.

6.2.6.2 Ozone-Induced Deoxyribonucleic Acid Damage

Studies utilizing a wide range of O_3 concentrations (0.1 to 20 ppm) have been performed to determine whether O_3 is genotoxic. Hamelin (1985) showed by a combination of agarose gel electrophoresis and electron microscopy that ozonation at 5 to 20 ppm caused single- and double-strand DNA breaks, nicking, relaxation, linearization, and then degradation of double-stranded plasmid pATl53 DNA molecules in solution. Hamelin also showed that ozonation of plasmid DNA reduced the transforming ability of this plasmid, and that *Escherichia coli* strains with mutations in DNA repair pathways (lexA, ozrA, and recA, but not uvrA) were less able to support the transforming ability of the ozonated plasmid. Hence, the lexA, ozrA, and recA gene products participate in repairing O_3-induced DNA breaks.

Similarly, Sawadaishi et al. (1985) showed that ozonolysis of supercoiled pBR322 DNA resulted in conversion of closed-circular DNA molecules to open-circular DNA and caused single-strand cleavage at specific sites. The concentrations of O_3 employed were not listed. Sawadaishi et al. (1986) further explored the specificity of O_3-induced damage to supercoiled plasmid pBR322 DNA by utilizing DNA sequencing techniques. The mechanistic data obtained, showing preferential degradation of thymine bases, are very interesting chemically, but the O_3 concentrations used were far too high (25,600 ppm) to be useful in assessing biologically relevant effects of O_3.

Exposure of naked DNA from HeLa cells to 2 ppm O_3 for 24 h resulted in the formation of hydroxymethyluracil, thymine glycol, and 8-hydroxyguanine (Cajigas et al., 1994). These results indicate a potential mutagenicity for O_3, although the question of the penetration of O_3 to the DNA of intact cells has not been explored carefully. Mura and Chung (1990) also studied the biological consequences of ozonation of DNA. They exposed phage T7 DNA to 5 ppm O_3 for periods of 5 to 15 min and found that O_3 decreased the template activity of the DNA. Both the rate of initiation of transcription and the length of the RNA chains transcribed were reduced. They concluded that O_3 induced abnormal changes in the structure of the phage T7 DNA and that these changes interfered with the ability of the DNA to be transcribed. In mammalian cells, Van der Zee et al. (1987) demonstrated that ozonation of murine L929 fibroblasts caused DNA strand breaks, DNA interstrand cross-links, and DNA protein cross-links, but the O_3 concentrations used were far too high (615 ppm) to be relevant to the ambient exposures that are the focus of this document.

Kozumbo and Agarwal (1990) conducted in vitro studies in which specific arylamines contained in tobacco smoke (l-naphthylamine, 2-naphthylamine, aniline, p-toluidine, o-toluidine, and m-toluidine) were exposed to 0.1 to 1.0 ppm O_3 for 1 h. When the reaction products were added to human lung fibroblasts and transformed human Type 2

cells in vitro, DNA damage occurred. This raises the possibility that smokers could incur DNA damage in their lung cells due to the interaction of O_3 with arylamines contained in tobacco smoke, but there are no data on whether such reactions occur in vivo.

A logical consequence of these findings (Table 6-15) is that O_3 could inhibit DNA replication in mammalian cells and induce cytotoxicity to these cells, and this was found by Rasmussen (1986). Rasmussen observed that DNA replication was inhibited in a concentration-dependent manner in Chinese hamster V79 cells by O_3 concentrations from 1 to 10 ppm following a 1-h exposure. These exposure regimens also induced cytotoxicity.

Table 6-15. Effects of Ozone on Deoxyribonucleic Acid Damage[a]

Ozone Concentration		Exposure Duration	Exposure Conditions	Cells	Observed Effects	Reference
ppm	$\mu g/m^3$					
0.1	196	1 h	15 μM l-napthylamine	Diploid human lung fibroblasts and transformed Type 2 cells	DNA breaks	Kozumbo and Agarwal (1990)
2.0	3,920	24 h	Phosphate buffered saline	Naked DNA from HeLa cells	Formation of hydroxymethyluracil, thymine glycol, and 8-hydroxyguanine in DNA	Cajigas et al. (1994)
1.0-10	1,960-19,600	1 h	Culture	Hamster (Chinese V79 cells)	Inhibition of DNA replication; cytotoxicity	Rasmussen (1986)
5.0	9,800	5-15 min	DNA at 50 μg/mL; O_3 at 0.5 L/min, room temperature	Ozonated T7 phage DNA	Decreased in vitro transcription	Mura and Chung (1990)
5.0-20	9,800-39,200	5-15 min	10 mM Tris/HC1 1 mM EDTA	Plasmid pAT153	Single-/double- strand breaks in DNA	Hamelin (1985)

[a]See Appendix A for abbreviations and acronyms.

Therefore, the available data show that O_3 causes single- and double-strand breaks in plasmid DNA in vitro, damages plasmid DNA so that its ability to serve as a template for transcription is decreased, and inhibits DNA replication and causes cytotoxicity in Chinese hamster V79 cells (Table 6-15).

6.2.6.3 Induction of Mutation by Ozone

Consistent with its ability to induce DNA damage, the very high concentration of 50 ppm O_3 also induced mutation to streptomycin resistance in *E. coli*, via both direct mechanisms and indirectly by the rec-lex error-prone DNA repair system, by a factor from two- to 35-fold in an exposure-time dependent manner (Table 6-16). However, no statistical analysis was performed on these data (Hamelin and Chung, 1975a,b; L'Herault and Chung, 1984). In assays designed to detect base substitution mutations by gases in *Salmonella typhimurium*, Victorin and Stahlberg (1988a) showed that O_3 alone at concentrations of 0.1 to 3.5 ppm did not induce mutation to histidine auxotrophy in Ames' strains TA100, TA102, or TA104. Ozone concentrations ≥ 2 ppm were cytotoxic. Victorin and Stahlberg (1988b) also showed that 0.5 and 1.0 ppm O_3, in combination with 1% vinyl chloride, and 1 ppm O_3, plus 0.1 or 1% butadiene, gave rise to a slight (approximately twofold) increase in mutation frequency. In these in vitro studies, as in many studies reviewed in this section, it must be pointed out that because O_3 is so reactive, placing vinyl chloride and butadiene in the experiments would result in exposure of the bacteria to reactive intermediates and reaction products resulting from the mixture, not exposure of the bacteria to O_3 alone. Further, these increases in mutation were small (no more than twofold) and not statistically analyzed, and the authors did not test strictly for concentration-dependent effects (Table 6-16). Thus, it is not clear that these small effects are reproducible.

More recently, Dillon et al. (1992) studied the ability of O_3 to induce mutation in the Ames' strains of *Salmonella* (Table 6-16). Ozone caused no mutation in *Salmonella* strains TA1535, TA98, TA100, and TA104. These authors found that 0.024- and 0.039-ppm O_3 exposures caused small increases in the mutation frequency in *Salmonella* tester strain TA102, which is uniquely sensitive to detecting mutation induced by oxygen radicals. These increases in mutation frequency were significant; however, the authors did not observe consistent concentration-dependent increases in the mutation frequency. At the higher concentrations of O_3, there appeared to be an inverse dependence for induction of mutation by O_3. The authors indicated that the cytotoxicity of O_3 complicated attempts to obtain a clear concentration response for mutagenicity. The presence of Arochlor 1254-induced rat liver S-9 metabolic activation did not affect the mutational responses in any of the strains tested. These authors did not observe reproducible increases in mutation frequency in *Salmonella* strains TA98, TA100, TA104, or TA1535. Dillon et al. (1992) therefore concluded that O_3 is a weak bacterial mutagen only under specific conditions utilizing noncytotoxic concentrations in TA102. However, because clear concentration-dependent responses for mutation could not be achieved, it is not clear that O_3 is definitively mutagenic in these studies.

Gichner et al. (1992) investigated whether O_3 could induce mutation in two mutagenicity assays in plants. The investigators found no induction of mutation in the *Nicotiana tabacum* leaf color reversion assay or in the *Tradescantia* stamen hair assay at 0.1 to 0.3 ppm O_3 (Table 6-16).

Dubeau and Chung (1979) showed that mutants of the yeast of *Saccharomyces cerevisiae* deficient in repair of single- and double-strand DNA breaks were more sensitive to the cytotoxicity of O_3 than wild-type cells, indicating that O_3 kills cells partly by generating these types of breaks. Dubeau and Chung (1982) also showed that treatment of *S. cerevisiae* with 50 ppm O_3 for 30 to 90 min resulted in (1) an 11- to 14-fold increased frequency of forward mutations, (2) an increase in reversions at six different loci by two- to threefold,

Table 6-16. Summary of Findings on the Mutagenicity of Ozone

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Exposure Conditions	Cells	Observed Effects	Reference
0.024	47	35 min	Culture	*S. typhimurium* TA102	At 0.024 ppm: 2.4-fold increase in mutation frequency	Dillon et al. (1992)
0.039	76	35 min			At 0.039 ppm: 1.6-fold increase in mutation frequency	
0.39	764	35 min			At 0.39 ppm: 1.3-fold increase in mutation frequency; no effects seen in *S. typhimurium* TA98, TA1535, TA100, or TA104	
0.1-0.3	196-588	5 or 11 h/day for 1-15 or 18 days		*Nicotiana tabacam Tradescantia*	No mutation at color locus	Gichner et al. (1992)
0.1-3.5	196-6,860	6 h	Culture	*S. typhimurium* TA100, TA102, or TA104	No mutation with or without metabolic activation	Victorin and Stahlberg (1988a)
0.5 1.0	980-1,960	6 h	Culture 1% vinyl chloride	*S. typhimurium* TA100	170% increase in mutation; statistical analysis not conducted	Victorin and Stahlberg (1988b)
1.0	1,960	6 h	Culture 0.1 or 1.0% butadiene	*S. typhimurium* TA100	170% increase in mutation; statistical analysis not conducted	Victorin and Stahlberg (1988b)
50	98,000	1-20 min	Culture	*E. coli*	Exposure-time-dependent mutation to streptomycin resistance, up to 35-fold increases in mutation frequency	L'Herault and Chung (1984)
50	98,000	30-90 min	Culture	*Saccharomyces cerevisiae*	Forward mutations, reversions, gene conversion, mitotic crossing over; no statistical analysis conducted	Dubeau and Chung (1982)

(3) an increase in gene conversions by two- to threefold, and (4) an increase in mitotic crossover by 1.3-fold. No statistical analysis was performed on these data, nor were concentration-response curves generated by the authors. These authors, therefore, demonstrated that O_3 was a mutagen and a recombination-inducing agent in *S. cerevisiae*. However, they also showed that its genotoxic activity was weak (20- to 200-fold less activity in terms of the frequency of mutants or recombinants induced) compared to the known mutagens ultraviolet light, X rays, and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG).

In this section, the inclusion of data on bacterial mutation utilizing exposures up to 50 ppm O_3 can be justified in order to help determine whether O_3 is genotoxic at all. The

available information shows that O_3 is not mutagenic in four *Salmonella* tester strains and may cause weak mutagenicity in *Salmonella* strain TA102, but these positive results are weakened by the lack of a concentration-response effect. The data also show O_3 is mutagenic in *E. coli*, is weakly mutagenic in *S. cerevisiae*, but is not mutagenic in *N. tabacam* or *Tradescantia*. Hence, because O_3 is mutagenic in three assays, but not in six others, and is weakly mutagenic in assays where the results are positive compared to known strong mutagens, O_3 should be considered a weak mutagen, at most (also reviewed in Victorin, 1992).

6.2.6.4 Induction of Cytogenetic Damage by Ozone

A number of investigators have studied whether O_3 induces cytogenetic damage. The previous air quality criteria document (U.S. Environmental Protection Agency, 1986) described a number of in vitro and in vivo studies in which O_3 exposure produced cytotoxic effects on cells and cellular components, including genetic material; very few newer studies have been reported. Cytogenetic and mutational effects of O_3 have been reported previously in isolated cultured cell lines, human lymphocytes, and microorganisms (Fetner, 1962; Hamelin et al., 1977a,b; Hamelin and Chung, 1975a,b; Scott and Lesher, 1963; Erdman and Hernandez, 1982; Guerrero et al., 1979; Dubeau and Chung, 1979, 1982). One of the earliest studies by Fetner (1962) demonstrated that in vitro exposure of human KB cells to 8 ppm O_3 for 5 and 10 min induced two- and sixfold increases in the number of chromatid deletions. Shiraishi et al. (1986) found that treatment of Chinese hamster V79 cells with 0.1 to 1.0 ppm O_3 inhibited growth of V79 cells by 10 to 70% and also induced a concentration-dependent increase in the number of sister chromatic exchanges (SCEs) per cell, up to a maximum of fourfold of that in untreated, control cells. The results indicate that if cells in culture are exposed to sufficiently high concentrations of O_3 for sufficiently long periods, chromosome damage will result.

In vivo exposure studies are of greater potential interest. Cytogenetic and mutational effects of O_3 in laboratory animals and humans are controversial. Lymphocytes isolated from animals exposed to O_3 were found to have significant increases in the numbers of chromosome (Zelac et al., 1971a,b) and chromatid (Tice et al., 1978) aberrations, after 4- to 5-h exposures to 0.2 and 0.43 ppm O_3, respectively. Single-strand breaks in DNA of mouse peritoneal exudate cells were measurable after a 24-h exposure to 1 ppm O_3 (Chaney, 1981). Gooch et al. (1976) analyzed the bone marrow samples from Chinese hamsters exposed to 0.23 ppm O_3 for 5 h and the leukocytes and spermatocytes from mice exposed for up to 2 weeks to 0.21 ppm O_3. No effect was found on the frequency of chromosome aberrations, nor were there any reciprocal translocations in the primary spermatocytes. These authors did show that there was a slight, but significant increase in the frequency of chromatid aberrations in human peripheral leukocytes exposed in vitro to 7.2 and 7.9 ppm O_3. The small increases observed in chromatid abnormalities in peripheral blood lymphocytes from humans exposed to 0.5 ppm O_3 for 6 to 10 h were not significant, possibly because of the small number ($n = 6$) of subjects studied (Merz et al., 1975). Subsequent investigations with improved experimental design and more human subjects, however, did not show cytogenetic effects after exposure to O_3 at various concentrations and for various times (McKenzie et al., 1977; McKenzie, 1982; Guerrero et al., 1979). Guerrero et al. (1979) showed no elevation in the frequency of SCEs in circulating lymphocytes of humans exposed to 0.5 ppm of O_3 for 2 h. However, these authors did find that exposure of diploid human fetal lung (WI38) cells to 0.25, 0.50, 0.75, and 1.0 ppm

O_3 for 1 h in vitro led to a concentration-dependent increase in SCEs in these cells. In addition, epidemiological studies have not shown any evidence of chromosome changes in peripheral lymphocytes of humans exposed to O_3 in the ambient environment (Scott and Burkart, 1978; Magie et al., 1982). Evidence now available, therefore, fails to demonstrate any cytogenetic or mutagenic effects of O_3 in humans when the exposure regimens are representative of exposures that the population might actually experience.

Finally, a study conducted by Erdman and Hernandez (1982) showed that treatment of *Drosophila virilis* with 30 ppm O_3 for 2 to 6 h resulted in an exposure time-dependent accrual of dominant lethals.

Therefore, O_3 does induce chromosomal aberrations in cultured cells, but the results in animals exposed to O_3 for chromosomal breakage are, at most, weak and their biological significance is controversial.

6.2.6.5 Induction of Morphological Cell Transformation by Ozone

Ozone has been studied in a number of mammalian cell culture systems to determine whether it can induce cell transformation (Table 6-17). The cell transformation assay in C3H/10T1/2 (10T1/2) mouse embryo cells is a standard assay that has been used by many investigators to detect cell tranformation activity as a potential indicator of carcinogenicity and to study molecular mechanisms of cell transformation induced by organic chemicals, carcinogenic metals, and radiation (reviewed in Landolph, 1985, 1989, 1990, 1994). Syrian hamster embryo (SHE) cells also are widely used to detect cell transformation by many classes of chemical carcinogens and radiation (Borek et al., 1986, 1989a,b). Borek et al. (1986) demonstrated that exposure of SHE cells and 10T1/2 mouse embryo cells to 5 ppm O_3 for 5 min inducted morphological transformation in both cell types. Also, in both cell types, there was a synergistic induction of morphological transformation when the cells were treated with 3 Grays of gamma radiation and 5 ppm O_3. These authors therefore concluded that O_3 acts as a direct cell transforming agent and as a co-cell transforming agent in the presence of gamma radiation. Borek et al. (1989a) also observed an additive amount of transformation when these cell types were treated with 6 ppm O_3 and 4 J/m^2 of ultraviolet light. A further study by Borek et al. (1989b) showed that exposure of 10T1/2 mouse embryo cells to 1 ppm O_3 for 5 min did not result in morphological transformation, but that increasing exposure to 5 ppm increased the transformation frequency by a factor of 15. Ozone and gamma radiation caused a synergistic increase in morphological transformation when O_3 was added to cells after the gamma radiation. When O_3 was added to cells before the gamma radiation, the transformation was not increased over that due to gamma radiation. These authors also showed by DNA transfection experiments that three O_3-induced transformed cell lines possessed dominantly acting transforming genes. In these studies, cells were incubated in phosphate-buffered saline during O_3 treatment and, hence, likely were exposed to reaction products of O_3 rather than O_3 itself.

Thomassen et al. (1991, 1992) exposed rats by inhalation to 0.14, 0.6, or 1.2 ppm O_3 for 6 h/day, 5 days/week, for a total of 1, 2, or 4 weeks. There was no increase in the frequency of preneoplastic transformation in cells removed from the tracheas and subsequently cultured. Cells incubated in serum-free medium and exposed to 0.7 or 10 ppm O_3 for 40 min in vitro also did not show an increased frequency of preneoplastic transformation. When rat tracheal epithelial cells were exposed in vitro to 0.7 ppm O_3,

Table 6-17. Effects of Ozone on Morphological Cell Transformation[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain/Cells) Age[b]	Observed Effects	Reference
0.14, 0.6, 1.2	274, 1,176, 2,352	6 h/day, 5 days/week for 1, 2, or 4 weeks	Rat, M (F344/N) 7-9 weeks old	No induction of preneoplastic variants in cultured tracheal epithelial cells	Thomassen et al. (1991)
0.7	1,372	40 min twice weekly for 5 weeks (in vitro)	Rat (Tracheal epithelial cells)	Twofold increase in frequency of preneoplastic variants; additive effects with MNNG	Thomassen et al. (1991)
0.7 10	1,372 19,600	40 min (in vitro)	Rat (Tracheal epithelial cells)	No induction of preneoplastic variants	Thomassen et al. (1991)
1.0 5.0	1,960 9,800	5 min (in vitro)	Mouse (C3H/10T1/2 embryo cells)	At 1.0 ppm: No morphological transformation alone; increased transformation by 0.4-Gray radiation by 1.7-fold like a co-carcinogen. At 5.0 ppm: 15-fold increase in morphological transformation and synergism with 4-Gray gamma radiation transformation.	Borek et al. (1989b)
5.0	9,800	5 min (in vitro)	Hamster (Primary diploid cells) Mouse (C3H/10T1/2 embryo cells)	In both cell lines, induction of morphological transformation and synergism with gamma rays	Borek et al. (1986)
6.0	11,760	10 min (in vitro)	Hamster (Syrian primary embryo cells) Mouse (C3H/10T1/2 embryo cells)	In both cell lines, induction of morphological transformation; additive transformation with UV light	Borek et al. (1989a)

[a]See Appendix A for abbreviations and acronyms.
[b]Age at start of exposure.

two times a week for 5 weeks, there was approximately a twofold increase in the frequency of preneoplastic variants detected. These authors also showed that treatment of rat tracheal epithelial cells with MNNG followed by exposure of cells to 0.7 ppm O_3 twice weekly for 5 weeks resulted in an approximately additive increase in the frequency of preneoplastic variants of the cells. The results of these studies should be interpreted cautiously because the changes are very small and because O_3 exposure followed by MNNG treatment yielded

negative results (Thomassen, 1992). In addition, it is likely that the culture conditions may significantly affect these results, particularly the volume of culture medium above the cells, and this variable has not been explored in sufficient detail. Further, the results of these experiments are somewhat variable. Recently, a system has been developed to culture human tracheobronchial epithelial cells and expose them to consistent and reproducible levels of O_3 (Tarkington et al., 1994).

In all these cell transformation experiments, the reactivity of O_3 makes it likely that secondary reaction products of O_3 formed in the aqueous medium, not O_3 itself, induced the cell transformation. Therefore, O_3 is able to induce morphological transformation in C3H/10T1/2 mouse embryo cells and in SHE cells at high concentrations (1.0, 5.0, and 6.0 ppm) but causes no significant effects in rat tracheal epithelial cells in vitro or in vivo.

6.2.6.6 Possible Direct Carcinogenic, Co-carcinogenic, and Tumor-Promoting Effects of Ozone as Studied in Whole Animal Carcinogenesis Bioassays

To investigate whether O_3 has carcinogenic, co-carcinogenic, or tumor-promoting effects, a number of investigators have conducted in vivo carcinogenesis bioassays with O_3 (Table 6-18). Some of the studies have used strain A mice (reviewed in Mustafa, 1990). The advantages and disadvantages in using strain A mice as a general screen for carcinogens by the ip route have been discussed in the literature (Stoner and Shimkin, 1985; Maronpot et al., 1986; Stoner, 1991; Maronpot, 1991). Strain A mice only rarely have been used in inhalation carcinogenesis assays. In addition, the A/J strain of mice has a high spontaneous incidence of benign pulmonary tumors (adenomas). This strain of mice has been shown to be very sensitive to tumor induction by polycyclic aromatic hydrocarbons (PAHs), carbamates, and aziridines and insensitive to aromatic amines, metal salts, and halogenated organic compounds administered by the ip route (Maronpot et al., 1986). In addition, carcinogenicity results in strain A mice did not correlate well with 2-year mouse and rat carcinogenicity results when the results of chemical testing in strain A/St mice (59 chemicals tested) were compared with strain A/J mice (30 chemicals tested) in a 2-year chronic bioassay (Maronpot et al., 1986). The chemicals chosen were heavily weighted with aromatic amines. The author concluded that "carcinogenicity test data are relevant only to the test model employed since there is no absolute reference for carcinogenicity." Maronpot (1991) also demonstrated a poor concordance between results of testing chemicals in the strain A assay and testing them in 2-year rat and mouse carcinogenicity assays at the National Cancer Institute (NCI). Stoner (1991), using the strain A mouse pulmonary assay, indicated that ip injection of PAHs, nitrosamines, nitrosureas, carbamates, aflatoxin, metals, and hydrazines induces tumors, but that the assay is not responsive to aromatic amines, aliphatic halides, and certain compounds carcinogenic in rodent liver or bladder. In this assay, an increase in lung tumor multiplicity (average number of lung tumors per mouse) caused by a chemical is considered as evidence for the carcinogenicity of a chemical.

Hassett et al. (1985) used inbred strain A/J mice, which are very sensitive to induction of pulmonary adenomas by chemical carcinogens. Exposure of A/J mice to 0.31 ppm O_3 for 103 h per week, every other week, for 6 mo, resulted in a 1.3-fold increase (not statistically significant) in the percent of mice with tumors (tumor incidence) and a statistically significant 1.4-fold increase in the number of tumors per mouse (tumor multiplicity). In this experiment, O_3 did not promote the carcinogenicity of urethane when

Table 6-18. Summary of Results on the Possible Carcinogenicity of Ozone[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effects	Reference
0.05 (sine wave from 0 to 0.1)	98 (sine wave from 0 to 96)	10 h/day for 13 mo	Rat, M (Wistar) 4 weeks old	Lung tumor response increased from 0% in BHPN- or O_3-treated animals, to 8.3% in animals treated with 0.5 g/kg BHPN + 0.05 ppm O_3 (not significant).	Ichinose and Sagai (1992)
0.31	608	103 h/week, every other week for 6 mo	Mouse, F (A/J) 7 weeks old	1.33-fold increase in percent of mice with adenomas, 1.42-fold increase in number of tumors per mouse. No promotion of carcinogenicity of urethane (2 mg/mouse before O_3).	Hassett et al. (1985)
0.5	980	102 h/first week of each month for 6 mo	Mouse, F (A/J) 7 weeks old	2.11-fold increase in percent of mice with tumors, 3.42-fold increase in number of tumors per mouse, interaction between O_3 and urethane (2 mg/mouse after each O_3 week).	Hassett et al. (1985)
0.4 0.8	784 1,568	8 h/day, 7 days/week for 18 weeks	Mouse, M (Swiss Webster and A/J) 8 weeks old	Urethane treatment before O_3 started. In Swiss Webster mice: No increase in lung tumor incidence; nonsignificant decrease in tumors per lung in urethane-treated animals. In A/J strain: No effect at 0.4 ppm. At 0.8 ppm: Threefold increase in percent mice with tumors, and 4.2-fold increase in number of tumors per mouse. Both 0.4 and 0.8 ppm O_3 decreased yield of tumors per mouse in urethane-treated mice, but had no effect on tumor incidence.	Last et al. (1987)
0.8	1,568	23 h/day 7 days/week for 6 mo	Hamster, M (Syrian Golden) 7-11 weeks old	No tumors observed in animals treated with 0.8 ppm O_3 only. In animals treated with 20 mg/kg DEN sc twice/week, 0.8 ppm O_3 did not increase tumors of lung, bronchus, trachea, or nasal cavity. Tumors of lung were decreased 50% (N.S.)	Witschi et al. (1993a,b)
0.12 0.5 1.0		6 h/day 5 days/week for 104 weeks (2 years)	Rat, M, F (F344/N)	No increase in neoplasms at any concentration tested.	National Toxicology Program (1994) Boorman et al. (1994)
0.5 1.0		6 h/day 5 days/week for 124 weeks (lifetime)	Rat, M, F (F344/N)	No increase in neoplasms at any concentration tested.	National Toxicology Program (1994) Boorman et al. (1994)
0.12 0.5 1.0		6 h/day 5 days/week 105 weeks (2 years)	Mouse, M, F ($B6C3F_1$)	No effects in males. In females: Increase in number of mice with neoplasms at 1.0 ppm (combined alveolar/bronchiolar adenoma or carcinoma in lung).	National Toxicology Program (1994)
0.5 1.0		6 h/day 5 days/week 130 weeks (lifetime)	Mouse, M, F ($B6C3F_1$)	In males: Increase in number of mice with carcinoma at 0.5 and 1.0 ppm, but not significant for change in number of mice with total neoplasms. In females: increase in number of mice with adenomas, but not carcinomas or total neoplasms.	National Toxicology Program (1994)

[a]See Appendix A for abbreviations and acronyms.
[b]Age at start of exposure.

O_3 exposure began 1 week after a single injection of animals with a total dose of 2 mg urethane/mouse. In a second experiment, exposure to 0.50 ppm O_3 (102 h during the first week of every month for 6 mo) caused a nonsignificant 2.1-fold increase in tumor incidence and a 3.2-fold increase in tumor multiplicity (statistics not shown) (Hassett et al., 1985). These authors reported that exposure to 0.5 ppm O_3, followed by urethane treatment (2 mg after each O_3 exposure set), resulted in an interaction between O_3 and urethane such that there were more animals with more than 16 lung tumors each. These authors concluded that exposure to 0.31 and 0.5 ppm O_3 increased the yield of pulmonary adenomas in A/J mice and that O_3 interacted with urethane to produce more lung tumors than urethane alone when O_3 was added before urethane.

The pulmonary adenomas induced in animals by chemical carcinogens are by definition benign tumors (Stoner, 1991; Maronpot, 1991); however, they do represent abnormal cell growth in the form of tumors and, hence, are significant biologically in that they are early steps in the pathway toward malignancy. It is not known if these tumors can progress to malignant tumors, because they are not as amenable to observation as are mouse skin tumors, where adenomas can be converted into adenocarcinomas at a frequency of approximately 8 to 10%. Similarly, it is not known with certainty whether pulmonary adenomas in humans progress to adenocarcinomas. It is clear, however, that lung tumors in mice are not equivalent to bronchogenic carcinomas in humans. However, because a shift is occurring, in that fewer squamous cell bronchogenic carcinomas and more peripheral adenocarcinomas are occurring in humans, the induction of peripheral adenomas and their progression to peripheral adenocarcinomas in mice may be a useful area for further mechanistic insight.

The study of Hassett et al. (1985) was reviewed extensively by scientists from EPA and the National Institutes of Environmental Health Sciences in 1985 and 1986 (Tilton, 1986). The consensus of these extensive reviews was that (1) the tumor yields in O_3-exposed mice were not statistically significantly different from the control animals, (2) any effects were marginally different from control values, and (3) the strain A mouse has a high spontaneous incidence of tumors, making it difficult to interpret the effects of O_3. Chemical induction of tumors in this assay system did not correlate well with the 2-year NCI carcinogenesis bioassay results. In addition, because Hassett et al. (1985) did not demonstrate a concentration-response effect in animals exposed to O_3, the consensus among the reviewers was that one could not conclude from these experiments that O_3 was a significant carcinogen or tumor promoter, and that rigorous inhalation carcinogenesis bioassays needed to be carried out with O_3-exposed animals to address this issue properly.

Last et al. (1987) also studied whether O_3 exposure could influence the yield of urethane-induced lung tumors in A/J and Swiss-Webster mice. Urethane treatment consisted of a single ip injection (1,000 mg/kg) 1 day before O_3 exposure began. In Swiss Webster mice, exposure to 0.4 or 0.8 ppm O_3 alone not only did not increase the tumor yield but actually decreased the yield of urethane-induced lung tumors per mouse, although the decrease was not statistically significant. In A/J mice, exposure to 0.4 ppm O_3 did not increase the lung tumor yield, but exposure to 0.8 ppm O_3 caused a threefold increase in tumor incidence and a 4.2-fold increase in lung tumor multiplicity. Exposure of urethane-treated mice to 0.4 or 0.8 ppm O_3 decreased lung tumor multiplicity but had no effect on tumor incidence. These differences in the strain A mouse were significant. The authors concluded that O_3 was not a tumor promoter or tumor-enhancing agent.

Ichinose and Sagai (1992) studied the ability of O_3 to interact with *N-bis*(2-hydroxypropyl) nitrosamine (BHPN) in the induction of lung tumors in Wistar rats. A single ip injection of BHPN (0.5 g/kg) did not cause any tumors in the rats. Rats were exposed for 10 h/day for 13 mo to a pattern of O_3 consisting of a sine curve from 0 to 0.1 ppm, with a mean concentration of 0.05 ppm. The 13-mo O_3 exposure started the day after BHPN injection, and the rats were examined 11 mo postexposure. No tumors were observed in the O_3-alone or control groups. When rats were exposed to 0.5 g/kg BHPN plus 0.05 ppm O_3, the lung tumor incidence increased to 8.3% (3/36), but this increase was not statistically significant. The tumors observed in this study cannot be stated definitively to have been induced by the treatment agent.

The data available on O_3 exposure and lung carcinogenesis up until 1988 have been reviewed by Witschi (1988). The chemical reactivity of O_3 and, in particular, its radiomimetic activity (ability to mimic radiation effects, such as causing cell-cycle arrest, chromosome breakage, etc.) also make O_3 a potential risk factor for human lung cancer. Nevertheless, as of 1988, there were no experimental studies conclusively linking lifelong exposure to O_3 with lung tumor induction in any animal species, nor was there conclusive epidemiological evidence to associate O_3 exposure with the development of lung cancer in humans. As of 1988, the only data implicating O_3 as a possible tumorigenic agent were from studies carried out in mice, where the tumors are adenomas derived from Type 2 alveolar cells or from Clara cells (reviewed in Witschi, 1988). In the A and Swiss-Webster mouse strains used to assay the carcinogenicity of O_3, the spontaneous incidence of lung tumors is very high. Hence, results of carcinogenicity experiments conducted on O_3 to date that utilize tumor incidence as an endpoint are not strongly positive, due to this high background. In strain A mice, in which the spontaneous multiplicity is usually less than one tumor per mouse, the tumor multiplicity is considered by many investigators to be a sensitive indicator of a carcinogenic effect. However, even using this indicator, the increase in tumor multiplicity after O_3 exposure is small, raising questions about the biological significance of the effects. In addition, the assay for inhalation carcinogenesis in strain A mice was not fully validated at this time. Whereas Hassett et al. (1985) concluded that O_3 increased the number of pulmonary adenomas in strain A mice, Witschi (1988) concluded that O_3 was not implicated unequivocally as a carcinogen in strain A/J mice, that no classical carcinogen bioassays had been conducted on O_3, and that a definitive judgment could not be made on the carcinogenicity of O_3.

A review (Witschi, 1991) of the available data on the carcinogenicity of O_3 and oxygen in mouse lungs indicated that oxidants can enhance or inhibit mouse lung tumorigenesis, depending on the experimental protocol employed, and the carcinogenicity of O_3 in mouse lung had not been established unequivocally. Exposure of strain A mice to O_3 induces hyperplasia of Type 2 alveolar cells, leading to expansion of the target cell population. It was speculated that this might have resulted in spontaneous transformation of these cells (Witschi, 1991). In lungs of animals treated with a carcinogen such as urethane and then exposed to O_3 before or after carcinogen administration, it was speculated that O_3 may cause cell proliferation and result in fixation of DNA damage (Witschi, 1991). The addition of O_3 after carcinogen exposure leads to a decreased tumor incidence compared to treatment with carcinogen alone; the reasons for this decrease with late O_3 exposure are not clear.

The effects of treating male Syrian Golden hamsters with dimethylnitrosamine (DEN) (20 mg/kg given subcutaneously twice per week) during the course of a 6-mo

exposure to 0.8 ppm O_3 were studied by Witschi et al. (1993a; see Table 6-18). The rationale for this study was to test the hypothesis that O_3 acts in a manner similar to hyperoxia in enhancing neuroendocrine lung tumors in this animal model. After exposure ceased, the animals were maintained in air for 1 mo. Ozone exposure did not increase the incidence of lung, bronchus, trachea, or nasal cavity tumors in the DEN-treated hamsters. There was a 50% decrease in the percent of animals with lung tumors in the DEN-plus-O_3-exposed animals compared to the DEN-plus-air-exposed animals, but this was not statistically significant. Ozone did not affect the incidence of DEN-induced liver tumors. The authors concluded that O_3 did not increase the number of DEN-induced respiratory tumors in hamsters and that O_3 exposure might have inhibited or delayed tumor development. Although reduction in tumor incidence caused by O_3 was not significant in this study alone, overall analysis of these data with other data from a 4-mo exposure of similar design (Witschi et al., 1993b) yielded significant results.

A definitive study of the carcinogenicity of O_3 and its ability to act as a co-carcinogen or tumor promoter was conducted by the U. S. National Toxicology Program (NTP) (National Toxicology Program, 1994; Boorman et al., 1994). Animals were exposed to air or O_3 for 6 h/day, 5 days/week for the number of weeks described below. Male and female F344/N rats were exposed to 0.12, 0.5, and 1.0 ppm O_3 for 104 weeks and to 0.5 and 1.0 ppm for 124 weeks ("lifetime" of the animals). Similar protocols were used for $B6C3F_1$ male and female mice, with the exception that the "2-year" study was 105 weeks and the lifetime study was 130 weeks. This study did not find any evidence of carcinogenic activity in male or female rats. There was a negative trend for mammary gland neoplasms in the female rats in the 2-year study, an effect that was not seen in the lifetime study. The NTP found "equivocal evidence"[1] of carcinogenic activity in O_3-exposed male mice and "some evidence" of carcinogenic activity in female mice exposed to O_3. In co-carcinogenesis experiments, male rats were treated with a known pulmonary carcinogen, 4-(*N*-methyl-*N*-nitrosomino)-1-(3-pyridyl)-1-butanone (NNK) (0.1 and 1.0 mg/kg, subcutaneous injection 3 times a week for first 20 weeks) and exposed to 0.5 ppm O_3 for 6 h/day, 5 days/week for 105 weeks. The NTP found "no evidence" that O_3 enhanced the incidence of NNK-induced pulmonary neoplasms. Table 6-19 shows the tumor incidences in mice. In the discussion to follow, all tumors described were at lung alveolar/bronchiolar sites. There was a decrease in the number of hepatocellular adenomas or carcinomas in female mice exposed to 1.0 ppm O_3 for 2 years and for hepatocellular carcinomas in the lifetime study. There was no statistically significant increase in tumors at any site other than the lung.

In male mice exposed to O_3 for 2 years, there were no statistically significant increases in the mice with alveolar/bronchiolar carcinomas or a combination of adenomas or carcinomas; at 0.5 ppm O_3, there was a small, twofold increase in the incidence of adenomas. In the lifetime studies of male mice, the incidence of mice with carcinomas increased 1.9-fold at 0.5 ppm O_3 and 2.3-fold at 1.0 ppm. The incidence of adenomas in

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[1]The NTP evaluates the strength of the evidence for conclusions regarding each carcinogenicity study, under the conditions of that particular study. There are five categories: two for positive results ("clear evidence" and "some evidence"), one for uncertain findings ("equivocal evidence"), one for no observable effects ("no evidence"), and one for experiments that cannot be judged because of major flaws ("inadequate study") (National Toxicology Program, 1994). This approach is very different from the weight-of-evidence approach used by EPA for cancer classification because the EPA approach considers all the available studies.

Table 6-19. Alveolar/Bronchiolar Tumor Incidence in $B6C3F_1$ Mice in the National Toxicology Program's Chronic Ozone Study

	Males			Females		
ppm	Adenomas[a]	Carcinomas[a]	Both[a]	Adenomas[a]	Carcinomas[a]	Both[a]
2-year exposure						
0.0	6/50	8/50	14/50	4/50	2/50	6/50
0.12	9/50 (p = 0.3)	4/50 (p = 0.15)	13/50 (p = 0.44)	5/50 (p = 0.55)	2/50 (p = 0.65)[b]	7/50 (p = 0.57)
0.5	12/50 (p = 0.06)	8/50 (p = 0.45)	18/50 (p =0.12)	5/49 (p = 0.52)	5/49 (p = 0.26)	9/49 (p = 0.33)
1.0	11/50 (p = 0.11)	10/50 (p = 0.27)	19/50 (p = 0.10)	8/50 (p = 0.24)	8/50 (p = 0.053)	16/50 (p = 0.02)
Lifetime exposure						
0.0	8/49	8/49	16/49	3/50	3/50	6/50
0.5	8/49 (p = 0.61)[b]	15/49 (p = 0.05)	22/49 (p = 0.14)	3/49 (p = 0.63)	5/49 (p = 0.33)	8/49 (p = 0.34)
1.0	9/50 (p = 0.47)	18/50 (p = 0.007)	21/50 (p = 0.15)	11/50 (p = 0.02)	2/50 (p = 0.50)[b]	12/50 (p = 0.10)
Combined						
0.0	14/99	16/99	30/99	7/100	5/100	12/100
0.5	20/99 (p = 0.16)	23/99 (p = 0.08)	40/99 (p = 0.06)	8/98 (p = 0.48)	10/98 (p = 0.14)	17/98 (p = 0.20)
1.0	20/100 (p = 0.14)	28/100 (p = 0.009)	40/100 (p = 0.04)	19/100 (p = 0.01)	10/100 (p = 0.14)	28/100 (p = 0.004)

[a]Number of animals with neoplasm/number of animals necropsied (p [probability] value, logistic regression test).
[b]Lower incidence.

Source: National Toxicology Program (1994).

male mice did not change significantly. In female mice exposed for 2 years, there were no statistically significant changes at 0.12 or 0.5 ppm O_3. However, at 1.0 ppm O_3, there was a fourfold increase in the frequency of female mice with carcinoma and a 2.7-fold increase in combined adenomas plus carcinomas. In female mice exposed for their lifetimes to O_3, 0.5 ppm caused no significant effects. At 1.0 ppm O_3, there was a 3.7-fold increase in the incidence of mice bearing pulmonary adenomas, a nonsignificant change in the frequency of mice with carcinomas, and a twofold ($p = 0.1$) increase in the incidence of combined adenomas and carcinomas.

When the results of the 2-year and lifetime O_3 carcinogenesis studies were combined and analyzed, for male mice, there was no statistically significant increase in the incidence of animals bearing adenomas, and, for carcinomas, there was a marginally significant increase at 0.5 ppm O_3 (1.4-fold increase, $p = 0.08$) and a significant, 1.7-fold increase at 1.0 ppm. The incidence of male mice bearing adenomas or carcinomas showed a marginally significant increase (1.3-fold, $p = 0.06$) at 0.5 ppm O_3 and a 1.3-fold increase at 1.0 ppm ($p = 0.045$). In the combined analysis of the 2-year and lifetime exposure of female mice, there were no statistically significant changes at 0.5 ppm O_3. At 1.0 ppm O_3, there was a 2.7-fold increase in the percent of mice bearing adenomas and a 2.3-fold increase in the frequency of mice with adenomas or carcinomas. There was no statistically significant increase in the carcinoma incidence.

The overall conclusions of the authors of the NTP O_3 inhalation carcinogenesis study were (1) there was no increased pulmonary tumor incidence in male or female F344/N rats exposed to 0.12, 0.5, or 1.0 ppm O_3; (2) male F344/N rats treated with the tobacco carcinogen NNK and exposed to 0.5 ppm O_3 did not have an increase in the pulmonary tumor incidence above that caused by NNK alone; (3) O_3 caused a slightly increased

incidence of alveolar/bronchiolar adenoma or carcinoma that yielded equivocal evidence of carcinogenicity of O_3 in male $B6C3F_1$ mice; and (4) O_3 increased the incidence of alveolar/bronchiolar adenoma or carcinoma in female $B6C3F_1$ mice, yielding some evidence of carcinogenic activity of O_3 in female mice.

Generally, in mice, adenomas appear to progress into carcinomas with time, and, thus, the incidence of mice having both adenomas and carcinomas is probably the more useful indicator of effects. The incidence of tumor-bearing mice was elevated significantly only in female mice exposed for 2 years to 1.0 ppm O_3. When both the 2-year and lifetime exposure studies were combined, there was a increased incidence of tumors in the males at 0.5 and 1.0 ppm O_3 and in females at 1.0 ppm. The NTP designated the data for male mice as equivocal for carcinogenesis because the combined tumor incidence in the 2-year study was within the historical range, and the combined incidence for the lifetime study was not significant, even though the carcinoma incidence was significant in the lifetime study. The evaluation of female mice resulted in NTP's finding of "some evidence of carcinogenic activity" because the combined pulmonary adenoma/carcinoma incidence was significantly increased and outside the range of the historical control tumor rates. When the lifetime and 2-year studies were combined, there were 28/100 adenomas plus carcinomas in the 1.0-ppm O_3 exposure group versus 12/100 in the controls ($p = 0.004$).

In summary, the strongest data on carcinogenicity come from the NTP study, which was ambiguous in male mice and positive only in female mice at high concentrations of O_3 (i.e., 1.0 ppm). This may represent a toxic or irritant effect, giving a nonspecific type of tumor due to mitogenesis. The carcinogenicity data are weak or equivocal in male mice, negative in F344/N male and female rats, and negative for co-carcinogenesis in male rats. Therefore, the potential for animal carcinogenicity is uncertain at the present time.

6.2.6.7 Possible Effects of Ozone on Injected Tumor Cells That Lodge in the Lung and Form Lung Colonies

To date, no rigorous studies have been conducted to examine the effects of O_3 on true lung tumors that would have metastasized. No studies have been conducted in which lung tumor cells detach themselves from primary lung or other tumors growing in organs and invade adjacent tissue, blood vessels, or lymphatics. A few studies have been conducted in which tumor cells are injected intravenously into animals and then lodge in the lung, forming lung colonies (Table 6-20). It must be stressed, however, that this experimental model is not an adequate model for lung tumor cell metastasis.

Kobayashi et al. (1987) showed that exposure of C3H/He mice for 1 or 14 (but not other) days to ≥ 0.1 ppm O_3 after mice were injected in the tail vein with the fibrosarcoma cell line (NR-FS) increased the number of metastatic lung tumors. Animals were exposed to O_3 for 14 days, then fibrosarcoma cells were injected into the tail vein of the animals, and pulmonary metastases were scored 14 days later. One day of exposure to 0.8 ppm O_3 gave the maximal enhancement of pulmonary metastases. This enhancement of pulmonary metastasis was concentration-dependent, in the range from 0.4 to 0.8 ppm O_3 from 1 to 14 days, but increases were small. This effect may arise in two ways: (1) by damage to the microvasculature and (2) by the differential sensitivity of various tumor cells to O_3 cytotoxicity (reviewed by Witschi, 1988). Richters (1988) reported that exposure of mice to 0.15 or 0.30 ppm O_3 for 60 days did not increase colonization of the lungs of mice injected iv with B16 melanoma cells.

Table 6-20. Effects of Inhaled Ozone on the Ability of Injected Tumor Cells To Colonize the Lungs of Mice[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effects	Reference
0.15 0.3	294 588	60 days	Mouse, M (C57B/6J) 5 weeks old	No increase in lung metastases from iv-injected B16 melanoma cells.	Richters (1988)
0.1 0.2 0.4 0.8	196 392 784 1,568	1-14 days	Mouse, M (C3H/He) 8-12 weeks old	Pulmonary metastases from iv-injected NR-FS fibrosarcoma cells. After 1 and 14 days of 0.1 ppm, 1.3-fold increases. After 5 and 7 days of 0.2 ppm, 1.3 and 2.3-fold increases. After 1 and 5 days of 0.4 ppm, 2.3- and 2.2-fold increases. After 1 day of 0.8 ppm, 4.6-fold increase.	Kobayashi et al. (1987)

[a]See Appendix A for abbreviations and acronyms.
[b]Age at start of exposure.

6.2.6.8 Summary and Conclusions

In summary, there are some weakly positive data and some negative data on the genotoxicity of O_3 (summarized in Table 6-21). Ozone at very high concentrations (5 to 20 ppm) causes DNA strand breakage in plasmid DNA (Hamelin, 1985). Ozone is, at most, weakly mutagenic in some assays and negative in others. Ozone is not mutagenic in *Tradescantia* or *N. tabacam* at concentrations of 0.1 to 0.3 ppm (Gichner et al., 1992); is weakly mutagenic in *E. coli* at 50 ppm, and *S. cerevisiae* at 50 ppm (L'Herault and Chung, 1984; Dubeau and Chung, 1982); and is nonmutagenic in three strains of *Salmonella* and, at most, marginally mutagenic in *Salmonella* strain TA102 at concentrations of 0.024, 0.039, and 0.39 ppm (Victorin and Stahlberg, 1988a,b; Dillon et al., 1992). Despite extensive studies by Dillon et al. (1992), the mutagenicity of O_3 in *Salmonella* TA102 is not conclusive because convincing concentration-dependent mutagenic effects have not yet been demonstrated, possibly due to the strong cytotoxicity of this compound. Ozone causes cytogenetic damage in cultured cells in vitro (e.g., Hamelin et al., 1977a,b; Dubeau and Chung, 1979, 1982), but no effects or small and conflicting effects when animals are exposed in vivo (Zelac et al., 1971a,b; Tice et al., 1978). Cell transformation studies have shown positive results on exposure of cells to O_3, but these studies were conducted with a fluid barrier above the cells that may have resulted in artifacts compared to an in vivo exposure (Borek et al., 1986, 1989a,b).

The in vitro studies are mechanistically interesting, but there are difficulties in the design of many of these studies. First, the concentrations used in these in vitro studies were typically orders of magnitude greater than those found in ambient air. Second, extrapolation of in vitro exposure concentrations to human exposure dose requires special methods that were not used in these studies. Third, direct exposure of isolated cells to O_3 is somewhat

Table 6-21. Summary of Data on the Genotoxicity of Ozone[a]

Assay System in Which Ozone Was Tested	Result[b]	Comments
Mutation to histidine prototrophy in *Salmonella* TA100	−	Small effects obtained, less than twofold; concentration-response effect was not shown at 0.024, 0.039, and 0.39 ppm O_3.
Mutation to histidine prototrophy in *Salmonella* TA102	+/−	Small effects obtained, and there was no direct exposure-response at 0.024, 0.039, and 0.39 ppm O_3.
Mutation to streptomycin resistance in *Eshcherichia coli*	+	Only 50 ppm O_3 was tested.
Mutation in *Saccharomyces cerevisiae*	+	Ozone caused mutation and recombination at 50 ppm, but this was a weak response compared to known strong mutagens (20- to 200-fold less mutagenic than UV light, X rays, and MNNG).
Mutation in *Nicotiana tabacam* in a leaf-color reversion assay	−	0.1 to 0.3 ppm O_3 was tested.
Mutation in *Tradescantia* in a stamen-hair assay	−	0.1 to 0.3 ppm O_3 was tested.
Chromosomal breakage in cultured mammalian cells	+	8 ppm O_3 in human KB cells, and 0.1 to 1.0 ppm O_3 in V79 cells.
Chromosomal breakage in animals	+/−	Results are, at best, weak and controversial; results in this assay are considered ambiguous and not definitively positive at present. Ozone was tested at 0.2, 0.43, 7.3, and 7.9 ppm.
Morphological transformation in C3H/10T1/2 mouse embryo cells and in Syrian hamster embryo cells	+	Experiments need to be conducted without or with only minimal amounts of fluid bathing the cells. Concentrations giving positive results are high (5 and 6 ppm O_3).
Induction of preneoplastic variants in rat tracheal epithelial cells	−	Both in vitro and in vivo exposures give negative or, at most, only twofold increases in cells exposed to 0.14, 0.6, 0.7, 1.2, or 10 ppm O_3.

Table 6-21 (cont'd). Summary of Data on the Genotoxicity of Ozone[a]

Assay System in Which Ozone Was Tested	Result[b]	Comments
Lung tumor induction in whole animals		
(a) Strain A/J mice, Swiss-Webster mice, Syrian Golden hamsters, Wistar rats	+/−	Positive results marginal, not statistically significant; experiments not designed to determine whether a concentration-response exists. Ozone was tested at 0.05, 0.31, 0.4, 0.5, 0.8, and 1.0 ppm.
(b) National Toxicology Program Studies— Male and female F344/N rats	−	No increased incidence of pulmonary adenomas or carcinomas in rats exposed to 0.12, 0.5, or 1.0 ppm O_3 for 2 years or with 0.5 or 1.0 ppm O_3 for animals' lifetimes.
Male $B6C3F_1$ mice (2-year study)	+/−	No effect at 0.12 ppm O_3, slight increases in the total pulmonary neoplasms at 0.5 and 1.0 ppm O_3, but they were not statistically significant.
Male $B6C3F_1$ (Lifetime studies)	+/−	Alveolar/bronchiolar carcinoma incidence increased twofold at 0.5 ppm O_3 ($p = 0.05$) and 1.0 ppm O_3 ($p = 0.007$); no increases in total pulmonary neoplasms.
Female $B6C3F_1$ mice (2-year study)	+	Fourfold increase in alveolar/bronchiolar carcinoma at 1.0 ppm O_3 ($p = 0.053$).
Female $B6C3F_1$ (Lifetime studies)	+	Fourfold increase in alveolar/bronchiolar adenomas and carcinomas at 1.0 ppm O_3 ($p = 0.02$).
	+	Threefold increase in alveolar/bronchiolar adenomas at 1.0 ppm O_3 ($p = 0.02$).
	+	Twofold increase in bronchiolar/alveolar adenomas and carcinomas at 1.0 ppm O_3, but not statistically significant.

[a]See Appendix A for abbreviations and acronyms.
[b]− = no effect; + = effect.

artifactual because it bypasses all the host defenses that would normally be functioning to protect the individual from the inhaled dose. Direct, in vitro O_3 exposure of isolated cells in tissue culture medium also results in chemical reactions between O_3 and culture media to generate chemical species that may not be produced in vivo. Therefore, for these reasons, the relevance and predictive value of in vitro studies to human health are questionable The most relevant data on the genotoxicity of O_3 should therefore be obtained from in vivo studies.

The earlier studies in whole animal carcinogenesis bioassays must be considered ambiguous at this time (Witschi, 1988, 1991). The NTP study utilized an inhalation model, assayed the carcinogenicity of O_3 in male and female F344/N rats and $B6C3F_1$ mice, and also tested whether O_3 could enhance the tumorigenicity of the tobacco-specific pulmonary carcinogen NNK (National Toxicology Program, 1994; Boorman et al., 1994). This study clearly showed that O_3 was not carcinogenic in female and male rats at 0.12, 0.5, and 1.0 ppm O_3 (6 h/day, 5 days/week for 2 years) or at 0.5 and 1.0 ppm O_3 (6 h/day, 5 days/week, lifetime). Exposure to 0.5 ppm O_3 did not enhance the carcinogenicity of NNK in male rats, leading to the conclusion that O_3 does not act as a co-carcinogen or tumor promoter in these animals. In the male mice, O_3 had equivocal effects at 0.5 and 1.0 ppm O_3 in the 2-year and lifetime inhalation studies. In the female mice, there was some evidence for the carcinogenicity of O_3 at 1.0 ppm (2.7-fold increase in total pulmonary neoplasms [$p = 0.02$] in the 2-year study; and twofold increase in total pulmonary neoplasms [$p = 0.1$] in the lifetime study; and 2.3-fold increase in total pulmonary neoplasms when the 2-year and lifetime study were combined [$p = 0.004$]).

Therefore, the earlier negative animal carcinogenesis studies, the negative carcinogenicity results in inhalation carcinogenesis studies in F344/N male and female rats, the ambiguous data in male $B6C3F_1$ mice, and the weak carcinogenicity of O_3 in female $B6C3F_1$ mice indicate that O_3 is carcinogenic only in female $B6C3F_1$ mice at high concentrations (1.0 ppm). The weak carcinogenicity of O_3 in female mice, the weak/ambiguous results in male mice, and the negative results in male and female F344/N rats point to, at best, a weak carcinogenicity of O_3 at very high concentrations.

6.3 Systemic Effects of Ozone

6.3.1 Introduction

Ozone has long been known to cause effects in organs and tissues outside the respiratory tract. The mechanisms are not known, but it is quite unlikely that O_3 itself enters the circulation (Pryor, 1992). Another possibility is that transported reaction products cause distant effects. Some effects may be secondary to effects on the lung (e.g., aversive behaviors that may result from lung irritation). The relatively few systemic studies reported since the last O_3 criteria document (U.S. Environmental Protection Agency, 1986) are discussed below. Some classes of effects (i.e., reproduction/development, endocrine system) were studied earlier and, hence, are cited briefly here in the introduction.

No reproductive toxicity studies of O_3 were found. Only two developmental studies provided sufficient details in the report to determine the exposures used. The only effect observed by Kavlock et al. (1979) in pregnant rats exposed to 0.44 to 1.97 ppm O_3 for the entire period of organogenesis or the three stages of gestation was an increased resorption of fetuses in rats exposed to 1.49 ppm in midgestation; no terata were found. A follow-up

study revealed that pups from dams exposed to 1 ppm O_3 during mid- or late gestation showed lower body weights 6 days after birth (Kavlock et al., 1980). A higher concentration (1.5 ppm) delivered during late gestation permanently runted 14% of the male pups.

Studies on the effects of O_3 on the endocrine system date to 1959. Generally, the body of work indicates that O_3 can affect the pituitary-thyroid-adrenal axis (U.S. Environmental Protection Agency, 1986). For example, a 1-day exposure to 1 ppm O_3 decreased serum levels of thyroid-stimulating hormone, thyroid hormones, and protein-bound iodine; prolactin levels increased (Clemons and Garcia, 1980a,b). Structural changes occurring in the parathyroid glands after a 4- to 8-h exposure to 0.75 ppm O_3 included hyperplasia of chief cells, but circulating hormone levels were not measured (Atwal and Wilson, 1974).

6.3.2 Central Nervous System and Behavioral Effects

Reports of headache, dizziness, and irritation of the nose, throat, and chest are common complaints that are associated with O_3 exposure in humans (see Chapter 7). Laboratory animal studies have been performed that demonstrate behavioral effects over a wide range of O_3 concentrations (0.08 to 1.0 ppm) and suggest that these behavioral changes may be analogous to the symptoms reported in humans. Although these behavioral changes may be indicative of O_3-induced symptoms, they are not indicative of neurotoxicity. Most of the studies prior to 1986 indicated that behavior could be suppressed with O_3 exposure. For example, Murphy et al. (1964) and Tepper et al. (1982) showed that running-wheel behavior was suppressed, and Peterson and Andrews (1963) and Tepper et al. (1983) showed that mice would alter their behavior to avoid O_3 exposure. Furthermore, Weiss et al. (1981) showed that bar-pressing responses for food reinforcement were suppressed, but greater O_3 concentrations were required to decrease this behavior than the concentrations needed to decrease running-wheel behavior.

Since 1986, several reports have extended the previous findings (Table 6-22). Tepper et al. (1985) compared the effects of a 6-h exposure to O_3 on the suppression of running-wheel behavior in rats and mice. The study indicated that the lowest effective concentration was about 0.12 ppm O_3 in the rat and about 0.2 ppm in the mouse. It also was observed that, with exposure to 0.5 ppm, recovery from O_3 required at least 3 h. In a follow-up study, Tepper et al. (1985) required mice to make a response that turned off the brief delivery (60 s) of O_3 at concentrations between 0.25 to 16 ppm. Mice learned to terminate O_3 exposures at 0.5 ppm. With each of three determinations of the concentration-response curve, mice got better at terminating O_3 exposure, rather than exhibiting an adaptation to exposure. The authors suggest that mice may have learned to use the odor of O_3 as a conditioned stimulus to initiate termination of exposure instead of responding directly to the irritant properties of O_3.

Because free-access wheel-running behavior was suppressed at 0.12 ppm O_3 (Tepper et al., 1982), and lever pressing for food reinforcement was reduced only at 0.5 ppm (Weiss et al., 1981), a series of experiments was performed to evaluate the behavioral determinants of the O_3 response (Tepper and Weiss, 1986). Food deprivation and response contingencies (having to perform a certain response to get a reward) were found to be relatively unimportant determinants of behavior because rats that had to run rather than press a lever to obtain food reinforcement showed behavioral suppression of running at 0.12 ppm. However, in another experiment, suppression of lever pressing was shown to be

Table 6-22. Effects of Ozone on Behavior[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Behavioral Conditions	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.08 0.12 0.25 0.5	157 980	6 h	Free-access wheel running	Rat, M (Long-Evans) 10 weeks old Mouse, M (Swiss-Webster) 5 weeks old	Mice less responsive than rats. Reduction in free-access wheel running at approximately 0.12 ppm in rats and 0.2 ppm in mice. Recovery from exposure to 0.5 ppm did not occur by 5 h postexposure in either mice or rats.	Tepper et al. (1985)
0.08 0.12 0.25 0.5	157 980	6 h	Wheel running for food	Rat, M (Long-Evans) 300 g	0.12 ppm O_3 decreased wheel running for food reinforcement.	Tepper and Weiss (1986)
0.08 0.12 0.25 0.5	157 980	6 h	Lever pressing for access to the running wheel	Rat, M (Long-Evans) 300 g	0.12 ppm O_3 decreased bar press for access to the running wheel. Two of the four animals were affected at 0.08 ppm.	Tepper and Weiss (1986)
0.12-1.5	235-2,940	6 h	Nose poke response for food	Rat, M (Long-Evans) 275 g	0.5 ppm O_3 decreased nose poking for food reinforcement. Effects were enhanced postexposure.	Tepper and Weiss (1986)
0.1-0.8	196-1,568	7 days continuous	Drinking, eating	Mice, M (ICR) 8-26 weeks old	Drinking, food consumption, and body weight initially decreased, but adapted with continued exposure, starting at 0.2 ppm.	Umezu et al. (1993)
0.25-16	490-31,360	60 s maximum	Nose poking terminated O_3 exposure	Mouse, M (Swiss-Webster) 30 g	At 0.5 ppm, mice learned to terminate O_3 exposure.	Tepper and Wood (1985)
0.4 1.2	784 2,352	13 days continuous	Home cage behavior	Mice	During first hour, rearing, grooming, sniffing, and social interactions increased, crossings and wall climbing decreased. These behaviors did not adapt with continued exposure.	Musi et al. (1994)
0.5 2.0	980 3,920	3 h	Lever pressing to avoid electric shock	Rat (Wistar) 300 g	Suppression of lever pressing began after 45 min of 2.0-ppm exposure and after 90 min of 0.5- or 1.0-ppm exposures.	Ichikawa et al. (1988)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

equally sensitive to O_3 exposure when pressing a lever allowed rats to have access to a running wheel. The authors concluded that increased physical activity, either used as the response to obtain reward, or as the reward, was an important behavioral variable in determining sensitivity to O_3 exposure. Ichikawa et al. (1988) demonstrated that behavior (lever pressing) maintained by the avoidance of electric shock, was even less sensitive to O_3 exposure (3 h, 1.0 ppm) than behaviors maintained by food reinforcement, as described above. Furthermore, the animals recovered quickly after O_3 exposure was terminated (60 to 120 min).

In mice exposed to O_3 continuously for 13 days (0.4 to 1.2 ppm), both increases and decreases in measured behaviors were observed (Musi et al., 1994). During the first hour of exposure to 0.8 or 1.2 but not 0.4 ppm O_3, increases in rearing, grooming, sniffing, and social interactions were observed, but locomotion and bar holding declined. With continued exposure (measurements on Days 3, 7, and 10), grooming and rearing were still increased but crossings and wall climbing remained depressed. The affected behaviors did not show adaptation. However, drinking, food consumption, and body weight were initially depressed, but abated with continued exposure, a finding previously reported in mice at O_3 concentrations as low as 0.2 ppm (Umezu et al., 1993).

In summary, the behavioral data indicate that transient changes in behavior occur in rodent models that are dependent on a complex interaction of factors such as (1) the type of behavior being measured, with some behaviors increased and others suppressed; (2) the factors motivating that behavior (differences in reinforcement); and (3) the sensitivity of the particular behavior (e.g., active behaviors are more affected than more sedentary behaviors).

6.3.3 Cardiovascular Effects

Several reports have demonstrated that O_3 exposure causes dramatic effects to the cardiovascular system in the rat (Table 6-23). Uchiyama et al. (1986) initially reported that heart rate (HR) and mean arterial blood pressure (MAP) were decreased by 53 and 29%, respectively, during a 3-h exposure to 1.0 ppm O_3. Arrhythmias, including atrioventricular block and premature atrial contractions, also were observed frequently. The effects appeared to be age- but not sex-dependent, with 11-week-old rats showing a greater response than did 8- or 4-week-old rats. Yokoyama et al. (1989b) showed that recovery from the effects of the 3-h, 1.0-ppm O_3 exposure was not complete by 5 h and that, with three consecutive daily exposures, both the HR and MAP responses were attenuated. Further investigations by the same group of authors (Uchiyama and Yokoyama, 1989) showed that, with exposures to 0.5 ppm O_3 for 6 h, HR and MAP decreased by 32 and 18%, respectively. A 4-week continuous exposure to 0.2 ppm initially resulted in a 12% decrease in HR, but this response was attenuated on Day 2 and was almost eliminated by Day 3. No further effects were observed during the rest of the 4-week exposure period. When these same animals were subsequently challenged with 0.8 ppm O_3 for 1.5 h, they also had an attenuated response when compared to rats that were O_3 naive. Additionally, some rats were instilled intratracheally with elastase to create an animal model of emphysema. This pretreatment, however, did not affect the outcome of either the HR or MAP responses to O_3 in any of the experiments, except in the 0.8-ppm challenge experiment. In this experiment, elastase-treated, O_3-exposed rats challenged with O_3 had a similar response to O_3 challenge as did O_3-naive rats, suggesting that the elastase treatment affected the ability of the rats to develop an adaptive lung response. In contrast, Tepper et al. (1990) did not observe an alteration in

Table 6-23. Effects of Ozone on the Cardiovascular System[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration and Conditions	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.1 0.2	196 392	5 days continuous	Rat, M (Wistar) 8 weeks old	0.1 ppm O_3 caused bradyarrhythmia up to 3 days of exposure; broadycardia occurred at 0.2 ppm during first 2 days of exposure. No effects on sleep-wakefulness patterns.	Arito et al. (1990)
0.2 0.5 1.0	392 980 1,960	4 weeks continuous 6 h 3 h	Rat, M (Wistar) 13 weeks old	At 0.2 ppm a 12% decrease in HR; response attenuated by 3 days. At 0.5 ppm, HR and MAP decreased by 32 and 18%, respectively.	Uchiyama and Yokoyama (1989)
0.25 1.0	490 1,960	2 h 18-20 °C 30-32 °C	Rat, M (F344) 13-16 weeks old	0.37 ppm O_3 caused bradycardia and bradyarrhythmia, ambient temperature of 30-32 °C blocked response.	Watkinson et al. (1993)
0.5 1.0	980 1,960	6 h 3 h	Rat, M (Wistar) 10 weeks old	0.5 and 1.0 ppm O_3 caused bradycardia and bradyarrhythmia. 1.0-ppm response was partially blocked by atropine.	Arito et al. (1992)
0.5 1.0	980 1,960	6 h 3 h, 3 days	Rat, M (Wistar) 10-11 weeks old	1.0 ppm O_3 caused bradycardia, bradyarrhythmia, and decreased MAP. The response to 1.0 ppm lasted >5 h postexposure and was attenuated with 3 consecutive daily exposures.	Yokoyama et al. (1989b)
1.0	1,960	3 h	Rat, M, F (Wistar) 4, 8, and 11 weeks old	1.0 ppm O_3 caused bradycardia, bradyarrhythmia, and decreased MAP. Older animals (11 weeks) were more affected than younger ones. No sex-related differences were noted.	Uchiyama et al. (1986)
1.0	1,960	135 min	Rat, M (F344) 90 days old	Ventilation stimulated with CO_2. No effect on mean blood pressure.	Tepper et al. (1990)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

blood pressure of rats exposed to 1.0 ppm O_3 for 135 min, even though their ventilation was increased by CO_2.

Arito et al. (1990) demonstrated bradycardic responses at 0.2 ppm O_3 during the first 2 days of a continuous 5-day exposure; bradyarrhythmia occurred during the first 3 days of a 0.1-ppm exposure. Simultaneously, these authors measured the sleep/wakefulness of the rats during exposure and found that more bradyarrhythmias occurred during wakefulness than during slow-wave or paradoxical sleep. Sleep/wakefulness patterns were not altered by this O_3 exposure. At high O_3 concentrations (1 ppm for 3 h), wakefulness and paradoxical sleep were suppressed, the amplitude of the electroencephalogram (EEG) was lowered, and slow-wave sleep was increased (Arito et al., 1992). These EEG changes appear to be temporally associated with the decrease in behavioral activity previously discussed (Tepper et al., 1982). Atropine sulfate blocked the suppression of wakefulness and bradycardia in a concentration-related manner and decreased slow-wave sleep, suggesting that some of the O_3 effects are parasympathetically mediated. The effects of O_3 on paradoxical sleep and the EEG amplitude were not affected by atropine administration. Watkinson et al. (1993) extended these findings by showing that the core temperature of rats was also reduced when HR fell at O_3 exposure concentrations between 0.37 and 1.0 ppm (2 h). Increasing ambient temperature to 30 to 32 °C attenuated the 1.0 ppm O_3-induced reduction in HR and core temperature.

In an attempt to synthesize the results from these studies, Watkinson and Gordon (1993) questioned the relevance of these parameters in the rat as compared to the human. Rats have different thermoregulatory responses than humans and typically respond to toxic insult by lowering core temperature. This response has been shown to increase survival value (Watkinson et al., 1989). Similar changes in core temperature and HR have not been reported in humans. This may be because of the large, and thus stable, thermal mass of humans, or, alternatively, these effects have not been observed because they were not measured, and because most O_3 exposure experiments are done using exercise, which may mask these responses. In support of this latter idea, Coleridge et al. (1993) reported that stimulation of bronchial C-fibers produces bradycardia. Ozone preferentially stimulates bronchial C-fibers and, as a result, induces bradycardia and tachypnea in the anesthetized, open-chest dog model. Furthermore, the tachypnea produced by O_3 exposure is inhibited by atropine administration (the effect on HR was not reported).

6.3.4 Hematological and Serum Chemistry Effects

Hematological effects reported in laboratory animals and humans after inhalation of O_3 indicate that the gas or, more likely, some reaction product can cross the blood-gas barrier. The effects of in vivo O_3 exposure in animals were summarized in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986). The hematologic parameters most frequently used to evaluate O_3 toxicity were morphologic and biochemical effects on erythrocytes (RBCs). These studies reported alterations in RBC morphology, increased RBC fragility, increased hemolysis, and decreased survival. The biochemical studies reported variable results, depending on the O_3 exposure concentration and the RBC enzyme under investigation.

More recent studies have stressed serum effects of O_3 exposure (Table 6-24). Exposure of rats for 2 h to 0.1 ppm O_3 increased plasma creatinine kinase activity, whereas no such effect was observed when exposure was to 0.05 and 0.25 ppm O_3 (Veninga and

Table 6-24. Hematology and Serum Chemistry Effects[a]

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.05 0.1 0.25	100 200 500	2 h	Rat, M (Wistar) 200 g	Increased plasma creatine kinase activity at 0.1 but not 0.05 and 0.25 ppm.	Veninga and Fidler (1986)
0.1 0.2 0.4 0.6	196 392 784 1,176	3 h	Rabbit, F (NZW) 2.5-3.5 years old	No change in plasma retinol, ascorbic acid, and α-tocopherol concentrations.	Canada et al. (1987)
0.4	784	Continuous for 14 days	Rat, M (Wistar) 20 weeks	Decrease in serum retinol concentration.	Takahashi et al. (1990)
0.8	1,568	18 h	Rat, M (F344)	Decrease in plasma lactic dehydrogenase isoenzyme activity.	Nachtman et al. (1988)
1.0	1,960	1 h	Guinea Pig, M (Hartley) 250-300 g	Increases in plasma concentrations of TXB_2, 6-keto-$PGF_{1\alpha}$, and PGE_1.	Miller et al. (1987)
1.0	1,960	1 h	Guinea Pig, M (Hartley) 250-300 g	Increases in plasma concentrations of TXB_2, 6-keto-$PGF_{1\alpha}$, and PGE_1.	Miller et al. (1988)
1.0	1,960	23 h/day for 2 weeks	Rat, M (CD) 400 ± 25 g	Heat-inactivated plasma increases DNA synthesis by lung fibroblasts and pneumocytes.	Tanswell et al. (1989, 1990) Tanswell (1989)
1.0	1,960	4 h	Mouse, M (CD-1) 8 weeks old	Inhibition of RBC deformability.	Morgan et al. (1988)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

Fidler, 1986). Decreased serum retinol concentrations were observed following continuous exposure of rats for 14 days to 0.4 ppm O_3 (Takahashi et al., 1990), but no changes in plasma retinol, ascorbic acid, and α-tocopherol were observed following exposure of rabbits for 3 h to O_3 ranging from 0.1 to 0.6 ppm (Canada et al., 1987). In similar studies, a decrease in plasma lactic dehydrogenase isoenzyme activity also was observed following exposure of rats for 18 h to 0.8 ppm O_3 (Nachtman et al., 1988).

Miller et al. (1987, 1988) investigated the effect of a 1-h exposure of guinea pigs to 1.0 ppm O_3 on plasma eicosanoid levels and observed increases in TXB_2, 6-keto-$PGF_{1\alpha}$, and PGE_1. These data suggest that some of the systemic effects of O_3 exposure, such as impairment of peritoneal AM phagocytosis (Canning et al., 1991), may be mediated by the immunosuppressive effects of the prostanoids (Oropeza-Rendon et al., 1979). Heat-inactivated plasma from rats exposed for 23 h/day for 2 weeks to 1.0 ppm O_3 also increases DNA synthesis by lung fibroblasts (Tanswell et al., 1989) and lung pneumocytes (Tanswell et al., 1990).

6.3.5 Other Systemic Effects

Previous studies suggest that O_3 has effects on the xenobiotic metabolism of the liver (U.S. Environmental Protection Agency, 1986). This effect has been observed in mice, rats, and hamsters as a prolongation of pentobarbital sleeping time (Graham et al., 1981). The effect appears to be sex dependent, with females having greater responses than males. Canada and Calabrese (1985) performed a similar experiment in both young (3- to 4-mo-old) and older (2-year-old) rabbits exposed for 3.75 h/day to 0.3 ppm O_3 for 5 consecutive days. They observed significant prolongation of the elimination of theophylline in older rabbits, but not in young rabbits, and the effect was more pronounced in females than in males. In a follow-up study, Canada et al. (1986) could not demonstrate increased pentobarbital sleeping in young (2.5-mo-old) mice or rats of comparable age to the study by Graham et al. (1981). However, effects were observed in older (18-mo-old) female mice and rats. Two other studies (Heng et al., 1987; Zidenberg-Cherr et al., 1991) from the same group of investigators indicate that liver antioxidant enzymes (Cu/Zn- and Mn-SOD and GSHPx) are decreased commensurate with the increase in these enzymes that is observed in the lung.

6.3.6 Summary

Several reports recently have appeared that extend previous observations in laboratory animals that indicate that ambient levels of O_3 can affect animal behavior. These effects are interpreted as analogous to O_3-induced symptoms in humans, rather than as evidence of neurotoxicity. The behavioral changes are transient but may persist several hours after acute exposure. Different types of behaviors appear to be variably sensitive to O_3 exposure, with active behaviors showing suppression at lower O_3 concentrations than do more sedentary behaviors or behaviors maintained by electric shock (Ichikawa et al., 1988; Tepper et al., 1985; Tepper and Weiss, 1986). For example, a 6-h exposure of rats to 0.12 ppm suppressed running-wheel behavior (Tepper et al., 1985). Furthermore, animals will respond to terminate a 1-min exposure to 0.5 ppm O_3, thus directly implicating the irritant properties of O_3 (Tepper and Wood, 1985). It appears that with additional training, animals can learn to terminate exposure using conditioned stimuli rather than relying directly on the aversive properties of O_3 (Tepper et al., 1985).

Ozone has been found to decrease HR, MAP, and core temperature profoundly in rats (Watkinson et al., 1993; Arito et al., 1990; Uchiyama and Yokoyama, 1989). During exposure, arrhythmias frequently occur. After a 3-h exposure to 1.0 ppm O_3, these effects appear to occur more in adult rats (11 weeks old) than in younger animals (4 and 8 weeks old), especially when the rats were awake (as measured by EEG) during the exposure (Uchiyama et al., 1986). The lowest exposures causing bradycardia in rats was 0.2 ppm for 48 h; 0.1 ppm for 24 h caused bradyarrhythmia (Arito et al., 1990). Similar effects have not been observed in humans or other species. In part, this may be because they have not been systemically examined or that human studies have been carried out during concurrent exercise, which may mask these effects. More likely, these effects represent species differences related to the magnitude and localization of reflex responses and differences in thermal mass.

6.4 Interactions of Ozone with Other Co-occurring Pollutants

6.4.1 Introduction

Most of the toxicological data for O_3 are derived from studies using O_3 alone. However, it is also important to evaluate responses to inhalation of typical pollutant combinations because ambient exposures involve mixtures. Such mixtures provide a basis for toxicological interactions, whereby combinations of chemicals may behave differently than would be expected from consideration of the action of each separate constituent. This section discusses toxicological studies of pollutant mixtures in which O_3 is one component. Discussions of many of these studies addressing the effect of O_3 alone on various organs or systems appear elsewhere in this chapter.

Evaluating the role of O_3 in observed responses to inhaled mixtures is not easy. In spite of the myriad of interpretative difficulties, it is essential to attempt to understand the potential for interactions because O_3 does not exist alone. One of the problems involves definitions of terms, and the study of toxicant interaction is complicated by the dilemma of attempting to characterize the effects from exposure to two or more chemicals. Most studies have employed a statistical definition, but this merely provides a description, and tells nothing about the mechanism of any interaction. But, in many cases, the mechanism of action of the individual components may not be understood fully, and information concerning the types of interactions may provide useful beginnings for studying mechanisms of action of the mixture components. Furthermore, any conclusion of interaction is highly dependent on the specific type of model used. Because the purpose of this chapter is to provide a toxicologic background for effects of O_3 in terms of public health significance, interaction will be defined as a departure from the additivity model (i.e., interaction is considered to occur when the response to a mixture is significantly different from the sum of the responses to the individual components). A less than additive interaction is antagonism, whereas synergism is an interaction that is more than additive. A subclassification of synergism, termed potentiation, is often used to describe an interaction in which response to a mixture is greater than the sum of the responses to the individual components, but where only one component produced a response different from control when administered alone. In many instances, however, potentiation and synergism have been used interchangeably. Although some synergistic interactions actually may serve to stimulate repair processes, or otherwise

reduce the harmful effects of O_3, and some antagonistic interactions eventually may increase the risk of disease development, synergism, as currently used, generally implies greater risk, and antagonism implies lesser risk. However, such assumptions eventually may be proven to be invalid in some instances. Also, interactions with the large number of natural air pollutants, such as microbes, spores, and dusts, that can produce considerable responses alone are not included in this section.

In most cases, the interaction of O_3 with other pollutants has been studied using mixtures that contained only one other copollutant (i.e., simple or binary mixtures). In such studies, the role played by each pollutant in eliciting measured responses can be elucidated with the appropriate experimental design, but most of the database involves exposures to the mixture and O_3 only, with no exposure to the copollutant alone. Although the O_3 concentration may have been varied among exposure groups or was present in one group and not in another (so its relative influence could be assessed to some extent), it cannot be determined in such cases whether the response to the mixture involved actual interaction or was merely additive.

The ambient atmosphere in most environments is generally a mixture of a number of pollutants, and assessing effects of such multicomponent atmospheres may serve to provide some indication of biological responses under conditions that better mimic ambient exposure. However, very few studies have used realistic combinations of pollutant concentrations when assessing interaction.

The ability to discern the contribution of O_3 to observed responses becomes even more difficult when such complex mixtures are studied. Even when binary mixtures are used, they often do not mimic the ambient pattern (e.g., NO_2 levels peak before O_3 levels do) or ambient concentrations (as absolute values or as ratios). Rarely are concentration-response mixture studies performed. This raises the possibility that an unrealistic experimental design may lead to masking the effect of a copollutant or to identifying a response that may not occur in the real world.

Another problem in assessing responses to mixtures involves the statistical basis for the conclusion of significant interaction. For example, a number of studies determined interaction by comparison of the response from exposure to only one component of the mixture with that from exposure to the complete mixture. On the other hand, some studies used statistical approaches specifically designed to indicate interactions. As another example, it may be relatively straightforward to study interactions when one exposure concentration of each of two pollutants is used, but it becomes much more difficult when there are multiple concentrations used, and even more difficult still when more than two pollutants are involved. Because variable criteria for conclusions of interaction have been used, the available database is one in which the statistical significance for determination of interaction varies in terms of its robustness.

6.4.2 Simple (Binary) Mixtures Containing Ozone

Tables 6-25 and 6-26 outline studies performed since publication of the last O_3 criteria document (U.S. Environmental Protection Agency, 1986) in which experimental animals were exposed to atmospheres containing O_3 with only one other copollutant. These tables provide the experimental details for the discussion that follows.

Table 6-25. Toxicological Interactions of Ozone and Nitrogen Dioxide[a]

Concentration[b] ppm	Concentration[b] $\mu g/m^3$	Pollutant	Exposure Duration	Species, Sex (Strain) Age[c]	Endpoints	Time of Endpoint Measurement	Response to Mixture	Interaction	Reference
0.05 0.04 0.05 0.4	98 75 98 752	O_3 NO_2 O_3 NO_2	NO_2: 24 h/day; O_3: Intermittent during hours 9-19/day following sine curve from 0-0.1 ppm (0.05 avg); total duration: 5-22 mo	Rat, M (Wistar) 7 weeks old	Lung protein content; lung lipid peroxides; antioxidant enzymes (G6PD, 6PGD, GR, GST, GSHPx, SOD)	0 PE	Protein: no change; peroxide: increase between 5 and 9 mo, return to control at >9 mo (greater effect with 0.4 ppm NO_2); enzymes: no change.	Protein: none (no effect of O_3 or NO_2 alone); peroxide: synergism (no change with O_3 or NO_2 alone for 9 mo); enzyme: none (no effect of O_3 or NO_2 alone).	Sagai and Ichinose (1991)
0.05 0.4	98 752	O_3 NO_2	O_3: Concentration ranged from 0 to 0.1 with sine curve over 9-19 h (0.05 avg); NO_2: 24 h/day; both 13 mo, 11 mo recovery	Rat, M (Wistar) 6 weeks old	Development of lung tumors from exposure (ingestion) of carcinogen, *N-bis*(2-hydroxypropyl) nitrosamine prior to O_3 and NO_2	0 PE	Increased tumor incidence (compared to air-exposed control).	Suggested synergism: no increase with O_3 alone (NO_2 alone not done).	Ichinose and Sagai (1992)
0.1 0.5 1.2 4.0 0.1 0.3 1.2 2.5 0.05 0.1 0.5 1.0 0.05 0.1 0.05 0.1	196 980 2,256 7,520 196 588 2,256 4,700 98 196 940 1,880 98 196 94 188	O_3 (baseline) O_3 (peak) NO_2 (baseline) NO_2 (peak) O_3 (baseline) O_3 (peak) NO_2 (baseline) NO_2 (peak) O_3 (baseline) O_3 (peak) NO_2 (baseline) NO_2 (peak) O_3 (baseline) O_3 (peak) NO_2 (baseline) NO_2 (peak)	15 day, continuous exposure to basal level; peaks: 1 h, twice daily, 5 days/week beginning after 64 h of continuous exposure	Mouse, F (CD-1) 4-6 weeks old	Bacterial infectivity (to *Streptococcus zooepidemicus* given after pollutant exposure)	Bacterial challenge given 0 or 18 h PE	No effect at low level; increased mortality at other levels.	Synergism: at 0.05 O_3 + 0.5 (1.0) NO_2, 0.1 O_3 + 1.2 (2.5) NO_2; marginal synergism at 0.1 O_3 + 1.2 (4) NO_2. Both O_3 and NO_2 increased mortality at two highest levels; only NO_2 increased at two lowest levels.	Graham et al. (1987)
0.1 0.3	196 564	O_3 NO_2	NO_2: 24 h/day; O_3: 8 h/day; 1, 3, 6, 18 mo	Rat, M (F344) 5 weeks old	Histopathology (LM, TEM)	0 PE	Connective tissue edema, Type 2 cell hypertrophy and enlarged lamellar bodies.	Changes more marked than with O_3 alone, NO_2 affected response to O_3 (no quantitation performed).	Terada et al. (1986)

Table 6-25 (cont'd). Toxicological Interactions of Ozone and Nitrogen Dioxide[a]

Concentration[b] ppm	Concentration[b] $\mu g/m^3$	Pollutant	Exposure Duration	Species, Sex (Strain) Age[c]	Endpoints	Time of Endpoint Measurement	Response to Mixture	Interaction	Reference
0.15 0.35	294 564	O_3 NO_2	7 h/day, 5 days/week for 12 weeks	Mouse, M (C57Bl/6J) 5 weeks	Colonization of lung by melanoma cells (injected after pollutant exposure)	Inject melanoma cells 0 PE and sacrifice 3 weeks PE	Increase in number of colonies/lung (compared to air control).	Not specified: no change with O_3, but previous study showed effect with NO_2.	Richters (1988)
0.2 4.0	392 7,520	O_3 NO_2	Continuous 1-2 mo	Rat, M (Wistar) 22 weeks old	Pulmonary xenobiotic metabolism, lung protein (homogenate)	0 PE	No effect on protein content, increase in selected enzymes.	Suggested antagonism for xenobiotic enzymes: O_3 induced increase in selected enzymes is lowered by addition of NO_2.	Takahashi and Miura (1989)
0.2 3.6 0.4 7.2 0.6 10.8 0.8 14.4	392 6,768 784 13,536 1,176 20,304 1,568 27,072	O_3 NO_2 O_3 NO_2 O_3 NO_2 O_3 NO_2	24 h/day for 3 days 12 h/day for 3 days 8 h/day for 3 days 6 h/day for 3 days	Rat, M (S-D) 10-12 weeks old	Protein (lavage), lavaged cells (epithelial PMN)	0 PE	Increased protein and cells.	Synergism: protein at 0.6 and 0.8 ppm O_3 mixtures, lavaged cells at 0.4-0.8 ppm O_3; others additive.	Gelzleichter et al. (1992a)
0.2 3.6 0.4 7.2 0.6 10.8	392 6,768 784 13,536 1,176 20,304	O_3 NO_2 O_3 NO_2 O_3 NO_2	6 h/day for 3 days	Rat, M (S-D) 10-12 weeks old	Protein (lavage), lavaged cell counts (epithelial, PMN, AM), DNA content of cell pellet	0 PE	Increased protein and cells, depending on concentration.	Synergism: cell counts at ≥0.4 ppm O_3 mixture, protein additive.	Gelzleichter et al. (1992b)
0.2 3.6 0.4 7.2 0.6 10.8 0.8 14.4	392 6,768 784 13,536 1,176 20,304 1,568 27,072	O_3 NO_2 O_3 NO_2 O_3 NO_2 O_3 NO_2	24 h/day for 3 days 12 h/day for 3 days 8 h/day for 3 days 6 h/day for 3 days	Rat, M (S-D) 250-275 g	Airway labeling index	4 days PE	Increased index in peripheral airways (TBs opening into ADs) and large airways at three highest doses, increased alveolar index at 0.8 + 14.4 ppm only.	Synergism for peripheral airways at highest dose and large airways at three highest doses only.	Rajini et al. (1993)
0.3 1.2	588 2,256	O_3 NO_2	Continuous for 3 days	Rat, M (S-D) 3 mo old	Lung enzymes (G6PD, 6PGD, ICD, GSHPx, GR, DR, GDT, NADPH-CR)	0 PE	Increased activity.	Synergism: 6PGD, ICD, GR, SOD; additive: GP, DR; others: effect same as O_3 only.	Lee et al. (1990)

Table 6-25 (cont'd). Toxicological Interactions of Ozone and Nitrogen Dioxide[a]

Concentration[b] ppm	Concentration[b] $\mu g/m^3$	Pollutant	Exposure Duration	Species, Sex (Strain) Age[c]	Endpoints	Time of Endpoint Measurement	Response to Mixture	Interaction	Reference
0.3 3.0	588 5,640	O_3 NO_2	2 h Nose-only	Rabbit, M (NZW) 4.5 mo old	Pulmonary eicosanoids (lavage) (PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, TXB_2, LTB_4)	0 or 24 h PE	Increases in PGE_2, $PGF_{2\alpha}$, and TXB_2 immediately PE (compared to air control).	Synergism: PGE_2, $PGF_{2\alpha}$; TXB_2 effect similar to O_3 alone.	Schlesinger et al. (1990)
0.3 3.0	588 5,640	O_3 NO_2	2 h/day for 14 days Nose-only	Rabbit, M (NZW) 4.5 mo old	Pulmonary eicosanoids (lavage) (PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, TXB_2)	0 PE after 7 or 14 exposures, or 24 h PE after 14 exposures	Decrease in PGE_2 (compared to air control) after 7 and 14 days and 24 h PE; 6-keto-$PGF_{1\alpha}$ decreased 24 h postexposure.	None: effects additive or similar to NO_2 alone.	Schlesinger et al. (1991)
0.4 0.4	784 752	O_3 NO_2	Continuous for 2 weeks	Mouse, M (ICR); Hamster, M (Golden); Rat, M (Wistar); Guinea pig, M (Hartley) all 10 weeks old	Lung lipid peroxides, antioxidant content, phospholipids, and fatty acids	0 PE	Variable increases to no effect, depending on species.	Not determinable: compared mixture vs. air control, no measure of single pollutants performed.	Sagai et al. (1987)
0.4 0.4	784 752	O_3 NO_2	24 h/day for 2 weeks	Mouse, M (ICR); Hamster, M (Golden); Rat, M (Wistar); Guinea pig, M (Hartley) all 10 weeks old	Lung lipid peroxides, antioxidant enzymes, total protein (homogenate)	0 PE	Increases, which were species dependent.	Not determinable: compared mixtures vs. air control, no measure of single pollutants performed.	Ichinose et al. (1988)
0.4 0.4	784 752	O_3 NO_2	Continuous for 2 weeks	Rat, M (Wistar) 10 weeks old; Guinea pig, M (Hartley) 10 weeks old	Lipid peroxides, lung antioxidants, and antioxidant enzymes	0 PE	Increased peroxides in guinea pig but not rat; increased antioxidants in rat but not guinea pig; enzymes increased or decreased in guinea pigs, increased to no change in rat.	Synergism for some endpoints, additive to no interaction for others; species dependent.	Ichinose and Sagai (1989)

Table 6-25 (cont'd). Toxicological Interactions of Ozone and Nitrogen Dioxide[a]

Concentration[b] ppm	Concentration[b] $\mu g/m^3$	Pollutant	Exposure Duration	Species, Sex (Strain) Age[c]	Endpoints	Time of Endpoint Measurement	Response to Mixture	Interaction	Reference
0.4 0.4	784 752	O_3 NO_2	Continuous for 2 weeks	Rat, M (Wistar) 10 weeks old; Guinea pig, M (Hartley) 10 weeks old	Lipid peroxides; lung antioxidants, and antioxidant enzymes	0 PE	Increased peroxides in guinea pig but not rat; increased antioxidants in rat but not guinea pig; enzymes increased or decreased in guinea pigs, increased to no change in rat.	Synergism for some endpoints, additive to no interaction for others; species dependent.	Ichinose and Sagai (1989)
0.45 4.8	882 7,520	O_3 NO_2	8 h/day for 7 days	Mouse, M (Swiss Webster) 2 mo old	Lung protein, DNA, sulfhydryl and nonsulfhydryl content; GR, GST, G6PD, 6PGD, ICD activities	0 PE	No change in protein or DNA; increase in activity of ICD, G6PD, 6PGD.	Synergism: enzyme activity.	Mustafa et al. (1985)
0.6 2.5 0.35 0.6	1,176 4,700 686 1,128	O_3 NO_2 O_3 NO_2	4 h (rest) 3 h (exercise)	Rat, M (S-D) 7 weeks old	Parenchymal histopathology	2 days PE	Increased focal lesions.	Synergism: ascribed to production of HNO_3 in exposure atmosphere.	Mautz et al. (1988)
0.6 2.5	1,176 4,700	O_3 NO_2	2 h (rest and exercise)	Rat, M (S-D) 47-52 days old	Epithelial permeability (tracheal, bronchoalveolar) (measured 1 and 24 h PE)	0, 1, 2 days PE	Rest: increased bronchoalveolar permeability at 1 and 24 h PE; exercise: increased bronchoalveolar permeability at 1 and 24 h PE (effects greater than with O_3 alone, no effect of NO_2).	Enhanced magnitude and duration of response (suggested potentiation).	Bhalla et al. (1987)
0.8 4.0	1,568 7,520	O_3 NO_2	Continuous for 3-56 days	Mouse, M (BALB/c) 8-10 weeks old	Antibody response to T-cell dependent and independent antigens in spleen	0 PE	Inconsistent pattern of increases and decreases of lung weight, thymus weight, or plaque formation.	Most responses similar to O_3 only; mixture affected some time points not affected by O_3 alone: implied nonadditive interaction, but specifics not determinable.	Fujimaki (1989)
0.8 14.4	1,568 27,072	O_3 NO_2	6 h/day for 3 days (concurrent) or sequential O_3 pre-NO_2; NO_2 pre-O_3; 6 h each	Rat, M (S-D) 10-12 weeks old	Protein (lavage); lavaged cell counts (epithelial, PMN, AM), DNA content of cell pellet	0 PE	Increased protein for concurrent or sequential, increase in cell counts for concurrent.	Synergism: protein and cell counts for concurrent, protein additive or antagonistic for sequential.	Gelzleichter et al. (1992b)

Table 6-25 (cont'd). Toxicological Interactions of Ozone and Nitrogen Dioxide[a]

Concentration[b] ppm	Concentration[b] $\mu g/m^3$	Pollutant	Exposure Duration	Species, Sex (Strain) Age[c]	Endpoints	Time of Endpoint Measurement	Response to Mixture	Interaction	Reference
0.8 14.4	1,568 27,072	O_3 NO_2	6 h/day for 45-79 days	Rat, M (S-D) 10/12 weeks old	Various biochemical and histological endpoints	0 PE	Increased lung DNA, protein, collagen, elastin; some deaths with mixture only at ≥55 days; decreased hydroxypyridinium.	Suggested synergism for hydroxyproline and hydroxypyridinium.	Last et al. (1993b)

[a]See Appendix A for abbreviations and acronyms.
[b]Grouped by pollutant mixture.
[c]Age or body weight at start of exposure.

Table 6-26. Toxicological Interactions to Binary Mixtures of Ozone with Acids and Other Pollutants[a]

Concentration[b] ppm	Concentration[b] $\mu g/m^3$	Pollutant	Exposure Duration	Species, Sex (Strain) Age[c]	Endpoints	Time of Endpoint Measurement	Response to Mixture	Interaction	Reference
0.1	196 125	O_3 H_2SO_4 (0.3 μm)	2 h/day, 5 days/week for up to 1 year Nose-only	Rabbit, M (NZW) 4.5 mo old	Tracheobronchial mucociliary transport, bronchial tree epithelial secretory cell numbers	Mucociliary transport during exposure; secretory cells 3 days after 4, 8, and 12 mo of exposure	Normal to accelerated clearance, increase in secretory cell numbers at early time points (4-mo exposure).	Clearance: No interaction; secretory cell numbers: synergism at 4 mo, antagonism at 8 and 12 mo.	Schlesinger et al. (1992a)
0.1 0.3 0.6	196 588 1,176 50 75 125	O_3 O_3 O_3 H_2SO_4 (0.3 μm) H_2SO_4 (0.3 μm) H_2SO_4 (0.3 μm)	3 h Nose-only	Rabbit, M (NZW) 4.5 mo old	Lavage cell counts; lavage LDH, PGE_2, $PGF_{2\alpha}$; AM phagocytosis; superoxide production; TNF activity	0 PE	No effects on lavage cell counts or LDH, PGE_2, $PGF_{2\alpha}$ or increase or decrease in TNF and phagocytosis depending on exposure concentration; no change in superoxide.	Antagonism: Phagocytosis, at all combinations; antagonism: Superoxide at 0.1 and 0.3 ppm O_3 and 75 and 125 $\mu g/m^3$ H_2SO_4; synergism: TNF at 125 $\mu g/m^3$ H_2SO_4 and 0.3 and 0.6 ppm O_3.	Schlesinger et al. (1992b)
0.12-0.64	235-1,254 40-1,000	O_3 H_2SO_4	6 h for 7 days (23.5 h/day)	Rat, M (S-D) 250-300 g	Lavageable protein	0 PE	Increase (compared to air control).	Synergism at ≥ 100 $\mu g/m^3$ H_2SO_4 and 0.2 ppm O_3 for 3 days.	Warren and Last (1987)
0.12-0.64	235-1,254 40-1,000	O_3 H_2SO_4	23.5 h/day for 5-9 days	Rat, M (S-D) 250-300 g	Lung tissue protein	0 PE	Increase (compared to air control).	Synergism at 1,000 $\mu g/m^3$ H_2SO_4 and 0.64 ppm O_3, ≥ 100 $\mu g/m^3$ H_2SO_4 and 0.20 ppm O_3.	Warren and Last (1987)
0.12-0.64	235-1,254 40-1,000	O_3 H_2SO_4	23.5 h/day for 7 days	Rat, M (S-D) 250-300 g	Rate of collagen synthesis	0 PE	Increase (compared to air control).	Synergism at ≥ 200 $\mu g/m^3$ H_2SO_4 and 0.64 ppm O_3, ≥ 500 $\mu g/m^3$ H_2SO_4 and 0.2 ppm O_3 (with suggestion at < 500 $\mu g/m^3$ H_2SO_4).	Warren and Last (1987)
0.15	294 300	O_3 H_2SO_4 (0.09 μm)	1 h to H_2SO_4, 2 h rest, then 1 h to O_3 Head-only (acid), whole-body (O_3)	Guinea pig, M (Hartley) 260-325 g	Pulmonary function	0 PE	Acid-induced decrease in DL_{CO} not affected by O_3.	None: O_3 did not alter acid effect.	Chen et al. (1991)

Table 6-26 (cont'd). Toxicological Interactions to Binary Mixtures of Ozone with Acids and Other Pollutants[a]

Concentration[b] ppm	Concentration[b] $\mu g/m^3$	Pollutant	Exposure Duration	Species, Sex (Strain) Age[c]	Endpoints	Time of Endpoint Measurement	Response to Mixture	Interaction	Reference
0.15	294 84	O_3 H_2SO_4 (layered on ZnO)	1 h to H_2SO_4, 2 h rest, then 1 h to O_3 Head-only (acid), whole-body (O_3)	Guinea pig, M (Hartley) 260-325 g	Pulmonary function	0 PE	Greater decrease in DL_{CO}, VC after O_3; no change in alveolar volume, TLC with mixture.	Suggested synergism (greater than additive) for DL_{CO}, but not VC.	Chen et al. (1991)
0.15	294 24	O_3 H_2SO_4 (layered on ZnO)	H_2SO_4 3 h/day for 7 days, O_3 on Day 9 Head-only (acid), whole-body (O_3)	Guinea pig, M (Hartley) 260-325 g	Pulmonary function	0 PE	Decrease in TLC, VC, DL_{CO} enhanced by O_3.	Suggested synergism (greater than additive).	Chen et al. (1991)
0.2	392 1,000	O_3 H_2SO_4	23.5 h/day for 7 days	Rat, M (S-D) 250-300 g	Rate of collagen synthesis	0 PE	Increase.	Possibly synergism: Effect different from O_3 alone.	Warren et al. (1988)
0.2	392 1,000	O_3 H_2SO_4	15 or 30 days	Rat, M (S-D) 250-300 g	Lung protein content	0 PE	Increase only at 15 days.	Suggested synergism.	Last (1991b)
0.64	1,254 1,000	O_3 H_2SO_4	23.5 h/day for 7 days	Rat, M (S-D) 250-300 g	Protein content (lavage)	0 PE	Increase.	None: Effect same as O_3 alone.	Warren et al. (1988)
0.64	1,254 1,000	O_3 H_2SO_4	23.5 h/day for 7 days	Rat, M (S-D) 250-300 g	Proximal acinar lesion volume	0 PE	Increase.	None: Effect same as O_3 alone.	Warren et al. (1988)
0.64	1,254 1,000	O_3 H_2SO_4	23.5 h/day for 7 days	Rat, M (S-D) 225-275 g	Lung protein and free proline content	0 PE	Increase with H_2SO_4 and O_3 only.	Synergism.	Last et al. (1986)
0.8	1,568 1,200	O_3 H_2SO_4 (0.63 μm)	O_3 for 2 h, followed by H_2SO_4 for 1 h	Guinea pig, F, M (Hartley) 1.5-2 mo old	Airway constriction (measured by trapped gas volume)	0 PE	Increase compared to air.	No interaction: Effect same as O_3 alone; H_2SO_4 had no effect.	Silbaugh and Mauderly (1986)
0.2	392 5	O_3 $(NH_4)_2SO_4$	23.5 h/day for 7 days	Rat, M (S-D) 250-300 g	Rate of collagen synthesis	0 PE	Increase.	Synergism: Effect greater than O_3; sulfate had no effect.	Warren et al. (1986)
0.2	392 5	O_3 $(NH_4)_2SO_4$	23.5 h/day for 2 days	Rat, M (S-D) 250-300 g	Lavageable protein	0 PE	Increase.	Synergism: Effect greater than O_3; sulfate had no effect.	Warren et al. (1986)
0.2	392 5	O_3 $(NH_4)_2SO_4$	23.5 h/day for 3 days	Rat, M (S-D) 250-300 g	Lavageable protein	0 PE	Increase.	No interaction: Effect same as O_3 alone.	Warren et al. (1986)

Table 6-26 (cont'd). Toxicological Interactions to Binary Mixtures of Ozone with Acids and Other Pollutants[a]

Concentration[b] ppm	Concentration[b] $\mu g/m^3$	Pollutant	Exposure Duration	Species, Sex (Strain) Age[c]	Endpoints	Time of Endpoint Measurement	Response to Mixture	Interaction	Reference
0.2- 0.64	392- 1,254 5	O_3 $(NH_4)_2SO_4$	23.5 h/day for 7 days	Rat, M (S-D) 250-300 g	Lung DNA content; tissue protein content; lavage LDH, acid phosphatase, N-acetyl-β-D-glucosaminidase	0 PE	No change in DNA, increase in tissue protein, increases in all enzyme levels.	No interaction: Effect on protein and enzymes same as O_3 alone.	Warren et al. (1986)
0.64	1,254 1,000	O_3 $(NH_4)_2SO_4$	23.5 h/day for 7 days	Rat, M (S-D) 225-275 g	Lung protein and free proline content	0 PE	No change.	None.	Last et al. (1986)
0.96	1,882 5,000	O_3 $(NH_4)_2SO_4$	23.5 h/day for 7 days	Rat, M (S-D) 225-275 g	Lung protein content, proline content, apparent collagen synthesis rate, fibroblast numbers in lesions, lesion volume	0 PE	Increase.	Synergism: Effect greater than O_3 alone; H_2SO_4 had no effect (previous study).	Last et al. (1986)
0.3 3.0	588 7,860	O_3 SO_2	5 h/day for 3 days Head-only	Sheep, F Adult (31 kg)	Tracheal mucus velocity	0 and 24 h PE	Decrease in velocity (compared to air control).	Not determinable: no measure of single pollutants performed.	Abraham et al. (1986)
0.15 0.1	294 250	O_3 HNO_3	4 h/day for 4 days Nose-only	Rat, M (F344) 250 g	Lavage cell population; protein (lavage); AM respiratory burst, LTC_4; elastase inhibitory capacity (lavage)	18 h PE	No change in any endpoint (compared to air control).	None.	Nadziejko et al. (1992)
0.6 0.4	1,176 1,000	O_3 HNO_3	4 h Nose-only	Rat, M (F344) 250 g	Lavage cell population; protein (lavage); AM respiratory burst, LTC_4; elastase inhibitory capacity (lavage)	18 h PE	Increased protein, PMN number, elastase inhibiting capacity; no effect on other endpoints (compared to air control).	Less than additive for protein, PMN number, elastase inhibitory capacity; no interaction for other endpoints.	Nadziejko et al. (1992)
0.4	784 380	O_3 HMSA[d] (0.32 μm)	4 h Nose-only	Rat, M (S-D) 7 weeks old	Breathing pattern, fatty acid composition of surfactant, nasal epithelium and parenchymal lesions, lavage protein (24-48 h PE)	Pulmonary function during exposure, others 23 h PE	Rapid breathing, increased protein, decreased fatty acid content, focal lesions with thickened alveolar septa and cellular infiltration in parenchyma.	None: Effect similar to O_3 alone.	Mautz et al. (1991)

Table 6-26 (cont'd). Toxicological Interactions to Binary Mixtures of Ozone with Acids and Other Pollutants[a]

Concentration[b] ppm	Concentration[b] $\mu g/m^3$	Pollutant	Exposure Duration	Species, Sex (Strain) Age[c]	Endpoints	Time of Endpoint Measurement	Response to Mixture	Interaction	Reference
0.2 0.4 0.8 1.0 0.4 0.3 1.1 3.3	392 784 1,568 1,230 784 369 1,353 4,059	O_3 O_3 O_3 HCHO O_3 HCHO HCHO HCHO	22 h/day for 3 days 22 h/day for 3 days	Rat, M (Wistar) 150-190 g	Nasal epithelial cell turnover, histopathology (LM)	Thymidine administered 2 h PE, sacrifice 4 h PE	Increased turnover (but some conditions produced decrease due to change in ventilation).	Synergism for turnover with 0.4 ppm O_3 and 1-3 ppm HCHO, depending on anatomical site (greater than the sum of individual responses to O_3 and HCHO); microscopic lesions similar to O_3 and/or HCHO alone.	Reuzel et al. (1990)
0.6 10 0.6 10	1,176 12,300 1,176 12,300	O_3 HCHO O_3 HCHO	3 h (rest) 3 h (exercise)	Rat, M (S-D) 7 weeks old	Nasal epithelial cell turnover, parenchymal histopathology	2 days PE	Increased focal parenchymal lesions with exercise, but no effect at rest; increased nasal cell turnover at rest or exercise.	Parenchyma: Synergism with exercise, antagonism at rest. Nasal: Synergism.	Mautz et al. (1988)
1.0 1.0	1,960 1,960	O_3 Cigarette smoke O_3 Cigarette smoke	O_3: 0.5 h, then 5 puffs smoke (sequential) O_3: 1.5 h, then 10 puffs smoke (sequential) O_3: chamber, smoke: head-only	Guinea pig, F (Hartley) 350-400 g	Airway responsivity (to metacholine challenge), tracheal vascular permeability, 0-24 h PE	0, 5, or 24 h PE	Increased responsivity and permeability immediately PE at both doses (magnitude, but not duration of effect).	Suggested synergism: No effect of O_3 or smoke alone at low dose; high O_3 increased responsivity and permeability; high smoke increased responsivity.	Nishikawa et al. (1992)
0.8	1,568 2,000-50,000	O_3 Silica (instilled)	Silica instilled on Day 1 followed by O_3 for 6 h/day, 5 days/week for 37 days beginning Day 4	Rat, M (S-D) 17 weeks old	Pulmonary fibrosis	24 h PE (last O_3)	No change in lung DNA, protein, or hydroxyproline content; increase in ratio of hydroxyproline to DNA, protein, or wet weight (compared to air control) at 50,000 μg.	None: No biological significance.	Shiotsuka et al. (1986)

Table 6-26 (cont'd). Toxicological Interactions to Binary Mixtures of Ozone with Acids and Other Pollutants[a]

Concentration[b] ppm	Concentration[b] $\mu g/m^3$	Pollutant	Exposure Duration	Species, Sex (Strain) Age[c]	Endpoints	Time of Endpoint Measurement	Response to Mixture	Interaction	Reference
0.8 1.5	1,568 2,940 10,000	O_3 O_3 Carbon black	4 h	Mouse, F (Swiss) 20-23 g	Cell counts in lavage; AM phagocytosis	20 h PE	Increase PMN counts compared to O_3 alone; greater depression of phagocytosis than O_3 alone.	At 0.8 ppm: No intereaction; at 1.5 ppm: Suggested synergism.	Jakab and Hemenway (1994)

[a]See Appendix A for abbreviations and acronyms.
[b]Grouped by pollutant mixture.
[c]Age or body weight at start of exposure.
[d]HMSA = hydroxymethanesulfonate.

6.4.2.1 Nitrogen Dioxide as Copollutant

The most commonly studied copollutant in binary mixtures with O_3 is NO_2. Studies discussed in the previous O_3 criteria document indicated that, although interaction may occur between these two pollutants, in general, O_3 often masked the effects of the NO_2 or accounted for most of the response. This is because, on a mole-to-mole basis, O_3 is considerably more toxic than NO_2, and the relative contribution of O_3 and NO_2 to pulmonary injury is driven by the exposure ratio of the two pollutants. Commonly studied endpoints for assessing effects of these mixtures were lung morphology, biochemistry, and resistance to bacterial infection.

To put the exposure concentrations of NO_2 into some perspective, short-term, 24-h averages are generally ≤ 0.17 ppm, and 1-h averages are generally ≤ 0.4 ppm in major metropolitan areas. However, hourly averages in most regions often exceed 0.2 ppm at least once during the year (Schlesinger, 1992).

An earlier study noted that the morphological response of the rat lung alveolar epithelium following 60 days of exposure to O_3/NO_2 mixtures (0.25 ppm O_3 + 2.5 ppm NO_2, or 0.9 ppm O_3 + 0.9 ppm NO_2) was due to the O_3 (Freeman et al., 1974). However, the duration of exposure may affect the contributory role of the copollutant. Thus, for example, Terada et al. (1986) exposed rats to O_3 alone, or to a mixture of 0.1 ppm O_3 + 0.3 ppm NO_2, with O_3 administered 8 h/day and NO_2 administered 24 h/day for up to 18 mo. Following 1 mo of exposure, observed lung lesions in the group exposed to the mixture were similar in severity to those noted with exposure to O_3 alone, but as the duration of exposure increased, the morphological changes in interstitial tissue appeared more marked in those animals exposed to the mixture. Edema of pulmonary connective tissue was more pronounced and alveolar Type 2 cells became swollen. Although this study was not quantitative, qualitative observations led the authors to conclude that the lesions were not due to O_3 alone but that NO_2 played some, albeit undefined, contributory role.

The effect of exposure duration on interaction was also noted in studies of Schlesinger et al. (1990, 1991). Rabbits exposed to 0.3 ppm O_3 + 3.0 ppm NO_2 for 2 h showed synergistic increases in certain BAL eicosanoids obtained immediately after exposure, whereas animals exposed to the same mixture for 2 h/day for 14 days showed no interaction for the same parameters.

A number of studies examined other biochemical responses to O_3/NO_2 mixtures (e.g., sulfhydryl metabolism and the activity of certain enzymes). Some of the studies discussed in the previous O_3 criteria document were found to involve synergism (e.g., Mustafa et al., 1984). More recent studies of lung biochemistry also suggest that O_3 and NO_2 interact synergistically. Ichinose and Sagai (1989) exposed rats and guinea pigs to 0.4 ppm O_3, 0.4 ppm NO_2, or a mixture of the two pollutants continuously for 2 weeks. No change in lung peroxide production was observed in rats, but the mixture synergistically increased peroxide levels in guinea pigs. The guinea pigs showed no change in lung antioxidant content following any exposure, whereas the mixture synergistically increased antioxidant levels in rat lung. The conclusion of a significant interaction was based on relative changes from air controls following exposure to the mixture, compared to changes following exposure to each pollutant alone, using the t-test. Synergism was defined as a change greater than the sum of the responses to individual pollutants; no specific test for interaction was performed.

Ichinose and Sagai (1989) also noted that levels of antioxidant enzymes in rat or guinea pig lungs were variously affected by exposure to the above mixture. For example, GST was decreased in both species exposed only to O_3, but the mixture produced a reduction

of this enzyme in guinea pigs and no change in rats. Thus, the occurrence of interaction was dependent on endpoint as well as species. This latter finding likely reflected interspecies differences in biochemical defenses against oxidant pollutants, given the results of a study by Sagai et al. (1987) with four animal species. This study suggested that observed species differences in lipid peroxide formation following exposure were related to the relative content of antioxidants and the specific composition of phospholipids and their fatty acids. The guinea pig was the most sensitive animal, and the hamster was the most resistant.

The effects of exposures to O_3/NO_2 mixtures on lung lipid peroxides and antioxidant activity have been examined in a number of other studies (Ichinose et al., 1988; Lee et al., 1990; Sagai and Ichinose, 1991), and the results generally confirm that noted above (i.e., such mixtures tend to produce synergistic interaction). However, there is also some evidence for antagonism. Takahashi and Miura (1989) examined effects on the pulmonary xenobiotic system of rats exposed for 1 or 2 mo to a mixture of 0.2 ppm O_3 + 4.0 ppm NO_2, as well as to each pollutant alone. Ozone induced an increase in lung cytochrome P-450 content, but the activity of these enzymes was reduced by the addition of NO_2 to the exposure atmosphere; that is, the mixture resulted in levels intermediate between those found with O_3 or NO_2 alone. However, the reduction in enzyme activity induced by NO_2 was restricted to those enzymes that had been increased by exposure to O_3 alone. The authors suggested that antagonism was due to the production of undefined secondary reaction products in the exposure atmosphere. A similar explanation was proposed to explain observed synergism of lung antioxidant activity in another study (Lee et al., 1990). Thus, the response to any secondary product likely depends on the endpoint examined, assuming that the same reaction products were formed in these two studies.

The role of exposure parameters in producing an interaction between simultaneously inhaled O_3 and NO_2 was examined by Gelzleichter et al. (1992a). Rats were exposed to various combinations of O_3 and NO_2 for various durations (6, 8, 12, and 24 h), such that the C $\times$ T products were identical for each of four exposure sets. As indicated in Table 6-24, as the exposure duration increased, the exposure concentration of each component of the mixture decreased. Lavaged protein levels and recovered cells were the endpoints. For each exposure combination, the additive response was predicted from the results of exposure to each pollutant alone, and then synergism was indicated when there was deviation from additivity. Responses to exposure to either O_3 or NO_2 alone for 6, 8, or 12 h showed that the product of C $\times$ T was a constant for the observed biological effects. However, less severe changes occurred when delivery was at the lowest dose rate (i.e., when the lowest concentration of each pollutant was delivered over the 24-h exposure duration). Exposure at higher dose rates (i.e., 6 to 12 h) increased the magnitude of the response. Thus, the degree of response to each pollutant alone was not a constant function of C $\times$ T throughout the entire range of dose rates, but was concentration driven, and was not identical at the highest and lowest rates. Responses following exposure to the mixture did not follow C $\times$ T, even over the range of dose rates in which C $\times$ T was constant following exposure to the pollutants individually. Thus, interaction, in this case, synergism, appeared to be concentration dependent, in that the response was disproportionately greater at the higher concentrations (higher dose rates) of the constituent pollutants in the mixture. The response following exposure to the mixtures appeared to be a function of peak concentration, rather than of cumulative dose. More recently, Rajini et al. (1993) noted that analysis of all kinetics following similar exposure to mixtures did not reflect a C $\times$ T relationship.

All of the studies described above involved simultaneous exposure to O_3 and NO_2. However, ambient exposure to these pollutants has temporal patterns, and exposure to one agent may alter the response to another, subsequently inhaled agent. The realism of these studies is somewhat dependent on their relationship to actual temporal patterns of pollutants in ambient air (i.e., whether one material is the precursor of the other, as is the case for O_3 and NO_2). As described in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986), Fukase et al. (1978) exposed mice for 7 days to 3 to 15 ppm NO_2 for 3 h/day, followed by 1 ppm O_3 for 3 h/day, and noted an additive effect on the level of lung GSH.

Yokoyama et al. (1980) exposed rats to 5 ppm NO_2 or 1 ppm O_3 for 3 h/day, or to NO_2 for 3 h followed by O_3 for 3 h/day, for various total durations up to 30 days, and assessed lung mechanics in postmortem lungs, lung histology, and enzyme activity in subcellular fractions of lung tissue. The activity of phospholipase A2 in the mitochondrial fraction was increased in those animals exposed to O_3 only or to O_3 after NO_2, and the response in the latter was significantly greater than that in the former. A decrease in activity of lysolecithin acyltransferase in the supernatent fraction was found only in those animals exposed to both NO_2 and O_3. Pulmonary mechanics showed a change in pulmonary resistance (as a function of elastic recoil pressure) in the O_3- and NO_2/O_3-exposed animals. Histologically, the lungs of the animals exposed to both NO_2 and O_3 appeared similar to those exposed to O_3 alone; however, a slight degree of epithelial necrosis in medium bronchi, not found with either NO_2 or O_3 alone, was seen in the animals exposed to both pollutants. In addition, damage at the bronchoalveolar junction appeared to be somewhat more marked in animals exposed to both gases than in those exposed to O_3 alone. This study suggested that sequential exposures produced responses that, in most cases, did not differ greatly from those due to O_3 alone.

Aside from sequential exposures, simulation of ambient exposure scenarios involving NO_2 and O_3 has been performed by examining the effects of a continuous baseline exposure to one concentration of both pollutants, with superimposed short-term peaks to a higher level of one or both gases. The endpoint generally examined in this regard has been bacterial resistance. Studies reported in the previous criteria document (e.g., Ehrlich et al., 1979; Ehrlich, 1983) in which mice were exposed to O_3 under various scenarios of baseline concentrations of NO_2 on which were superimposed daily peak exposures to NO_2 or a combination of NO_2 and O_3 suggested that exposure with peaks can enhance response to pollutant mixtures, and that the sequence of peak exposures was important in producing reduced resistance to infection that was different from that due to exposure to the baseline concentration only.

As a comparison, toxicologic interactions for infectivity involving simultaneous exposure to NO_2 and O_3 discussed in the previous O_3 criteria document were found generally to be additive following acute exposures, with each pollutant contributing to the observed response when its concentration reached the threshold at which the gas would have affected bacterial resistance when administered alone (Goldstein et al., 1974). If the exposure level of either NO_2 or O_3 was below this threshold, then the response was due solely to the constituent inhaled at the more toxic concentration (Ehrlich et al., 1977).

More recently, Graham et al. (1987) examined resistance to respiratory infection (as measured by bacterial-induced mortality) in mice continuously exposed (for 15 days, 24 h/day) to baseline levels of an NO_2/O_3 mixture with two daily, 1-h peaks of the mixture at very high, high, intermediate, and low exposure concentrations (see Table 6-25 for

concentrations). Animals were also exposed to the same baseline levels of either NO_2 or O_3 onto which were superimposed two daily, 1-h peaks of the same single gas in concentrations as above. At the low concentration, only NO_2 increased mortality. At the intermediate exposure level, the mixture was synergistic; NO_2 alone increased mortality and O_3 had no effect. At the high exposure level, the combined exposure was again synergistic; exposure to each gas separately increased mortality. A similar effect was seen at the very high level, although the combined exposure just missed statistical significance for synergism. These results are consistent with those of the earlier studies reported in the previous O_3 criteria document and support the conclusion that response depends on the specific exposure pattern. The results of Graham et al. (1987) are also consistent with those from the earlier studies with simultaneous exposures.

The relationship between exposure and response is very complex and seems to depend on exposure duration, the ratio of O_3 and NO_2 concentrations, and other factors that may include the production of secondary reaction products within the exposure atmosphere. This complexity was highlighted by the study of Gelzleichter et al. (1992b), who examined effects of combined or sequential exposures of rats to mixtures of O_3 and NO_2 at various concentrations ranging from 0.2 to 0.8 ppm O_3 and 3.6 to 14.4 ppm NO_2. Sequential exposures consisted of 6 h of O_3 at night, followed by 6 h of NO_2 during the day, or vice versa; concurrent exposures were for 6 h/day for 3 days. Various endpoints were examined, and it was noted that sequential and concurrent exposures did not result in the same response. Thus, lavage protein levels were increased additively with sequential exposure (in any pollutant order) but were found to be greater than additive with concurrent exposure. An increase in the number of lavaged epithelial cells was additive for the O_3 night/NO_2 day sequence, antagonistic for the NO_2 night/O_3 day sequence, and additive for concurrent exposure. An increase in the number of lavaged PMNs was additive for both sequential conditions and was synergistic for concurrent exposure. It was concluded that production of synergism depended on the concentration of each pollutant within the mixture, and additivity would result for any endpoint when the concentration of each component of the mixture fell below a certain threshold level. However, these threshold concentrations were endpoint specific, with some endpoints being more sensitive than others; it was speculated that the least sensitive assays were based on changes that were reversible, whereas the most sensitive ones were irreversible. The authors also noted that the extent of chemical reaction within the O_3/NO_2 mixture atmosphere was related to the extent of toxicological interaction, suggesting that interaction was due to the production of some secondary reaction product, which, in this case, was suggested to be nitrogen pentoxide. This particular chemical also had been suggested in earlier studies to be responsible for interactions following exposure to NO_2/O_3 atmospheres (e.g., Diggle and Gage, 1955).

The concentrations at which synergism occurred in the study of Gelzleichter et al. (1992b) discussed above ($\geq$0.4 ppm O_3 and $\geq$7.2 ppm NO_2) were higher than those that generally are found in ambient air. However, the threshold concentration for interaction was dependent on exposure dose rate, with higher rates leading to lower threshold concentrations for synergism.

Although interaction is clearly modulated by environmental exposure factors, such as concentration, duration of exposure, or specific exposure regime, host factors also may play a role. Mautz et al. (1988) examined the effect of exercise on rats exposed to mixtures of O_3 and NO_2. Exercise modified the toxic interactions of combined pollutants, resulting in synergistic interaction occurring at lower exposure concentrations of the constituent pollutants

than with exposure at rest. Thus, a similar magnitude of response, an increase in the extent of focal lesions in lung parenchyma, was noted 2 days following a 4-h exposure to 0.6 ppm O_3 + 2.5 ppm NO_2 at rest or with a shorter (3-h) exposure to lower concentrations, 0.35 ppm O_3 + 0.6 ppm NO_2, with exercise. In both cases, the response was different from that due to either pollutant given alone. Furthermore, a greater response was noted with a 3-h exposure to 0.6 ppm O_3 + 2.5 ppm NO_2 with exercise than to the same mixture for the same exposure duration at rest. Thus, exercise also increased the response at similar concentrations compared to rest. The effect of exercise was ascribed to an increase in delivered dose or dose rate, due to increased $\dot{V}_E$. The ability of exercise to enhance response to a pollutant mixture also was noted by Bhalla et al. (1987).

The study of Mautz et al. (1988) above also provided further evidence suggesting that chemical reactions within the exposure atmosphere may play some role in toxicologic interaction. In this case, nitric acid (HNO_3) vapor was noted at concentrations ranging from 0.02 to 0.73 ppm, depending on the concentrations of the primary constituents. As discussed below, acids have been found to interact with O_3. This study also found interaction to occur at a concentration of one of the components, NO_2, that had no effect when administered alone. Although this appears to contrast with the conclusions of Graham et al. (1987) above, the endpoints in these two studies were quite different.

Another aspect of pollutant interaction involves the ability of O_3/NO_2 mixtures to affect the course of other lung changes (e.g., malignant tumor colonization). Richters (1988) exposed mice to a mixture of 0.15 ppm O_3 + 0.35 ppm NO_2 for 7 h/day, 5 days/week for 12 weeks, following which the mice were injected (iv) with viable melanoma cells. The mice were sacrificed 3 weeks later, and the lungs were examined for melanoma colonies. Although exposure to O_3 alone produced no change in the percentage of animals with colonies or in the average number of colonies per lung (compared to air control), exposure to the mixture produced an increase in the latter, suggesting to the authors that the mixture faciliated cancer cell colonization. However, the exact role played by O_3 in the mixture is not clear because a previous study had indicated that NO_2 alone facilitates blood-borne cancer cell spread to the lungs (Richters and Kuraitis, 1981). Furthermore, the experimental model used is not generally accepted as representing metastatic mechanisms.

Ichinose and Sagai (1992) also examined the ability of an O_3/NO_2 mixture to promote primary lung tumor development. Rats were injected (ip) with BHPN and then were exposed for 13 mo to a mixture of 0.05 ppm O_3 + 0.4 ppm NO_2, O_3 alone, or to clean air (chamber control). Although the NO_2 exposure was continuous, the O_3 exposure was intermittent, with the concentration altered between 0 and 0.1 ppm following a sine curve from 9 to 19 h of each day (resulting in a daily mean concentration of 0.05 ppm). One other group of rats served as a room control, maintained in a clean room for 24 mo following injection of BHPN. After an 11-mo recovery period, all animals were autopsied. Compared to clean-air-exposed animals, lung tumor incidence was increased in mice exposed to the mixture; O_3 alone did not increase the tumor incidence. However, the authors noted that tumor incidence in the room control group was not different from that in the group exposed to the mixture, and suggested that the clean air (chamber control) group should be used as a control in interpreting the data from the pollutant-exposed animals. The enhanced incidence in mixture-exposed animals was suggested to be due to synergistic increases in lipid peroxidation, which was noted in other studies (see Table 6-25). A complication in interpreting this study is that a previous study (Sagai and Ichinose, 1991) had suggested tumor development in animals exposed to an O_3 and NO_2 mixture without BHPN, although

this latter study involved a longer exposure duration and somewhat higher pollutant concentrations.

6.4.2.2 Acidic Compounds as Copollutants

Binary mixtures containing acids comprise another type of commonly examined exposure atmosphere. Most of these mixtures included acidic sulfate aerosols as the copollutant. Peak (1-h) ambient levels of sulfuric acid (H_2SO_4) are estimated at 75 $\mu g/m^3$, with longer (12-h) averages about one-third of this concentration (Spengler et al., 1989).

Earlier studies that employed simultaneous single, repeated, or continuous exposures of various animal species to mixtures of acid sulfates and O_3 found responses for several endpoints, including tracheobronchial mucociliary clearance, alveolar clearance, pulmonary mechanics, and lung morphology, to be due solely to O_3 (U.S. Environmental Protection Agency, 1986; Cavender et al., 1977; Moore and Schwartz, 1981; Phalen et al., 1980; Juhos et al., 1978). However, synergism was noted for bacterial infectivity in mice (Grose et al., 1982), for response to antigen in mice (Osebold et al., 1980), and for effects on lung protein content and the rate of collagen synthesis in rats (Last et al., 1983, 1984a; Last and Cross, 1978). More recent studies, performed since publication of the previous O_3 criteria document, support the earlier finding of synergism between O_3 and acid sulfates on lung biochemistry, and provide possible explanations for underlying mechanisms.

Last et al. (1986) exposed rats for 7 days to O_3 alone (at 0.96 ppm) and to mixtures of O_3 with one of three aerosols, sodium chloride, sodium sulfate, or ammonium sulfate [$(NH_4)_2SO_4$] (all at 5 mg/m^3); only the $(NH_4)_2SO_4$ was acidic. Lung protein content, proline content, collagen synthesis rate, fibroblast numbers in parechymal lesions, and the volume of parenchymal lesions were examined following exposure. Mixtures of O_3 with sodium chloride or sodium sulfate produced changes that did not differ from those found with O_3 alone. On the other hand, mixtures of $(NH_4)_2SO_4$ with O_3 resulted in increases in all of the measured parameters, and the increases were greater in magnitude than those due to O_3 alone; synergism was concluded, although there has been some question concerning the statistical approach used (Last, 1991a). These results suggested that acidity was necessary for synergism of the aerosols with O_3. This conclusion was further supported by demonstrating that significant interaction of O_3 with H_2SO_4, which is much more acidic than $(NH_4)_2SO_4$, occurred at lower concentrations than was noted for mixtures of O_3 and $(NH_4)_2SO_4$ (Warren and Last, 1987); interaction was suggested with H_2SO_4 concentrations as low as 40 $\mu g/m^3$ (with 0.2 ppm O_3) for some lung biochemical endpoints. The studies above did not use any specific statistical test for interaction, and conclusions of interaction were based on findings of significant differences from responses following exposure to O_3 alone and the absence of detectable responses following exposure to H_2SO_4 aerosols at the same and higher concentrations.

Warren et al. (1986) found synergistic interaction with the above endpoints following 7 days of exposure to 0.2 ppm O_3 + 5 mg/m^3 $(NH_4)_2SO_4$. However, exposure for only 3 days produced responses that were not different from those noted with O_3 alone. This seems to indicate that the duration of exposure is a factor affecting the occurrence of any interaction. However, exposure duration may also affect the type of interaction. In a study by Schlesinger et al. (1992a) in which rabbits were exposed to a mixture of 0.1 ppm O_3 + 125 $\mu g/m^3$ H_2SO_4 for 2 h/day, 5 days/week, a synergistic increase in bronchial epithelial secretory cell number was noted after 4 mo of exposure, whereas antagonism was noted following 8 mo of continued exposure.

The mechanism underlying interaction between acid sulfates and O_3 is not known. Last et al. (1986) noted that similar sites of deposition for O_3 and acid aerosols favored synergism. A synergistic response of biochemical indices in rat lung with exposure to 1,000 $\mu g/m^3$ H_2SO_4 + 0.6 ppm O_3 was found when the acid droplet diameter was 0.5 μm, whereas no increase compared to the O_3-only response was noted when the droplet diameter was 0.02 μm. Apparently, the larger particles that deposited to a greater extent within the bronchoalveolar junction, the major target site for O_3, were most interactive.

Observed synergism between O_3 and acid sulfates in rats also was suggested to be due to a shift in the local microenvironmental pH of the lung following deposition of acid, enhancing effects of O_3 by producing a change in the reactivity or residence time of reactants, such as free radicals, involved in O_3-induced tissue injury (Last et al., 1984a). If this were the only explanation, then the effects of O_3 should be enhanced consistently by the presence of acid in an exposure atmosphere. However, in the study of Schlesinger et al. (1992b) in which rabbits were exposed for 3 h to combinations of O_3 at 0.1, 0.3, and 0.6 ppm + H_2SO_4 (0.3 μm) at 50, 75, and 125 $\mu g/m^3$, antagonism was noted in the evaluation of stimulated production of superoxide anion by AMs harvested by lavage immediately after exposure to 0.1 or 0.3 ppm O_3 in combination with 75 or 125 $\mu g/m^3$ H_2SO_4 and also for AM phagocytic activity at all of the O_3/acid combinations. Mixtures of O_3 (0.6 ppm) and another acid, HNO_3 vapor (1,000 $\mu g/m^3$), also produced antagonism for certain aspects of the function of AMs harvested from acutely exposed rats (Nadziejko et al., 1992). Although the deposition sites of both acid and O_3 should be comparable in these two studies, perhaps the particular cellular endpoints examined are subject to this type of interaction.

Last (1989) observed an apparent all-or-none response in rats exposed to the acid sulfate/O_3 mixtures. That is, there was no concentration-response relationship between the concentration of acid in the mixture and the extent of change in various endpoints, compared to effects observed with O_3 alone. In the study of Schlesinger et al. (1992b), a similar phenomenon was noted, but, in this case, the concentration of O_3 in the mixture did not always influence the response compared to that seen with acid alone. Thus, exposure-concentration-response relationships noted with individual pollutants may not necessarily hold following exposure to their mixtures. This is consistent with the results of Gelzleichter et al. (1992a) for mixtures of O_3 and NO_2.

The above studies involved simultaneous exposures to O_3 and acidic pollutants, but some studies involving sequential exposures to O_3 and acid sulfate aerosols were described in the previous O_3 criteria document. For example, Gardner et al. (1977) found an additive increase in infectivity when mice were exposed to 0.1 ppm O_3 for 3 h prior to a 2-h exposure to 900 $\mu g/m^3$ H_2SO_4, whereas no difference from air control was noted when the acid was administered prior to O_3. Grose et al. (1980) noted a reduction in ciliary activity in isolated tracheal sections obtained from hamsters exposed to 0.1 ppm O_3 for 3 h, followed by exposure to 1,090 $\mu g/m^3$ H_2SO_4 for 2 h, that was less in magnitude than that found with exposure to acid alone; O_3 alone had no effect.

Silbaugh and Mauderly (1986) examined the ability of O_3 to increase susceptibility to a subsequent exposure to H_2SO_4 in terms of producing airway constriction. Guinea pigs were exposed to 0.8 ppm O_3 for 2 h followed by H_2SO_4 (12 mg/m^3 for 1 h). An increased volume of trapped gas in the lungs (the metric of constriction) was seen with both O_3 alone and with the mixture, but the response to the latter did not differ from that due

to the former, and acid alone had no effect. Thus, in this case, preexposure to O_3 did not affect response to a subsequent exposure to acid.

Chen et al. (1991) examined the reverse exposure scenario, whether exposure to H_2SO_4 affected subsequent response to O_3. Guinea pigs were exposed to H_2SO_4 or ultrafine zinc oxide (ZnO) particles coated with H_2SO_4. A 1-h exposure to 0.15 ppm O_3 following a 1-h exposure to acid (300 $\mu g/m^3$, 0.09 μm) did not alter the response seen with acid alone, a decline in DL_{CO}. However, when single (1-h) or multiple (3-h/day, 7-day) exposures to acid-coated ZnO (24 or 84 $\mu g/m^3$ equivalent H_2SO_4) were followed by a 1-h exposure to 0.15 ppm O_3, the effect on DL_{CO} appeared to be greater than additive, although no specific statistical test for interaction was performed. This study suggested that prior exposure to acid increased the susceptibility of the guinea pig to subsequent exposure to O_3, but it also showed that the manner in which the acid was delivered affected whether or not any interaction occurred. It is likely that the number of particles was greater in the ZnO-H_2SO_4 aerosol than in the H_2SO_4 aerosol, and the interaction may reflect this greater particle number.

6.4.2.3 Other Copollutants

Although the bulk of the database for binary mixtures of O_3 involves NO_2 or acids, a few studies examined responses to combinations of O_3 with other pollutants.

Reuzel et al. (1990) exposed rats to mixtures of O_3 (0.2, 0.4, or 0.8 ppm) + HCHO (0.3 to 3.0 ppm). Although exposure to the mixtures did not alter the nature or extent of histological lesions, (cilia loss and epithelial hyperplasia) compared to exposure to each pollutant alone, a site-specific synergistic increase in turnover of nasal epithelial cells was found with all concentrations of HCHO together with 0.4 ppm O_3. A lack of such response with 0.8 ppm O_3 was ascribed to an O_3-induced alteration in breathing pattern, which reduced the delivered dose. It was, however, noted that interaction occurred only when one constituent of the mixture was administered at cytotoxic concentrations, an exposure scenario that rarely occurs in ambient air. In any case, the authors concluded that because cell proliferation likely plays a role in carcinogenesis, and that if mixtures potentiate cell proliferation, then exposure to pollutant mixtures may increase cancer risk.

Mautz et al. (1988) exposed rats for 3 h, both at rest and with exercise, to a mixture of 0.6 ppm O_3 + 10 ppm HCHO. A synergistic increase in nasal epithelial cell turnover followed exposure with exercise, whereas exposure at rest resulted in no difference from that seen with HCHO alone. Likewise, exposure to the mixture with exercise resulted in an increase in the number of focal lesions in lung parenchyma compared to either O_3 or HCHO alone, but exposure at rest resulted in a lower incidence of lesions than seen with O_3 alone. This latter observation was ascribed to an effect of HCHO on breathing pattern, producing a change in inhaled dose of O_3 that did not occur with exercise.

Nishikawa et al. (1992) examined the effect of sequential exposure to cigarette smoke and O_3 in altering airway responsivity to inhaled bronchoconstrictor challenge and tracheal vascular permeability in guinea pigs. Animals were exposed to 1 ppm O_3 for 0.5 h, followed by 5 puffs of cigarette smoke, or to 1 ppm O_3 for 1.5 h, followed by 10 puffs. Exposure to O_3 and five puffs increased responsivity and vascular permeability immediately after exposure, whereas no effect on either endpoint was noted with either pollutant given alone. Exposure to O_3 and 10 puffs also increased responsivity and permeability, but to the same extent as did the lower concentration mixture or exposure to O_3 alone, whereas exposure to 10 puffs of smoke only increased responsivity. Thus, sequential exposure to

O_3 and cigarette smoke enhanced the magnitude of response compared to either pollutant alone, but the duration of response was not altered.

The potential role of O_3 in enhancing fibrotic lung disease by interaction with silica was examined by Shiotsuka et al. (1986), who exposed rats with developing silica-induced fibrosis to O_3 at 0.8 ppm for 6 h/day, 5 day/week for 37 exposure days. Silica had been instilled (2, 12, or 50 mg) on Day 1 of the study and exposure to O_3 began on Day 3 or 4 postinstillation. There was found to be no interaction between silica and O_3 in development of fibrosis, as assessed biochemically (lung content of hydroxyproline) or histopathologically. Although an increase was found in the ratio of hydroxyproline to total protein in the group exposed to the mixture and instilled with the highest amount of silica, this was not considered by the authors to be biologically significant.

6.4.3 Complex (Multicomponent) Mixtures Containing Ozone

Ambient pollution in most areas is a complex mix of more than two chemicals, and a number of studies have examined the effects of exposure to multicomponent atmospheres containing O_3. Some of these attempted to simulate photochemical reaction products occurring under actual atmospheric conditions. However, the results of these studies are often difficult to interpret due to chemical interactions between the components, as well as the resultant production of variable amounts of numerous secondary reaction products, and a lack of precise control over the ultimate composition of the exposure environment. In addition, the role of O_3 in the observed biological responses is often obscure.

One type of experimental multicomponent atmosphere that has been examined is ultraviolet-irradiated and nonirradiated automobile exhaust mixtures. Irradiation leads to the formation of photochemical reaction products that are biologically more active than those in nonirradiated mixtures. Such mixtures are characterized by total oxidant concentrations (expressed as O_3) in the range of 0.2 to 1.0 ppm. Although the effects described following exposure were not necessarily uniquely characteristic of O_3, and, although O_3 could have been responsible for some, or even most of them, in most cases, the biological effects have been difficult to associate with any one particular component. Effects of exhaust mixtures on different species have been discussed in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986). Pulmonary function changes were demonstrated in guinea pigs after short-term exposures to irradiated exhaust and in dogs after long-term exposure to both irradiated and nonirradiated exhaust mixtures.

Additional studies of complex mixtures have been performed since publication of the previous O_3 criteria document. Kleinman et al. (1985) exposed rats (Sprague-Dawley, male, 7 weeks old, nose-only) for 4 h to atmospheres designed to represent photochemical pollution and consisting of 0.6 ppm (1,180 $\mu g/m^3$) O_3 + 2.5 ppm (4,700 $\mu g/m^3$) NO_2 + 5.0 ppm (13,100 $\mu g/m^3$) sulfur dioxide (SO_2) + particles. The particulate phase consisted of 1,000 $\mu g/m^3$ of either H_2SO_4 or $(NH_4)_2SO_4$ laced with iron sulfate [$Fe_2(SO_4)_3$] and manganese sulfate ($MnSO_4$). The metallic salts act as catalysts for the conversion of sulfur IV into sulfur VI and for the incorporation of gases into the aerosol droplets. The respiratory region was examined for morphological effects. A confounding factor in these studies was the production of HNO_3 vapor in atmospheres that contained O_3 and NO_2, a phenomenon discussed previously, and nitrate in those that contained O_3 and $(NH_4)_2SO_4$, but not NO_2. Nevertheless, a significant enhancement of tissue damage was noted with exposure

to atmospheres containing H_2SO_4 or secondarily produced HNO_3 compared to those containing $(NH_4)_2SO_4$, a less acidic compound. In addition, there was some suggestion that the stronger acidic atmospheres resulted in a greater area of the parenchyma becoming involved in lesions, which were characterized by a thickening of alveolar walls, cellular infiltration in the interstitium, and an increase in free cells within alveolar spaces. An increased rate of nasal epithelial cell turnover was noted following exposure to atmospheres containing particulate acids compared with exposure to either O_3 alone or to a mixture of O_3 + NO_2. Furthermore, exercise seemed to potentiate the nasal and parenchymal responses to the complex mixtures containing strong acids (Kleinman et al., 1989), a finding similar to that with the previously discussed mixtures of O_3 and NO_2 or O_3 and HCHO.

Bhalla et al. (1987) examined the effects of a seven-component atmosphere (similar to that above) on epithelial permeability of rat lungs (Sprague-Dawley, male, 47 to 52 days old). The animals were exposed for 2 h (chambers, relative humidity [RH] = 85%) to the following: O_3 (0.6 ppm) + NO_2 (2.5 ppm) + SO_2 (5.0 ppm) + ferric oxide (Fe_2O_3) (241 $\mu g/m^3$) + $(NH_4)_2SO_4$ (308 to 364 $\mu g/m^3$) + $Fe_2(SO_4)_3$ (411 to 571 $\mu g/m^3$) + $MnSO_4$ (7 to 9 $\mu g/m^3$). The response to this mixture was compared to that following exposure to O_3 (0.6 ppm) + NO_2 (2.5 ppm), O_3 alone (0.6 or 0.8 ppm), or NO_2 alone (6 or 12 ppm). As above, the complex mixture was found to result in production of HNO_3, in this case at measured concentrations of 1,179 to 2,558 $\mu g/m^3$ (0.46 to 1.02 ppm); the O_3 + NO_2 atmosphere also resulted in some HNO_3 vapor formation. Epithelial permeability was found to increase immediately following exposure to O_3, O_3 + NO_2, or to the complex mixture. Although the magnitude of this change was similar following exposure to O_3 alone or in combination with other pollutants, there was increased persistence of effect after exposure to either the binary or complex mixture.

Prasad et al. (1988) used a similar multicomponent atmosphere and examined effects on AM surface receptors. Rats (Sprague-Dawley, male, 200 g) were exposed for 4 h/day, for 7 or 21 days to a mixture of O_3 (0.3 ppm) + NO_2 (1.2 ppm) + SO_2 (2.5 ppm) + $(NH_4)_2SO_4$ (270 $\mu g/m^3$) + $Fe_2(SO_4)_3$ (220 $\mu g/m^3$) + $MnSO_4$ (4 $\mu g/m^3$) + Fe_2O_3 (150 $\mu g/m^3$), or to O_3 alone. Both the mixture and O_3 alone resulted in a decrease in Fc receptor activity beginning immediately after the last exposure. Exposure to the complex atmosphere for 7 days resulted in a response similar to that seen with O_3 alone, but continued exposure to this mixture for up to 21 days resulted in an even greater reduction in receptor function compared to O_3 alone. However, as with most studies of complex mixtures, although the response to the mixture was different from that found with O_3, the role of other constituents was not clear. Phagocytic function of AMs was also examined following exposure to the mixture, but there were no O_3-only controls for comparison.

Mautz et al. (1985a) examined the effects of a complex mixture on pulmonary mechanics in exercising dogs. Exposures (nose-only) were for 200 min to a mixture of O_3 (0.45 to 0.7 ppm) + SO_2 (4.8 to 5.2 ppm) + H_2SO_4 (800 to 1,200 $\mu g/m^3$, 0.2 μm) + catalytic salts of $Fe_2(SO_4)_3$ and $MnSO_4$. A greater increase in resistance and decrease in compliance was found with the complex atmosphere than with O_3 alone, but the effect was ascribed to the presence of H_2SO_4. Although synergism was implied, it could not be concluded definitively because the mixture was not tested without O_3.

Mautz et al. (1991) further examined the ability of components of acidic fogs to alter the response to O_3. Rats (Sprague-Dawley, male, 7 weeks old, n = 12/group) were exposed for 4 h (nose-only; temperature = 22 to 23 °C, RH = 82 to 83%) to 0.4 ppm

O_3 or to a mixture of 0.4 ppm O_3 + 670 $\mu g/m^3$ HNO_3 vapor + 610 $\mu g/m^3$ H_2SO_4 particles (0.32 μm). Exposure to either O_3 or the mixture resulted in comparable changes: development of a rapid, shallow breathing pattern; a decrease in fatty acid composition of pulmonary surfactant; and focal parenchymal lesions with thickened alveolar septa and cellular infiltration. The lack of any modulation of the O_3-induced effects by acids prompted the authors to raise the question of the sensitivity of rats to inhaled acids. Although responses to any pollutant are somewhat species dependent, there is some evidence that rats are not the most sensitive species to acidic aerosols (U.S. Environmental Protection Agency, 1989). As discussed previously, the extent of interaction within any one species of animal is endpoint dependent, and it is likely that the sensitivity of various endpoints is species dependent. Thus, rats do show biochemical changes (e.g., in collagen metabolism) with exposure to fairly low levels of acidic aerosols in combination with O_3 (see Table 6-10), although these involved longer duration exposures. In any case, the underlying reasons for the lack of interaction in the complex-mixture study above remain unclear.

Kleinman et al. (1989) exposed rats (Sprague-Dawley, male, 7 weeks old, nose-only) to a mixture of O_3 (0.8 ppm) + SO_2 (5.0 ppm) + H_2SO_4 or $(NH_4)_2SO_4$ (1,000 $\mu g/m^3$) at high RH (85%) and noted a delay in early clearance of inert particles from the lungs, compared to air-exposed controls. However, it is difficult to relate any effects to the O_3 because responses to O_3 alone were not examined.

The ideal complex mixture is one that actually exists in the ambient environment. Saldiva et al. (1992) exposed rats (Wistar, male, 2 mo old) for 6 mo to actual atmospheres of São Paulo, Brazil, with controls maintained for the same period of time in a clean, rural environment. The mean pollution levels over the exposure period were as follows: 0.011 ppm O_3, 1.25 ppm CO, 29.05 $\mu g/m^3$ SO_2, and 35.18 $\mu g/m^3$ particles. The animals exposed to the urban air showed evidence of bronchial secretory cell hyperplasia, ciliary structural changes, increased viscosity of mucus, and impaired mucociliary clearance. Although chronic exposure to air pollution may result in pulmonary dysfunction, the specific components producing the response could not be determined.

Inhalation exposures to air pollutants are, of course, the ideal way to assess interaction, but in vitro exposures may provide indications of potential interactions. Shiraishi and Bandow (1985) exposed Chinese hamster V79 cells for 2 h to photochemical reaction products produced from the reaction of propylene and NO_2 in a smog chamber. The resultant exposure atmospheres consisted of various proportions of propylene (0.07 to 0.16 ppm), NO_2 (0.22 to 0.28 ppm), O_3 (0.09 to 0.38), PAN (0.04 to 0.41 ppm), and HCHO (0.23 to 1.50 ppm). Exposures to NO_2 and O_3 alone also were performed. All of the complex mixtures resulted in an increased frequency of sister chromatid exchange and growth inhibition. The effects of the mixture were greater than those due to either O_3 or NO_2 alone for sister chromatid exchange, but growth inhibition was similar to that induced solely by O_3. The authors concluded that the observed effects were not due to any single compound within the mixture, but rather to various compounds producing multiple effects.

6.4.4 Summary

It is difficult to summarize the role that O_3 plays in response to exposure to binary mixtures, and it is even harder to determine its role in response to multicomponent atmospheres. One of the problems in understanding interactions is that, although the specific mechanisms of action of the individual pollutants within a mixture may be known, the exact

bases for toxic interactions have not been elucidated clearly. There are, however, certain generic mechanisms that may underlie pollutant interactions. One is physical, involving adsorption of one pollutant onto another and subsequent transport to more or less sensitive sites or to sites where one of the components of the mixture normally would not deposit in concentrated amounts. This, however, probably does not play a major role in O_3-related interactions. A second mechanism involves production of secondary products that may be more toxicologically active than the primary materials. This has been demonstrated or suggested in a number of studies as a basis for interaction between O_3 and NO_2. A third mechanism involves biological or chemical alterations at target sites that affect response to O_3 or the copollutant. This has been suggested to underlie interactions with mixtures of O_3 and acid sulfates. A related mechanism is an O_3- or copollutant-induced physiological change, such as alteration in ventilation pattern, resulting in changes in the penetration or deposition of one pollutant when another is present. This has been implicated in enhanced responses to various O_3-containing mixtures with exercise.

Evaluation of interactions between O_3 and copollutants is a complex procedure. Responses are dependent on a number of host and environmental factors, such that different studies using the same copollutants may show different types or magnitudes of interactions. The occurrence and nature of any interaction is dependent on the endpoint being examined and is also highly related to the specific conditions of each study, such as animal species, health status, exposure method, dose, exposure sequence, and the physicochemical characteristics of the copollutants. Because of this, it is difficult to compare studies, even those examining similar endpoints, that were performed under different exposure conditions. Thus, any description of interactions is really valid only for the specific conditions of the study in question and cannot be generalized to all conditions of exposure to a particular chemical mixture. Furthermore, it is generally not possible to extrapolate the effect of pollutant mixtures from studies on the effects of each component when given separately. In any case, what can be concluded from the database is that interactions of O_3-containing mixtures are generally synergistic (antagonism has been noted in a few studies), depending on the various factors noted above, and that O_3 may produce more significant biological responses as a component of a mixture than when inhaled alone. Furthermore, although most studies have shown that interaction occurs only at higher than ambient concentrations with acute exposure, some have demonstrated interaction at more environmentally relevant levels (e.g., 0.05 to 0.1 ppm O_3 with NO_2) with repeated exposures.

6.5 Summary and Conclusions

6.5.1 Introduction

In the past 30 years, thousands of research studies on the effects of O_3 in laboratory animals have been reported in the literature. This body of evidence presents a clear picture of the types of alterations O_3 can cause on respiratory tract host defense mechanisms, biochemistry, structure, and lung function. Less is known about carcinogenic potential and effects on organs distant from the lungs. These types of effects are observed in many animal species from mice to nonhuman primates, lending credence to the qualitative extrapolation of these effects to humans. The major issue is what levels, durations, and patterns of exposure are capable of causing these effects in humans. Extrapolation is discussed in Chapter 8. Suffice it to say here that the animal toxicological studies assist in

interpreting observations made in O_3-exposed humans and extend the knowledge of potential human hazards that never can be studied adequately in humans.

This summary and conclusion section deals exclusively with the effects of O_3, alone and in mixture. Other photochemical oxidants either have been evaluated elsewhere (NO_2 and HCHO; U.S. Environmental Protection Agency, 1993; Grindstaff et al., 1991) or in an earlier O_3 criteria document (U.S. Environmental Protection Agency, 1986). This section is organized by molecular mechanisms of effects, respiratory tract effects, systemic effects, and effects of mixtures. Generally, it is an interpretative, factual summary of the array of effects observed in animals. Chapter 8 presents the current state of extrapolation of these effects to humans, and Chapter 9 integrates knowledge from animal toxicology, epidemiology, and human clinical studies.

Together, this chapter and the animal toxicological chapter in the 1986 document (U.S. Environmental Protection Agency, 1986), contain more than 1,000 references. Although all of them contribute to choosing and understanding the key issues to be summarized here, there obviously must be a highly selective choice made as to which references to include here. Generally, the papers discussed here were selected either because they represent the lowest effective concentration for an endpoint or they significantly influenced a particular conclusion.

6.5.2 Molecular Mechanisms of Effects

Molecular mechanisms (the manner in which chemical reactions of O_3 are translated into biological effects) are alluded to in different sections of this document. Studies that link O_3 chemistry with O_3 effect measurements would greatly strengthen the theoretical basis for understanding the biological effects of O_3. They also would allow examination of the similarity between animals and humans, thus strengthening interspecies extrapolations. Ozone has been shown to react directly with a variety of biomolecules that are present in both animals and humans. Most of the attention has been centered on polyunsaturated fatty acids and carbon-carbon double bonds, although reactions with sulfhydryl, amino, and some electron-rich compounds may be equally important. Free radicals may be involved, and antioxidant defenses appear to lessen the effect of these reactions. A "molecular target" for O_3 (the biomolecules most affected by reaction with O_3 or most crucial in mediating the observed responses) has not been identified for any of the endpoints studied. In fact, the target may be different for different endpoints.

An important concept in evaluating molecular targets was elucidated recently by Pryor (1992), who suggests (based on reaction and diffusion rate data) that the O_3 molecule does not penetrate through cell membranes or even the surfactant layer of the lung. Instead, a "reaction cascade" forms intermediates (organic or oxygen-free radicals, lipid hydroperoxides, aldehydes, hydrogen peroxide, etc.), which penetrate into the cells, causing the biological effects observed (Pryor et al., 1991). Confirmation of such O_3-induced free-radical autoxidation of lipids has been sought in vivo, but the indirect nature of the measurement methods produced equivocal results. More direct evidence has been obtained by Kennedy et al. (1992), who used electron spin-trapping methods to measure a concentration-related increase in radical adducts of the lipid fraction of lungs from O_3-exposed rats. Increased radical signals were detected after a 2-h exposure to ≥ 0.5 ppm O_3, but because the rats' respiration was stimulated by CO_2, the effective dose would be greater than it appears. Oxidized (oxygenated) biomolecules that result from reaction with

O_3 also may mediate the effects of O_3. Studies by Hatch et al. (1994) show that crude fractions of the lung lining layer become labeled with oxygen-18 after exposure to oxygen-18-labeled O_3. The label is concentrated in the airway lining layers, and the amount of oxygen-18 incorporation in this layer appears to be correlated with effects of O_3 (permeability and inflammation) in both rats and humans. These findings are consistent with the hypothesis that O_3 reacts with the lining of the lung, that the same types of interactions occur in both animals and humans, and that these reactions lead to similar effects.

6.5.3 Respiratory Tract Effects

6.5.3.1 Effects on Host Defenses

Several systems defend the respiratory tract of the host against infectious and neoplastic disease as well as nonviable inhaled particles; all of these systems can be affected by O_3. The mucociliary clearance system moves particles deposited on the mucous layer (either through deposition from the air stream or entry of cells or cellular debris from the alveoli) upwards and out of the lower respiratory tract. The nasal passages also have an effective clearance system. Concentrations as low as 0.15 ppm O_3 (8 h/day, 6 days) caused structural changes in the nasal respiratory epithelium (e.g., ciliated cell necrosis, shortened cilia) of monkeys (Harkema et al., 1987). Ciliated cells also are lost or damaged in the conducting airways of the lower respiratory tract after short exposures (e.g., 0.96 ppm O_3, 8 h, monkeys; Hyde et al., 1992). Mucous chemistry also is changed (McBride et al., 1991). Sufficient morphologic damage would be expected to have functional consequences. Acute exposures (0.6 ppm O_3, 2 h) slow mucociliary particle clearance in rabbits, but repeated exposures (up to 14 days) caused no effects. Alveolar clearance is slower and involves clearance of particles through interstitial pathways to the lymphatic system or movement of particle-laden AMs up to the bottom of the mucociliary escalator. Effects on alveolar clearance are concentration-dependent. A single 2-h exposure of rabbits to 0.1 ppm O_3 accelerated clearance up to 14 days postexposure, exposure to 0.6 ppm caused no effect, and a higher concentration (1.2 ppm) slowed alveolar clearance (Driscoll et al., 1986). Alveolar clearance of asbestos particles was slowed by a 6-week exposure to an urban pattern of O_3 (Pinkerton et al., 1989).

Alveolar macrophages are the first line of defense against microbes and nonviable particles that reach the pulmonary region of the lung. They phagocytize particles, kill microbes, and interact with lymphocytes in the development of an immune response. Thus, their proper functioning is critical. Alveolar macrophages from several species of animals exposed acutely to O_3 can exhibit decreased phagocytosis; decreased lysosomal enzyme activities and superoxide anion radical production, both of which function in killing bacteria; alterations in membrane morphology; chromosomal damage; decreased cytotoxicity to tumor cells; increased release of PGE_2 and $PGF_{2\alpha}$; and alterations in the number of AMs. Phagocytic changes are the most investigated. Exposure of rabbits to a level as low as 0.1 ppm O_3 (2 h/day) decreased nonspecific phagocytosis (of latex microspheres) after 2 or 6 but not 13 days of exposure (Driscoll et al., 1987). Recovery from the single 2-h exposure to 0.1 ppm O_3 was complete by 7 days postexposure. This pattern of response was confirmed in mice for Fc-receptor mediated phagocytosis (Gilmour et al., 1991; Canning et al., 1991).

The humoral- and cell-mediated immune system of the lung also is affected by O_3. Generally, T-cell-dependent immunity is more susceptible than B-cell-dependent immunity, but most immune functions examined have exhibited effects. However, because relatively few studies have been conducted, it currently is not possible to adequately interpret the impacts of O_3-induced alterations on the immune system (e.g., decreases in mitogenic responses of T cells, alterations in T:B-cell ratios in the MLN). Only a few studies have attempted to correlate immunological changes and infectious disease outcome. Van Loveren et al. (1988) infected rats with Listeria, exposed them to O_3 for 1 week (0.26 to 1.02 ppm) and measured several endpoints. Ozone concentrations of 1.02 and 0.77 ppm, respectively, increased Listeria-induced mortality and severity of pathologic lesions in the lung and liver. They interpreted these findings as due to O_3-induced impaired clearance of the bacteria caused by decreased AM function and decreased cellular immunity (e.g., decreased delayed-type hypersensitivity and decreased T:B-cell ratios in MLN).

A reasonably large body of evidence indicates that the impact of O_3 on one or several host defense mechanisms leads to the inability of animals to fight bacterial infection and alters the course of viral infection. Antibacterial models are more commonly used. Mice exposed for 3 h to 0.4 ppm O_3 have decreased intrapulmonary killing of *S. zooepidemicus* (Gilmour et al., 1993a; Gilmour and Selgrade, 1993). Similar results have been obtained for *S. aureus* at a slightly higher concentration (Goldstein et al., 1971b). Correlations have been made between O_3 exposure and decreases in AM phagocytosis, decreases in bactericidal activity, growth of bacteria in the lungs, presence of bacteria in the blood, and mortality in mice (Coffin and Gardner, 1972; Gilmour and Selgrade, 1993). The lowest O_3 exposure causing increased streptococcal-induced mortality is 0.08 ppm for 3 h in mice (Coffin et al., 1967; Coffin and Gardner, 1972; Miller et al., 1978). However, prolonged intermittent exposure to 0.1 ppm O_3 for 15 weeks only slightly increased the mortality (Aranyi et al., 1983), and continuous exposure for 15 days to 0.1 ppm with two daily 1-h peaks (5 days/week) to either 0.3 or 0.5 ppm did not enhance mortality in the same model system (Graham et al., 1987). Prolonged exposure (1 to 2 weeks) also did not affect bactericidal activity to *S. aureus* (Gilmour et al., 1991).

Generally, short-term exposure to O_3 does not affect viral titers in the lungs of mice infected with influenza virus; however, reduced numbers of lung tissue T and B cells will reduce antibody titers to the virus, and mortality, lung pathology, and increased lung wet weight do occur (Selgrade et al., 1988; Jakab and Hmieleski, 1988). Ozone also enhances postinfluenzal alveolitis and structural changes that begin at 30 days postinfection (Jakab and Bassett, 1990). The complexity of the interaction of viral infection and O_3 exposure is further illustrated by Selgrade et al. (1988), who found that the effects of O_3 on influenza virus infection were dependent on the temporal relationship of O_3 exposure and day of infectious challenge. Also, interferon, which can be induced by viral infection, mitigates the O_3-induced lung lessons in mice, raising the possibility that certain stages of viral infection may have interactions with the lung that are different from other stages (Dziedzic and White, 1987b).

6.5.3.2 Effects on Inflammation and Permeability

The barrier function of the respiratory tract is disrupted by O_3, allowing cellular and fluid components from the blood to enter the lung and allowing certain types of substances in the lung to enter the blood. Markers of inflammation generally included increased proteins and PMNs in BAL. Concurrent with these events, but not necessarily

interdependently, AMs liberate more arachidonic acid, which results in the production of biologically active LTs and PGs. Similar responses are observed in mice, rats, rabbits, guinea pigs, hamsters, and nonhuman primates. After acute exposure, the lowest effective concentration that increases BAL protein and number of PMNs is 0.12 ppm O_3 (mice, 24 h of exposure, BAL immediately after exposure) (Kleeberger et al., 1993a). However, the increase in BAL protein typically is maximal roughly 16 to 24 h postexposure. In rats exposed to 0.8 ppm O_3 for 6 h and examined by lavage and morphometry several times postexposure, the increase in nasal PMNs occurs sooner and wanes about the time that these cells are increasing in number in the lungs (Hotchkiss et al., 1989a,b). It should be recognized that BAL can enable measurement of the protein and cells accessible by lavage, including the resident material (i.e., may include protein from O_3-induced cellular destruction) and the material entering from the tissue or circulation. Thus, interstitial inflammation, which has been observed in several species microscopically, is not detectable by BAL.

Several C $\times$ T studies have been conducted in mice using BAL protein as an endpoint. In two studies, there was combination of various Cs (0.1 to 2.04 ppm O_3) and Ts (1 to 12 h), resulting in a number of different C $\times$ T products (Rombout et al., 1989; Highfill et al., 1992). Both of these studies showed that the influence of T increased as C increased (i.e., there was no simple relationship of $C^a \times T^a$ = constant product; however, at the lowest C $\times$ T products, there was a more equivalent influence of C and T). Gelzleichter et al. (1992b) used a single C $\times$ T product composed of a variety of Cs and Ts for up to 3 days of exposure. The 24 h/day exposure group had less response than the other groups that responded equivalently. Effects of longer term exposure on permeability and inflammation are more complex to interpret (also see subsequent discussion on lung structure). Histological examination of rat lungs exposed to 0.5 ppm O_3 (2.25 h/day) showed more inflammatory cells in the alveoli after 5 days of exposure than after 1 day of exposure (Tepper et al., 1989). In contrast, the increase in BAL PMNs that occurred after Day 1 of exposure of rats had resolved by Day 4 (7 h/day) (Donaldson et al., 1993).

Some studies suggest that, although protein and PMN increases are observed concurrently, this may be more a function of experimental design than the actual biological sequence of events. For example, in rats depleted of PMNs with anti-PMN serum, O_3 did not increase BAL PMNs, but BAL protein still was increased (Pino et al., 1992b). Also Young and Bhalla (1992) observed an increase in tracheal protein earlier than increased tracheal PMNs. They interpreted this and other related results to suggest that the recruited PMNs may serve to sustain an increase in permeability.

6.5.3.3 Effects on Structure, Function, and Biochemistry

Theoretically, and in some cases empirically, lung structure, function, and biochemistry are linked. Correlations are not exact because of differences in available measurement methods (e.g., most lung function tests used do not measure sensitively the function of the smallest airways, where the "classical" O_3 lesion is observed) and some independence of effects (e.g., a transient change in breathing frequency would not be morphologically detectable). Also, most biochemical measurements are made of whole lung, rather than focal areas of damage, and only some enzyme activities measured would be expected to be correlated to structure or function (e.g., collagen metabolism, antioxidant metabolism).

After acute exposure to O_3, the most commonly observed effect in several species is tachypnea (increased f and decreased V_T) with little (if any) change in $\dot{V}_E$; the lowest exposure causing tachypnea was 0.2 ppm O_3 for 3 h in rats (Mautz and Bufalino, 1989). Other effects reported after acute exposure to ≤ 1 ppm include increased R_L and decreased Cdyn, TLC, VC, FRC, RV, FVC, DL_{CO}, and the multibreath N_2 slope (e.g., Fouke et al., 1991; Mautz et al., 1985b; Miller et al., 1988). However, these changes are not observed in all studies, probably due to differences in animal species, measurement method, and exposure protocols. With rare exception, concentrations well in excess of 1 ppm O_3 are required to increase airway reactivity.

Two C $\times$ T studies of pulmonary function using acute exposure periods have been performed. Costa et al. (1989) found that FVC, DL_{CO}, and the multibreath N_2 slope decreased with increasing C $\times$ T products in rats and that the influence of T is greater at higher Cs. In guinea pigs, Nishikawa et al. (1990) observed that airway responsiveness to methacholine increased at higher C $\times$ T products (e.g., at 90 but not at 30 ppm $\cdot$ min); the authors concluded that T was an important factor in the O_3 response.

When rats were exposed for 5 days (2.25 h/day, with CO_2 to stimulate ventilation equivalent to light exercise in humans) to 0.35, 0.5, and 1.0 ppm O_3, the change in shape of the flow-volume curve occurred and tachypnea peaked on Days 1 and 2, but by Day 5, there was no difference from control (except at 1 ppm) (Tepper et al., 1989). This attenuation is similar to that observed in humans. However, in other, similar groups of animals, histological changes in the lung progressed, and BAL protein remained elevated. Other similarities between laboratory animals and humans in their pulmonary function responses to short-term O_3 exposure are explored in Chapter 8.

Ozone causes similar types of alterations in lung morphology in all laboratory animal species studied. The most affected cells are the ciliated epithelial cells of the airways and Type 1 cells in the gas exchange region. Within the nasal cavity, anterior portions of the respiratory and transitional epithelium are affected. Cilia are lost or damaged; some ciliated cells become necrotic, are lost, and are replaced with nonciliated cells. Mucus-secreting cells are affected.

The CAR (the junction of the conducting airways and the gas exchange region) is a primary target, possibly because it receives the greatest dose of O_3 delivered to the lower respiratory tract (see Chapter 8) and has Type 1 epithelial cells covering a large surface area. Even though there are significant interspecies differences in the structure of the CAR (e.g., primates, including humans, have RBs, which are rudimentary or absent in laboratory animals such as rats or mice), it is the target in all species studied. Exposure to O_3 causes loss of cilia or necrosis of the ciliated cells, leaving a bare basement membrane that is replaced by nonciliated bronchiolar cells, which may become hyperplastic after longer exposures. Mucous secreting cells can be affected, but not as significantly as ciliated cells. Type 1 cells also are damaged and can be sloughed from the surface; Type 2 cells, which are thicker, replace them. Sometimes, Type 2 cells differentiate into Type 1 cells. This epithelial remodeling is accompanied by an inflammatory response in the CAR, primarily consisting of an increase in number of PMNs in the earlier stages and an increase in number of AMs in later stages; interstitial edema occurs. With increased duration of exposure, alveolar septa in the CAR thicken due to increased matrix, basement membrane, collagen, and fibroblasts and a thickened alveolar epithelium.

These patterns of change have different relationships to duration of exposure, as illustrated by Dungworth (1989) (see Figure 6-3; Section 6.2.4.5). Inflammatory changes

peak after a few days of O_3 exposure; are still observable, but to a much lesser degree, in tissue during months of exposure; and begin to return to control values after exposure ceases. In contrast, epithelial hyperplasia rapidly increases during about the first week of exposure, plateaus as exposure continues, and begins to decrease slowly when exposure stops. Interstitial fibrosis requires months of exposure to be observed microscopically and increases slowly, but when exposure ceases, interstitial fibrosis still can persist or continue to increase. Numerous studies using several different species and experimental approaches support these findings. Only a few of the studies (primarily those using more sensitive morphometric measurements) are used here to illustrate key points and to show correlations with pulmonary function and lung biochemistry. Only rat and nonhuman primate studies are discussed because most investigations were conducted on them. At equivalent exposures, nonhuman primates appear to be more responsive than rats (Section 6.2.4).

Generally, short-term exposures to concentrations ≤ 0.2 ppm O_3 do not cause changes detectable by LM in the nasal cavities of rats or nonhuman primates, except for inflammation and an occasional delayed postexposure finding of mild hyperplasia. For example, Hotchkiss et al. (1989a) reported inflammation in the nasal epithelium of rats up to 66 h after a 6-h exposure to levels as low as 0.12 ppm O_3; there was no necrosis, loss of cilia, or hyperplasia even at 1.5 ppm. After 3 days (22 h/day) of exposure, ≥ 0.4 ppm caused loss of cilia and hyperplasia and metaplasia of the nasal epithelium of rats (Reuzel et al., 1990). Nonhuman primates appeared to be more responsive. Harkema et al. (1987) observed that exposure to 0.15 or 0.3 ppm O_3 for 6 or 90 days (8 h/day) caused necrosis of ciliated cells, shortened cilia, and increased mucous granule cells in the respiratory epithelium; alterations in cell numbers also were found in the transitional epithelium.

Within the CAR, a number of alterations occur. In rats and monkeys, ciliated and Type 1 cells become necrotic and are sloughed from the epithelium as early as the first 2 to 4 h of an exposure to about 0.5 ppm O_3 (Stephens et al., 1974a,b). Repair, as shown by increased DNA synthesis by nonciliated bronchiolar and Type 2 cells, begins by about 18 to 24 h of exposure (Evans et al., 1976a,b; Stephens et al., 1974a; Castleman et al., 1980), although cell damage continues (Castleman et al., 1980). The lesion is fully developed by about 3 days of continuous exposure, after which the rate of repair exceeds the rate of damage. The increase in antioxidant enzyme activities (e.g., succinate oxidase, G6PD, and 6PGD) parallels the increase in Type 2 cells, which are rich in these enzymes; the increase in the Type 2 cell population is probably responsible for these biochemical changes (Bassett et al., 1988a; U.S. Environmental Protection Agency, 1986).

Lesions in the CAR are one of the hallmarks of O_3 toxicity, having been well established. The study by Chang et al. (1992) provides examples of some of the patterns of cellular alterations. Chang et al. (1992) exposed rats to an urban pattern of O_3 (0.06 ppm background, 7 days/week on which were superimposed 9-h peaks [5 days/week] slowly rising to 0.25 ppm) for 78 weeks and made periodic examinations of the CAR TB and proximal alveoli by TEM morphometry during and after exposure. Type 1 cells had a larger volume at Week 13 and increased numbers at Weeks 13 and 78; there were no such changes at 17 weeks after exposure ceased. Type 2 cell volume per area of basement membrane increased immediately after Week 78 and was still increased 17 weeks after exposure ceased. Interstitial cells and matrix were increased after Weeks 1, 13, and 78, but returned to control by 17 weeks after exposure ceased. However, epithelial and endothelial basement membrane were thickened and accompanied by increased collagen fibers at the later examination times and 17 weeks after the 78-week exposure ended. In TBs, surface areas of ciliated and

nonciliated cells decreased during exposure. Pulmonary function studies conducted in identically exposed groups of rats were consistent with the morphometric findings (Tepper et al., 1991). Generally, expiratory resistance was increased (suggesting central airway narrowing), but it was only significantly different from control at 78 weeks. Tidal volume was reduced at all evaluation times. Overall, breathing frequency was reduced, but no single evaluation time was significant. Monkeys exposed to a higher concentration of O_3 (0.64 ppm, 1 year) also showed increased resistance and decreased flows, which were interpreted as central and peripheral airway narrowing; during a 3-mo postexposure period, decreases in static lung compliance persisted (Wegner, 1982).

Several studies have demonstrated distal airway remodeling. This bronchiolization of CAR alveoli is so named because bronchial epithelium replaces the Type 1 and 2 cells typical of ADs, resulting in the appearance of RBs in rats and increased volume fraction and volume of RBs in monkeys. This has been observed at exposures as low as 0.5 ppm O_3 (50 days) in rats (Moore and Schwartz, 1981) and as low as 0.25 ppm (8 h/day, 18 mo) in monkeys (Tyler et al., 1988). Inflammation occurs concurrently, perhaps indicating an influence on remodeling. In monkeys, such bronchiolization can persist 6 mo after the end of a 1-year (8 h/day) exposure to 0.64 ppm (Tyler et al., 1991b).

Exposure regimens can have unexpected impacts on experimental outcomes. Several investigations of combinations of O_3 "episodes" or O_3 "seasons" with clean-air periods have been examined. In the first of these, Last et al. (1984b) compared air control rats to two groups of rats exposed to 0.96 ppm. One group received a 90-day (8 h/day) exposure ("daily"); the other group had intermittent units of 5 days of O_3 (8 h/day) and 9 days of air, such that there were 35 O_3 exposure days over the 90-day period (episodic). Both groups had equivalent increases in lung collagen. Using a similar exposure regimen, Barr et al. (1990) found equivalent CAR remodeling and volumes of CAR lesions in both groups. In contrast, RB thickness increased in the daily group only, and the CAR interstitium increased in thickness only in the episodic group. Monkeys were studied more extensively after a daily (8 h/day) exposure to 0.25 ppm for 18 mo and a seasonal exposure only during the odd months of the 18-mo period (Tyler et al., 1988). Most morphometric measurements were similar between the two groups (e.g., both had respiratory bronchiolitis). However, only the daily group had an increased number of AMs in the lumen and interstitium. Only the seasonal group had increased lung collagen content; increased chest wall compliance, suggesting delayed lung maturation; and increased inspiratory capacity. This body of work indicates that under these types of exposure circumstances, the simple product of $C \times T$ does not predict the outcome. Indeed, half the O_3 (on a $C \times T$ basis) caused equivalent or more effects than a "full" O_3 exposure.

The complexity of understanding $C \times T$ relationships is further illustrated by Chang et al. (1991), who compared two different exposure regimens (one a square wave and the other an urban pattern) on the basis of $C \times T$ products. There was a linear relationship between $C \times T$ products and the increase in Type 1 cell volume in the CAR; a similar observation on Type 2 cell volumes was less robust. There was no such relationship for other morphometric endpoints in the same animals. Cell proliferation in the nasal epithelium does not increase linearly with increasing $C \times T$ but does increase linearly with increasing C (Henderson et al., 1993).

Long-term exposure also thickens CAR alveolar septa, due to an increase in inflammatory cells, fibroblasts, and amorphous extracellular matrix (Fujinaka et al., 1985; Barry et al., 1985; Zitnik et al., 1978). There is some morphological evidence of mild

fibrosis (i.e., local increase in collagen) in CAR interalveolar septa (Last et al., 1979; Boorman et al., 1980; Chang et al., 1992; Pickrell et al., 1987b; Freeman et al., 1974; Moore and Schwartz, 1981). Biochemical evidence supports these findings, even though biochemical approaches would be expected to be less sensitive because the whole lung (rather than focal lesions) is examined. Last et al. (1979) directly demonstrated the correlation by observing increased collagen histologically and biochemically (collagen synthesis rate) in rats similarly exposed to 0.5 to 2.0 ppm O_3 for 7 to 21 days. The increase became greater with increasing concentration and duration of exposure. Similar correlations were observed at a higher concentration by Pickrell et al. (1987b). The increased collagen content can persist after exposure ceases (Chang et al., 1992; Hussain et al., 1976a,b; Last et al., 1984b), but some studies suggest that higher concentrations (>0.5 ppm) may be required for such persistence (Last and Greenberg, 1980; Pickrell et al., 1987b). Collagen cross-links were studied in monkeys exposed to 0.61 ppm O_3 for 1 year (8 h/day) (Reiser et al., 1987). Earlier examination of these same monkeys revealed that collagen content was increased (Last et al., 1984b). When specific collagen cross-links were measured, the increase in "abnormal" cross-links observed immediately after exposure remained in the lungs at 6 mo postexposure.

These morphologic/morphometric and biochemical findings of fibrotic changes are supported by some pulmonary function studies. For example, rats exposed for up to 78 weeks, using the same urban exposure protocol as Chang et al. (1992), exhibited reduced lung volume and hastened N_2 washout patterns, consistent with a "stiffer" lung (i.e., restrictive lung disease) (Costa et al., 1994).

The chronic O_3 study by the NTP and the Health Effects Institute (HEI) (Last et al., 1994; Szarek, 1994; Radharkrishnarmurthy, 1994; Parks and Roby, 1994; Harkema and Mauderly, 1994; Harkema et al., 1994; Chang et al., 1995; Pinkerton et al., 1995; Catalano et al., 1995a,b) further illustrates some of the complex interrelationships between lung structure, function, and biochemistry. All of these endpoints were evaluated in a collaborative project using rats exposed 6 h/day, 5 days/week for 20 mo to 0.12, 0.50, or 1.00 ppm O_3. Although lung biochemistry and structure were affected at the higher O_3 concentrations ($\geq$0.50 ppm), there were no observed effects on pulmonary function. This is consistent with the relative sensitivity of the tests used and suggests that the observed effects were not sufficient to overcome the reserve function of the lung.

Combined analyses of the NTP/HEI collaborative studies showed that 0.50 and 1.00 ppm O_3 caused a variety of structural and biochemical effects; 0.12 ppm O_3 did not cause any major effects, although a few specific endpoints were altered. Hallmarks of chronic rhinitis (e.g., inflammation, mucous cell hyperplasia, decreased mucous flow) were observed in focal regions of the nasal cavity. Structural and biochemical changes included some, but not many hallmarks of airway disease. Typical O_3-induced changes (e.g., bronchiolarization, increased interstitial matrix) observed in the tracheobronchial region and in the CAR were characteristic of centriacinar fibrosis; however, diffuse pulmonary fibrosis was not observed.

An integrative, multiple endpoint analysis (Catalano et al., 1995a) utilizing median polish techniques produced composite variables for disease surrogates that were tested for trends across all three O_3 concentrations. Trends for centriacinar fibrosis, airway disease, and chronic rhinitis were examined for 10, 18, and 3 endpoints, respectively, from the individual NTP/HEI studies. A statistically significant trend was noted for the association between chronic rhinitis and increasing O_3 concentration. The differences between control

and exposed rats were statistically significant at 0.50 and 1.00 ppm O_3. Marginally significant and significant trends were found for the association between centriacinar fibrosis or airway disease and increasing O_3 concentration; however, no statistically significant differences were found between control and O_3-exposed rats.

As discussed above, long-term O_3 exposure can cause lung fibrotic changes; however, there is no evidence that O_3 causes emphysema, using the currently accepted morphological definition of human emphysema (U.S. Environmental Protection Agency, 1986).

6.5.3.4 Genotoxicity and Carcinogenicity of Ozone

A significant amount of research has been conducted to determine whether O_3 is genotoxic or carcinogenic. Many of the early experiments have flaws in experimental design or have used O_3 concentrations far above levels that could occur in ambient air.
In evaluating the data, a number of conclusions can be made. In vitro exposure of naked plasmid DNA to very high O_3 concentrations results in single and double-strand breaks in the DNA, as confirmed by gel electrophoresis and electron microscopy studies (Hamelin, 1985). Testing of O_3 in various mutagenesis assays has led to marginal or small results in a number of assays and negative results in other assay systems. Ozone is not mutagenic in *Salmonella* strains TA98, TA100, TA104, and TA1535 and causes, at most, weak effects in strain TA102 that are not strictly concentration dependent (Dillon et al., 1992; Victorin and Stahlberg, 1988a,b). Extremely high concentrations of O_3 (50 ppm) caused mutation to streptomycin resistance in *E. coli* and caused various types of mutations in the yeast *S. cerevisiae*, but O_3 was a weak mutagen compared to known strong mutagens in the yeast system (L' Herault and Chung, 1984; Dubeau and Chung, 1982). Ozone was not mutagenic in the *N. tabacam* or *Tradescantia* mutation assay systems (Gichner et al., 1992). Hence, overall, the data on the mutagenicity of O_3 are mixed: negative in six assays, marginally positive in one assay, and weakly positive in two assays. The present data indicate that O_3 is, at most, a weak mutagen, but further data are needed in mammalian cell systems to draw definitive conclusions regarding this point. There are some data indicating that O_3 may cause chromosome breakage in cultured cells, but in vivo animal studies are conflicting (Zelac et al., 1971a,b; Tice et al., 1978). A human study with an appropriate experimental design was negative (McKenzie et al., 1977; McKenzie, 1982).

Regarding carcinogenicity, O_3 has been shown to induce morphological transformation in cultured C3H/10T1/2 mouse embryo cells and in SHE cells and to cause a synergistic morphological transformation in cells treated also with gamma radiation (Borek et al., 1986, 1989b). However, these results could be due to interactions of O_3 with the culture medium that generate chemical species different from those produced in vivo. Whole animal carcinogenesis assays performed in strain A mice have demonstrated marginal increases in tumor yield that were not statistically significant or concentration dependent (Hassett et al., 1985; Last et al., 1987). The NTP study demonstrated that O_3 was not a tumor promoter or a co-carcinogen when NNK-treated male F344/N rats were exposed for 2 years to 0.5 ppm O_3 (National Toxicology Program, 1994). In the NTP study, rats and mice were exposed to 0.12, 0.5, or 1.0 ppm O_3 for 6 h/day, 5 days/week for two years or a lifetime. This NTP study showed no evidence of carcinogenic activity in male or female F344/N rats, equivocal evidence of carcinogenic activity in male $B6C3F_1$ mice, and some evidence of carcinogenic activity in female $B6C3F_1$ mice at a high concentration (1.0 ppm).

Hence, O_3 has been shown to be a weak pulmonary carcinogen only in female $B6C3F_1$ mice at toxic concentrations in one experiment.

At present, O_3 is shown to be nonmutagenic in some assay systems; at most, weakly mutagenic in a few assay systems; and clastogenic in vitro but not in vivo. Ozone can transform cells in vitro. Ozone does not cause concentration-dependent tumor induction that is statistically significant in hamsters, Wister male rats, F344/N male or female rats, male or female A/J mice, or Swiss-Webster male mice. There are ambiguous data for pulmonary carcinogenesis in male $B6C3F_1$ mice and weak carcinogenesis data in female $B6C3F_1$ mice from chronic exposure to 1.0 ppm O_3. Therefore, O_3 has been shown to be a carcinogen only in female $B6C3F_1$ mice in one experiment. Because a chronic exposure to 1 ppm O_3 was required to induce pulmonary tumors in female mice, it is possible that pulmonary toxicity, which occurs only at high O_3 concentrations (1.0 ppm) and does not occur at lower levels, contributed to the tumor development. Hence, the potential for animal carcinogenicity is uncertain at the present time.

6.5.3.5 Factors That Influence Ozone Exposure

Factors that increase the delivered dose of O_3, decrease biochemical defense mechanisms, or increase cellular sensitivity can increase the impact of a given O_3 exposure. The most commonly studied factors include exercise, age, and nutrition.

As discussed in Chapter 8, exercise increases the dose of O_3 delivered to the respiratory tract and alters the distribution of O_3. As would be expected, exercise during exposure enhances the effect of O_3. This has been demonstrated by Mautz et al. (1985b), who showed that exercising rats had more extensive lung lesions than rats exposed at rest. Similarly, Tepper et al. (1990, 1994) found that rats were more responsive to O_3 when coexposed to CO_2 to increase ventilation, simulating exercise.

A number of studies have been conducted to compare the effects of O_3 on various ages of mice and rats, from 1 day old to older adults. Interpretation of these studies is difficult because, prior to weaning, the huddling behavior of the neonates with their dams as well as the bedding material (present in some studies) may have affected the concentration of O_3 in the breathing zone and hence the subsequent delivered dose. Generally, in short-term exposure biochemical studies of antioxidant metabolism, there was a decrease or no change in enzyme activity in neonates. As age increased after weaning, the typical increase in antioxidant metabolism became greater with age (Elsayed et al., 1982; Tyson et al., 1982; Lunan et al., 1977; Mustafa et al., 1985). Stephens et al. (1978) found that morphological effects did not occur in animals exposed prior to weaning at 21 days of age. This may explain the results of Barry et al. (1985, 1988), who found no morphometric differences in the CAR and TB in rats that started a 42-day exposure at ages of 1 day and 42 days. In identically exposed rats, however, Raub et al. (1983) found more, though admittedly subtle, pulmonary function changes in the youngest group of animals. Yokoyama et al. (1984) did not detect any age-related differences in lung function of rats at 4, 7, and 10 weeks of age. Although O_3-induced increases in BAL protein and PMNs do not show age dependence, BAL prostaglandins increased sooner and more leukocytes were dead in younger (13-day-old) rats, compared to adults (e.g., 16 weeks old) (Gunnison et al., 1990, 1992a). Age (5 weeks versus 9 weeks) did not influence the O_3-induced decrease in lung bactericidal activity (Gilmour et al., 1993a).

The literature on O_3-exposed pregnant animals is extremely sparse. Exposure of rats (1 ppm O_3 6 h) on Day 17 of pregnancy or Days 3, 13, and 20 of lactation caused a

greater increase in lung permeability and inflammation than that observed in nonpregnant rats (Gunnison et al., 1992b).

Numerous reports document that animals made vitamin E deficient are more susceptible to the biochemically detected effects of O_3 (e.g., lipid changes, antioxidant metabolism changes) (U.S. Environmental Protection Agency, 1986; Pryor, 1991). Generally, the research shows that, although vitamin E deficiency enhances susceptibility to lung biochemical changes, there is not a proportionate relationship between vitamin E supplementation (above normal levels) and protection from O_3. Also, vitamin E deficiency did not alter the impact of O_3 on lung structure (Chow et al., 1981). Vitamin C deficiency also has an influence. Guinea pigs deficient in vitamin C had a greater increase in BAL protein (compared to vitamin C-normal animals) when exposed acutely to 0.5 but not 1.0 ppm O_3 (Slade et al., 1989).

6.5.4 Systemic Effects

Theoretical analyses (Pryor, 1992) indicate that the O_3 molecule does not penetrate to the blood, yet there are numerous reports of systemic effects (i.e., effects on lymphocytes, erythrocytes, serum, central nervous system, parathyroid gland, circulatory system, and liver). Possibly one or several of the reaction products of O_3 (see Section 6.2.1) penetrates the lung tissue, or perhaps some systemic responses are secondary to pulmonary effects. Although a variety of clinical chemistry changes occur after O_3 exposure, they cannot be interpreted and will not be discussed here (see U.S. Environmental Protection Agency, 1986, and Section 6.3). Effects on systemic immunity are discussed in Section 6.5.3.1.

6.5.4.1 Central Nervous System and Behavioral Effects

Acute exposure to O_3 caused transient changes in behavior. The lowest exposure causing effects was 0.12 ppm O_3 for 6 h in rats; wheel-running activity decreased (Tepper et al., 1985; Tepper and Weiss, 1986). Because exercising animals were exposed in these studies (i.e., they received a higher dose of O_3), it is not surprising that higher O_3 concentrations (0.5 ppm, 6 h) are required to affect sedentary behavior (e.g., operant behaviors such as lever pressing for food reinforcement) (Weiss et al., 1981). Mice show aversive responses to O_3 (0.5 ppm, 60 s) by terminating O_3 exposure (Tepper et al., 1985). The lowest exposures causing effects are impacted by the type of reward. For example, O_3 had less effect on behaviors to avoid electric shock (Ichikawa et al., 1988) than on behaviors to obtain food or access exercise (Tepper et al., 1982, 1985; Weiss et al., 1981).

6.5.4.2 Cardiovascular Effects

In rats, O_3 can cause bradyarrhythmia at exposures as low as 0.1 ppm for 3 days; bradycardia, at exposures as low as 0.2 ppm for 2 days; and decreased mean arterial blood pressure, at exposures as low as 0.5 ppm for 6 h (Arito et al., 1990, 1992; Uchiyama and Yokoyama, 1989; Watkinson et al., 1993; Yokoyama et al., 1989b; Uchiyama et al., 1986). There is an interaction between some of these responses and thermoregulation in the rat. For example, when heart rate decreased, the core temperature of the exposed rats also decreased, and when exposures were conducted at higher ambient temperatures, there was no change in core temperature or heart rate (Watkinson et al., 1993). Such interactions add to the

complexity of extrapolating this type of response to humans, and therefore, without more information, qualitative extrapolation would be highly speculative.

6.5.4.3 Reproductive and Developmental Effects

No reports of "classical" (e.g., 2-generation studies) reproductive assays with O_3 were found. Kavlock et al. (1979, 1980) performed several developmental toxicity experiments in rats. Pregnant rats exposed intermittently (8 h/day) to 0.44 to 1.97 ppm O_3 during early, mid-, or late gestation or during the entire period of organgensis (Days 6 to 15) had no significant teratogenic effects. Continuous exposure during mid-gestation increased the resorption of embryos. Postnatal growth and behavioral development also were investigated. There was no effect on neonatal mortality (up to 1.5 ppm). Pups from dams exposed continuously to 1 ppm during mid- or late gestation weighed less 6 days after birth. Pups from pregnant rats exposed continuously to 1 ppm during late gestation had delays in behavioral development (e.g., righting, eye opening).

6.5.4.4 Other Systemic Effects

A number of investigations have shown the effects of O_3 on the pituitary-thyroid-adrenal axis, as evidenced by changes in circulating hormones and morphological changes in the thyroid and parathyroid glands (U.S. Environmental Protection Agency, 1986). No more recent studies could be found.

Several approaches have been used to study the effects of O_3 on the liver: increase in sleeping time following the injection of drugs (e.g., pentobarbital) metabolized by the liver, drug pharmacokinetics, and changes in liver enzymes. The lowest exposure causing increased sleeping time from pentobarbital was 0.1 ppm O_3 for at least 15 or 16 days (3 h/day) in female mice (Graham et al., 1981). In three species of animals, only females were affected (Graham et al., 1981). Pentobarbital pharmacokinetics was marginally ($p = 0.06$) slowed in mice exposed to 1 ppm O_3 for 3 h (Graham et al., 1985); theophylline clearance was slowed in older rabbits exposed to 0.3 ppm O_3 for 5 days (3.75 h/day) (Canada and Calabrese, 1985). Ozone has caused both increases, decreases, and no changes in liver xenobiotic metabolism, depending on the exposure and enzyme being measured (U.S. Environmental Protection Agency, 1986).

6.5.5 Effects of Mixtures

Humans in the real world are exposed to complex mixtures of gases and particles. Sufficient evidence exists to know that the health outcome is dependent on the mixture, but the relative role (or even the exact identity) of the "major" components is not known. Because of this, it is crucial to evaluate the health effects of O_3 in light of epidemiological, human clinical, and animal toxicological studies. For the purposes of this document, an interaction is considered to occur when the response to the mixture is statistically significantly higher (synergism) or lower (antagonism) than the sum of the individual pollutants. Most animal toxicological studies of O_3 interactions have been conducted with binary mixtures (predominantly NO_2 and H_2SO_4). The rarer reports on complex mixtures are interesting, but less helpful because often the studies did not include a group exposed only to O_3, and therefore knowledge of the role of O_3 is confounded. Thus, only the binary mixture studies will be summarized here. This research has demonstrated that exposure to O_3 in combination with another chemical can result in antagonism, additivity, or synergism,

depending on the animal species, exposure regimen, and endpoint studied. Interpretation is further complicated by the fact that most studies used exposure regimens unlike the real world in terms of ratios of pollutant concentrations, "natural" sequencing of exposure patterns, and other factors. For example, when O_3 and NO_2 exposures were sequential (in any order), there was an additive increase in BAL protein, as compared to a synergistic increase when the exposures were concurrent (Gelzleichter et al., 1992a).

A range of interactions has been shown with O_3 and NO_2 combinations. For example, a 2-week exposure to an O_3-NO_2 mixture (0.4 ppm of both) synergistically increased antioxidants in the lungs of rats but not guinea pigs, peroxide levels were synergistically increased in guinea pigs but not rats, and GST activity was decreased in guinea pigs and unchanged in rats (Ichinose and Sagai, 1989). Most of the interaction studies using lung biochemical endpoints display synergism. A rare exception was the antagonism to the increase in lung cytochrome P-450 content caused by 0.2 ppm O_3 (1 to 2 mo) when the rats were coexposed to 4 ppm NO_2 (Takahashi and Miura, 1989). Combinations of various acute exposure durations and of O_3 and NO_2 concentrations did not follow a $C \times T$ relationship for increased lung permeability, but were synergistic at higher $C \times T$ products (Gelzleichter et al., 1992b). For pulmonary host defenses against bacterial infection, the interaction is dependent on the exposure pattern. Graham et al. (1987) showed that a 15-day exposure of mice to mixtures of O_3 and NO_2, each having a baseline level with two daily 1-h peaks of the pollutant, resulted in synergism only when exposure to either gas alone caused an increase in bacterial-induced mortality.

Both synergistic and antagonistic interactions have been found with combinations of O_3 and acidic sulfates. Warren et al. (1986) reported that with 3 days of exposure to 0.2 ppm O_3 + 5 mg/m^3 $(NH_4)_2SO_4$, O_3 alone was responsible for increasing BAL protein, collagen synthesis rate, and other parameters, but, by 7 days of exposure, synergism occurred. When rabbits were exposed for 4 mo (2 h/day, 5 days/week) to 0.1 ppm O_3 + 125 μg/m^3 H_2SO_4, there was a synergistic increase in epithelial secretory cell number, whereas 8 mo of exposure resulted in antagonism (Schlesinger et al., 1992a). Antagonism also was observed for effects or certain AM functions after acute exposures to O_3-H_2SO_4 mixtures (Schlesinger et al., 1992b). Sequential exposures to O_3 and H_2SO_4 also have been examined. Exposure to O_3 did not influence the subsequent effects of H_2SO_4 on bronchoconstriction in guinea pigs (Silbaugh and Mauderly, 1986). Gardner et al. (1977) found an additive increase in bacterial infectivity when mice were exposed acutely to 0.1 ppm O_3 before (but not after) H_2SO_4.

In summary, the animal toxicological studies clearly demonstrate the major complexities and potential importance of interactions, but do not provide a scientific basis for predicting the results of interactions under untested ambient exposure scenarios.

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7

Human Health Effects of Ozone and Related Photochemical Oxidants

7.1 Introduction

In the previous chapter, results of ozone (O_3) studies in laboratory animals were presented in order to understand the wide range of potential effects that might occur in exposed human populations and to expand the understanding of the mechanisms of O_3 toxicity and the basic exposure-response relationships for O_3. The concept of quantitatively extrapolating results from laboratory animals to humans is further explored in Chapter 8. Whenever possible, however, risk assessment of pollutants should be based on direct evidence of their health effects in human populations. Information on human health responses to O_3 can be obtained through controlled human exposure studies on volunteer subjects or through field and epidemiological studies of populations that are exposed to ambient air containing O_3. Controlled human studies typically use fixed concentrations of O_3 under carefully regulated environmental conditions, whereas realistic O_3 exposure conditions occur in field and epidemiology studies, but are more variable. The primary purpose of all these studies, however, is to obtain exposure-response data for O_3. This chapter will summarize the results of controlled human, field, and epidemiologic studies on the health effects of exposure to O_3 that have been published or accepted for publication in the peer-reviewed literature. Further evaluation of the most important key information from this chapter, as it relates to the rest of the document, will be provided in Chapter 9, where the overall database on O_3 health effects is integrated and summarized.

Most of the scientific information selected for review and comment in this chapter comes from the literature published since completion of the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986). Some of these newer studies were briefly reviewed in the supplement to that document (U.S. Environmental Protection Agency, 1992), but more thorough evaluation of these studies is included here. In order to give a broader overview of the known human health effects of O_3, the older literature is summarized, and specific studies whose data were judged to be significant because of their usefulness in deriving the current National Ambient Air Quality Standards (NAAQS) are discussed briefly. The reader is, however, referred to the more extensive discussion of these key studies in the previous document. Other, older studies also are briefly discussed in this chapter if they are (1) open to reinterpretation because of newer data or (2) potentially useful as criteria for the O_3 NAAQS reevaluation. To further aid in the development of this chapter, summary tables of the relevant O_3 literature are included for each of the major subsections. In summarizing the human health effects literature, changes from control are described if they were

statistically significant at a probability (p) value less than 0.05. A specific p value is provided, however, if it aids understanding of the data, particularly trends toward significance, or if major effects need to be emphasized. Where appropriate, critique of a statistical procedure also is mentioned.

7.2 Controlled Human Exposure Studies

7.2.1 Pulmonary Function Effects of One- to Three-Hour Ozone Exposures

7.2.1.1 Healthy Subjects

Introduction

The pulmonary responses observed in healthy human subjects exposed to ambient O_3 concentrations consist of decreased inspiratory capacity; mild bronchoconstriction; rapid, shallow breathing pattern during exercise; and subjective symptoms of cough and pain on deep inspiration. In addition, O_3 has been shown to result in airway hyperresponsiveness as demonstrated by an increased physiological response to a nonspecific stimulus. The decrease in inspiratory capacity results in a decrease in forced vital capacity (FVC) and total lung capacity (TLC) and, in combination with bronchoconstriction, contributes to a decrease in the forced expiratory volume in 1 s (FEV_1). However, it is important to stress that in many of the studies reporting the effects of ambient ranges of O_3 concentrations (i.e., <0.3 ppm), the observed decrements in FEV_1, to a large extent, reflect decrements in FVC of a similar magnitude (i.e., a decreased inspiratory capacity) and, to a lesser extent, increases in central and peripheral airway resistance (R_{aw}).

The majority of controlled human studies have been concerned with the effects of various O_3 concentrations in healthy subjects performing continuous exercise (CE) or intermittent exercise (IE) for variable periods of time. These studies have two weaknesses: (1) the failure to detect short-term effects with long-term consequences and (2) their use of small numbers that are not generally representative of the general population. Controlled human exposure studies of this type have provided the strongest and most quantifiable concentration-response data on the health effects of O_3. As a result of these studies, a large body of data regarding the interaction of O_3 concentration (C), minute ventilation ($\dot{V}_E$), and duration of exposure (T) is available. The most salient observations from these studies are (1) O_3 concentration is more important than either $\dot{V}_E$ or T in determining pulmonary responses and (2) normal, healthy subjects exposed to O_3 concentrations ≥ 0.12 ppm (the level of the current NAAQS) develop significant reversible, transient decrements in pulmonary function if $\dot{V}_E$ or T are increased sufficiently. There is typically a large intersubject variability in physiologic and symptomatic responses to O_3; however, with most individuals these responses tend to be reproducible. The relationship among response variables such as spirometry, resistance measurements, symptoms, and nonspecific bronchial responsiveness is yet to be fully determined, but the generally weak associations suggest that several response mechanisms may be operant. In addition, a growing number of studies are beginning to provide insight into the relationship between regional dosimetry (see Chapter 8), mechanisms of pulmonary responses elicited by acute O_3 exposure, and tissue level events within the airways. This type of information promises to provide further insight into the health effects relevance of O_3-induced pulmonary responses in determining which individuals are at greatest risk from ambient O_3 exposure.

In this section, the effects of acute (single 1- to 3-h) O_3 exposures on pulmonary function in healthy subjects are examined by reviewing studies that investigate (1) the O_3 exposure-response relationship; (2) intersubject variability, individual sensitivity, and the association between responses; and (3) mechanisms of pulmonary function responses and the relationship between tissue-level events and functional responses. Unless otherwise stated, the term "significant" is used in this section to denote statistical significance at $p < 0.05$. Recent, single O_3 exposure studies of greater than 3 h duration are reviewed in Section 7.2.2. These single-exposure, longer duration studies are beginning to provide important insights into the $C \times T \times \dot{V}_E$ interaction related to a significant pulmonary response. Key studies of less than 3 h duration that have contributed to the exposure-response database and other studies that have contributed to a better understanding of O_3-induced pulmonary responses in healthy individuals are summarized in Table 7-1. Table 7-1 summarizes studies reviewed in the previous air quality criteria document (U.S. Environmental Protection Agency, 1986), as well as studies published since completion of this earlier document. Not reviewed in this section are studies that examine changes in airway responsiveness induced by O_3 inhalation (see Section 7.2.3). All of the studies discussed here used appropriate controls and therefore, for simplicity, the text will not indicate for each study that subjects were also exposed under similar conditions to filtered air (FA [reported at 0 ppm O_3]).

The Ozone Concentration-Response Relationship

At-Rest Exposures. No new studies examining the acute effects of a single exposure to O_3 concentrations below 1 ppm in resting humans have been published since the 1986 U.S. Environmental Protection Agency (EPA) criteria document (U.S. Environmental Protection Agency, 1986). Seven studies (Young et al., 1964; Bates et al., 1972; Silverman et al., 1976; Folinsbee et al., 1978; Horvath et al., 1979; Kagawa and Tsuru, 1979; König et al., 1980) examining 2-h, at-rest exposures were discussed in the 1986 EPA criteria document (U.S. Environmental Protection Agency, 1986) involving 91 healthy subjects (74 males, 17 females) exposed to O_3 concentrations ranging from 0.1 to 1.0 ppm. The lowest concentration at which significant reductions in FVC and FEV_1 were reported was 0.5 ppm (Folinsbee et al., 1978; Horvath et al., 1979). Reports of increases in R_{aw} are inconsistent in resting human subjects exposed to O_3 concentrations below 1.0 ppm.

Exposure with Exercise. Bates et al. (1972) and Hazucha et al. (1973) were the first investigators to examine the effect on pulmonary function responses of increasing ventilation via exercise during O_3 inhalation. The IE protocol used consisted of the subjects alternating rest and light exercise on a cycle ergometer at a rate sufficient to double resting $\dot{V}_E$ for 15 min during a period of 2 h.

Hazucha et al. (1973) observed significant decreases in forced expiratory endpoints at 0.37 ppm O_3 ($p < 0.05$) and 0.75 ppm O_3 ($p < 0.001$), with subjects exposed to 0.75 ppm having the greatest decrements. After exposures, all subjects complained to varying degrees of substernal soreness, chest tightness, and cough. The important findings from these early studies were that the exercise-induced increase in $\dot{V}_E$ accentuated the observed pulmonary response at any given O_3 concentration and lowered the minimum O_3 concentration at which significant pulmonary responses were observed. Subsequently, the interaction between O_3 concentration and $\dot{V}_E$ was examined by using similar IE protocols in which both O_3 concentration and level of $\dot{V}_E$ were varied.

Table 7-1. Controlled Exposure of Healthy Human Subjects to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Healthy Adult Subjects at Rest							
0.25 0.50 0.75	490 980 1,470	2 h	NA	8 M 5 F	Young, healthy adults, 21 to 22 years old	FVC decreased with 0.50- and 0.75-ppm O_3 exposure compared with FA; 4% nonsignificant decrease in mean $\dot{V}O_{2max}$ following 0.75 ppm O_3 compared with FA exposure.	Horvath et al. (1979)
0.37 0.50 0.75	726 980 1,470	2 h	NA	20 M 8 F	Young, healthy adults, 19 to 29 years old	Decrease in FEV_1, $V_{25\%VC}$, and $V_{50\%VC}$ with 0.75 ppm O_3 exposure compared to FA.	Silverman et al. (1976)
0.50	980	2 h	NA	40 M	Young, healthy adults, 18 to 28 years old	Decrease in forced expiratory volume and flow.	Folinsbee et al. (1978)
Healthy Exercising Adult Subjects							
0.08 0.10 0.12 0.14 0.16	157 196 235 274 314	2 h IE (4 × 15 min at $\dot{V}_E$ = 68 L/min)	Tdb = 32 °C RH = 38%	24 M	Young, healthy adults, 18 to 33 years old	No significant changes in pulmonary function measurements.	Linn et al. (1986)
0.10 0.15 0.20 0.25	196 294 392 490	2 h IE (4 × 14 min treadmill at mean $\dot{V}_E$ = 70.2 L/min)	Tdb = 22 °C RH = 50%	20 M	Young, healthy NS, 25.3 ± 4.1 (SD) years old	FVC, FEV_1, $FEF_{25-75\%}$, SG_{aw}, IC, and TLC all decreased with (1) increasing O_3 concentration, and (2) increasing time of exposure; threshold for response was above 0.10 ppm but below 0.15 ppm O_3.	Kulle et al. (1985)
0.12 0.18 0.24	235 353 470	1 h competitive simulation exposures at mean $\dot{V}_E$ = 87 L/min	Tdb = 23 to 26 °C RH = 45 to 60%	10 M	10 highly trained competitive cyclists, 19 to 29 years old	Decrease in FVC and FEV_1 for 0.18- and 0.24-ppm O_3 exposure compared with FA exposure; decrease in exercise time for subjects unable to complete the competitive simulation at 0.18 and 0.24 ppm O_3, respectively.	Schelegle and Adams (1986)
0.12 0.18 0.24 0.30 0.40	235 353 470 588 784	2.5 h IE (4 × 15 min treadmill exercise [$\dot{V}_E$ = 65 L/min])	Tdb = 22 °C RH = 40%	20 M 22 M 20 M 21 M 20 M 29 M	Young, healthy adults, 18 to 30 years old	Significant decrease in FVC, FEV_1, and $FEF_{25-75\%}$ at 0.12 ppm O_3; decrease in V_T and increase in f and SR_{aw} at 0.24 ppm O_3.	McDonnell et al. (1983)

Table 7-1 (cont'd). Controlled Exposure of Healthy Human Subjects to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Healthy Exercising Adult Subjects (cont'd)							
0.12 0.18 0.24 0.30 0.40	235 353 470 588 784	2 × 2.5 h IE (4 × 15 min treadmill exercise [$\dot{V}_E$ = 35 L/min/m^2 BSA]). Exposure separated by 48 ± 30 days and 301 ± 77 days	Tdb = 22 °C RH = 40%	8 M 8 M 5 M 5 M 6 M	Young, healthy adults, 18 to 30 years old	Pulmonary function variables SR_{aw} and $\dot{V}_E$ were not significantly different in repeat exposures, indicating that the response to 0.18 ppm O_3 or higher is reproducible.	McDonnell et al. (1985b)
0.12 0.18 0.24 0.30 0.40	235 353 470 588 784	2 × 2.5 h IE (4 × 15 min treadmill exercise [$\dot{V}_E$ = 35 L/min/m^2 BSA])	Tdb = 22 °C RH = 40%	290 M	Young, healthy adults, 18 to 32 years old	O_3 concentration and age predicted FEV_1 decrements; it was concluded that age is a significant predictor of response (older subjects being less responsive to O_3).	McDonnell et al. (1993)
0.12 0.18 0.24 0.30 0.40	235 353 470 588 784	2.5 h IE (4 × 15 min treadmill exercise [$\dot{V}_E$ = 25 L/min/m^2 BSA])	Tdb = 22 °C RH = 40%	17 WM/15 BM/15 WF/ 15BF 15 WM/15 BM/15 WF/ 16BF 15 WM/17 BM/17 WF/ 15BF 16 WM/15 BM/17 WF/ 16BF 15 WM/15 BM/15 WF/ 15BF 15 WM/15 BM/15 WF/ 15BF	Young, healthy whites and blacks, 18 to 35 years old	Decreases in FEV_1 for all levels of O_3 as compared with FA; increase in SR_{aw} with 0.18 ppm O_3 and greater compared with FA; black men and women had larger FEV_1 decrements than white men, and black men had larger FEV_1 decrements than white women.	Seal et al. (1993)
0.12 0.20	235 392	1 h CE (mean $\dot{V}_E$ = 89 L/min)	Tdb = 31 °C	15 M 2 F	Highly trained competitive cyclists, 19 to 30 years old	Decrease in $\dot{V}_{Emax}$, $\dot{V}O_{2max}$, V_{Tmax}, work load, ride time, FVC, and FEV_1 with 0.20 ppm O_3 exposure during maximal exercise conditions, but not significant with 0.12 ppm O_3 exposure, as compared to FA exposure.	Gong et al. (1986)

Table 7-1 (cont'd). Controlled Exposure of Healthy Human Subjects to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Healthy Exercising Adult Subjects (cont'd)							
0.16 0.24 0.32	314 470 627	1 h CE (mean $\dot{V}_E$ = 57 L/min)	Tdb = 32 °C RH = 42 to 46%	42 M 8 F	Competitive bicyclists, 26.4 ± 6.9 (SD) years old	Small decrements in FEV_1 at 0.16 ppm with larger decrements at 0.24 ppm O_3.	Avol et al. (1984)
0.20	392	4 h IE (4 × 50 min cycle ergometry or treadmill running [$\dot{V}_E$ = 40 L/min])	Tdb = 20 °C RH = 50%	11 M 3 F (FA exposure); 9 M 3 F (O_3 exposure)	Adult, healthy NS, 19 to 41 years old	Decrease in FVC, FEV_1, V_T, and SR_{aw} and increase in f with O_3 exposure compared with FA; total cell count and LDH increased in isolated left main bronchus lavage and inflammatory cell influx occurred with O_3 exposure compared to FA exposure.	Aris et al. (1993a)
0.20 0.30	392 588	30 to 80 min CE cycle ergometry ($\dot{V}_E$ = 33 or 66 L/min)	Tdb = 20 to 24 °C RH = 40 to 60%	8 M	Aerobically fit, 22 to 46 years old	O_3 effective dose was significantly related to pulmonary function decrements and exercise ventilatory pattern changes; multiple regression analysis showed that O_3 concentration accounted for the majority of the pulmonary function variance.	Adams et al. (1981)
0.20 0.35	392 686	1 h CE or competitive simulation (mean $\dot{V}_E$ = 77.5 L/min)	Tdb = 23 to 26 °C RH = 45 to 60%	10 M	Well-trained distance runners, 19 to 31 years old	Decrease in FVC, FEV_1, and $FEF_{25-75\%}$ with 0.20 and 0.35 ppm O_3 exposure compared with FA; V_T decreased and f increased with continuous 50-min O_3 exposures; three subjects unable to complete continuous and competitive protocols at 0.35 ppm O_3.	Adams and Schelegle (1983)
0.21	412	1 h CE (75% $\dot{V}O_{2max}$)	Tdb = 19 to 21 °C RH = 60 to 70%	6 M 1 F	Well-trained cyclists, 18 to 27 years old	Decrease in FVC, FEV_1, $FEF_{25-75\%}$, and MVV with 0.21 ppm O_3 compared with FA exposure.	Folinsbee et al. (1984)
0.21	412	1 h CE cycle ergometry (mean $\dot{V}_E$ = 80 L/min)	Tdb = 22.5 °C RH = 58.8%	14 M 1 F	Highly fit endurance cyclists, 16 to 34 years old	No significant differences in the effects of albuterol on metabolic data, pulmonary function, airway reactivity, and exercise performance vs. placebo; decrease in $\dot{V}_{Emax}$ during O_3 conditions.	Gong et al. (1988)
0.25	490	1 h CE (mean $\dot{V}_E$ = 63 L/min)	Tdb = 20 °C RH = 70%	19 M 7 F	Active nonathletes	FVC, FEV_1, and MVV all decreased with 0.25 ppm O_3 exposure compared with FA.	Folinsbee et al. (1986)
0.25	490	1 h CE cycle ergometer ($\dot{V}_E$ = 30 L/min/m^2 BSA)	NA	5 M 2 F	Young, healthy NS, 22 to 30 years old	12.4% decrease in FEV_1. Significant elevation of substance P and 8-epi-$PGF_{2\alpha}$ in segmental airway washing, but not bronchoalveolar lavage fluid.	Hazbun et al. (1993)

Table 7-1 (cont'd). Controlled Exposure of Healthy Human Subjects to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Healthy Exercising Adult Subjects (cont'd)							
0.30	588	1 h CE cycle ergometry (mean $\dot{V}_E$ = 60 L/min)	NA	5 M	Normal	Decrease in FVC and FEV_1 and increase in SR_{aw} 1 h post-O_3 exposure; increase in percent PMNs at 1, 6, and 24 h post-O_3 exposure compared with FA in first aliquot "bronchial" sample. PMNs peaked at 6 h post-O_3 in "bronchial" sample. Percent PMNs elevated at 6 and 24 h post-O_3 in pooled aliquots.	Schelegle et al. (1991)
0.30	588	1 h CE cycle ergometry ($\dot{V}_E$ = 60 L/min) and 2 h IE cycle ergometry ($\dot{V}_E$ = 45 to 47 L/min)	Tdb = 21 to 25 °C RH = 45 to 60%	12 M	Moderately fit, young and healthy	Decrease in FEV_1 equivalent for all protocols.	McKittrick and Adams (1995)
0.35	686	1 h CE cycle ergometry (mean $\dot{V}_E$ = 60 L/min)	Tdb = 21 to 25 °C RH = 45 to 60%	14 M	Moderately fit, young, healthy adults, 18 to 34 years old	Significant decreases in FVC and FEV_1 with O_3 exposure compared to FA exposure; FVC and FEV_1 decreases with O_3 exposure were attenuated significantly with indomethacin compared to no drug and placebo; SR_{aw} increases were not affected by indomethacin.	Schelegle et al. (1987)
0.37 0.50 0.75	726 980 1,470	2 h IE cycle ergometry ($\dot{V}_E$ = 2.5 × rest)		20 M 8 F	Young, healthy adults, 19 to 29 years old	Decrease in FVC with 0.50 ppm and FEV_1 with 0.50 and 0.75 ppm O_3 compared to FA; decrease in $V_{25\%VC}$ with 0.37 and 0.75 ppm and $V_{50\%VC}$ with 0.37, 0.50, and 0.75 ppm O_3 exposure compared to FA.	Silverman et al. (1976)
0.40	784	2 h IE treadmill exercise ($\dot{V}_E$ = 50 to 75 L/min)	Tdb = 22 °C RH = 40%	8 M	Young, healthy NS, 18 to 27 years old	Decreases in FVC, FEV_1, V_T, and TLC and increases in SR_{aw} and f with O_3 exposure compared with FA. Atropine pretreatment abolished O_3-induced increase in SR_{aw} and attenuated FEV_1 and $FEF_{25-75\%}$ response.	Beckett et al. (1985)
0.40	784	1 h CE treadmill exercise; ($\dot{V}_E$ = 20 L/min/m^2 BSA)	NA	20 M	Young, healthy NS	V_T fell by 25%, and O_3 uptake efficiency in the lower respiratory tract fell by 9% during O_3 exposure.	Gerrity et al. (1994)
0.40	784	2 h IE (4 × 15 min heavy treadmill exercise [$\dot{V}_E$ = 35 L/min/m^2 BSA])	NA	11 M	Young, healthy NS, 18 to 35 years old	No correlation between pulmonary function and inflammatory endpoints measured in BAL fluid obtained 18 h after exposure; increase in percentage of PMNs, total protein, albumin, IgG, and neutrophil elastase; decrease in percentage of macrophages with O_3 exposure compared to FA exposure.	Koren et al. (1989a)

Table 7-1 (cont'd). Controlled Exposure of Healthy Human Subjects to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Healthy Exercising Adult Subjects (cont'd)							
0.40	784	2 h IE (4 × 15 min heavy treadmill exercise [$\dot{V}_E$ = 35 L/min/m^2 BSA])	Tdb = 22 °C RH = 40%	10 M	Young, healthy NS, 18 to 35 years old	PMN, PGE_2, and IL-6 were higher in BAL fluid obtained 1 h post-O_3 exposure than 18 h; fibronectin and urokinase-type plasminogen activator were higher 18 h post-O_3 exposure than 1 h.	Koren et al. (1991)
0.40	784	2 h IE (4 × 15 min bicycle ergometry [$\dot{V}_E$ = 30 L/min/m^2 BSA])	Tdb = 22 °C RH = 50%	13 M	NS, 18 to 31 years old	Indomethacin pretreatment and O_3 exposure resulted in a significantly smaller decrease in FVC and FEV_1 than O_3 exposure alone; airway hyperresponsiveness was not significantly affected by indomethacin pretreatment.	Ying et al. (1990)
0.40	784	1 h CE (treadmill exercise; $\dot{V}_E$ = 20 L/min/m^2 BSA)	Tdb = 22 °C RH = 40%	22 M	Young, healthy NS, 18 to 35 years old	Significant decreases in FVC, FEV_1, FEV_1/FVC, and $FEF_{25-75\%}$. The half-width of an expired aerosol bolus was significantly increased, suggesting an ozone-induced change in small airway function.	Keefe et al. (1991)
0.40 0.60	784 1,176	2 h IE (4 × 15 min cycle ergometry at 100 W for males and 83 W for females)	Tdb = 71.5 °C RH = 55%	7 M 3 F	Healthy NS, 23 to 41 years old	Increase in airway responsiveness to methacholine challenge, in mean percentage of neutrophils, and in $PGF_{2\alpha}$, TXB_2, and PGE_2 concentrations measured in BAL fluid 3 h after 0.40- and 0.60-ppm O_3 exposure compared with FA exposure.	Seltzer et al. (1986)
0.50	980	2 h IE (4 × 15 min treadmill exercise; $\dot{V}_E$ = 40 L/min)	Tdb = 21 °C RH = 40%	18 M	Healthy, young adults, 20 to 30 years old	Decrease in VC, V_T, and maximal transpulmonary pressure, and increase in SR_{aw} and f with O_3 exposure compared to FA exposure; lidocaine inhalation partially reversed the decrease in VC.	Hazucha et al. (1989)
0.75	1,470	2 h IE (4 × 15 min light [50 W] cycle ergometry)	NA	13 M	4 light S, 9 NS, 19 to 30 years old	Decrease in FVC, FEV_1, ERV, IC, and $FEF_{50\%}$ after 1 h exposure to 0.75 ppm O_3; decrease in $\dot{V}O_{2max}$, V_{Tmax}, V_{Emax}, maximal workload, and heart rate following 0.75-ppm O_3 exposure compared with FA.	Folinsbee et al. (1977)

[a]See Appendix A for abbreviations and acronyms.
[b]Grouped by rest and exercise; within groups listed from lowest to highest O_3 concentration.

Silverman et al. (1976) and Folinsbee et al. (1975) exposed a group of 20 males and 8 females to 0.37, 0.50, or 0.75 ppm for 2 h while resting or exercising intermittently. The IE protocol used alternated 15 min of rest with 15 min of exercise, sufficient to increase the $\dot{V}_E$ value at rest by a factor of 2.5. The submaximal exercise responses of the subjects were tested postexposure using a three-stage cycle ergometer test, with loads adjusted to 45, 60, and 75% of maximum oxygen uptake ($\dot{V}O_{2max}$) (Folinsbee et al., 1975). Pulmonary function responses were related to the total inhaled dose or the "effective dose" of O_3 calculated as the product of $C \times T \times \dot{V}_E$. Neither submaximal exercise oxygen uptake ($\dot{V}O_2$) nor $\dot{V}_E$ were affected significantly by any level of O_3 exposure; however, a significant increase in respiratory frequency (f) and a significant decrease in tidal volume (V_T) at the 75% $\dot{V}O_{2max}$ workload were observed. The relationship between the effective dose of O_3 and the mean percent change in selected measures of lung function was analyzed using linear regression. Forced vital capacity, maximum expiratory flow at 25 and 50% of FVC ($\dot{V}_{max25\%}$ and $\dot{V}_{max50\%}$, respectively), and FEV_1 were found to have a significant linear correlation with the effective dose. The description of the relationship between O_3 pulmonary function decrements and effective dose was apparently improved by the use of a second-order polynomial model in which effective dose was used as the independent variable.

Although the investigations of Silverman et al. (1976) and others (Bates et al., 1972; Hackney et al., 1975; Hazucha et al., 1973) clearly demonstrate the potentiating effects of exercise on O_3 responses, the level of exercise used in these studies was low, requiring increases in $\dot{V}_E$ of only 2 to 2.5 times resting, a level of exercise lower than that of a subject walking at 5.5 km/h (DeLucia and Adams, 1977). In order to address this concern, DeLucia and Adams (1977) exposed six healthy nonsmoking male subjects on 12 separate occasions to FA and 0.15 and 0.30 ppm O_3 for 1 h, while at rest and while exercising continuously at workloads that required 25, 45, and 65% of the subjects' $\dot{V}O_{2max}$. They observed a significant time-dependent increase in f during the 65% $\dot{V}O_{2max}$, 0.30-ppm O_3 exposure, and, immediately following this same exposure, there was a significant decrease in FEV_1 and forced expiratory flow at 25 to 75% of FVC ($FEF_{25-75\%}$).

These initial studies, which clearly demonstrated the potentiating effects of exercise on human responses to acute O_3 exposure, provided the impetus for a series of studies (Adams et al., 1981; Folinsbee et al., 1978; McDonnell et al., 1983; Kulle et al., 1985; Linn et al., 1986) designed to define more precisely O_3 exposure-response relationships. These investigations utilized both IE (Folinsbee et al., 1978; McDonnell et al., 1983) and CE (Adams et al., 1981) of varying intensity. Folinsbee et al. (1978) exposed four groups of 10 subjects each to FA, 0.1, 0.3, and 0.5 ppm O_3 for 2 h. One group was exposed while at rest, and the other three groups were exposed while performing IE at levels requiring a ventilation of 30, 50, or 70 L/min. These combinations of ventilation and O_3 concentration ($C \times T \times \dot{V}_E$) resulted in a range of total inhaled effective dose of 0.00 to 4.41 mg O_3. Adams et al. (1981) exposed eight trained male subjects to FA, 0.2, 0.3, and 0.4 ppm O_3 while they exercised continuously at two different workloads (35 and 62% of $\dot{V}O_{2max}$) for durations ranging from 30 to 80 min. Each subject completed all 18 protocols with at least 3 days between each. The findings from these two studies confirmed that significant pulmonary responses occurred at 0.3 ppm when subjects exercised at moderately heavy workloads. It was further demonstrated, by multiple regression analysis, that the O_3 effective dose was a better predictor of response than O_3 concentration, $\dot{V}_E$, or duration of exposure, alone. Multiple regression analysis also revealed that the majority of variance

for pulmonary function responses was accounted for by O_3 concentration, followed by $\dot{V}_E$. In the Adams et al. (1981) study, in which both workload and duration of exposure were varied, duration of exposure was observed to be the poorest predictor of response for all parameters analyzed. However, the minor impact of changes in exposure duration could have been an artifact of the limited combinations of ventilation and durations of exposure used by these investigators.

McDonnell et al. (1983) conducted a study with the primary purpose of discerning the lowest concentration of O_3 at which group mean decrements in pulmonary function occur in heavily exercising healthy men. In order to determine a concentration-response relationship, six groups of subjects (n = 20 to 29) were exposed to either an FA control or one of five O_3 concentrations (0.12, 0.18, 0.24, 0.30, or 0.40 ppm) at a $\dot{V}_E$ of 67 L/min and exposure duration of 2.5 h (15-min rest, 15-min exercise). These investigators observed small significant changes in FVC, FEV_1, $FEF_{25-75\%}$, and cough at 0.12 ppm O_3 and concentration-dependent responses in all variables measured (FVC, FEV_1, $FEF_{25-75\%}$, specific airway resistance [SR_{aw}], f, V_T, and subjective symptoms) at O_3 concentrations >0.24 ppm.

Kulle et al. (1985) also conducted a similar study on healthy, nonsmoking men performing IE at a $\dot{V}_E$ of 70 L/min for an exposure duration of 2 h, (16-min rest, 14-min exercise). Twenty subjects were exposed to an FA control or one of four O_3 concentrations (0.10, 0.15, 0.20, or 0.25 ppm). These investigators observed a significant C × T interaction at 0.15 ppm O_3 for FVC, FEV_1, $FEF_{25-75\%}$, and in all variables measured (FVC, FEV_1, $FEF_{25-75\%}$, SR_{aw}, f, V_T, and subjective symptoms) at O_3 concentrations greater than 0.15 ppm.

Linn et al. (1986) exposed 24 healthy, well-conditioned male subjects (18 to 33 years of age) for 2 h to 0.00, 0.08, 0.10, 0.12, 0.14, or 0.16 ppm O_3, using an IE protocol (15-min rest, 15-min exercise; $\dot{V}_E$ = 68 L/min) combined with an ambient heat stress (32 °C and 38% relative humidity [RH]). They observed no statistically significant changes in forced expiratory endpoints and symptoms after exposure to O_3 concentrations from 0.08 to 0.14 ppm. These authors observed a small (−2.3%) but significant ($p < 0.05$) reduction in FEV_1, which was not associated with symptoms of respiratory discomfort, following the 2-h 0.16-ppm O_3 exposure.

More recently, Seal et al. (1993) examined whether gender or race differences exist in responsiveness to O_3. The authors exposed 372 white and black, males and females (n > 90 in each gender-race group) once for 2.33 h to 0.0, 0.12, 0.18, 0.24, 0.30, or 0.40 ppm O_3 using an IE protocol (15-min rest, 15-min exercise; $\dot{V}_E$ = 25 L/min/m^2 body surface area [BSA]). Statistical analysis (nonparametric two-factor analysis of variance) of the percent changes from baseline for FEV_1, SR_{aw}, and cough responses demonstrated no significant differences in responsiveness to O_3 between the gender-race groups studied. Changes in FEV_1, SR_{aw}, and cough were first noted at 0.12, 0.18, and 0.18 ppm O_3, respectively, for the group as a whole. It is difficult to compare the results from this study with other studies that have examined the O_3 concentration-response relationship in healthy adult males because the authors did not present a separate analysis of male responses. For further evaluation of the influence of gender and race on O_3 responsiveness, see Section 7.2.1.3.

The observation of significant decrements in pulmonary function in heavily exercising healthy subjects at O_3 concentrations of 0.2 ppm and lower has been confirmed by numerous investigators (Adams and Schelegle, 1983; Avol et al., 1984; Folinsbee et al.,

1984; Gong et al., 1986) who utilized 1-h continuous heavy exercise exposure protocols. Adams and Schelegle (1983) and Folinsbee et al. (1984) observed significant decrements in FVC and FEV_1 in well-trained subjects exposed to 0.2 ppm O_3 while exercising with a $\dot{V}_E$ of approximately 80 L/min. Avol et al. (1984) observed small but significant decrements in FVC and FEV_1 in a group of 50 competitive cyclists (42 males, 8 females) exposed to 0.16 ppm O_3 while exercising with a $\dot{V}_E$ of 57 L/min in combination with added heat stress (32 °C). Similarly, Gong et al. (1986) observed modest but significant decrements in FVC and FEV_1 in a group of 17 top-caliber endurance cyclists exposed to 0.12 ppm O_3 while exercising at approximately 70% of their $\dot{V}O_{2max}$ (mean $\dot{V}_E$ = 89 L/min) with an added heat stress (32 °C). In addition to the above studies that used continuous exercise, Schelegle and Adams (1986) observed significant reductions in FVC and FEV_1 and increased symptoms of respiratory discomfort following exposure to 0.18 but not 0.12 ppm O_3 in a group of competitive endurance athletes exposed while performing a competitive simulation consisting of a 30-min warm-up followed by a 30-min competitive bout (mean $\dot{V}_E$ over entire protocol = 87 L/min).

The studies reviewed above demonstrate that in healthy young adults performing moderate to severe IE and CE of 1 to 3 h duration, an O_3 concentration of 0.12 to 0.18 ppm is required to elicit statistically significant decrements in pulmonary function and subjective respiratory symptoms.

Retrospective analysis by Hazucha (1987) confirmed the previously reported (Adams et al., 1981; Folinsbee et al., 1978) dominant role that O_3 concentration plays in determining O_3-induced responses. Hazucha (1987) analyzed data from studies that utilized IE protocols of 2 h in duration. While controlling for ventilation, this investigator found that the data best fit a model that was a quadratic function of O_3 concentration. Based on this analysis, Hazucha (1987) also concluded that an O_3 concentration below which no pulmonary function response would be elicited could not be defined.

The studies reviewed in this subsection used different patterns (i.e., CE or IE) of exercise during their exposure protocols. An important question to ask is to what extent are the results of these studies comparable when total inhaled doses are the same but exercise pattern differs. A recent study by McKittrick and Adams (1995) addresses this question. These investigators exposed 12 aerobically trained men to 0.30 ppm O_3 (three protocols) and FA (three protocols) on six occasions. These protocols consisted of a 1 h CE (O_3 and FA) and two 2-h IE (2 × O_3 and 2 × FA) protocols delivered in random sequence separated by a minimum of 3 days. Lung function FEV_1 decrements of 17.6, 17.0, and 17.9% were obtained for the 1-h CE and the two 2-h IE, 0.3-ppm O_3 protocols, respectively. These values were significantly different from the FA values, but were not significantly different from each other. The O_3 CE protocols resulted in greater postexposure values for subjective symptoms than obtained with either of the O_3 IE protocols. However, the overall symptom severity during the last minute of exercise for the two IE protocols was not significantly different from the CE postexposure value. McKittrick and Adams (1995) concluded that when the total inhaled dose of O_3 is equivalent at a given O_3 concentration, there is no difference between pulmonary function responses induced by CE and IE protocols of 2-h or less duration, although subjective symptoms are reduced slightly during the last rest period of IE.

Intersubject Variability, Individual Sensitivity, and the Association Between Responses

Bates et al. (1972) noted that variation in sensitivity and response was evident for various symptoms and pulmonary functions assessed following O_3 exposure. This observation of large intersubject variability in response to O_3 also has been reported by numerous other investigators (Adams et al., 1981; Folinsbee et al., 1978; McDonnell et al., 1983; Kulle et al., 1985) and is illustrated by data from Kulle et al. (1985) plotted in Figure 7-1. The description of the factors that contribute to intersubject variability is important for the understanding of individual responses, mechanisms of response, and health risks associated with acute exposures. The effect of this large intersubject variability on the ability to predict individual responsiveness to O_3 was recently demonstrated by McDonnell et al. (1993). These investigators analyzed the data of 290 white male subjects (18 to 32 years of age) who inhaled either 0.00, 0.12, 0.18, 0.24, 0.30, or 0.40 ppm O_3 for 2 h while performing an IE protocol ($\dot{V}_E$ = 35 L/min/m^2 BSA) to identify personal characteristics (i.e., age, height, baseline pulmonary functions, presence of allergies, and past smoking history) that might predict individual differences in FEV_1 response. Of the personal characteristics studied, only age contributed significantly to intersubject responsiveness (younger subjects were more responsive), accounting for 4% of the observed variance. Interestingly, O_3 concentration accounted for only 31% of the variance, clearly demonstrating the importance of as yet undefined individual characteristics that determine responsiveness to O_3.

McDonnell et al. (1985b) examined the reproducibility of individual responses to O_3 exposure in healthy human subjects exposed twice, with from 21 to 385 days separating exposures (mean = 88 days). This investigation was conducted in order to determine whether the observed intersubject variability is due primarily to real differences in O_3 responsiveness among subjects, or whether it can be accounted for by other sources of variability. The authors examined FVC, FEV_1, $FEF_{25\text{-}75\%}$, SR_{aw}, cough, shortness of breath (SB), pain on deep inspiration (PDI), V_T, and f responses induced by O_3 exposure to concentrations ranging from 0.12 to 0.40 ppm. Reproducibility was assessed using the intraclass correlation coefficient (R), which incorporates into a single measure all the information contained in the correlation coefficient, slope, and intercept obtained in linear regression analysis. Similar to the more routinely used correlation coefficient, R is equal to one when two identical measurements occur in the same subject; and the "worst" possible coefficient is equal to $1/(n - 1)$, which approaches zero for a large n. The ranking of most to least reproducible for the responses studied was FVC (R = 0.92), FEV_1 (R = 0.91), $FEF_{25\text{-}75\%}$ (R = 0.83), cough (R = 0.77), SB (R = 0.60), SR_{aw} (R = 0.54), PDI (R = 0.37), f (R = −0.20), and V_T (R = −0.03). The value of R was significantly different from zero for FVC, FEV_1, $FEF_{25\text{-}75\%}$, cough, SB, and SR_{aw}. McDonnell et al. (1985b) concluded that the reported large intersubject variability in magnitude of response was due to large differences in the intrinsic responsiveness of individual subjects to O_3 exposure. However, the factors that contribute to this large intersubject variability remain undefined.

The examination of intersubject variability is complicated by a poor association between the various O_3 responses. In their study investigating O_3 exposure-response relationships, McDonnell et al. (1983) observed very low correlation between changes in SR_{aw} and FVC (r = −0.16) for 135 subjects exposed to O_3 concentrations ranging from 0.12 to 0.40 ppm for 2.5 h.

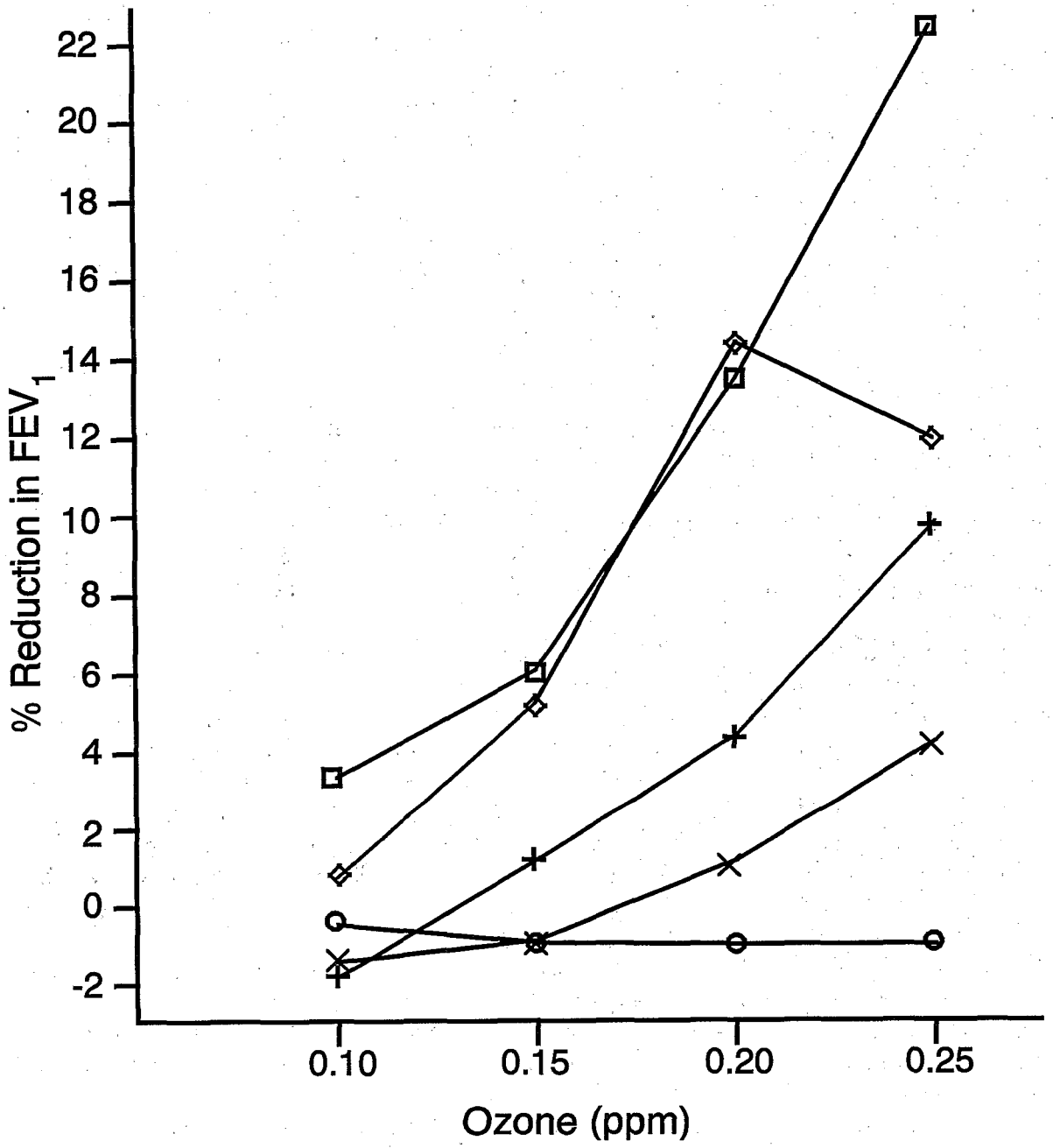

Figure 7-1. Individual concentration-response curves for five separate subjects exposed to 0.10, 0.15, 0.20, and 0.25 ppm ozone (O_3) for 2 h with moderate intermittent exercise. Illustrates the wide variability in responsiveness to O_3 from individual to individual.

Source: Kulle et al. (1985).

Mechanisms of Acute Pulmonary Responses

The pulmonary responses observed during and following acute exposure to O_3 at concentrations between 0.1 and 0.5 ppm in normal healthy human subjects include decreases in TLC, IC, FVC, FEV_1, $FEF_{25-75\%}$, and V_T; and increases in SR_{aw}, f, and airway responsiveness. Ozone exposure also has been shown to result in the symptoms of cough, PDI, SB, throat irritation, and wheezing. When viewed as a whole, changes in these specific parameters can be categorized into four general responses: alterations in (1) lung volumes, (2) airway caliber, (3) bronchomotor responsiveness, and (4) symptoms. The absence of consistent associations among the various responses from individual to individual suggests that the functional responses observed are the result of multiple interactions within the respiratory tract. These interactions may be the result of O_3 action on the biochemical, anatomical, and physiological systems of the respiratory tract. In turn, these factors determine O_3 dose distribution and the resulting cellular and reflex responses.

Bates et al. (1972) observed that the most significant decrement in pulmonary function was the reduction in the transpulmonary pressure at maximal inspiratory volume without a concomitant decrease in static compliance. This would suggest an inhibition of maximal inspiratory effort after O_3 exposure that may result in reductions in IC. These authors speculated that this inhibition is an early result of stimulation of rapidly adapting pulmonary stretch receptors, or "irritant receptors", located in the major bronchi. Since 1972, when this hypothesis was first published, numerous studies have examined the underlying mechanisms leading to the functional responses observed in human subjects. These mechanistic studies have used both animal models and human subjects. This discussion of mechanisms will focus on studies that used human subjects but also will cover those animal studies that have direct relevance to O_3-induced functional responses.

The acute inhalation of ambient concentrations of O_3 by healthy human subjects has been shown to result in a concentration-dependent increase in R_{aw} (Folinsbee et al., 1978; McDonnell et al., 1983; Kulle et al., 1985; Seal et al., 1993). This O_3-induced increase in R_{aw} has been shown to be poorly correlated with changes in forced expiratory endpoints (McDonnell et al., 1983). Ozone-induced increases in R_{aw} have a rapid onset (Beckett et al., 1985) compared with the gradual development of decrements in forced expiratory endpoints (Kulle et al., 1985). Ozone-induced increases in R_{aw} also appear to be greater in atopic subjects as a group (Kreit et al., 1989; McDonnell et al., 1987), although this does not appear to be the case for O_3-induced decrements in FVC and symptoms. Taken together, these observations suggest that different pathways lead to O_3-induced decrements in IC and to O_3-induced increments in R_{aw}.

Increases in R_{aw} induced by O_3 have been shown to be blocked by atropine sulfate pretreatment in human subjects (Beckett et al., 1985; Adams, 1986). This inhibition suggests that the release of acetylcholine from parasympathetic postganglionic fibers that innervate airway smooth muscle plays a role in this response. However, the observation that a 2-h, 0.6 ppm O_3 inhalation also results in a hyperresponsiveness to methacholine, a cholinergic agent (Holtzman et al., 1979), suggests the possibility that acute O_3 exposure also can increase the sensitivity of airway smooth muscle to acetylcholine independent of a reflex mechanism involving cholinergic postganglionic nerves. The role that an increase in airway smooth muscle sensitivity to the endogenous release of acetylcholine might play in O_3-induced increases in R_{aw} has not been studied.

Analyses by Colucci (1983) have suggested that the increase in R_{aw} is not as large as would be expected when O_3 exposure is combined with moderate to heavy exercise.

However, the observation that circulating epinephrine levels increase as a function of the relative workload in exercising human subjects (Galbo, 1983; Warren and Dalton, 1983) suggests that stimulation of airway smooth muscle beta-adrenoreceptors may counteract airway smooth muscle contraction induced by O_3 exposure. The observations by Beckett et al. (1985) that the beta-agonists abolish O_3-induced bronchoconstriction is consistent with this possibility.

Another question to be addressed with regard to O_3-induced increases in R_{aw} is where along the airway (central versus peripheral airways) is the increase in resistance produced? Studies of acutely and subchronically exposed animals have demonstrated tissue damage in the centriacinar region (Castleman et al., 1977; Mellick et al., 1977), as well as increases in peripheral resistance and reactivity (Gertner et al., 1983a,b,c; Beckett et al., 1988). Keefe et al. (1991) examined the possibility of an effect on small airways using an inhaled aerosol bolus dispersion technique in 22 healthy, nonsmoking male subjects exposed to 0.4 ppm O_3 for 1 h using a CE protocol ($\dot{V}_E$ = 20 L/min/m^2 BSA). The bolus dispersion technique is not dependent on vital capacity maneuvers and compares the profile of a bolus of small (0.5- to 1.0-μm) aerosol particles injected into the inspired airstream (at a fixed lung volume) with the profile of the bolus during expiration. Dispersion of the bolus during expiration can be affected by increases in turbulence within the airway, the development of asymmetries in ventilation due to unequal regional time constants within the lung, and an increase in aerosol deposition in the small airways. Keefe et al. (1991) observed that O_3 exposure in their subjects resulted in a significant increase in dispersion of an aerosol bolus (without an increased aerosol deposition) that was not correlated with changes in SR_{aw}. These findings suggest that exposure to 0.4 ppm O_3 under the conditions of this experiment results in changes in small airway function that are not detectable by more conventional techniques.

Ozone-induced alterations in ventilatory pattern have been observed in exercising dogs (Lee et al., 1979) and humans (Adams et al., 1981; Folinsbee et al., 1978; McDonnell et al., 1983; Kulle et al., 1985). In exercising humans, O_3 exposure has been shown to result in a decrease in V_T and an increase in f in the absence of any change in $\dot{V}_E$. A rapid, shallow breathing pattern is consistent with the maintenance of an appropriate ventilation with a reduced VT. Reduction of VT is probably related to the reduction of IC and is anecdotally related to reduction in breathing discomfort caused by PDI.

Lee et al. (1979), who produced a reversible vagotomy by cooling the vagus nerves to 0 °C, abolished the rapid, shallow breathing induced by O_3 inhalation in conscious dogs. More recently, Schelegle et al. (1993) have shown in anesthetized dogs exposed to O_3 that cooling the cervical vagus nerves to 7 °C did not abolish the observed O_3-induced rapid, shallow breathing pattern and bronchoconstriction, but cooling the vagus nerves to 0 °C did abolish both the rapid, shallow breathing and the bronchoconstriction. These findings suggest that O_3 stimulates nonmyelinated C fiber afferents arising from the lung, whose conduction is not totally blocked at 7 °C but is blocked totally at 0 °C. This conclusion is consistent with the findings of Coleridge et al. (1993) that bronchial C fibers are the only receptors that are stimulated directly during O_3 inhalation in anesthetized dogs. If similar bronchial C fibers were stimulated or sensitized in humans exposed to O_3, this could explain the O_3-induced rapid, shallow breathing observed during exercise, as well as the subjective symptoms associated with taking a deep inspiration.

Hazucha et al. (1989) exposed 11 healthy normal volunteers to FA and 0.5 ppm O_3 for 2 h while they were performing moderate IE. Ozone exposure induced a significant

decrement in FVC, which was associated with a marked fall in IC without an increase in residual volume. Spraying of the upper airway with lidocaine aerosol in these subjects was immediately followed by return of FVC toward control values. Hazucha et al. (1989) concluded that O_3 inhalation stimulates lidocaine-sensitive tracheal and laryngeal airway receptors, which leads to an involuntary inhibition of full inspiration, a reduction in FVC, and a concomitant decrease in maximal expiratory flow rates in humans.

The airway afferents blocked by lidocaine in the Hazucha et al. (1989) investigation remain undefined. However, it seems likely that the lung afferents involved are the same ones that result in O_3-induced rapid, shallow breathing in dogs (i.e., bronchial C fibers). When stimulated with exogenous chemicals in animal experiments, bronchial C fibers induce a reflex apnea (Coleridge and Coleridge, 1986). In dogs, this reflex apnea involves the inhibition of inspiratory neurons, expiratory neurons, and γ- and α-motoneurons in the intercostal nerves (Koepchen et al., 1977; Schmidt and Wellhoner, 1970). Such a reflex response in humans would explain the reflex inhibition of maximal inspiration consequent to acute O_3 exposure.

Data consistent with an O_3-induced stimulation of bronchial C fibers in human subjects recently has been published by Hazbun et al. (1993). These investigators observed a significant increase in substance P, the neurotransmitter released from the afferent endings of bronchial C fiber during excitation, in segmental airway washings of seven (2 female/5 male) healthy, nonsmoking subjects after a 1 h CE ($\dot{V}_E$ = 30 L/min/m^2 BSA) exposure to 0.25 ppm O_3. Substance P was not elevated in bronchoalveolar lavage (BAL) fluid after air exposure. In addition, the segmental airway substance P levels were significantly correlated ($r^2 = 0.89$; $p < 0.05$) with an elevated airway concentration of 8-epi-prostaglandin $F_{2\alpha}$, a marker of oxidative free radical reactions. These results are consistent with (1) an increased release of substance P secondary to an increased discharge of bronchial C fibers induced by O_3 inhalation, and (2) an O_3-induced inhibition of neutral endopeptidase, the enzyme that degrades substance P within the airways.

Lung C fibers have been shown to be stimulated by prostaglandin E_2 and other lung autacoids (Coleridge et al., 1978, 1976). Interestingly, Schelegle et al. (1987), Eschenbacher et al. (1989), and Ying et al. (1990) have shown that pretreatment with the cyclooxygenase inhibitor indomethacin reduces and, in some cases, totally abolishes O_3-induced pulmonary function decrements in human subjects. Schelegle et al. (1987) examined whether O_3-induced pulmonary function decrements could be inhibited by the prostaglandin synthetase inhibitor indomethacin in healthy human subjects. Fourteen college-age males completed six 1-h exposure protocols consisting of no drug, placebo, and indomethacin pretreatments, with FA and O_3 (0.35 ppm) exposure within each pretreatment. Pretreatments were delivered weekly in random order in a double-blind fashion. Exposures consisted of 1 h exercise on a cycle ergometer with work loads set to elicit a mean $\dot{V}_E$ of 60 L/min. Statistical analysis revealed significant effects for FVC and FEV_1 across pretreatment, with no drug versus indomethacin and placebo versus indomethacin comparisons being significant. These findings suggest that cyclooxygenase products of arachidonic acid, which are reduced by indomethacin inhibition of cyclooxygenase, play a role in the development of pulmonary function decrements. These and similar findings by Eschenbacher et al. (1989) and Ying et al. (1990) suggest that the release of some cyclooxygenase product consequent to O_3 inhalation plays a role in O_3-induced pulmonary function decrements. This idea is supported by the findings of Koren et al. (1991), who obtained a positive correlation between O_3-induced pulmonary function decrements and the

level of prostaglandin E_2 (PGE_2) in BAL fluid collected within 1 h after the end of exposure in human subjects who varied greatly in O_3 responsiveness.

The release of cyclooxygenase products of arachidonic acid from injured airway epithelium can thus be viewed as a link in a cascade of events, which begins with the initial reaction of O_3 with the tissues and ends with the observed pulmonary function responses. The apparent components of this chain of events include factors that influence (1) O_3 delivery to the tissue (e.g., the inhaled concentration, breathing pattern, and airway geometry); (2) O_3 reactions with components in airway surface liquid and epithelial cell membranes; (3) local tissue responses, including injury and inflammation; and (4) stimulation of neural afferents (bronchial C fibers) and the resulting reflex responses. It still is not understood how each event in this cascade contributes to the pulmonary responses induced by acute O_3 inhalation.

The influence that individual responsiveness has on this cascade of events has not been determined; however, recent data suggest that individual O_3 responsiveness may feed back and influence the distribution of O_3 dose within the lung. Gerrity et al. (1994) tested the hypothesis that O_3-induced rapid, shallow breathing helps to limit the dose of O_3 reaching the lower respiratory tract. They found that the degree of O_3-induced rapid, shallow breathing (25% decrease in V_T) was significantly correlated with a decrease in O_3 uptake efficiency of the lower respiratory tract. This observation may explain the recent data of Schelegle et al. (1991) and Aris et al. (1993a) that suggest individual responsiveness to O_3 as measured by FEV_1 decrements may be negatively correlated with the number of neutrophils (PMNs) present in BAL samples. However, the interrelationship among the responsiveness to O_3, the distribution of dose within the airway, and resulting airway inflammation is still poorly understood.

7.2.1.2 Subjects with Preexisting Disease

Introduction

Ten studies (König et al., 1980; Linn et al., 1982a; Koenig et al., 1985; Linn et al., 1978, 1983a; Solic et al., 1982; Kehrl et al., 1985; Superko et al., 1984; Silverman, 1979; Kulle et al., 1984) examining the pulmonary responses to acute O_3 exposures of less than 3 h in patients with preexisting disease were discussed in the 1986 criteria document (U.S. Environmental Protection Agency, 1986). This section examines the effects of O_3 exposure on pulmonary function in subjects with preexisting disease by reviewing O_3 exposure studies that utilized subjects with (1) chronic obstructive pulmonary disease (COPD), (2) asthma, (3) allergic rhinitis, and (4) ischemic heart disease. Because of their important health implications, all of the available studies are reviewed and summarized in Table 7-2. Unless otherwise stated, the term "significant" is used to denote statistical significance at $p < 0.05$.

Subjects with Chronic Obstructive Pulmonary Disease

In five of the studies cited above, the O_3-induced pulmonary function responses of patients with mild to moderate COPD were examined (König et al., 1980; Linn et al., 1982a, 1983a; Solic et al., 1982; Kehrl et al., 1985). No significant changes in pulmonary function or symptoms were reported in any of the studies of the effects of O_3 in patients with COPD. Four of these studies (Linn et al., 1982a, 1983a; Solic et al., 1982; Kehrl et al., 1985) examined the effects of O_3 concentrations between 0.1 and 0.3 ppm O_3 in 66 mild to moderate COPD patients using mild IE exposure protocols of 1 to 2 h duration. The total

Table 7-2. Ozone Exposure in Subjects with Preexisting Disease[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity	Exposure Condition	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Subjects with Chronic Obstructive Pulmonary Disease							
0.12	236	1 h IE (2 × 15 min light bicycle ergometry)	Tdb = 25 °C RH = 50%	18 M, 7 F	8 smokers, 14 ex-smokers, 3 nonsmokers; FEV_1/FVC = 32 to 66%	No significant changes in pulmonary function measurements; small significant decrease in arterial O_2 saturation.	Linn et al. (1982a)
0.18 0.25	353 490	1 h IE (2 × 15 min light bicycle ergometry)	Tdb = 25 °C RH = 50%	15 M, 13 F	15 smokers, 11 ex-smokers, 2 nonsmokers; FEV_1/FVC = 36 to 75%	No significant changes in pulmonary function measurements; no significant change in arterial O_2 saturation.	Linn et al. (1983a)
0.20	392	2 h IE (4 × 7.5 min light treadmill running)	Tdb = 22 °C RH = 40%	13 M	8 smokers, 4 ex-smokers, 1 nonsmoker; productive cough; FEV_1/FVC = 46 to 70%	No significant changes in pulmonary function measurements; small significant decrease in arterial O_2 saturation.	Solic et al. (1982)
0.30	588	2 h IE (4 × 7.5 min light treadmill running)	Tdb = 22 °C RH = 40%	13 M	9 smokers, 4 nonsmokers; FEV_1/FVC = 37 to 65%	No significant changes in pulmonary function measurements or arterial O_2 saturation.	Kehrl et al. (1985)
0.41	804	3 h daily (1 × 15 min light bicycle ergometry during each exposure) for 5 days	Tdb = 22 °C RH = 50%	17 M, 3 F	All smokers; productive cough; FEV_1/FVC = 56 to 82% and/or FEV_3/FVC = 75 to 93%	Decrease in FVC and FEV_3 with 0.41 ppm O_3 compared with FA exposure.	Kulle et al. (1984)
Subjects with Heart Disease							
0.20 0.30	392 588	40 min CE treadmill walking	NA	6 M	Coronary heart disease with angina pectoris threshold	No significant changes in pulmonary function measurements, exercise ventilatory pattern, oxygen uptake, or cardiovascular parameters.	Superko et al. (1984)

Table 7-2 (cont'd). Ozone Exposure in Subjects with Preexisting Disease[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Subjects with Allergic Rhinitis							
0.18	353	2 h IE (4 × 15 min)	NA	26 M	History of allergic rhinitis	Increased respiratory symptoms, SR_{aw}, and reactivity to histamine with O_3 exposure and decreased FVC, FEV_1, and $FEF_{25-75\%}$ with O_3 exposure compared to FA.	McDonnell et al. (1987)
0.50	980	4 h rest	Tdb = 20 to 24 °C RH = 40 to 48%	6 M, 6 F	History of seasonal allergic rhinitis; acute response to nasal challenge with antigen	Increase in upper and lower respiratory symptom scores, cell influx, epithelial cells with O_3 exposure compared to FA; no effect on acute allergic response to nasal antigen challenge between O_3 and FA exposure.	Bascom et al. (1990)
Adult Subjects with Asthma							
0.10 0.25 0.40	196 490 784	1 h light IE (2 × 15 min on treadmill, $\dot{V}_E$ = 27 L/min)	Tdb = 21° C RH = 40%	12 M, 9 F, 19 to 40 years old	Stable mild asthmatics with FEV_1 > 70% and methacholine responsiveness	No significant differences in FEV_1 or FVC were observed for 0.10 and 0.25 ppm O_3-FA exposures or postexposure exercise challenge; 12 subjects exposed to 0.40 ppm O_3 showed significant reduction in FEV_1.	Weymer et al. (1994)
0.12	236	1 h rest	NA	7 M, 8 F	Never smoked, mild stable asthmatics with exercise-induced asthma	Exposure to 0.12 ppm O_3 did not affect pulmonary function. Preexposure to 0.12 ppm O_3 at rest did not affect the magnitude or time course of exercise-induced bronchoconstriction.	Fernandes et al. (1994)
0.12	236	0.75 h IE $\dot{V}_E$ = 30 L/min (15 min rest, 15 min exercise, 15 min rest) followed by 15 min exercise inhaling 0.10 ppm SO_2	Tdb = 22° C RH = 75%	8 M, 5 F, 12 to 18 years old	Asthmatics classified on basis of positive clinical history and methacholine challenge. Asymptomatic at time of study.	Filtered air followed by SO_2 and O_3 alone did not cause significant changes in pulmonary function. Ozone followed by SO_2 resulted in significant decrease in FEV_1 (8%) and $\dot{V}_{max50\%}$ (15%) and a significant increase in R_T (19%).	Koenig et al. (1990)

Table 7-2 (cont'd). Ozone Exposure in Subjects with Preexisting Disease[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Adult Subjects with Asthma (cont'd)							
0.12 0.24	236 472	1.5 h IE, $\dot{V}_E$ = 25 L/min	Tdb = 22° C RH = 65%	4 M, 4 F (nonasthmatics); 18 to 35 years old; 5 M, 5 F (asthmatics); 18 to 41 years old	Physician-diagnosed asthma confirmed with methacholine challenge test. All nonsmokers and asymptomatic at time of study. Nine were atopic.	No significant changes in pulmonary and nasal function measurements in either asthmatics or nonasthmatics. Significant increase in nasal lavage white cell count and epithelial cell following O_3 exposure in asthmatics only.	McBride et al. (1994)
0.12	236	6.5 h/day IE (6 × 50 min) (2 days of exposure), $\dot{V}_E$ = 28 L/min (asthmatic), $\dot{V}_E$ = 31 L/min (healthy)	NA	8 M, 7 F (nonasthmatics); 22 to 41 years old; 13 M, 17 F (asthmatics); 18 to 50 years old	Asthmatics classified on basis of positive clinical history, previous physician diagnosis, and low PD_{20}. Mild to severe asthmatics.	Significant increase in bronchial reactivity to methacholine in both asthmatics and nonasthmatics. FEV_1 decreased 8.6% in asthmatics and 1.7% in nonasthmatics, with difference not being significant.	Linn et al. (1994)
0.12	236	1 h rest	NA	4 M, 3 F, 21 to 64 years old	Mild, stable asthma	Increase in bronchial responsiveness to allergen; no change in baseline airway function.	Molfino et al. (1991)
0.20	392	2 h IE (4 × 15 min at 2× rest $\dot{V}_E$ cycle ergometry)	Tdb = 31 °C RH = 35%	20 M, 2 F, 19 to 59 years old	Physician diagnosed asthma; 6 smokers, 9 ex-smokers, 7 nonsmokers	No significant changes in pulmonary function measurements; significant blood biochemical changes.	Linn et al. (1978)
0.25	490	2 h rest	NA	5 M, 12 F, 20 to 71 years old	Nonsmoking asthmatics selected from a clinical practice	No significant changes in pulmonary function measurements.	Silverman (1979)

Table 7-2 (cont'd). Ozone Exposure in Subjects with Preexisting Disease[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Adult Subjects with Asthma (cont'd)							
0.40	784	2 h IE (4 × 15 min cycle ergometry)	Tdb = 22 °C RH = 50%	4 M, 5 F (normals), 19 to 31 years old; 4 M, 5 F (asthmatics), 18 to 34 years old	Asthmatics as diagnosed by a physician; history of chest tightness and wheezing	Decrease in FVC and IC with O_3 in asthmatics; increase in airway responsiveness to methacholine in asthmatics with O_3 and FA; asthmatic subjects had significantly greater decreases in FEV_1 and $FEF_{25-75\%}$ with O_3 exposure than did normal subjects.	Kreit et al. (1989) Eschenbacher et al. (1989)
Adolescent Subjects with Asthma							
0.12	235	1 h rest	Tdb = 22 °C RH ≥ 75%	4 M, 6 F (normals), 13 to 18 years old; 4 M, 6 F (asthmatics), 11 to 18 years old	Asthmatics had a history of atopic extrinsic asthma and exercise-induced bronchospasm	Decrease in FRC with O_3 exposure in asthmatics; no consistent significant changes in pulmonary functional parameters in either group or between groups.	Koenig et al. (1985)
0.12	235	1 h IE (2 × 15 min treadmill walking at mean $\dot{V}_E$ = 32.5 L/min)	Tdb = 22 °C RH ≥ 75%	5 M, 8 F (normals), 12 to 17 years old; 9 M, 3 F (asthmatics), 12 to 17 years old	Asthmatics selected from a clinical practice and had exercise- induced bronchospasm	Decrease in maximal flow at 50% of FVC in asthmatics with O_3 exposure compared to FA; no significant changes with combined O_3-NO_2 exposure.	Koenig et al. (1988)
0.12 0.18	235 353	40 min IE (1 × 10 min treadmill walking at mean $\dot{V}_E$ = 32.5 L/min)	NA	4 M, 9 F (normals), 14 to 19 years old; 8 M, 8 F (asthmatics), 12 to 19 years old	Asthmatics had allergic asthma, positive responses to methacholine, and exercise- induced bronchospasm	Decrease in FEV_1 and increase in R_T in normals and asthmatics with 0.12 and 0.18 ppm O_3 exposure compared to FA; no consistent differences between normals and asthmatics.	Koenig et al. (1987)

[a]See Appendix A for abbreviations and acronyms.
[b]Grouped by rest and exercise; within groups listed from lowest to highest O_3 concentration.

exercise time in all four of these studies was 30 min, with intensity being variable (exercise $\dot{V}_E$ approximately 14 to 28 L/min). Linn et al. (1982a) observed a small but significant reduction in arterial oxygen saturation in 25 mild to moderate COPD patients at the end of the 0.12 ppm O_3 exposure for 1 h (absolute mean difference = 1.3%, $p < 0.05$). Similarly, Solic et al. (1982) observed a small reduction in arterial oxygen saturation in 13 mild to moderate COPD patients at the end of a 0.2-ppm O_3 exposure for 2 h (absolute mean difference = 0.48%, $p < 0.008$). In contrast, Kehrl et al. (1985) did not find a significant effect on arterial oxygen saturation in 13 mild to moderate COPD patients after exposure to 0.3 ppm O_3 using the same IE exposure protocol used by Solic et al. (1982). Similarly, Linn et al. (1983a) found no significant effect on arterial oxygen saturation in 28 mild to moderate COPD patients exposed to 0.18 and 0.25 ppm O_3 for 1 h. The combined observations of these studies indicate that persons with COPD are not responsive to O_3 concentrations of 0.3 ppm and lower in combination with mild exercise. However, this conclusion should be viewed within the context of the low total inhaled dose of O_3 involved in the above studies, in that studies in healthy subjects using similar total inhaled doses also have not shown significant pulmonary function effects. Interpretation of these studies also is complicated by the wide range of the pulmonary function impairment of the patients studied (FEV_1/FVC from 0.3 to 0.7), their variable smoking history, and the fact that these patients are older ($\approx$60 years of age). The inconsistency of the observed small decreases in arterial oxygen saturation makes the interpretation of the clinical significance of this data difficult and uncertain.

Despite similar limitations, Kulle et al. (1984) observed small (<4%), statistically significant decreases in FVC and FEV_3 in 20 smokers (age range 31 to 51 years) diagnosed with mild chronic bronchitis exposed to 0.4 ppm O_3 for 3 h using an IE protocol (one 15-min exercise period beginning 1 h prior to end of exposure, $\dot{V}_E$ approximately 29 to 38 L/min). In addition, Kulle et al. (1984) observed that repeated daily exposure over a 5-day period led to an attenuation of these forced expiratory endpoints, and that this attenuation did not last longer than 4 days. The pulmonary responses induced by O_3 exposure in this study were associated only with mild symptoms.

Subjects with Asthma

Three studies examining the pulmonary responses to acute O_3 exposures in adult (Linn et al., 1978; Silverman, 1979) and adolescent (Koenig et al., 1985) asthmatics were discussed in the earlier criteria document (U.S. Environmental Protection Agency, 1986). Significant decrements in group mean pulmonary function were not observed for adult asthmatics exposed for 2 h at rest (Silverman, 1979) or with light IE (Linn et al., 1978) to O_3 concentrations of 0.25 ppm or less. However, it should be noted that, although group mean pulmonary function responses were not significantly affected in these studies, there were responsive asthmatic subjects who had obvious decrements in pulmonary function.

Koenig and co-workers (Koenig et al., 1985, 1987, 1988) conducted a series of studies examining the pulmonary responses of adolescent asthmatics and nonasthmatics (11 to 19 years of age) exposed to low levels of O_3. Koenig et al. (1985) found no significant changes in pulmonary function or symptoms in 10 adolescent normal and asthmatic subjects (four male, six female) who inhaled 0.12 ppm O_3 for 1 h at rest. The asthmatic subjects in this study were characterized as having histories of atopic (Type I, immunoglobulin E [IgE]-mediated) asthma and exercise-induced bronchospasm. Subsequently, in two separate studies of similar groups of adolescent asthmatics and nonasthmatics, Koenig et al. (1987, 1988)

observed no significant changes in pulmonary function or symptoms following exposure to 0.12 and 0.18 ppm O_3 with moderate IE up to 1 h, although a small significant decrease in flow at 50% of FVC was observed in the adolescent asthmatics exposed to 0.12 ppm O_3.

Kreit et al. (1989) and Eschenbacher et al. (1989) have demonstrated that exposure to 0.4 ppm O_3 with heavy IE (exercise $\dot{V}_E$ = 30 L/min/m^2 BSA) for 2 h elicits a significant decrease in FVC, FEV_1, FEV_1/FVC, and $FEF_{25\text{-}75\%}$ in both normal and asthmatic subjects. In these studies, O_3 exposure caused significantly greater decrements in FEV_1, FEV_1/FVC, and $FEF_{25\text{-}75\%}$ in asthmatic subjects. In contrast, Kreit et al. (1989) and Eschenbacher et al. (1989) found no significant difference between asthmatic and normal subjects in FVC and subjective symptoms. In addition, the effect of O_3 exposure on bronchial responsiveness as measured by the concentration of methacholine needed to increase SR_{aw} 100% ($PC_{100SRaw}$) was also studied. The asthmatic subjects had a significant decrease in $PC_{100SRaw}$ following FA and O_3 exposure. In comparison, the normal subjects had a significant decrease in $PC_{100SRaw}$ following O_3 exposure, with the percent decrease in mean $PC_{100SRaw}$ after O_3 exposure being similar in normal and asthmatic subjects, although the asthmatic patients' baseline $PC_{100SRaw}$ was significantly lower than that of the normal subjects. The findings of this study indicate that if the total inhaled dose is increased sufficiently by either increasing $\dot{V}_E$ during exposure or O_3 concentration, mild to moderate asthmatics will respond with a greater obstructive response than will normal subjects.

Linn et al. (1994) have reported responses of healthy (n = 15) and asthmatic (n = 30) subjects to 0.12 ppm O_3 and 100 μg/m^3 of respirable sulfuric acid (H_2SO_4) aerosol (MMAD = 0.5 μm; geometric standard deviation [σ_g] = 2), alone and in combination using the EPA prolonged-exposure protocol (see Section 7.2.2). These investigators observed a significant O_3-induced reduction in FEV_1 that was statistically significant and an increase in airway responsiveness to methacholine for all subjects combined. The asthmatic subjects demonstrated a statistically significant decrease in FEV_1 as a function of exposure duration regardless of pollutant exposure. In addition, there was a greater reduction in FEV_1 following O_3 alone in the asthmatics as compared to the nonasthmatics (−8.6% versus −1.7%), although this difference was not statistically significant. Despite the lack of a significant difference between asthmatics' and nonasthmatics' group mean FEV_1 responses with O_3 exposure, the responses observed in the asthmatics may be considered more important because their average FEV_1 was already significantly depressed by the underlying illness.

The findings of the above studies comparing the pulmonary function responses following O_3 exposure in asthmatic and nonasthmatic subjects suggest that asthmatics are at least as sensitive, if not more sensitive, to the acute effects of O_3 inhalation. The underlying mechanism that would explain a possible increased responsiveness of asthmatic subjects to O_3 is undefined. One possible mechanism could be that asthmatic subjects have an exaggerated airway inflammatory response to acute O_3 exposure. A study conducted by McBride et al. (1994) would support this hypothesis. McBride et al. (1994) exposed 10 asymptomatic asthmatic subjects with histories of allergic rhinitis and 8 nonallergic healthy subjects to FA and 0.12 and 0.24 ppm O_3 for 90 min using a light IE protocol ($\dot{V}_E$ = approximately 25 L/min). Pulmonary function tests, posterior rhinomanometry, and nasal lavage (NL) were performed before exposure and 10 min and 6 and 24 h after exposure. No significant changes in pulmonary or nasal function were found in either the allergic asthmatic or nonallergic nonasthmatic subjects. The allergic asthmatic subjects had a significant increase in the number of white blood cells in NL fluid 10 min and 24 h following

the 0.24-ppm O_3 exposure. In addition, a significant increase in epithelial cells was present 10 min after exposure to 0.24 ppm O_3 in the asthmatic subjects. No significant cellular changes were observed in the nonasthmatic subjects. These data indicate that the upper airways of asthmatic individuals are more sensitive to the acute inflammatory effects of O_3 than those of nonallergic nonasthmatic subjects.

The above studies compared the effects of O_3 inhalation on pulmonary function in asthmatic and normal subjects, but do not address the effect of preexposure to ambient concentrations of O_3 on the responsiveness of asthmatic subjects to other respiratory challenges, including other irritant gases, allergens, and exercise. Koenig et al. (1990) reported an increase in the bronchial response to an SO_2 challenge in a group of 13 asymptomatic adolescent asthmatic subjects following inhalation of 0.12 ppm O_3 for 45 min using a light to moderate IE protocol ($\dot{V}_E$ = approximately 30 L/min).

Molfino et al. (1991) investigated whether resting exposure to 0.12 ppm O_3 for 1 h potentiates the airway response to inhaled allergen in seven patients with mild asthma with seasonal symptoms of asthma and positive skin tests for ragweed or grass. This study was conducted over four week-long periods during the winter when ambient allergen levels were low. In each week, there were 3 consecutive study days. On Days 1 and 3, subjects underwent methacholine challenges, whereas, on Day 2, the subjects received one of four combined challenges in a single-blind design: (1) air breathing followed by inhalation of allergen diluent, (2) O_3 exposure followed by inhalation of allergen diluent, (3) air breathing followed by inhalation of allergen, and (4) O_3 exposure followed by inhalation of allergen. Molfino et al. (1991) observed no significant differences in baseline FEV_1 after O_3 exposure, but did observe a significant reduction in the provocative concentration of allergen required to reduce FEV_1 15%. This study was limited by its small number of subjects, and the results were confounded by possible ordering effects with the "O_3 exposure followed by allergen protocol" being the last protocol for all but one subject. Despite these limitations, the findings suggest that O_3 concentrations as low as 0.12 ppm may increase the bronchial responsiveness to allergen in atopic subjects.

In order to examine whether preexposure to O_3 results in exacerbation of exercise-induced asthma, two studies were conducted recently (Fernandes et al., 1994; Weymer et al., 1994). Fernandes et al. (1994) preexposed 15 stable mild asthmatics with exercise-induced asthma to 0.12 ppm O_3 for 1 h at rest followed by a 6-min exercise challenge test and found no significant effect on either the magnitude or time course of exercise-induced bronchoconstriction. Similarly, Weymer et al. (1994) observed that preexposure to either 0.10 or 0.25 ppm O_3 for 60 min while performing light IE did not enhance or produce exercise-induced asthma in 21 otherwise healthy adult subjects with stable mild asthma. Although the results of these studies would suggest that preexposure to O_3 neither enhances nor produces exercise-induced asthma in asthmatic subjects, the relatively low total inhaled doses used in the above studies limit the ability to draw any definitive conclusions.

Subjects with Allergic Rhinitis

McDonnell et al. (1987) exposed 26 adults (18 to 30 years of age) with allergic rhinitis to clean air and 0.18 ppm O_3 for 2 h using an IE protocol ($\dot{V}_E$ = 64 L/min at 15-min intervals). The study subjects with allergic rhinitis did not have a history of asthma-like symptoms. Following O_3 exposure, the subjects with allergic rhinitis exhibited significant increases in respiratory symptoms, airway reactivity to histamine, and SR_{aw} and

significant decreases in FVC, FEV_1, and $FEF_{25-75\%}$ when compared to clean air exposure. When compared to normal subjects without allergic rhinitis similarly exposed to 0.18 ppm O_3, the subjects with allergic rhinitis were no more responsive to O_3, based on symptoms, forced expiratory parameters, or airway reactivity to histamine aerosols, although subjects with allergic rhinitis did have a small but significantly greater increase in SR_{aw}. The data on subjects with allergic rhinitis and asthmatic subjects suggest that both of these groups have a greater rise in R_{aw} to O_3 with a relative order of airway responsiveness to O_3 being normal < allergic < asthmatic.

Bascom et al. (1990) conducted a study to characterize the upper respiratory response to acute O_3 inhalation, nasal challenge with antigen, and the combination of the two. Bascom et al. (1990) exposed 12 resting asymptomatic subjects with histories of allergic rhinitis in a randomized, crossover design on each of 2 days, separated by 2 weeks, to clean air or 0.5 ppm O_3 for 4 h. Following exposure, subjects underwent nasal challenge with four doses of antigen (1, 10, 100, and 1,000 protein nitrogen units of ragweed or grass). Upper and lower airway symptoms were rated and NL was performed before and after clean air and 0.5 ppm O_3 exposure, and following each antigen challenge. Exposure to O_3 caused significant increases in upper and lower airway symptoms, a mixed inflammatory cell influx with a sevenfold increase in NL PMNs, a 20-fold increase in eosinophils and a 10-fold increase in mononuclear cells as well as an apparent sloughing of epithelial cells. There was a significant increase in NL albumin concentration following O_3 exposure. When expressed as a change from the postexposure values, there was no significant difference between O_3 and clean air exposure in antigen-induced upper and lower airway symptoms, cells, albumin and mediators (histamine and TAME-esterase activity). These results suggest that acute exposure to O_3 does not alter the acute response to nasal challenge with antigen.

Subjects with Ischemic Heart Disease

One study has been conducted examining the cardiopulmonary effects of acute O_3 inhalation in patients with ischemic heart disease. Superko et al. (1984) exposed six middle-aged males with angina-symptom-limited exercise tolerance for 40 min to FA and to 0.2 and 0.3 O_3 while they were exercising continuously according to a protocol simulating their angina-symptom-limited exercise training prescription (mean $\dot{V}_E$ = 35 L/min). No significant pulmonary function impairment or evidence of cardiovascular strain induced by O_3 inhalation was observed. The low workloads were dictated by the patients' angina-symptom-limited exercise tolerance, and these low workloads acted to "protect" them from O_3-induced effects by limiting the total inhaled dose.

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

Gender Differences

As was noted in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986), the pulmonary function responses to O_3 of only a small number of female subjects have been evaluated under controlled laboratory conditions. Although the database on females has expanded (see Table 7-3), there are still fewer data than for males. Most studies involving mixed groups of male and female subjects include too few female subjects to allow for meaningful comparisons between the responses of the sexes, or fail to consider the question at all. There are, however, a few studies that utilize only female subjects. Several studies cited in the 1986 O_3 criteria document suggested that females might be more responsive to O_3 than males (Horvath et al., 1979; Gliner et al., 1983; Gibbons and Adams,

Table 7-3. Gender Differences in Pulmonary Function Responses to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity	Exposure Conditions[c]	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.12 0.18 0.24 0.30 0.40	235 353 470 588 784	2.33 h $\dot{V}_E$ = 25 L/min/m^2 BSA (one exposure/subject)	Mean T = 22 °C Mean RH = 4% treadmill	30 to 33 F and 30 to 33 M in each concentration group; total of 372 individuals participated	Healthy NS, 18 to 35 years old, blacks and whites	Decrements in FEV_1, increases in SR_{aw} and cough, correlated with O_3 concentration. There were no significant differences between the responses of males and females.	Seal et al. (1993)
0.18	353	1 h (mouthpiece) CE $\dot{V}_E \approx$ 47 L/min	T = 21 to 25 °C RH = 45 to 60% cycle	14 F	Mean FVC = 5.11 ± 0.53 L, NS, 20 to 24 years old	Significant concentration-response effect on FVC and FEV_1; lung size had no effect on percentage decrements in FVC or FEV_1.	Messineo and Adams (1990)
0.30	588			14 F	Mean FVC = 3.74 ± 0.30 L, NS, 19 to 23 years old		
0.20 0.30	392 588	1 h (mouthpiece) IE (20 min exercise) $\dot{V}_E \approx$ 28 L/min for men $\dot{V}_E \approx$ 23 L/min for women	T ≈ 22 °C RH ≥ 75% treadmill	9 M 10 F	NS, 55 to 74 years old NS, 56 to 74 years old	No changes in spirometry in men or women. Women had significant 13% increase in R_T following exposure, which was sustained at 20 min postexposure.	Reisenauer et al. (1988)
0.30	588	1 h (mouthpiece) CE $\dot{V}_E \approx$ 70 L/min for men $\dot{V}_E \approx$ 50 L/min for women	T = 21 to 25 °C RH = 45 to 60% cycle	20 M 20 F	NS, 18 to 30 years old NS, 19 to 25 years old	Significant decrements in FVC, FEV_1, and $FEF_{25-75\%}$ following O_3 exposure. No significant differences between men and women for spirometry or SR_{aw}.	Adams et al. (1987)

Table 7-3 (cont'd). Gender Differences in Pulmonary Function Responses to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity	Exposure Conditions[c]	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.45	882	2 h IE $\dot{V}_E \approx 27.9$ L/min for men $\dot{V}_E \approx 25.4$ L/min for women	T = 24 °C RH = 58% cycle	8 M 8 F	Healthy NS, 51 to 69 years old Healthy NS, 56 to 76 years old	Range of responses in FEV_1: 0 to −12% (mean = −5.6%). No significant difference in responses of men and women. Tendency for women to have greater effects.	Drechsler-Parks et al. (1987a,b)
0.45	882	2 h IE Mean $\dot{V}_E$ = 28.5 L/min for men Mean $\dot{V}_E$ = 26.1 L/min for women	Mean T = 23.1 °C Mean RH = 46.1% cycle/treadmill	10 M 6 F	Healthy NS, 60 to 89 years old Healthy NS, 64 to 71 years old	Mean decrement in FEV_1 = 5.7%. Decrements in FVC and FEV_1 were the only pulmonary functions significantly altered by O_3 exposure. No significant differences between responses of men and women.	Bedi et al. (1989)
0.48	941	2 h IE $\dot{V}_E \approx 25$ L/min	T = 21 °C WBGT cycle	10 F	Healthy NS, 19 to 36 years old	Mean decrement in FEV_1 = 22.4%. Significant decrements in all spirometry measurements. Results not significantly different from a similar study on males (Drechsler-Parks et al., 1984).	Horvath et al. (1986)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.
[c]WBGT = 0.7 $T_{wet\ bulb}$ + 0.3 $T_{dry\ bulb\ or\ globe}$.

1984; Lauritzen and Adams, 1985). DeLucia et al. (1983), on the other hand, did not find significant differences in the responses of young men and young women to O_3 exposure.

Messineo and Adams (1990) hypothesized that differences previously observed between the responses of males and females exposed to O_3 were related to differences in lung size between the sexes. They addressed this issue by selecting two groups of 14 women each. One group had a mean FVC of 5.11 L, and the other group had a mean FVC of 3.74 L. All subjects were 19 to 24 years of age and were healthy nonsmokers who had not lived in a high-air-pollution area for at least 6 mo. The subjects completed three 1-h CE ($\dot{V}_E$ = 47 L/min) exposures: (1) FA, (2) 0.18 ppm O_3, and (3) 0.30 ppm O_3. The mouthpiece exposures were presented in random order, at least 4 days apart, and all were performed when the subject was in the follicular phase of her menstrual cycle. Two subjects in the small-lung group and one in the large-lung group were unable to complete the 0.30 ppm O_3 exposure. Both groups had similar O_3-induced percentage decrements (9 to 10% following exposure to 0.18 ppm O_3 and 23 and 26% following exposure to 0.30 ppm O_3 for the small- and large-lung groups, respectively) in all measures of lung function, regardless of lung size, leading to the conclusion that lung size, per se, is not systematically related to percentage decrements in FEV_1 consequent to O_3 exposure.

Horvath et al. (1986) exposed 10 healthy, young, nonsmoking females, 19 to 36 years of age (mean age 23.6 years) to 0.48 ppm O_3 or FA for 2 h while they exercised intermittently at a target ventilation of 25 L/min. The subjects engaged in three 20-min cycle ergometer exercise periods alternated with four 15-min rest periods. The exposures were a minimum of 1 week apart. The responses of these subjects were compared with those of a group of 10 young males who earlier had completed the same protocol (Drechsler-Parks et al., 1984). There were no statistically significant differences in the responses based on gender. The female subjects had decrements of 18.8, 22.4, and 30.8% in FVC, FEV_1, and $FEF_{25\text{-}75\%}$, respectively, compared to 19.8, 25.0, and 31.9% for the male subjects. On an individual basis, 4 of the 10 males and 3 of the 10 females had decrements of 30% or more in FEV_1 following the exposure to 0.48 ppm O_3. One male subject did not respond to the O_3 exposure. It was noted, however, that the female subjects inhaled an absolute dose of O_3 about 22% less than the male subjects due to a slightly lower exercise $\dot{V}_E$ and the inherently lower resting $\dot{V}_E$ of females compared to males. However, when O_3 dose was related to BSA or to FVC, the females inhaled slightly higher relative doses of O_3 than the males.

Adams et al. (1987) compared the responses of 20 young men (18 to 30 years of age) and 20 young women (19 to 35 years of age) exposed to 0.3 ppm O_3 via mouthpiece. All subjects were healthy nonsmokers with clinically normal pulmonary function. None had a history of significant allergies, and none had resided in a high-air-pollution area for at least 3 mo. The subjects completed 1-h CE exposures (mean $\dot{V}_E \approx$ 70 L/min for males and 50 L/min for females) to FA and 0.3 ppm O_3. The exposures were given in random order and were separated by a minimum of 5 days. Ozone exposure induced significant decrements in FVC, FEV_1, and $FEF_{25\text{-}75\%}$ compared to FA exposure. Three females and four males were unable to complete the O_3 exposure. Females experienced mean decrements of 14.2, 20.3, and 24.5% in FVC, FEV_1, and $FEF_{25\text{-}75\%}$, respectively, compared to mean decrements of 15.8% in FVC, 23.8% in FEV_1, and 35.7% in $FEF_{25\text{-}75\%}$ for males. There were no statistically significant differences between the spirometry or SR_{aw} responses related to gender. Because the female subjects inhaled a substantially smaller absolute dose of O_3 due to the considerably lower exercise $\dot{V}_E$, yet had similar decrements in pulmonary

function compared to men, the authors concluded that females are more responsive to O_3 than males. In this study, the female subjects inhaled a lower relative dose of O_3 compared to males, when expressed on the basis of BSA, but a similar relative dose when expressed on the basis of FVC.

Seal et al. (1993) reported on 372 healthy black and white men and women between 18 and 35 years of age who each were assigned to complete one 2.33-h exposure to FA, 0.12, 0.18, 0.24, 0.30, or 0.40 ppm O_3. Subjects exercised intermittently on a motor-driven treadmill at a work load inducing a $\dot{V}_E$ of about 23 L/min/m^2 BSA for women and about 24.5 L/min/m^2 BSA for men. Although female subjects inhaled about 22% less total dose of O_3 than males in each exposure-concentration group, there were no significant differences in the changes in FEV_1 (see Figure 7-2), SR_{aw}, or cough ratings between males and females among either blacks or whites. Women also inhaled a lower absolute dose of O_3 than men.

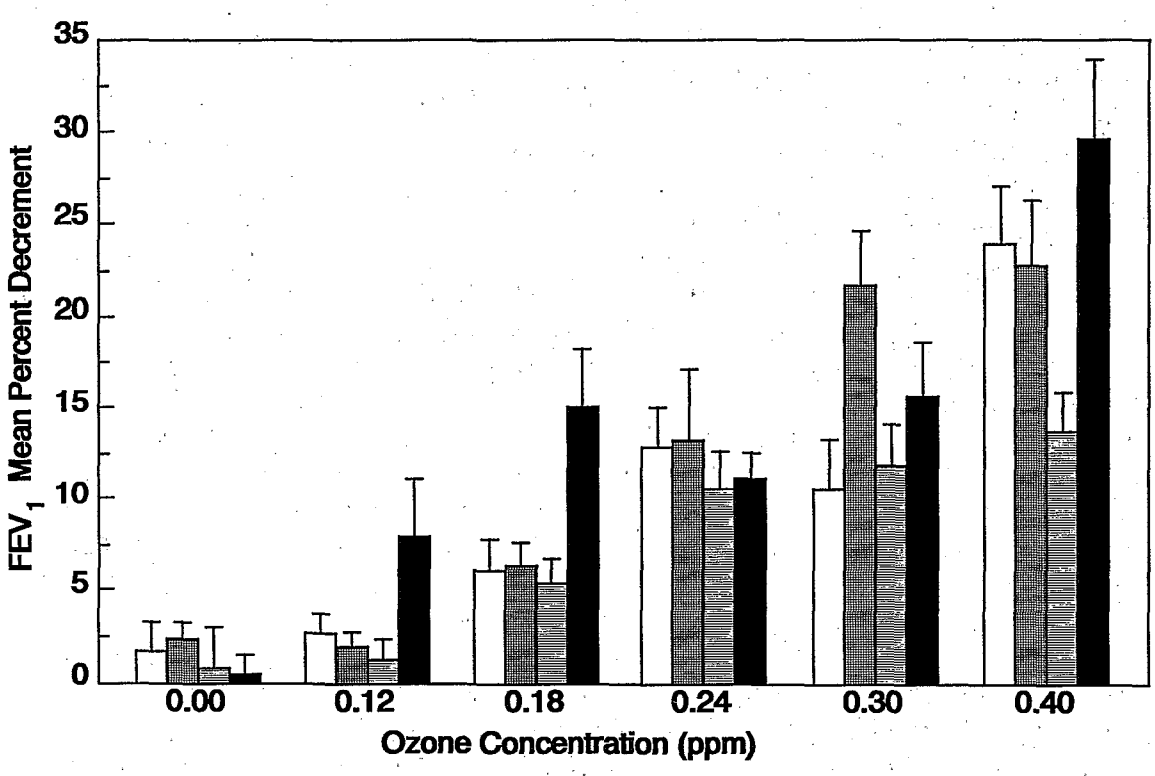

Figure 7-2. Mean percent change (± standard error of the mean) in post-minus prevalues of forced expiratory volume in 1 s (FEV_1) for each gender-race group. Open bars = white women; cross-hatched bars = black women; hatched bars = white men; solid bars = black men.

Source: Seal et al. (1993).

Drechsler-Parks et al. (1987a,b) compared the responses of eight men and eight women between 51 and 76 years of age to FA and 0.45 ppm O_3. The subjects were all healthy nonsmokers who were long-term residents of a relatively low pollution area. The subjects participated in 2-h IE (20 min rest/20 min exercise at $\dot{V}_E$ = 25 L/min) exposures that were presented in random order and were separated by at least 1 week. Except for FEV_3, there were no statistically significant differences between the responses of the men and women subjects, although women had slightly larger mean decrements in FVC and FEV_1 than men. Individual decrements in FVC and FEV_1 ranged from 0 to about 12% for both male and female subjects. Based on FEV_1, two females and three males had no response to the O_3 exposure. Male subjects inhaled a somewhat larger absolute effective dose of O_3 due to higher exercise and resting $\dot{V}_E$. When $\dot{V}_E$ was normalized to BSA, females inhaled a larger dose of O_3 than males. When $\dot{V}_E$ was normalized to FVC, the relative inhaled doses of O_3 were similar.

Reisenauer et al. (1988) reported on the pulmonary function responses of 9 men and 10 women between 55 and 74 years of age who were exposed to 0.0, 0.2, and 0.3 ppm O_3. The three exposures were presented in random order and at the same time of day for each subject. The subjects were exposed via mouthpiece for 1 h, during which seven men exercised for 10 min and rested for 50 min, and the other two men and all of the women alternated two 20-min rest periods and two 10-min exercise periods. Ventilation rates were about 28 L/min for men and 23 L/min for women, although, when $\dot{V}_E$ was normalized to BSA, the relative $\dot{V}_E$ for males and females was similar. All data were pooled, regardless of the total exercise time. There were no significant changes in any parameter of pulmonary function in the males. Females had no significant changes in any spirometric parameter, but, following the 0.3-ppm O_3 exposure, did have a small (13%) increase in total respiratory resistance (R_T), which remained at this level 20 min postexposure.

Bedi et al. (1989) reported on the responses of 10 men and 6 women (60 to 89 years of age) exposed for 2 h to FA or 0.45 ppm O_3 for 3 consecutive days. Only the first O_3 day results will be discussed in this section; the issue of repeated exposures is addressed in Section 7.2.1.4. Exposures were conducted at the same time of day, on consecutive days, with the FA exposure always conducted first. The subjects alternated 20-min exercise periods (mean $\dot{V}_E$ = 28.5 L/min for men and 26.1 L/min for women) and 20-min rest periods throughout the 2-h chamber exposures. When $\dot{V}_E$ was normalized to BSA, women inhaled slightly higher relative doses of O_3; but when normalized to FVC, women inhaled a slightly lower relative dose of O_3 than men. There were no statistically significant group mean differences between the responses of men and women subjects. The mean decrements in FVC and FEV_1 following the O_3 exposure for the 16 subjects were 2.8 and 5.7%, respectively. In an exploratory analysis, the subjects were divided into two groups based on whether their decrement in FEV_1 following the first O_3 exposure compared to the FA exposure was $\geq$5% or <5%. There were eight subjects in each group, with the sensitive group consisting of two females and six males. The mean post-O_3 exposure decrement in FEV_1 was 320 mL for the sensitive group, versus 21 mL for the nonresponsive group. Similar patterns of response were evident in FVC and FEV_3. There were no significant changes in any flow parameter, maximum voluntary ventilation (MVV), expiratory reserve volume, or functional residual capacity.

The question as to whether there is a difference in sensitivity to O_3 between men and women remains unresolved. Different conclusions depend on whether $\dot{V}_E$ is normalized to body or lung size in calculating the inhaled doses of O_3. The subgroups studied by Bedi

et al. (1989) included six males and two females, suggesting that older males may be more sensitive to O_3 than older females. However, Reisenauer et al. (1988) found a significant increase in R_T only in women. Horvath et al. (1986), Adams et al. (1987), and Drechsler-Parks et al. (1987a,b) suggested that because their female subjects had similar pulmonary function responses to their male subjects, even though the females inhaled less O_3, females were more sensitive than males. Messineo and Adams (1990) suggested that some factor other than absolute lung size accounted for observed differences between males and females; their two groups of females with widely different lung sizes experienced similar decrements in pulmonary function following equivalent exposures. Although the currently available literature suggests that females may be somewhat more sensitive to O_3 than males, the question is not settled. Further, comparative studies have included only small subject groups, except for Seal et al. (1993), and often only group mean data are presented, with little information about individual responses.

Hormonal Influences

Seal et al. (1995) compared the pulmonary function responses of 48 white and 55 black women (18 to 35 years of age) whose menstrual phase was known at the time of a single 2.3-h exposure to 0.18, 0.24, 0.30, or 0.40 ppm O_3. Subjects performed intermittent treadmill exercise ($\dot{V}_E$ = 20 L/min/m^2 BSA) during the first 2 h of exposure. There were no significant effects for SR_{aw} or cough that could be related to menstrual cycle. There was a race × menstrual phase interaction for FEV_1. However, when the groups of black and white women were analyzed separately, there was no significant primary effect for menstrual cycle phase. The significance of the observed interaction between race and menstrual cycle phase is unknown.

Weinmann et al. (1995) compared the pulmonary function responses of six healthy, nonsmoking women to a 130-min exposure to 0.35 ppm O_3, 4 to 8 days after the onset of menses and 4 to 8 days after ovulation. Subjects performed intermittent exercise at a workload that induced a $\dot{V}_E$ of 10 × FVC. Ovulation was confirmed by a blood progesterone test. Spirometry was performed pre- and 25-min post-O_3 exposure. Although resting $\dot{V}_E$ was the same during both exposures, exercise load had to be reduced 30% during the luteal phase in order to match the ventilatory response to exercise during the follicular phase. There were no significant effects related to phase of the menstrual cycle. The authors concluded that menstrual phase does not need to be considered in experimental design. One problem with the study is that the postexposure measurements were made 25 min after the conclusion of the exposure. Typically, pulmonary function decrements begin to reverse once exposure ends; thus, any pulmonary function changes that did occur could be expected to be reduced at 25-min post-O_3 exposure, compared to immediately after exposure.

Acute O_3 exposure has been shown to cause short-term airway inflammation (see Section 7.2.4) induced by PGs, among other inflammatory substances. It also has been demonstrated that progesterone inhibits PG production in the uterine endometrium, which fluctuates as the progesterone concentration varies throughout the menstrual cycle. Fox et al. (1993) investigated the hypothesis that O_3 exposure during the follicular phase, when progesterone concentration is lowest, might result in greater pulmonary function responses due to reduced anti-inflammatory influences of progesterone. Nine nonsmoking women completed 1-h mouthpiece exposures to FA and 0.3 ppm O_3 while exercising continuously ($\dot{V}_E$ about 50 L/min) during both the follicular and mid-luteal phases of two to four ovulatory

menstrual cycles. There were no differences in any pulmonary function responses to FA related to menstrual phase, nor was there a difference in the mean FVC decrements following the follicular or mid-luteal phase O_3 exposures. The O_3-induced decrements in FEV_1 and $FEF_{25-75\%}$ were significantly larger during the follicular phase (17.3 and 23.1%, respectively) than during the mid-luteal phase (13.4 and 15.3%, respectively). The authors speculated that the difference between the FEV_1 and $FEF_{25-75\%}$ responses to the two O_3 exposures could be due to differences in circulating progesterone and the effect of progesterone on prostaglandin activity.

Available data (see Table 7-4) do not permit a conclusion regarding the influence of the menstrual cycle on responses to O_3 exposure. Two of the three studies available, Fox et al. (1993) and Weinmann et al. (1995), were performed with small groups of subjects and resulted in opposite conclusions. Seal et al. (1995) compared race (black versus white) and menstrual phase, obtaining a significant interaction between race and phase, but post-hoc analysis failed to establish a basis for the interaction, leaving the implications of the study unclear.

Age Differences

It has been hypothesized that age may be a factor in responsiveness to O_3. Although children make up a large proportion of the population, few controlled laboratory studies of the pulmonary function effects of any air pollutant have been reported on subjects under age 18. Field and epidemiological studies (see Section 7.4) attempting to relate ambient air pollutant exposure to pulmonary function in children have suggested that children may be more responsive to ambient air pollution than young adults.

The previous O_3 criteria document (U.S. Environmental Protection Agency, 1986) included only one laboratory exposure study in which children were the subjects. McDonnell et al. (1985a) evaluated the pulmonary function responses of 23 boys between 8 and 11 years of age to 0.00 and 0.12 ppm O_3 in random order. The boys alternated 15-min rest and exercise periods ($\dot{V}_E$ = 35 L/min/m^2 BSA) for the first 120 min of the 150-min exposure. Forced expiratory spirometry and respiratory symptoms were measured before exposure and at 125 min of exposure, whereas R_{aw} was measured before exposure began and after 145 min of exposure. The group mean decrement in FEV_1 following the O_3 exposure was 3.4%, compared to 4.3% for a group of young adult males who earlier had completed the same protocol (McDonnell et al., 1983). It should be noted that the absolute $\dot{V}_E$ for the children (39.4 L/min) and adults (65.0 L/min) was similar when normalized for BSA (about 35 L/min/m^2 BSA). Assuming that adjusting ventilation for differences in BSA is an appropriate normalizing technique, these children appeared to experience O_3-induced pulmonary effects similar to adults. The children reported no symptoms, but the adults reported a small, but statistically significant, increase in cough following O_3 exposure.

Although controlled laboratory studies of the effects of exposure to air pollutants are rarely performed with children as subjects, a few, more recent studies are discussed below (see Table 7-5). Avol et al. (1987) have reported on the pulmonary function responses of 33 healthy boys and 33 healthy girls having a mean age of 9.4 years. The children completed exposures to purified air and outdoor ambient air that was drawn into an environmental chamber. Ambient temperature averaged about 33 °C. Exposures were 1 h in duration, were separated by a minimum of 2 weeks, and were conducted from June through September, beginning in the early afternoon when ambient air pollutant concentrations generally peak. The subjects performed continuous exercise throughout the

Table 7-4. Hormonal Influences on Pulmonary Function Responses to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	References
0.12 0.24 0.30 0.40	235 470 588 784	2.3 h IE $\dot{V}_E$ = 20 L/min/m^2 BSA	NA	48 WF, 55 BF	Healthy NS, 18 to 35 years old	Significant menstrual cycle phase × race interaction for FEV_1. No significant menstrual cycle phase effect when blacks and whites were analyzed separately. No significant menstrual phase effects for SR_{aw} or cough score.	Seal et al. (1995)
0.30	588	1 h CE $\dot{V}_E \approx$ 50 L/min	NA	9 F	Healthy NS, regular menstrual cycles, 20 to 34 years old	FEV_1 decreased 13.1% during the mid-luteal phase and 18.1% during the follicular phase. Decrement in $FEF_{25\text{-}75\%}$ was significantly larger during the follicular phase than the mid-luteal phase. Changes in FVC were similar in both phases.	Fox et al. (1993)
0.35	686	130 min	NA	9 F	Healthy NS, regular menstrual cycles, 18 to 35 years old	Changes in FVC, FEV_1, $FEF_{25\text{-}75\%}$, $\dot{V}_{max50\%}$, and $\dot{V}_{max25\%}$ were similar during both the follicular and luteal phases.	Weinmann et al. (1995)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.

Table 7-5. Age Differences in Pulmonary Function Responses to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.113[c] + other ambient pollutants	221	1 h CE $\dot{V}_E \approx 22$ L/min	T = 32.7 °C RH ≈ 43% cycle	33 M, 33 F	NS for both groups, mean age = 9.4 years old	No differences in responses of boys and girls. Similar decrements (<5% on average) following both purified air and ambient air (O_3 at 0.11 ppm) exposures.	Avol et al. (1987)
0.12	235	1 h (mouthpiece) IE $\dot{V}_E$ = 4 to 5 × resting	T = 22 °C RH = 75% treadmill	5 M, 7 F	Healthy NS, 12 to 17 years old	No significant changes in any pulmonary function in healthy subjects.	Koenig et al. (1988)
0.12 0.18	235 353	40 min (mouthpiece) IE 10 min exercise at $\dot{V}_E$ = 32.6 L/min; 40 min (mouthpiece) IE 10 min exercise at $\dot{V}_E$ = 41.3 L/min	NA treadmill	3 M, 7 F 4 M, 6 F	Healthy NS, 14 to 19 years old	No significant change in FEV_1; increased R_T with exposure to 0.18 ppm O_3. Some subjects responded to 5 to 10 mg/mL methacholine after 0.18-ppm O_3 exposure, whereas none responded to 25 mg/mL methacholine at baseline bronchochallenge.	Koenig et al. (1987)
0.18 0.24 0.30 0.40	353 470 588 784	2.3 h IE $\dot{V}_E$ = 20 L/min/m^2 BSA	NA	48 WF, 55 BF	Healthy NS, 18 to 35 years old, black and white	Older women had smaller changes in FEV_1 than younger women. No age- related differences in SR_{aw} or cough score.	Seal et al. (1993)
0.20 0.30	392 588	1 h (mouthpiece) IE (20 min) $\dot{V}_E \approx 28$ L/min for men $\dot{V}_E \approx 23$ L/min for women	T ≈ 22 °C RH ≥ 75% treadmill	9 M, 10 F	Healthy NS, 55 to 74 years old	No change in any spirometry measure. Women had 13% increase in R_T after 0.30-ppm exposure.	Reisenauer et al. (1988)

Table 7-5 (cont'd). Age Differences in Pulmonary Function Responses to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.45	882	2 h IE $\dot{V}_E \approx 26$ L/min	T ≈ 23 °C RH = 53% cycle	8 M 8 F	Healthy NS, 51 to 76 years old	Mean decrement in $FEV_1 = 5.6 \pm 13\%$; range of decrements = 0 to 12%.	Drechsler-Parks et al. (1987a,b)
0.45	882	2 h IE Mean $\dot{V}_E$ = 28.5 L/min for men Mean $\dot{V}_E$ = 26.1 L/min for women	T = 23 °C RH = 46% cycle/treadmill	10 M 6 F	Healthy NS, 60 to 89 years old	Mean decrement in $FEV_1 = 5.7\%$; eight subjects had a 5% or greater difference between their response to O_3 and FA, and the other eight had less than a 5% difference between their responses to FA and 0.45 ppm O_3.	Bedi et al. (1989)
0.45	882	2 h IE $\dot{V}_E \approx 26$ L/min	T ≈ 24 °C RH = 63% cycle	8 M 8 F	Healthy NS, 51 to 69 years old Healthy NS, 56 to 76 years old	13 subjects had decrements in FEV_1 on three separate exposures to 0.45 ppm within 5% of their mean response to the three exposures. The other three subjects were not reproducible. Symptom reports did not correlate well with pulmonary function changes.	Bedi et al. (1988)
0.45	882	1 h CE $\dot{V}_E \approx 26$ L/min 2 h IE $\dot{V}_E \approx 26$ L/min	T ≈ 23 °C RH = 58% cycle/treadmill	7 M 5 F	Healthy NS, 60 to 79 years old (all in 60s except one 79 years old)	Comparison of 1-h CE protocol and 2-h IE protocol indicated no difference between the changes in pulmonary function following the two protocols.	Drechsler-Parks et al. (1990)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.
[c]Ozone concentration is the mean of a range of ambient concentrations.

hour of exposure. Boys and girls exercised at similar $\dot{V}_E$, 22 to 23 L/min. It should be noted that the ambient exposure included the full range of air pollutants present in the outdoor air mix on the days of the exposures, except for small fractions of O_3 and particles lost in the inlet duct. Concentrations of O_3, nitrogen dioxide (NO_2), total suspended particulate (TSP), particulate nitrate, particulate sulfate, particulate sodium, and particulate ammonium were measured throughout the exposures. The O_3 concentration during the ambient air exposures averaged 0.113 ppm, whereas it averaged 0.003 ppm during the purified air exposures. The children consistently had similar declines in pulmonary function with time, following both FA and ambient air exposures. Typical mean decrements in FVC and FEV_1 were 50 mL or less. The investigators also have published similar studies on adolescents and adults (Avol et al., 1984, 1985a). The responses of the adolescents and adults to both exposures were not substantially different from those of the children whose results are reported here. The authors further noted that the children seemed to have difficulty performing consistent, reproducible pulmonary function tests, a factor that could have impacted on these results.

Several studies comparing the pulmonary function responses of healthy and asthmatic adolescents to O_3 exposure have appeared in the literature. The responses of the asthmatics are presented in Section 7.2.1.2; only data on normal adolescent subjects will be discussed in this section.

Koenig et al. (1987) reported on 20 adolescents, 14 to 19 years of age, who were exposed for 40 min to air or 0.12 or 0.18 ppm O_3 via a mouthpiece system. Ten subjects were exposed to each O_3 concentration, but not all subjects were exposed to both concentrations. None of the healthy subjects had a history of asthma or allergies, and all had pulmonary function within the predicted range, based on age, sex, and height. There was a 5- to 7-min break in exposure for pulmonary function test performance following 30 min of resting exposure, followed by a 10-min exercise period ($\dot{V}_E = 32.6 \pm 6.4$ L/min for the 0.12-ppm O_3 exposure and 41.3 ± 9.3 L/min for the 0.18-ppm O_3 exposure). Changes in FEV_1 were not significant following any exposure. After exposure to 0.18 ppm O_3, R_T was increased 15%.

Koenig et al. (1988) also have reported on the pulmonary function responses of another group of 12 healthy adolescents (12 to 17 years of age) to 1-h exposures to air and 0.12 ppm O_3. The subjects were exposed by mouthpiece to air and 0.12 ppm O_3 while alternating 15-min periods of exercise ($\dot{V}_E = 32.8 \pm 6.0$ L/min) with 15-min periods of rest ($\dot{V}_E = 8.8 \pm 1.2$ L/min). Tests of pulmonary function included forced expiratory spirometry and R_T. Healthy subjects had no significant alterations in any parameter of pulmonary function consequent to exposure to air or 0.12 ppm O_3.

Although few data are available on the responses of healthy adolescents exposed to O_3, the limited existing data do not identify adolescents as being either more or less responsive than young adults.

At the time the 1986 O_3 criteria document was released, no studies specifically evaluating the pulmonary function responses of older adults had been reported. Several studies (Folinsbee et al., 1985; Adams et al., 1981) that included a few middle-aged individuals among the subjects were suggestive that there might be a decrease in O_3 responsiveness with advancing age. Several reports have since appeared, collectively suggesting that, collectively, healthy older adults (i.e., over 50 years of age) generally are minimally responsive to O_3, although some individuals remain responsive to O_3.

Drechsler-Parks et al. (1987a) reported on eight men and eight women between 51 and 76 years of age who were exposed for 2 h to FA or 0.45 ppm O_3. The subjects were healthy nonsmokers with normal baseline pulmonary function. The chamber exposures involved alternating 20-min rest and exercise periods ($\dot{V}_E$ averaged 27.9 $\pm$ 0.29 L/min for men and 25.4 $\pm$ 0.80 L/min for women). Exposures were presented in random order at least 1 week apart. The only significant difference related to sex was in FEV_3, in which women had larger decrements than men. There were no significant decrements in any other parameter of pulmonary function related to O_3 exposure, except when the data of all 16 subjects were pooled, significant mean decrements of 5.3 $\pm$ 1.3% in FVC, and 5.6 $\pm$ 1.3% in FEV_1 were observed. The range of individual decrements in FEV_1 was from 0 to 12%. Two women and three men had no response following the O_3 exposure. The subjects reported more symptoms following the O_3 exposure than the FA exposure. Seven subjects reported cough, nine reported sore throat, and six reported chest tightness. The authors compared their results on older adults to the results from a group of young adults who had completed the same protocol. All of the older subjects inhaled slightly higher doses of O_3 than the younger subjects (10.23×10^{-4} L for older men versus 10.12×10^{-4} L for younger men and 8.48×10^{-4} L for older women versus 7.94×10^{-4} L for younger women). However, older men had a mean decrement in FEV_1 of 4.2% versus 23.7% for younger men, whereas older women had a mean decrement in FEV_1 of 7.0% versus 14.7% for younger women. The decrements of the older subjects also were compared to published values for the young adults, with the older subjects studied consistently showing smaller changes in pulmonary functions than the young adults. These comparisons indicated that these older adults were less responsive to O_3 exposure than typical young adults, in terms of pulmonary function changes and symptom reports.

Reisenauer et al. (1988) reported on the pulmonary function responses of 19 healthy adults between 55 and 74 years of age. All were nonsmokers with baseline pulmonary function within the predicted normal range. None had a history of asthma, atopy, or cardiovascular disease, and none responded to a baseline methacholine bronchochallenge. Subjects were exposed by mouthpiece to 0.0, 0.20, or 0.30 ppm O_3 for 1 h. Seven men rested for 50 min and exercised for 10 min, whereas the other two men and all women alternated two 20-min rest periods with two 10-min exercise periods; $\dot{V}_E$ was approximately 28 L/min for men and 23 L/min for women. The three exposures were presented in random order at the same time of day for each individual, but the separation between exposures is not stated. The only significant change in pulmonary function was a 13% increase in R_T with exposure to 0.30 ppm O_3 in women only, which was sustained for at least 20 min postexposure. The authors concluded, based on the increase in R_T in the women, that older adults were at increased risk for pulmonary function changes with near-ambient O_3 exposure. However, R_T is a highly variable parameter, and no other changes were significant. Given the large number of variables tested, this isolated result possibly is related to the large number of statistical tests performed. In contrast, the results of Koenig et al. (1987) from 10 healthy adolescents exposed to 0.18 ppm O_3 using a similar protocol, reported a mean decrement in FEV_1 of 2% and an increase of 10.5% in R_T at 2 to 3 min postexposure. At 7 to 8 min postexposure, the increase in R_T was 15.3%. Comparison of these results to those of Reisenauer et al. (1988) at 0.20 ppm O_3 supports the contention that younger individuals are more responsive to O_3 than older individuals, in that no changes in spirometry were noted in the older adults exposed to 0.30 ppm O_3, although older women

showed increased R_T with 0.30 ppm O_3 exposure, whereas the adolescents had a mean decrement of 1% in FEV_1 and a mean increase of 16% in R_T with exposure to 0.18 ppm O_3.

Bedi et al. (1988) reported that older men and women 51 to 76 years of age who completed three exposures to 0.45 ppm O_3 did not respond equivalently to each of three exposures. The subjects were healthy nonsmokers with baseline pulmonary function within predicted normal limits. The subjects alternated 20-min exercise ($\dot{V}_E$ was approximately 26 L/min) and 20-min rest periods throughout the 2-h chamber exposures. There was a minimum of 1 week between exposures, but separations ranged from 1 to 4 weeks between exposures 1 and 2, and between 1 and 7 weeks between exposures 2 and 3. Analysis of variance indicated no difference between the group mean responses to the three exposures. The data were then subjected to a correlation analysis, which led to the conclusion that the responses within an individual subject were not reproducible. McDonnell et al. (1985b), on the other hand, found good reproducibility of pulmonary function responses after exposure to various concentrations of O_3 between 0.12 and 0.40 ppm in young adult males between 18 and 30 years of age.

Seal et al. (1993) compared the pulmonary function responses of 48 white and 55 black women (18 to 35 years) who each completed a 2.3-h exposure to 0.18, 0.24, 0.30, or 0.40 ppm O_3. The subjects participated in only one exposure each while exercising intermittently ($\dot{V}_E$ = 20 L/min/m^2 BSA) during the first 2 h of the exposure. Older subjects within the age range tested had smaller decrements in FEV_1 than younger subjects.

One simple method to estimate the O_3 exposure dose is to calculate the product of O_3 concentration (parts per million), $\dot{V}_E$ (liters per minute), and exposure duration (minutes). Research on young adults (Folinsbee et al., 1978; Adams et al., 1981) has demonstrated that the order of relative importance of the three factors is O_3 concentration, $\dot{V}_E$, and exposure duration. Drechsler-Parks et al. (1990) investigated the relative role of the three components of effective dose in 12 healthy, nonsmoking adults between 61 and 79 years of age. The subjects were exposed to both FA and 0.45 ppm O_3, once while they performed a 1-h continuous exercise protocol and once while they performed a 2-h IE protocol in which they alternated 20-min exercise periods and 20-min rest periods. Mean $\dot{V}_E$ ranged from 25.2 to 27.3 L/min among the four exposures. Exposures were separated by at least 1 week. Regardless of protocol, O_3 exposure induced significant decrements in $FEV_{0.5}$, FEV_1 (7.7 and 10.6% for the 1- and 2-h exposures, respectively), FEV_3, and peak expiratory flow rate (PEFR) compared to FA exposure. There were significant decrements in $FEF_{25\text{-}75\%}$ (0.37, 0.46, 0.49, and 0.47 L/s, respectively), $FEF_{50\%}$, $FEF_{25\%}$, and MVV following all four exposures. The only significant difference between the responses to the 1- and 2-h O_3 protocols was in $FEV_{0.5}$. The total number of symptoms reported was 10 for the 1-h FA exposure, 6 for the 1-h O_3 exposure, and 12 for both the 2-h FA and 2-h O_3 exposures. It appears that resting ventilation during the 2-h protocol had a smaller effect compared to exercise ventilation. This supports earlier reports that the O_3 concentration is the most significant factor among the three factors that contribute to effective dose (Adams et al., 1981; Folinsbee et al., 1978; Hackney et al., 1975).

Available data, although on a limited number of subjects, consistently indicate that responsiveness to O_3 is decreased in persons over 50 years of age compared to younger adults. Although there are few data available on adults in their thirties and forties, the statistical modeling study of McDonnell et al. (1993) on subjects from 18 to 32 years of age suggests that responsiveness to O_3 is already diminishing by age 30, and that the most responsive individuals are likely to be less than 25 years of age. The results of Bedi et al.

(1988) suggest that older adults may be less reproducible in their responses to O_3 than younger adult males (McDonnell et al., 1985b); however, this finding is based on only 16 subjects and should be confirmed before being considered conclusive.

Ethnic and Racial Factors

Young white males have been the most frequently studied population in published reports on pulmonary function responses to O_3. There is concern, however, that responses to O_3 may be influenced by ethnic differences based on the observation that blacks have smaller lungs than whites for a given standing or sitting height (Rossiter and Weill, 1974; McDonnell and Seal, 1991). Thus, an equivalent inhaled volume of O_3 could result in a larger O_3 dose per unit of lung tissue in blacks compared to whites, potentially inducing greater effects in blacks than whites exposed to O_3 under the same conditions. Seal et al. (1993) evaluated the pulmonary function responses of 372 individuals, black, white, male, and female ($n > 90$ per group), between 18 and 35 years of age who were exposed to 0.00, 0.12, 0.18, 0.24, 0.30, or 0.40 ppm O_3. Each subject was assigned randomly to an exposure group and participated in only one experimental session. The protocol involved a 2.33-h exposure to the assigned condition. During the first 2 h of exposure, the subjects alternated 15-min rest periods and 15-min exercise periods ($\dot{V}_E = 25$ L/min/m^2 BSA). Spirometric and plethysmographic measurements were made at 5 and 20 min following the final exercise period. The initial nonparametric analysis of the percentage changes in FEV_1 indicated that FEV_1 responses increased with increasing O_3 concentration, and a group effect occurred that was independent of O_3 concentration. There was an O_3 effect, but no group effect or group $\times$ O_3 interaction for SR_{aw}, indicating an increase in SR_{aw} with increasing O_3 concentration. Both group and O_3 effects were significant for cough, but the interaction was not significant. A post hoc analysis, using a different statistical method on the absolute changes in FEV_1, indicated that the black males experienced significant decrements in FEV_1 following exposure to 0.12 ppm O_3, whereas black women and white men and women did not have significant decrements in FEV_1 at O_3 concentrations below 0.18 ppm. These results are not easily explained because there was no gender difference among whites and no racial difference among women. Furthermore, the black men had significantly greater decrements in FEV_1 at only some of the O_3 concentrations studied (see Figure 7-2). Although the results can be considered suggestive of an ethnic difference, more subjects must be studied before the issue of ethnic difference in O_3 responsiveness can be more definitive. It should be noted that, although this study included a large number of subjects, each subject participated in only one experiment. Thus, the range of individual responsiveness could have been different between groups.

Environmental Factors

A number of environmental factors, such as ambient temperature and humidity, season of the year, route of inhalation, and smoking history have been hypothesized to potentially impact on responses to O_3 exposure in additive or synergistic ways. None of these potentially interacting agents has been addressed adequately in the extant O_3 literature. Although O_3 concentrations in Los Angeles, for example, generally are highest on hot, dry days, most research on responses to O_3 exposure has been conducted under temperature and humidity conditions not substantially different from those typical of indoor environments. The few studies that included temperature and humidity as experimental factors have produced equivocal results (see Chapter 10, Section 10.2.9 in the 1986 O_3 criteria

document). No new reports of temperature or humidity effects have appeared since the 1986 O_3 criteria document (U.S. Environmental Protection Agency, 1986).

Earlier studies discussed in the 1986 O_3 criteria document have suggested that cigarette smokers are less responsive to O_3 than nonsmokers. Since then, the question as to whether reactivity to O_3 returns with cessation of cigarette smoking has been addressed by Emmons and Foster (1991). Thirty-four individuals with no history of asthma or obvious respiratory disease who enrolled in a smoking cessation program were assigned randomly to an O_3 group (n = 18) or an FA group (n = 16). The subjects ranged from 24 to 58 years of age and had a group mean smoking history of 33.9 $\pm$ 13 pack-years. Most of the subjects had baseline pulmonary functions somewhat below predicted values, based on age, height, and sex. Subjects completed 2-h exposures to 0.42 ppm O_3 or FA, as assigned, prior to beginning the smoking cessation program. The subjects rested during the exposures, except for 5 min of exercise at 150 kg · m/min (no $\dot{V}_E$ given) at the beginning of the last 30 min of exposure. Nine subjects in the O_3 group and six in the FA group completed the 6-mo smoking cessation program and repeated their assigned exposures at the end of the program. Prior to beginning the smoking cessation program, both the FA and O_3 groups had pre- to postexposure changes in FVC, FEV_1, and $FEF_{25-75\%}$ within the variability of repeated tests. The O_3 group had a significant mean change in $FEF_{25-75\%}$ of -22.5%, comparing post- to preexposure, whereas the FA group had a nonsignificant -12% change. Changes in FVC and FEV_1 were not significant in either group. It should be noted that smoking cessation led to a group mean improvement in baseline $FEF_{25-75\%}$ of 22.9%. The post-O_3 exposure values for $FEF_{25-75\%}$ were similar following the initial and the post-smoking cessation exposures. Thus, the difference in the $FEF_{25-75\%}$ decrement with O_3 exposure post-smoking cessation was largely due to the improvement that ensued from 6 mo of abstinence from smoking. The results of Emmons and Foster (1991) suggest that active smoking blunts responsiveness to O_3 and that cessation of smoking for 6 mo leads to improved baseline pulmonary function and possibly the reemergence of O_3 responsiveness.

7.2.1.4 Repeated Exposures to Ozone

Repeated daily exposure to O_3 in the laboratory setting leads to attenuated changes in spirometry and symptom responses that were initially termed "adaptation" (Hackney et al., 1977a). A series of repeated exposure studies, performed in various laboratories, was reviewed in the previous criteria document (U.S. Environmental Protection Agency, 1986). The spirometric responses to repeated O_3 exposure typically showed that the response was increased on the second exposure day to concentrations in the range of 0.4 to 0.5 ppm O_3 in exposures accompanied by moderate exercise (see Table 7-6). Thus, the response was enhanced on the second consecutive day. Mechanisms for enhanced responses had not been established, although it was hypothesized that persistence of O_3-induced damage for greater than 24 h may have contributed to the larger Day 2 response. An enhanced Day 2 response was less obvious or absent in exposures that were repeated at lower concentrations or that caused relatively small group mean O_3-induced decrements in spirometry. Two reports (Bedi et al., 1985; Folinsbee et al., 1986) indicated that enhanced spirometric responsiveness was present within 12 h, lasting for at least 24 h and possibly 48 h, but was clearly absent after 72 h. After 3 to 5 days of consecutive daily exposures to O_3, responses were markedly diminished or absent. One study (Horvath et al., 1981) suggested that the rapidity of this decline in response was related to the magnitude of the subjects' initial responses to O_3 or their "sensitivity". Finally, the persistence of the attenuation of spirometric and symptom

Table 7-6. Changes in Forced Expiratory Lung Volume After Repeated Daily Exposure to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity[c]	Number and Gender of Subjects	Percent Change in FEV_1 on Consecutive Exposure Days: First	Second	Third	Fourth	Fifth	References[d]
0.12	235	6.6 h, IE (40)	17 M	−12.79	−8.73	−2.54	−0.6	0.2	Folinsbee et al. (1994)
0.20	392	2 h, IE (30)	10 M	+1.4	+2.7	−1.6	—	—	Folinsbee et al. (1980)
0.20	392	2 h, IE (18 and 30)	8 M, 13 F	−3.0	−4.5	−1.1	—	—	Gliner et al. (1983)
0.20	392	2 h, IE (18 and 30)	9[e]	−8.7	−10.1	−3.2	—	—	Gliner et al. (1983)
0.20	392	1 h, CE (60)	15 M	−5.02	−7.8	—	—	—	Brookes et al. (1989)
0.25	490	1 h, CE (63)	4 M, 2 F	−20.2	−34.8	—	—	—	Folinsbee et al. (1986)
			5 M, 2 F	−18.8	—	−22.3	—	—	
0.35	686	2 h, IE (30)	10 M	−5.3	−5.0	−2.2	—	—	Folinsbee et al. (1980)
0.35	686	1 h, CE (60)	8 M	−31.0	−41.0	−33.0	−25.0	—	Foxcroft and Adams (1986)
0.35	686	1 h, CE (60)	10 M	−16.1	−30.4	—	—	—	Schonfeld et al. (1989)
			10 M	−14.4	—	−20.6	—	—	
0.35	686	1 h, CE (60)	15 M	−15.9	−24.6	—	—	—	Brookes et al. (1989)
0.40	784	3 h, IE (4-5 × resting)	13 M[f]	−9.2	−10.8	−5.3	−0.7	−1.0	Kulle et al. (1982)
0.40	784	3 h, IE (4-5 × resting)	11 F[f]	−8.8	−12.9	−4.1	−3.0	−1.6	Kulle et al. (1982)
0.40	784	2 h, IE (65)	8 M	−18.0	−29.9	−21.1	−7.0	−4.4	Folinsbee et al. (1995)
0.42	823	2 h, IE (30)	24 M	−21.1	−26.4	−18.0	−6.3	−2.3	Horvath et al. (1981)
0.45	882	2 h, IE (27)	1 M, 5 F	−13.3	—	−22.8	—	—	Bedi et al. (1985)
0.45	882	2 h, IE (27)	10 M, 6 F	−5.8	−5.6	−1.9	—	—	Bedi et al. (1989)
0.47	921	2 h, IE (3 × resting)	8 M, 3 F[g]	−11.4	−22.9	−11.9	−4.3	—	Linn et al. (1982b)
0.50	980	2 h, IE (30)	8 M	−8.7	−16.5	−3.5	—	—	Folinsbee et al. (1980)
0.50	980	2.5 h, IE (2 × resting)	6	−2.7	−4.9	−2.4	−0.7	—	Hackney et al. (1977a)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.
[c]Exposure duration and intensity of IE or CE were variable; $\dot{V}_E$ (number in parentheses) given in liters per minute or as a multiple of resting ventilation.
[d]For a more complete discussion of these studies, see Table 7-7 and U.S. Environmental Protection Agency (1986).
[e]Subjects were especially sensitive on prior exposure to 0.42 ppm O_3 as evidenced by a decrease in FEV_1 of more than 20%. These nine subjects are a subset of the total group of 21 individuals used in this study.
[f]Bronchial reactivity to a methacholine challenge also was studied.
[g]Seven subjects completed entire experiment.

responses has been studied (Horvath et al., 1981; Linn et al., 1982b; Kulle et al., 1982). These studies indicate that the attenuation of response is relatively short-lived, being partially reversed within 3 to 7 days and typically abolished within 1 to 2 weeks. Repeated exposures separated by 1 week (for up to 6 weeks) apparently do not cause any lessening of the spirometric response (Linn et al., 1982b).

Folinsbee et al. (1995) (also see Devlin et al., 1995) exposed a group of 15 healthy males to 0.4 ppm O_3 for 2 h/day on 5 consecutive days. Subjects performed heavy IE ($\dot{V}_E$ = 60 to 70 L/min, 15 min rest/15 min exercise). Decrements in FEV_1 averaged 18.0, 29.9, 21.1, 7.0, and 4.4% on the 5 exposure days. Baseline preexposure FEV_1 decreased from the first day's preexposure measurement and was depressed by an average of about 5% on the third day. This study illustrates that, with high-concentration and heavy-exercise exposures, spirometry and symptom responses are not completely recovered within 24 h.

Besides the absence of pulmonary function responses after several days of O_3 exposure, symptoms of cough and chest discomfort usually associated with O_3 exposure generally are absent (Folinsbee et al., 1980, 1994; Linn et al., 1982b; Foxcroft and Adams, 1986). In addition, airway responsiveness to methacholine is increased with an initial O_3 exposure (Holtzman et al., 1979; Folinsbee et al., 1988), may be further increased with subsequent exposures (Folinsbee et al., 1994), and shows a tendency for the increased response to diminish with repeated exposure (Kulle et al., 1982; Dimeo et al., 1981). A number of possible explanations for the initially enhanced and then lessened response may be related to changes that are occurring in pulmonary epithelia as a consequence of O_3 exposure. Inflammatory responses (Koren et al., 1989a), epithelial damage, and changes in permeability (Kehrl et al., 1987) could be invoked to explain at least a portion of these responses. By blocking spirometric and symptom responses with indomethacin pretreatment, Schonfeld et al. (1989) demonstrated that in the absence of an initial spirometric response such effects were not enhanced by repeated exposure. However, the mechanisms of these responses with regard to repeated exposures in humans remains to be elucidated.

Recent studies of repeated O_3 exposures have addressed some other features of the responses (see Table 7-7). A series of reports from the Rancho Los Amigos group in California have examined changes in response to O_3 as a result of the season of the year in the South Coast Air Basin of Los Angeles, CA. The purpose of this research (Linn et al., 1988; also Hackney et al., 1989; Avol et al., 1988) was to determine whether responsive subjects (n = 12), identified during an initial screening following a period of low ambient O_3 exposure, would remain responsive after regular ambient exposure during the "smog season". Responses of so-called "nonresponsive" subjects (n = 13) also were examined across the year. The subjects were exposed to 0.18 ppm O_3 on four occasions, spring, fall, winter, and the following spring. Only 17 subjects (8 responders) participated in the final spring exposures. The marked difference in FEV_1 response between responsive and nonresponsive subjects seen initially (−12.4% versus +1%) no longer was present after the summer smog season (fall test) or 3 to 5 mo later (winter test). However, when the reduced subset of subjects was exposed during the following spring, the responsive subjects again had significantly larger changes in FEV_1. Seasonal changes in FEV_1 response to O_3 in the responsive and nonresponsive subjects are shown below.

Table 7-7. Pulmonary Function Effects with Repeated Exposures to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.12	235	6.6 h 50 min exercise/10 min rest, 30 min lunch $\dot{V}_E$ = 38.8 L/min	18 °C 40% RH five consecutive daily exposures	17 M	Healthy NS	FEV_1 responses were maximal on first day of exposure (−13%), less on second day (−9%), absent thereafter. Symptom responses only the first 2 days. Methacholine airway responsiveness was at least doubled on all exposure days, but was highest on the second day of ozone. Airway responsiveness was still higher than air control after 5 days of ozone exposure. Trend to lessened response, but it was not achieved after 5 days.	Folinsbee et al. (1994) (also see Table 7-9)
0.18	353	2 h IE (heavy) $\dot{V}_E \approx$ 60 to 70 L/min (35 L/min/m^2 BSA)	31 °C 35% RH (screen exposures in spring 1986; second exposures in summer/fall 1986 and winter 1987 and spring 1987 for responders and nonresponders only)	59 adult Los Angeles residents 12 responsive 13 nonresponsive	Responders: Age = 19 to 40 years; 6 atopic, 2 asthmatic, 4 normal Nonresponders: Age = 18 to 39 years, 13 normal	Responders had ΔFEV_1 = 12.4% after initial screening; nonresponders had no change. Responders had nonsignificant response in late summer or early winter, but were responsive again in early spring (spring 1986, −385 mL; Autumn 1986, −17 mL; winter 1987, +16 mL; spring 1987, −347 mL). Nonresponders did not change with season. Suggests that responders responses may vary with ambient exposure, but nonresponders generally remain nonresponsive.	Linn et al. (1988) (also see Hackney et al., 1989)
0.20/0.20 0.35/0.20 0.35/0.35	392/392 686/392 686/686	1 h CE at 60 L/min	21 to 25 °C 40 to 60% RH (three 2-day sets of exposures)	15 M	Healthy aerobically trained NS, FVC = 4.24 to 6.98 L	Consecutive days of exposure to 0.20 ppm produced similar responses on each day (−5.02, −7.80); 0.35/0.20 ppm pair caused increased response to 0.20 ppm on second day (−8.74); 0.35/0.35 ppm caused much increased response on Day 2 (−15.9, −24.6). Symptom responses were worse on the second exposure to 0.35 ppm, but not with second exposure to 0.20 ppm.	Brookes et al. (1989)

Table 7-7 (cont'd). Pulmonary Function Effects with Repeated Exposures to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.35	686	≈1 h CE (see paper for details)	22 to 25 °C 35 to 50% RH (1 day FA; 1 day O_3; 4 days consecutive exposure to O_3)	8 M	Aerobically trained healthy NS (some were known O_3 sensitive), age = 22.4 ± 2.2 years	Largest FEV_1 decrease on second of 4 days O_3 exposure (−40% mean decrease). Trend for adaptation not complete in 4 days. $\dot{V}O_{2max}$ decreased with single acute O_3 exposure (−6%) but was not significant after 4 days of O_3 exposure (−4%). Performance time was less after acute O_3 (211 s) exposure than after FA (253 s).	Foxcroft and Adams (1986)
0.35	686	60 min CE ≈ $\dot{V}_E$ = 60 L/min	21 to 25 °C 40 to 60% RH (two exposures for each subject separated by 24, 48, 72, or 120 h)	40 M (4 groups of 10)	NS; nonallergic, non-Los Angeles residents for >6 mo; age ≈ 25 years	No differences between responses to exposures separated by 72 or 120 h. Enhanced FEV_1 response at 24 h (−16.1% vs. −30.4%). Possible enhanced response at 48 h (−14.4% vs. −20.6%). Similar trends observed for respiratory pattern and SR_{aw}.	Schonfeld et al. (1989)
0.45	882	2 h IE (3 × 20 min exercise) $\dot{V}_E$ = 26 L/min	23.3 °C 62.5% RH (three exposures with a minimum 1-week interval)	8 M, 8 F	Healthy NS, 61 years old for M and 65 years old for F (FVC = 4.97 L for M and 3.11 L for F)	Spirometric changes were not reproducible from time to time after ozone exposure ($r < 0.50$). Repeat exposures to air yielded consistent responses.	Bedi et al. (1988)
0.45	882	2 h IE (3 × 20 min exercise) $\dot{V}_E$ = 27 L/min	23.3 °C 63% RH Exposed for 3 consecutive days, not exposed for 2 days, then exposed to 0.45 ppm again for 1 day	10 M, 6 F	Healthy NS, 60 to 89 years old (median age = 65 years; mean FVC = 3.99 L; mean FEV_1 = 3.01 L; FEV_1/FVC range = 61 to 85%)	Overall increase in symptoms, but no single symptom increased significantly. FVC decreased 111 mL and 104 mL on Days 1 and 2, respectively. FEV_1 fell by 171 and 164 mL, and FEV_3 fell by 185 and 172 mL. No significant changes on Days 3 and 4 or with FA. FEV_1 changes were −5.8, −5.6, −1.9, and −1.7% on the four O_3 days.	Bedi et al. (1989)

Table 7-7 (cont'd). Pulmonary Function Effects with Repeated Exposures to Ozone[a]

Ozone Concentration[b]		Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
ppm	$\mu g/m^3$						
0.45 (+ 0.30 PAN)	882	2 h IE (20 min rest, 20 min exercise) $\dot{V}_E$ = 27 L/min	22 °C 60% RH 5 days consecutive exposure to PAN + O_3	3 M, 5 F	Healthy NS, Mean age = 24 years	FEV_1 decreased ≈19% with O_3 alone, ≈15% on Day 1 of O_3 + PAN, ≈5% on Day 5 of O_3 + PAN, ≈7% 3 days after 5 days of O_3 + PAN, ≈15% after 5 days of O_3 + PAN. Similar to O_3 adaptation studies, O_3 responses peaked after 2 days, were depressed 3 days later, and responses returned 7 days later. PAN probably had no effect on adaptation to O_3.	Drechsler-Parks et al. (1987b) (also see Table 7-13)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.

	ΔFEV_1 Spring (mL)	ΔFEV_1 Fall (mL)	ΔFEV_1 Winter (mL)	ΔFEV_1 Spring (mL)
Responders	−385	−17	+16	−347
Nonresponders	+28	+90	+34	+81

These results suggest a seasonal variability in response that may be attributed to increased ambient O_3 exposure during the summer months. It must be noted that the responders included subjects who had a history of complaints from ambient air pollution. Furthermore, this group included a significant proportion of allergic individuals whose seasonal allergies could have contributed to their varying responses. Historically, however, studies with the subjects drawn from the population of Los Angeles have reported reduced responses to O_3 exposure in the laboratory compared to nonresidents (Hackney et al., 1976, 1977b).

Brookes et al. (1989) reexamined a hypothesis previously tested by Gliner et al. (1983), that repeated exposure to one concentration can alter response to subsequent exposure to a different O_3 concentration. Gliner et al. (1983) previously had shown that the response to 0.40 ppm O_3 was not influenced by previously being exposed to 0.20 ppm O_3 for 2 h on 3 consecutive days. Brookes et al. (1989) tested whether exposure to 0.20 or 0.35 ppm O_3 would change subsequent response to 0.20 or 0.35 ppm O_3. They found increased responses to 0.20 ppm for both preexposures ($\Delta FEV1 = -5.02$, -7.80, and -8.74% for 0.20 ppm acutely, 0.20 ppm after 0.20 ppm, and 0.20 ppm after 0.35 ppm, respectively), but this trend was significant only for the higher concentration. Although not statistically significant, the response increase seen on the second exposure day at 0.20 ppm is similar to that seem by Gliner et al. (1983). These observations suggest that, although preexposure to low concentrations of O_3 may not influence response to higher concentrations, preexposure to a high concentration of O_3 may significantly increase response to a lower concentration on the following day.

Schonfeld et al. (1989) confirmed previous observations of Bedi et al. (1985) and Folinsbee et al. (1986) that the period of enhanced responsiveness to O_3 following an initial exposure persists for about 24 to 48 h but is absent by 72 h after the initial exposure. In a series of paired exposures to 0.35 ppm with continuous heavy exercise separated by intervals of 1, 2, 3, or 4 days, they found that the responses to the second exposure were clearly increased at 24 h ($\Delta FEV1 = -16.1$ and -30.4% for the first and second exposures, respectively) and possibly also at 48 h ($\Delta FEV1 = -14.4$ and -20.6%). Similar trends were observed for other physiological variables such as SRaw and respiratory pattern during exercise. With a 3- or 4-day interval between exposures, the responses to the two exposures were similar.

Foxcroft and Adams (1986) demonstrated that decrements in exercise performance seen after a 1-h exposure to 0.35-ppm (continuous heavy exercise) were less after 4 consecutive days of O_3 exposure than they were after a single acute exposure. Maximal aerobic power and performance time on a progressive bicycle exercise test were reduced 6% and 42 s, respectively, from FA control, after a single 0.35-ppm exposure. After 4 consecutive days of 1-h exposures, the maximal aerobic power was reduced only 4% and the performance time by only 14 s; these differences from FA control were not statistically

significant. Despite the change in exercise performance, Foxcroft and Adams (1986) did not show the attenuation of FEV_1 response seen in many previous studies (Folinsbee et al., 1980; Linn et al., 1982b). However, these investigators selected known O_3-sensitive subjects whose FEV_1 decrements exceeded 30% on the first 3 days of exposure. The large magnitude of these responses, the trend for the responses to decrease on the third and fourth day, the decreased symptom responses, and the observations of Horvath et al. (1981) that O_3-sensitive subjects adapt slowly, suggest that attenuation of response would have occurred if the exposure series had been continued for another 1 or 2 days. These observations support the contention advanced by Horvath et al. (1981) that the progression of attenuation of response is a function of "O_3 sensitivity". Furthermore, these results suggest that exercise responses after O_3 exposure may be limited, either voluntarily or involuntarily, more by subjective symptoms than by alterations in gas exchange consequent to changes in ventilatory function.

Bedi et al. (1989) examined the responses of elderly subjects (median age, 65 years) to four exposures to 0.45 ppm O_3 for 2 h with mild IE. The first three exposures were on consecutive days, with the fourth exposure following the third by 3 days. Changes in FEV_1 on the first two exposure days averaged −5.8 and −5.6%, about half the response expected in a group of healthy young males (−12.7%; Folinsbee et al., 1978). There were no significant changes in FEV_1 on the third (−1.9%) and fourth (−1.7%) exposure days. Symptom responses were negligible, although there was an overall increase in symptoms on the first day of O_3 exposure compared to air exposure. Despite the high concentration of the exposure, there was no enhancement of the spirometry response on the second day of exposure. Although similar observations have been made in previous studies producing small changes in spirometry (Folinsbee et al., 1980, 1994) with repeated exposures, the responses of older subjects are not sufficiently understood to explain these responses. Bedi et al. (1988) had previously reported that responses to O_3 in the older subjects tended to be less reproducible, although this factor alone could not explain these responses.

Drechsler-Parks et al. (1987b) examined the response to repeated exposures to 0.45 ppm O_3 plus 0.30 ppm peroxyacetyl nitrate (PAN). Exposures to O_3 and O_3 plus PAN yielded similar changes in spirometry (ΔFEV_1 = −19 and −15%, respectively). Thus, PAN did not increase responses to O_3. Repeated exposure to the PAN plus O_3 mixture resulted in similar changes to those seen with O_3 exposure alone. Responses in FEV_1 exceeded −30% on the second exposure and fell to less than −5% after the fifth day. The attenuation of response persisted 3 days after the repeated exposures, but was absent after 7 days. These observations suggest that PAN does not influence the attenuation of response to repeated O_3 exposure. If the PAN responses are considered negligible, this study confirms the observation that the attenuation of O_3 responses with chamber exposures lasts no longer than 1 week.

Repeated multihour exposure to low concentrations of O_3 has been examined (Horvath et al., 1991; Folinsbee et al., 1994; Linn et al., 1994). Horvath et al. (1991) exposed subjects for 2 consecutive days to 0.08 ppm using the 6.6-h prolonged-exposure protocol (see Section 7.2.2). They observed small pre- to postexposure changes in FEV_1 (−2.5%) on the first exposure, but no change on the second day. Linn et al. (1994) observed a 1.7% decrease in FEV_1 in healthy subjects after a 6.5-h exposure to 0.12 ppm. A second consecutive exposure yielded even smaller (<1%) responses. With exposure to a mixture of O_3 plus 100 $\mu g/m^3$ of H_2SO_4 aerosol, there was a 4.2% decrease in FEV_1 on the first exposure day. In a group of asthmatics exposed under similar conditions, the FEV_1 response on the first day was −8.6% (O_3) and −11.6% (O_3 plus acid). After adjustment for

the exercise effect (−4.6%), the responses (−4 and −7%) were still greater than those of nonasthmatics. Responses were slightly reduced on the second day of exposure.

Folinsbee et al. (1994) exposed 17 subjects to 0.12 ppm O_3 for 6.6 h on 5 consecutive days. Spirometry responses were typified by changes in FEV_1 that reached −13% on the first day and −9% on the second day of exposure. No significant differences in spirometry responses between FA and subsequent O_3 exposures were observed. Symptom responses were also greatest on the first exposure day and were largely absent from the third day on. Methacholine responsiveness was tested using a single dose of methacholine and then by comparing changes in R_{aw} as the ratio of SR_{aw} after methacholine aerosol to that after saline aerosol. The responses to FEV_1 and methacholine testing are shown below.

	Day 1	Day 2	Day 3	Day 4	Day 5	Clean Air
$\Delta\%FEV_1$	−12.79	−8.73	−2.54	−0.6	+0.2	+1.1
SR_{aw} Ratio	3.67	4.55	3.99	3.24	3.74	2.22

Methacholine responsiveness was increased (over the clean air response) throughout the 5 days of O_3 exposure, although it reached a peak on the second day, and, in some subjects, there was a trend for responsiveness to decrease after 5 days. These results suggest that repeated exposure to low levels of O_3, despite the attenuation of symptoms and pulmonary function changes, is not without hazard. It is likely that some epithelial damage persists that contributes to the enhanced response to methacholine throughout the exposure series. However, it must be noted that, in this study, subjects initially were selected based on their FEV_1 response to 0.16 ppm O_3 for 4 h. This may in part explain the greater FEV_1 responses seen in this study, but there was no correlation between individual FEV_1 decrements and changes in methacholine responsiveness. Furthermore, the Horvath et al. (1991) subjects were exposed only to 0.08 ppm, and they were somewhat older than the Folinsbee et al. (1994) subjects; the Linn et al. (1994) subjects, on the other hand, had lower ventilation during exercise and were residents of Los Angeles accustomed to exposure to these levels of O_3 (see Chapter 4 for typical O_3 concentrations).

Based on studies cited here and in the previous criteria document (U.S. Environmental Protection Agency, 1986), several conclusions can be drawn about repeated 1- to 2-h O_3 exposures. Repeated exposures to O_3 can cause an enhanced (i.e., greater) response on the second day of exposure. This enhancement appears to be dependent on the interval between the exposures (24 h causes the greatest increase) and is absent with intervals ≥3 days. An enhanced response also appears to depend to some extent on the magnitude of the initial response. Small responses to the first O_3 exposure are less likely to result in an enhanced response on the second day of O_3 exposure. Repeated daily exposure also results in attenuation of spirometric responses, typically after 3 to 5 days of exposure. This attenuated response persists for less than 1 or as long as 2 weeks. In temporal conjunction with the spirometry changes, symptoms induced by O_3, such as cough and chest discomfort, also are attenuated with repeated exposure. Ozone-induced changes in airway responsiveness attenuate more slowly than spirometric and symptom responses. Attenuation of the changes in airway responsiveness also persist longer than changes in spirometry, although this has been studied only on a limited basis. In longer-duration, lower-concentration studies that do

not cause an enhanced second-day response, the attenuation of response to O_3 appears to proceed more rapidly.

7.2.1.5 Effects on Exercise Performance

Introduction

An early epidemiological study examining race performances in high school cross-country runners (Wayne et al., 1967) suggested that exercise performance is depressed by inhalation of ambient oxidant air pollutants. Wayne et al. (1967) suggested that the detrimental effects of oxidant air pollutants on race performance may have been related to increased R_{aw} or to the associated discomfort in breathing, thus limiting runners' motivation to perform at high levels. The effects of acute O_3 inhalation on exercise performance have been evaluated in numerous controlled human studies. These studies can be divided into two categories: (1) those that examine the effects of acute O_3 inhalation on maximal oxygen uptake and (2) those that examine the effects of acute O_3 inhalation on the ability to complete strenuous continuous exercise protocols up to 1 h in duration. Five studies (Folinsbee et al., 1977; Horvath et al., 1979; Folinsbee et al., 1984; Adams and Schelegle, 1983; Savin and Adams, 1979) examining the effects of acute O_3 exposures on exercise performance were discussed in the 1986 EPA criteria document (U.S. Environmental Protection Agency, 1986). This section summarizes the studies reviewed in that document and reviews more recent studies that examine the effect of acute O_3 inhalation on maximal oxygen uptake and endurance performance. Studies are also summarized in Table 7-8.

Effect on Maximal Oxygen Uptake

Three studies (Folinsbee et al., 1977; Horvath et al., 1979; Savin and Adams, 1979) examining the effects of acute O_3 exposures on $\dot{V}O_{2max}$ were discussed in the 1986 EPA criteria document (U.S. Environmental Protection Agency, 1986). Of these studies, only Folinsbee et al. (1977) observed that $\dot{V}O_{2max}$ was significantly decreased (10.5%) following a 2-h exposure to 0.75 ppm O_3 with light IE. Reductions in $\dot{V}O_{2max}$ were accompanied by a 9.5% decrease in maximum attained workload, a 16% decrease in maximum ventilation, and a 6% decrease in maximum heart rate. The 16% decrease in maximum ventilation was associated with a 21% decrease in V_T. In addition, the O_3 exposure resulted in a 22.3% decrease in FEV_1 and subjective symptoms of cough and chest discomfort. In contrast, Horvath et al. (1979) did not observe a change in $\dot{V}O_{2max}$ or other maximum cardiopulmonary endpoints in male and female subjects exposed at rest to 0.75 ppm O_3 for 2 h, although FVC was significantly decreased (10%). Similarly, Savin and Adams (1979) observed no effect on maximum attained workload or $\dot{V}O_{2max}$ in nine subjects exposed to 0.3 ppm O_3 while performing a progressively incremented exercise test to volitional fatigue lasting 30 min. In addition, Savin and Adams (1979) observed no significant effect on pulmonary function, performance time, maximum heart rate, or anaerobic threshold, although maximum ventilation was significantly reduced 7%.

More recent findings of Foxcroft and Adams (1986) and Gong et al. (1986) support the earlier observations of Folinsbee et al. (1977). Foxcroft and Adams (1986) observed significant ($p < 0.05$) reductions in performance time (16.7%), $\dot{V}O_{2max}$ (6.0%), maximum ventilation (15.0%), and maximum heart rate (5.6%) in eight aerobically trained males during a rapidly incremented $\dot{V}O_{2max}$ test following 50-min exposure to 0.35 ppm O_3 with CE (exercise $\dot{V}_E$ = 60 L/min). Similarly, Gong et al. (1986) found significant

Table 7-8. Ozone Effects on Exercise Performance[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.06-0.07 0.12-0.13	120-140 245-260	CE ($\dot{V}_E$ = 30 to 120 L/min) 16 to 28 min progressive maximum exercise protocol	Tdb = 23 to 24.5 °C RH = 50 to 53%	12 M, 12 F	Athletic	Reduced maximum performance time and increased respiratory symptoms during O_3 exposure.	Linder et al. (1988)
0.12 0.18 0.24	235 353 470	1 h competitive simulation exposures at mean $\dot{V}_E$ = 87 L/min	Tdb = 23 to 26 °C RH = 45 to 60%	10 M	Highly trained competitive cyclists	Decrease in exercise time of 7.7 min and 10.1 min for subjects unable to complete the competitive simulation at 0.18 and 0.24 ppm O_3, respectively; decrease in FVC and FEV_1 for 0.18- and 0.24-ppm O_3 exposure compared with FA exposure.	Schelegle and Adams (1986)
0.12 0.20	235 392	1 h CE $\dot{V}_E$ = 89 L/min	Tdb = 31 °C	15 M, 2 F	Highly trained competitive cyclists	Decrease in $\dot{V}_{Emax}$, $\dot{V}O_{2max}$, V_{Tmax}, workload, ride time, FVC, and FEV_1 with 0.20 ppm O_3 exposure, but not significant with 0.12-ppm O_3 exposure, as compared to FA exposure.	Gong et al. (1986)
0.20 0.35	392 686	1 h CE or competitive simulation at mean $\dot{V}_E$ = 77.5 L/min	Tdb = 23 to 26 °C RH = 45 to 60%	10 M	Well-trained distance runners	V_T decreased and f increased with continuous 50-min O_3 exposures; decrease in FVC, FEV_1, and $FEF_{25-75\%}$ from FA to 0.20 ppm and FA to 0.35-ppm O_3 exposure in all conditions; three subjects unable to complete continuous and competitive protocol at 0.35 ppm O_3.	Adams and Schelegle (1983)
0.21	412	1 h CE at 75% $\dot{V}O_2max$	Tdb = 19 to 21 °C RH = 60 to 70%	6 M, 1 F	Well-trained cyclists	Decrease in FVC, FEV_1, $FEF_{25-75\%}$, and MVV with 0.21 ppm O_3 compared with FA exposure.	Folinsbee et al. (1984)
0.25	490	1 h CE $\dot{V}_E$ = 63 L/min	Tdb = 20 °C RH = 70%	19 M, 7 F	Active nonathletes	FVC, FEV_1, and MVV all decreased with 0.25-ppm O_3 exposure compared with FA.	Folinsbee et al. (1986)
0.25 0.50 0.75	490 980 1,470	2 h rest	NA	8 M, 5 F		FVC decreased with 0.50- and 0.75-ppm O_3 exposure compared with FA; 4% nonsignificant decrease in mean $\dot{V}O_{2max}$ following 0.75 ppm O_3 compared with FA exposure.	Horvath et al. (1979)
0.35	686	50 min CE $\dot{V}_E$ = 60 L/min	NA	8 M	Trained nonathletes	V_T decreased, f increased with 50-min O_3 exposures; decrease in FVC, FEV_1, $FEF_{25-75\%}$, performance time, $\dot{V}O_{2max}$, $\dot{V}_{Emax}$, and HR_{max} from FA to 0.35-ppm O_3 exposure.	Foxcroft and Adams (1986)

Table 7-8 (cont'd). Ozone Effects on Exercise Performance[a]

Ozone Concentration[b]		Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
ppm	$\mu g/m^3$						
0.75	1,470	2 h IE (4 × 15 min light [50 W] bicycle ergometry)	NA	13 M	4 light S, 9 NS	Decrease in FVC, FEV_1, ERV, IC, and $FEF_{50\%}$ after 1-h 0.75-ppm O_3 exposure; decrease in $\dot{V}O_{2max}$, $\dot{V}_{Tmax}$, $\dot{V}_{Emax}$, maximal workload, and heart rate following 0.75-ppm O_3 exposure compared with FA.	Folinsbee et al. (1977)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.

reductions in performance time (29.7%), $\dot{V}O_{2max}$ (16.4%), maximum ventilation (18.5%), and maximum workload (7.8%) in 17 top-caliber endurance cyclists during a rapidly incremented $\dot{V}O_{2max}$ test following 1-h exposure to 0.2 ppm O_3 with very heavy CE ($\dot{V}_E$ = 90 L/min) and the addition of ambient heat stress (31 °C). In both studies (Foxcroft and Adams, 1986; Gong et al., 1986), the reductions in maximal exercise endpoints were accompanied by significant decrements in pulmonary function and marked subjective symptoms of respiratory discomfort. More recently, Linder et al. (1988) observed small decrements in performance time during a progressive maximal exercise test at O_3 concentrations as low as 0.06 ppm. These small effects were associated with increased respiratory symptoms and small, inconsistent changes in FEV_1. Hence, it appears that maximal oxygen uptake is reduced if it is preceded by an O_3 exposure entailing a sufficient total inhaled dose of O_3 to result in significant pulmonary function decrements or subjective symptoms of respiratory discomfort.

Effect on Endurance Exercise Performance

Two studies (Adams and Schelegle, 1983; Folinsbee et al., 1984) that addressed the effects of acute O_3 exposures on the ability of highly trained subjects to complete strenuous continuous exercise protocols were discussed in the 1986 EPA criteria document (U.S. Environmental Protection Agency, 1986).

Adams and Schelegle (1983) exposed 10 well-trained distance runners to FA and 0.20 and 0.35 ppm O_3 while the runners exercised on a bicycle ergometer at workloads simulating either a 1-h steady-state "training" bout or a 30-min warm-up followed immediately by a 30-min "competitive bout". The exercise levels in the steady-state training bout were of sufficient magnitude (68% of their $\dot{V}O_{2max}$) to increase mean $\dot{V}_E$ to 80 L/min. The $\dot{V}_E$ averaged over the entire competitive simulation was also 80 L/min, whereas the mean $\dot{V}_E$ during the 30-min competitive bout was 105 L/min. Subjective symptoms increased as a function of O_3 concentration for both training and competitive protocols. In the competitive protocol, four runners exposed to 0.20 ppm O_3 and nine exposed to 0.35 ppm O_3 indicated that they could not have performed maximally. Three subjects were unable to complete both the training and competitive protocols at 0.35 ppm O_3, and a fourth failed to complete only the competitive ride.

Folinsbee et al. (1984) exposed six well-trained men and one well-trained woman to 0.21 ppm O_3 while they exercised continuously on a bicycle ergometer for 1 h at 75% of their $\dot{V}O_{2max}$ ($\dot{V}_E$ = 81 L/min). Following O_3 exposure, FVC and FEV_1 were reduced significantly and the subjects reported symptoms of laryngeal and tracheal irritation and chest soreness and tightness when taking deep breaths. Anecdotal reports obtained from the cyclists suggested that their performance would have been limited if they experienced similar symptoms during competition.

Avol et al. (1984) exposed 50 well-conditioned cyclists to 0.00, 0.08, 0.16, 0.24, and 0.32 ppm O_3 for 1 h in ambient heat (32 °C) while they exercised continuously ($\dot{V}_E$ = 57 L/min). Reductions in FEV_1 and symptoms, initially detected at 0.16 ppm O_3, increased in a concentration- dependent manner. Three and 16 cyclists could not complete the 1-h exposure to 0.16 and 0.24 ppm O_3, respectively, without a reduction in workload. Similarly, in their study of the effects of O_3 exposure on $\dot{V}O_{2max}$, Gong et al. (1986) reported that 6 of 17 highly trained endurance cyclists were not able to complete 1-h exposure to 0.2 ppm O_3 with very heavy CE ($\dot{V}_E$ = 90 L/min) and the addition of ambient heat stress (31 °C).

In a study designed to determine the effects of the inhalation of low ambient O_3 concentrations on simulated competitive endurance performance, Schelegle and Adams (1986) exposed 10 highly trained endurance athletes to 0.12, 0.18, and 0.24 ppm O_3 while they were performing a 1-h "competitive" protocol. The competitive protocol used in this study was similar to that used by Adams and Schelegle (1983) except that the workload during the final 30-min competitive bout was more intense; it was selected based on the maximum workload (approximately 86% of their $\dot{V}O_{2max}$, mean $\dot{V}_E$ = 120 L/min) each subject could maintain for 30 min while breathing FA. All subjects completed the FA exposure, whereas one, five, and seven subjects could not complete the 0.12-, 0.18-, and 0.24-ppm O_3 exposures, respectively. Following 0.18- and 0.24-ppm O_3 exposures, FVC and FEV_1 were reduced significantly ($p < 0.05$), and subjective symptoms were elevated significantly ($p < 0.05$). No significant effect of O_3 was found for metabolic or ventilatory pattern responses. Similarly, Folinsbee et al. (1986) found that highly trained runners experienced a reduced run time on a treadmill (speed and grade set at approximately 80% of their subjects $\dot{V}O_{2max}$) when exposed to 0.18 ppm O_3 compared with FA. These subjects did have significantly elevated symptoms of respiratory discomfort and significantly decreased FVC and FEV_1, whereas arterial oxygen saturation at the end of the run was not affected by O_3 exposure.

Determining the mechanisms leading to the observed decrements in maximal oxygen uptake and the inability to complete strenuous exercise protocols is problematic. As stated by Astrand and Rodahl (1977) "the capacity for prolonged rhythmic muscular exercise is limited by an interrelated composite of cardiorespiratory, metabolic, environmental, and psychological factors." Many investigators cited above have concluded that the observed reductions in exercise performance appeared to be due to symptoms limiting the ability of their subjects to perform. However, in every case, this is a conclusion achieved by exclusion and not by the demonstration of a causal relationship. Other factors could also contribute to O_3-induced decrements in exercise performance. One possibility is that stimulation of neural receptors in the airways may result in an inhibition of alpha-motor nerve activity to respiratory muscles during inspiration (Koepchen et al., 1977; Schmidt and Wellhoner, 1970), resulting in the observed decrease in V_T and, at the same time, increasing the subject's sensation of respiratory effort. This mechanism would not be directly related to symptoms of discomfort but, because of the common role of airway neural afferents, may be difficult to discern from the effects of symptoms of respiratory discomfort. Indeed, a reflex inhibition of the ability to inspire would be consistent with the reduced V_T following O_3 exposure in subjects performing maximal exercise and would be consistent with the development of a physiologically induced ventilatory limitation to maximal oxygen uptake.

7.2.2 Pulmonary Function Effects of Prolonged (Multihour) Ozone Exposures

Since 1988, a series of studies has described the responses of subjects exposed to relatively low (0.08 to 0.16 ppm) O_3 concentrations for durations of 4 to 8 h (see Table 7-9). These studies have demonstrated statistically significant changes in spirometry, R_{aw}, symptoms, and airway responsiveness during and after exposures. As in studies conducted at higher concentrations of O_3 for shorter periods of time, there is broad variability in response.

The only related study cited in the previous criteria document (U.S. Environmental Protection Agency, 1986) was that of Kerr et al. (1975), who exposed

Table 7-9. Pulmonary Function Effects After Prolonged Exposures to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.08 0.10 0.12	157 196 235	6.6 h IE (6 × 50 min) $\dot{V}_E \approx 39$ L/min	18 °C 40% RH	22 M	Healthy NS, 18 to 33 years old	FVC and FEV_1 decreased throughout the exposure; FEV_1 decrease at end exposure was 7.0, 7.0, and 12.3%, respectively. FEV_1 change >15% occurred in 3, 5, and 9 subjects at 0.08, 0.10, and 0.12 ppm, respectively. Methacholine responsiveness increased by 56, 89, and 121%, respectively.	Horstman et al. (1990)
		See Horstman et al. (1990) and Folinsbee et al. (1988)				A lognormal model was fitted to FEV_1 data. Model parameters indicate O_3 concentration had greater effect than $\dot{V}_E$ or duration (estimated exponent for $[O_3] \approx 4/3$).	Larsen et al. (1991)
0.08 0.10	157 196	6.6 h IE (6 × 50 min) $\dot{V}_E = 40$ L/min	18 °C 40% RH	38 M	Healthy NS, mean age 25 years old	FEV_1, decreased 8.4% at 0.08 ppm and 11.4% at 0.10 ppm. Symptoms of cough, PDI, and SB increased with O_3 exposure.	McDonnell et al. (1991)
0.08	157	6.6 h IE (6 × 50 min) $\dot{V}_E = 35$ to 38 L/min (1 day of air, 2 days of O_3)	25 °C 48% RH	5 F, 6 M	Healthy NS, 30 to 45 years old	FVC decreased 2.1%, FEV_1 decreased 2.2% on first day of O_3 exposure; no change on second O_3 day.	Horvath et al. (1991)
0.12	235	6.6 h IE (6 × 50 min) $\dot{V}_E = 42.6$ L/min	18 °C 40% RH (1 exposure to clean air; 1 exposure to O_3)	10 M	Healthy NS, 18 to 33 years old	FEV_1 decreased by 13% after 6.6 h. FVC dropped 8.3%. Cough and PDI increased with O_3 exposure. Airway responsiveness to methacholine doubled after O_3 exposure.	Folinsbee et al. (1988)
(a) 0.12 (b) Varied from 0.0 to 0.24 (increased by 0.06 ppm/h then decreased by 0.06 ppm/h)	235	8 h IE (8 × 30 min) $\dot{V}_E = 40$ L/min	22 °C 40% RH <3 $\mu g/m^3$ TSP	23 M	Healthy NS, 20 to 35 years old	(a) FEV_1 decreased 5% by 6 h and remained at this level through 8 h. (b) FEV_1 change mirrored O_3 concentration change with a lag time of ≈ 2 h. Max decrease of 10.2% after 6 h. FEV_1 change was reduced in last 2 h of exposure.	Hazucha et al. (1992)

Table 7-9 (cont'd). Pulmonary Function Effects After Prolonged Exposures to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.12	235	6.5 h/day IE (6 × 50 min) (2 days of exposure) $\dot{V}_E$ = 28 L/min (asthmatic) $\dot{V}_E$ = 31 L/min (healthy)	21 °C 50% RH	15 (8 M, 7 F) 30 (13 M, 17 F)	Healthy NS, 22 to 41 years old Asthmatic NS, 18 to 50 years old	Bronchial reactivity to methacholine increased with O_3 exposure in healthy subjects. FEV_1 decreased 2% (pre- to postexposure) in healthy subjects and 7.8% in asthmatics. Responses were generally less on the second day. Two healthy subjects and four asthmatics had FEV_1 decreases >10%.	Linn et al. (1994)
0.12	235	6.6 h IE (6 × 50 min) $\dot{V}_E$ = 38.8 L/min	18 °C 40% RH (5 consecutive days of exposure to O_3, 1 day exposure to CA)	17 M	Healthy NS, mean age 25 ± 4 years old	FEV_1 decreased by 12.8, 8.7, 2.5, and 0.6 and increased by 0.2 on Days 1 to 5 of O_3 exposure, respectively. Methacholine airway responsiveness increased by >100% on all exposure days. Symptoms increased on the first O_3 day, but were absent on the last 3 exposure days.	Folinsbee et al. (1994)
0.16	314	4 h IE (4 × 50 min) $\dot{V}_E \approx$ 38.9 L/min	18 °C 40% RH (one exposure to O_3, no control exposure)	15 M	Healthy NS, mean age 25 ± 4 years old	FVC decreased 9.5% and FEV_1 decreased 16.6%. FEV_1/FVC ratio decreased from 0.79 to 0.73.	

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.

subjects for 6 h to 0.5 ppm O_3, with only two brief 15-min periods of moderate exercise ($\dot{V}_E$ = 44 L/min) during the exposure. Small changes in spirometry were observed. Because of the minimal extent of exercise and the high O_3 concentration, these results cannot be compared to the more recent studies.

The first prolonged O_3 exposure study involving low concentrations and a substantial amount of "moderate exercise"[1] was reported by Folinsbee et al. (1988). The basic protocol used by these investigators has been used in a number of subsequent investigations and therefore merits describing in some detail. The exposures lasted 6 h and 35 min ($\approx$6.6 h). Except for a 35-min lunch break (during which O_3 exposure continued at rest) after 3 h, the subjects exercised at a moderate level (with a ventilation of about 40 L/min) for 50 min of each hour. Pulmonary function tests were conducted during the 10-min rest period and at the beginning and end of exposure. The exposure was intended to simulate a day of heavy outdoor work or play. For convenience, this protocol is referred to as the EPA prolonged-exposure protocol.

In this study (Folinsbee et al., 1988), a group of 10 subjects was exposed to clean air and 0.12 ppm O_3 for 6.6 h. Forced vital capacity and FEV_1 decreased in a roughly linear fashion throughout the exposure and had fallen by 8.3 and 13%, respectively, by the end of the exposure. Symptoms of cough and chest discomfort were increased, and airway responsiveness to methacholine was approximately doubled after O_3 exposure. There was a wide range of response, three subjects had FEV_1 decrements of 25% or greater, and the three least sensitive subjects had less than 5% change in FEV_1.

In order to extend these initial observations, Horstman et al. (1990) used the same protocol to expose a group (n = 22) of subjects to clean air and three different O_3 concentrations (0.08, 0.10, and 0.12 ppm). At 0.12 ppm O_3, responses were similar to those observed in the previous study, with the exception that the symptom responses were smaller in the new group of subjects. A similar (but of smaller magnitude) pattern of response in spirometry, R_{aw}, and airway responsiveness was seen at the two lower concentrations. The mean FEV_1 responses during the four exposures are shown in Figure 7-3. The responses were dependent on concentration and exposure duration (ventilation was not varied) and averaged 7, 8, and 13% at the three O_3 concentrations. Larsen et al. (1991) used these data (Horstman et al., 1990) to develop a "dose-response" relationship for percent change in FEV_1 as a function of O_3 concentration and exposure duration. The lognormal multiple linear regression model suggested that FEV_1 responses were approximately linear with duration of exposure but that O_3 concentration plays a slightly more important role. The exponent of approximately 4/3 suggests that doubling O_3 concentration would be similar to increasing exposure duration by about 2½ times.

A series of additional exposures were conducted at 0.08 and 0.10 ppm O_3 to study changes in cells and inflammatory mediators from BAL (see Section 7.2.4), but pulmonary function was measured as well. McDonnell et al. (1991) reported an 8.4% decrease in FEV_1 at 0.08 ppm and an 11.4% decrease at 0.10 ppm. These responses were slightly larger than those seen in the previous Horstman et al. (1990) study. The duration-FEV_1 response data were fit to a three-parameter logistic model, which significantly improved the amount of

[1]The "moderate" exercise descriptor is based on previously published EPA guidelines for representative types of exercise (see Table 10-3, U.S. Environmental Protection Agency, 1986). Note, however, that exercise continued at this level (40 L/min) for 6 to 8 h should be considered as "heavy" or "strenuous work or play".

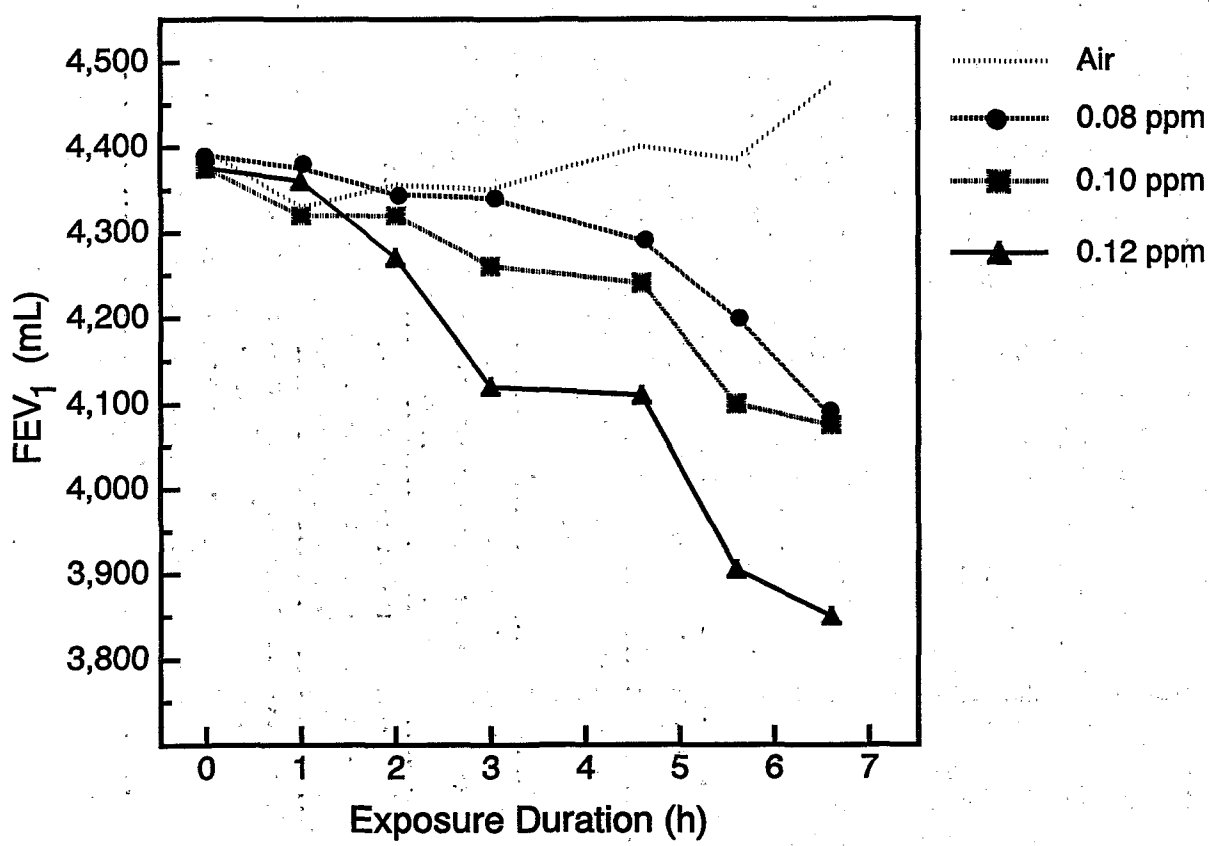

Figure 7-3. The forced expiratory volume in 1 s (FEV_1) is shown in relation to exposure duration at different ozone concentrations. A 35-min resting exposure period was interposed between the end of the third hour and the beginning of the fourth hour. There were six 50-min exercise periods (minute ventilation ≈ 39 L/min) during the exposure; these measurements were made 5 min after the end of each exercise. The total exposure duration was 6.6 h. The standard error of the mean (not shown) for these FEV_1 averages ranged from 120 to 160 mL.

Source: Horstman et al. (1990).

variance explained by the model compared to a linear model; this is consistent with exploratory analyses in the Folinsbee et al. (1988) report. The reasonably good fit to the logistic model suggests that the O_3-pulmonary function response relationship may have a sigmoid shape. The primary importance of this observation is that it suggests that there is a response plateau. That is, for a given O_3 concentration and exercise ventilation level (i.e., dose rate), and after a certain length of exposure, the FEV_1 response tends not to increase further (i.e., plateau) with increasing duration of exposure.

In the fourth study in this series (Folinsbee et al., 1994), 17 subjects were exposed to 0.12 ppm O_3 for 6.6 h on 5 consecutive days. Subjects who were not responsive

to O_3 were not selected to participate in this study. Responses in FEV_1 on the first of these exposures averaged −12.8%. Again, symptom responses were modest with a significant increase in lower respiratory symptoms on the first exposure day. A significant increase in airway responsiveness to methacholine also was shown. The response to the repeated exposures is discussed in Section 7.2.1.4. In addition, 15 subjects were exposed to 0.16 ppm for 4 h using the same hourly exposure protocol as described above. In these subjects, FVC decreased 9.5%, and FEV_1 declined 16.6%.

Folinsbee et al. (1991) took the FEV_1 response data from all four studies conducted at the EPA Health Effects Research Laboratory, using the same prolonged-exposure protocol, and examined the distribution of responses among the subjects at the three concentrations. This response distribution is illustrated graphically in Figure 7-4, which illustrates that FEV_1 decrements as large as 30 to 50% have been observed with prolonged exposure to O_3 concentrations ≤0.12 ppm. This response distribution allows one to determine the number or percentage of subjects with responses in excess of a certain level. The proportion of subjects with an FEV_1 decrease in excess of 10% is shown in Figure 7-4. With air exposure, no one exceeded this response level; however, 46% of the subjects exposed to 0.12 ppm O_3 had a >10% drop in FEV_1 after 6.6 h.

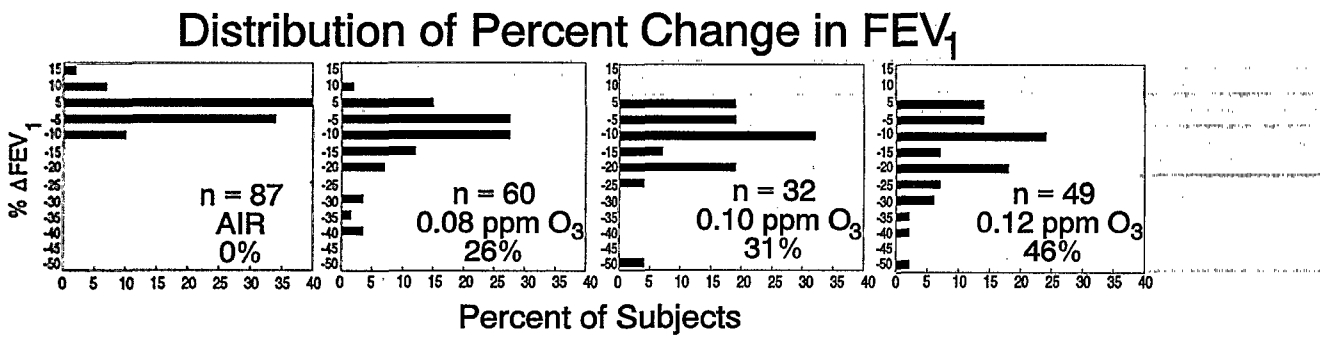

Figure 7-4. The distribution of response for 87 subjects exposed to clean air and at least one of 0.08, 0.10, or 0.12 ppm ozone (O_3) is shown here. The O_3 exposures lasted 6.6 h, during which time the subjects exercised for 50 min of each hour with a 35-min rest period at the end of the third hour. Decreases in forced expiratory volume in 1 s (FEV_1) are expressed as percent change from baseline. For example, the bar labeled "−10" indicates the percent of subjects with a decrease in FEV_1 of >5% but ≤10%, and the bar labeled "5" indicates improvement in FEV_1 of >0% but ≤5%. Each panel of the figure indicates the percentage of subjects at each O_3 concentration with a decrease of FEV_1 in excess of 10%.

This response distribution also illustrates the wide range of response to O_3 under these exposure conditions and reinforces the observation by others (McDonnell et al., 1983; Horvath et al., 1981) of a substantial range of individual response to O_3.

Horvath et al. (1991) examined the responses of healthy men and women (ages 30 to 45) to 0.08 ppm O_3 for 6.6 h using the EPA prolonged-exposure protocol. When

compared with the clean air exposure, FEV_1 decreased about 5% with O_3 exposure. However, the variability of this response among this small heterogeneous group of subjects was enough to preclude statistical significance of this observation. No significant changes were observed with a second exposure on the next day. On the first day of O_3 exposure, 7 of the 11 subjects reported chest tightness. The authors point out that the range of variability in response in their study was similar to that reported by Folinsbee et al. (1988) and Horstman et al. (1990), although fewer subjects experienced large negative changes in FEV_1. One possible explanation for the differences between the findings of Horvath et al. (1991) and Horstman et al. (1990) may be that the subjects in Horvath's study were significantly older, which may result in reduced responses to O_3, as Drechsler-Parks et al. (1987a) have shown (see Section 7.2.1.3). The ventilation during exercise (37 to 39 L/min) was similar to that reported by Horstman et al. (1990). An additional FEV_1 measurement was made in this study at the end of the lunch period (i.e., after 40 min of rest). At this time, the small decrements in FEV_1 seen after the third exercise were reversed, and the FEV_1 was similar to the response in FA at the same time point. Although spirometry was not measured at this time in the other prolonged-exposure studies (Folinsbee et al., 1988; Horstman et al., 1990), it was noted that the decline in FEV_1 was attenuated between the third and fourth postexercise measurement. These observations suggest that the subjects' lung function may indeed have improved during the lunch rest period.

Linn et al. (1994) have reported responses of 45 healthy and asthmatic subjects to 0.12 ppm O_3 using the EPA prolonged-exposure protocol. In healthy subjects, they observed a small (1.7%) decrease in FEV_1, which was statistically significant, and an increase in airway responsiveness to methacholine. The functional responses in asthmatics (e.g., a 7.8% decrease in FEV_1) were greater than those of the healthy subjects. They observed smaller responses on a second consecutive day of exposure, as did Horvath et al. (1991) and Folinsbee et al. (1994). The ventilation averaged 31 and 28 L/min in the healthy and asthmatic subjects, respectively. The FEV_1 responses observed in this study, although statistically significant, are much lower than those observed by EPA investigators (Folinsbee et al., 1988; Horstman et al., 1990; Folinsbee et al., 1994). The smaller responses may be due to previous ambient exposures, lower ventilations, or a larger proportion of O_3-insensitive subjects in Los Angeles. Only 1 of 15 healthy subjects experienced an FEV_1 decrement in excess of 10%, whereas 9 of 30 asthmatics had FEV_1 decrements in excess of 10%. Asthmatic responses ranged from 12% to −35%.

To further explore the factors that determine responsiveness to O_3, Hazucha et al. (1992) designed a protocol to examine the effect of varying, rather than constant, O_3 concentrations. In this study, subjects were exposed to a constant level of 0.12 ppm O_3 for 8 h and to an O_3 level that increased linearly from 0 to 0.24 ppm for the first 4 h and then decreased linearly from 0.24 to 0 over the second 4 h of the 8 h exposure (triangular concentration profile). Subjects performed moderate exercise for the first 30 min of each hour. The overall exposure dose for these two exposures, calculated as the $C \times T \times \dot{V}_E$, was almost identical (difference < 1%). With exposure to the constant 0.12 ppm O_3, the FEV_1 declined approximately 5% by the fifth hour of exposure and remained at that level for the remainder of the exposure. These responses are illustrated in Figure 7-5. This observation clearly indicates a response plateau, suggested in other studies (Horstman et al., 1990), with an exposure regimen that produces relatively small changes in lung function.

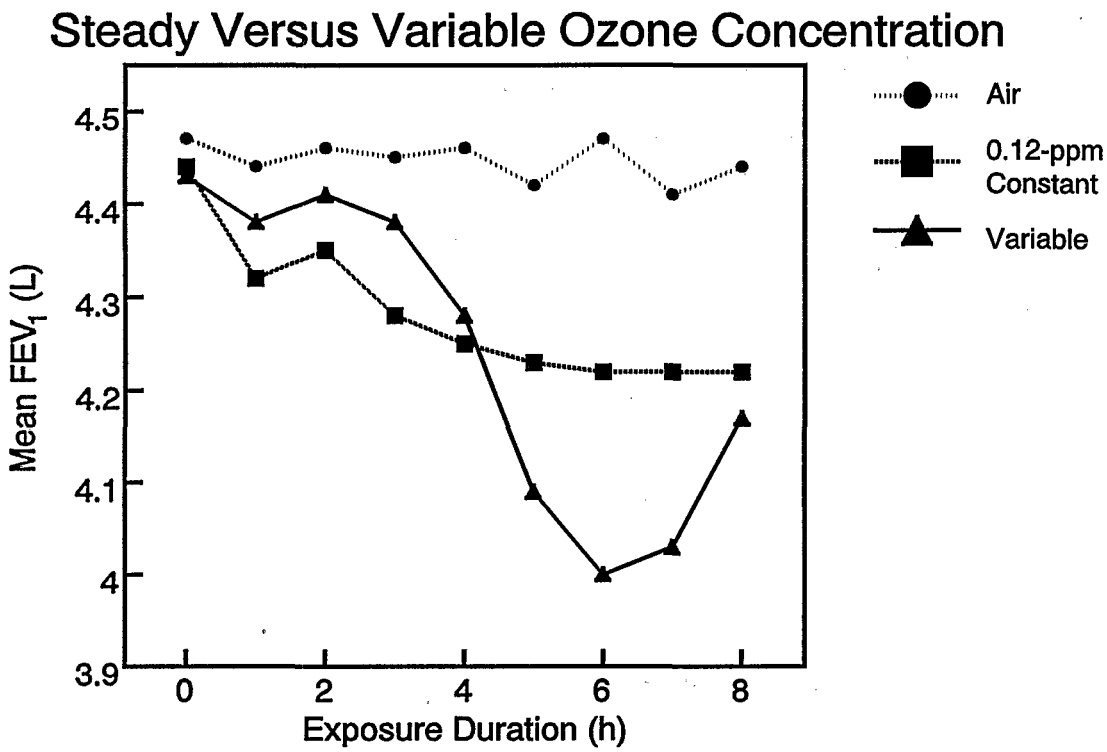

Figure 7-5. The forced expiratory volume in 1 s (FEV_1) is shown in relation to exposure duration (hours) under three exposure conditions. Subjects exercised (minute ventilation ≈ 40 L/min) for 30 min during each hour; FEV_1 was measured at the end of the intervening rest period. Standard error of the mean for these FEV_1 averages (not shown) ranged from 120 to 150 mL.

Source: Hazucha et al. (1992).

With the triangular O_3 concentration profile, the FEV_1 decreased almost twice as much after 6 h of exposure. The initial response over the first 3 h was minimal, and then there was a substantial decrease in FEV_1, corresponding to the higher average O_3 concentration, that reached a nadir after 6 h. Despite continued exposure to a lower O_3 concentration (<0.12 ppm), the FEV_1 began to improve and was reduced by only 5.9% at the end of the 8-h exposure. (However, note that the average O_3 concentration in the eighth hour was 0.03 ppm). This study illustrates two important points. First, a response plateau occurs. It is intuitively obvious that there must be a limit to the acute decrease that can occur in FEV_1. However, from this study, it is also clear that the response plateau must be dependent on the O_3 concentration because much larger decreases in FEV_1 occur with exposure to O_3 concentrations higher than 0.12 ppm. Second, the response to O_3 exposure is dependent on the dose rate (some function of C and $\dot{V}_E$) and the cumulative dose (some function of dose rate and T), at least when the O_3 concentration is varied. This study also affirms the observation (Folinsbee et al., 1978; Adams et al., 1981; Hazucha, 1987; Larsen

et al., 1991) that O_3 concentration is a more important factor in determining O_3 responses than is either exposure duration or the volume of air breathed during the exposure.

7.2.3 Increased Airway Responsiveness

Increased airway responsiveness indicates that the airways are predisposed to bronchoconstriction induced by a variety of stimuli (e.g., specific allergens, SO_2, cold air, etc.). Airway responsiveness is usually measured by having the individual forcefully exhale into a spirometer designed to measure expiratory flow rates (e.g., FEV_1) or, less commonly, by measuring R_{aw} in a body plethysmograph. In order to determine the level of airway responsiveness, airway function is measured before and immediately after the inhalation of small amounts of an aerosolized bronchoconstrictor drug (e.g., methacholine or histamine). The dose of the bronchoconstrictor drug is increased in a step-wise fashion until a predetermined degree of airway response (e.g., a 20% drop in FEV_1 or a 100% increase in R_{aw}) has occurred. The dose of the bronchoconstrictor drug that produced the aforementioned response is often referred to as the "PD_{20}" (i.e., the provocative dose that produced a 20% drop in FEV_1) or the "PD_{100}" (i.e., the provocative dose that produced a 100% increase in R_{aw}).

A high level of bronchial responsiveness is a hallmark of asthma. However, varying degrees of increased airway responsiveness may occur in other lung disease (e.g., chronic bronchitis or viral respiratory infections) or in healthy asymptomatic individuals. The range of nonspecific bronchial responsiveness, as expressed by the PD_{20} for example, is at least 1,000-fold from the most sensitive asthmatics to the least sensitive healthy subjects (see Figure 7-6). The average PD_{20} for healthy subjects is 10 to 100 times that of mild to moderate asthmatics (Chatham et al., 1982; Cockcroft et al., 1977). Atopic or allergic individuals without asthma (intermediate in responsiveness between healthy subjects and mild asthmatics) typically have a lower PD_{20} than healthy individuals (Townley et al., 1975; Cockcroft et al., 1977). Increasing severity of asthma, as indicated by increasing symptoms or medication usage, is associated with decreasing PD_{20}. Mild asthmatics may have a PD_{20} that is 10 times higher than that of moderate or severe asthmatics (Cockcroft et al., 1977). A low PD_{20} in nonasthmatics also is associated with increased symptoms and a reduced baseline FEV_1 (Kennedy et al., 1990). The average changes in airway responsiveness induced by O_3 range from 150 to 500%. This means that, in a healthy subject exposed to O_3, a PD_{20} of 20 units would decrease to a PD_{20} between 13 and 4 units. Therefore, with a pronounced O_3-induced change in airway responsiveness, a healthy subject could move from the normal range into the upper half of the mild asthmatic range of airway responsiveness.

Increases in airway responsiveness are an important consequence of exposure to O_3. Results of studies reporting changes in airway responsiveness following O_3 exposure are summarized in Table 7-10. These studies vary with regard to exposure regimens, type and dose of bronchoconstrictive agent, and subject population. Increased airway responsiveness associated with O_3 exposure was first reported by Golden et al. (1978), who studied histamine-responsiveness in eight healthy men after exposure to 0.6 ppm O_3 for 2 h at rest and found that the histamine-induced ΔR_{aw} for the group was 300% greater 5 min after O_3 exposure than at baseline. Two of their subjects, however, had an increased response to histamine 1 week or greater after exposure, raising the possibility that high O_3 levels can result in more persistent increases in airway responsiveness. Later, Holtzman et al. (1979) found in 16 nonasthmatic subjects that a 10-breath methacholine or histamine challenge

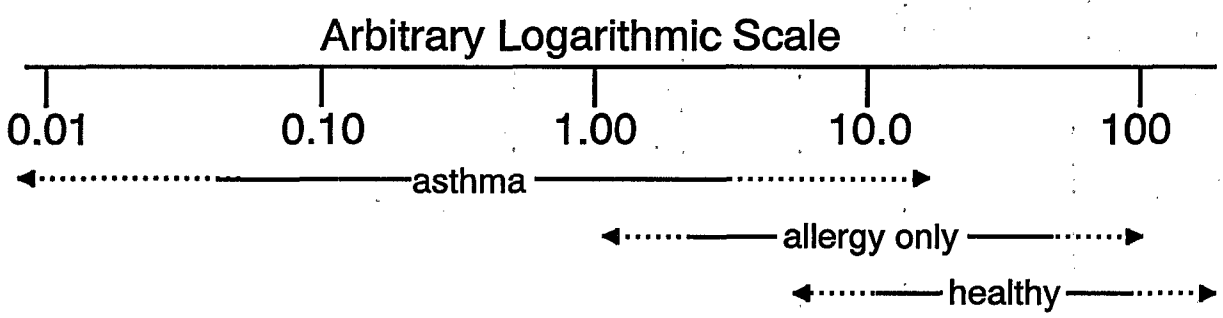

Figure 7-6. Airway function can be measured before and immediately after the inhalation of an aerosolized bronchoconstrictor drug like methacholine. The provocative dose that produces a 20% drop in forced expiratory volume in 1 s has been used to express the range of nonspecific bronchial responsiveness.

increased SRaw almost twice as much after O_3 as after air exposure, but this effect resolved after 24 h. Atopic subjects showed similar increases in responsiveness to histamine after O_3 exposure. The authors concluded that the increased nonspecific bronchial responsiveness after O_3 exposure was not related to atopy. König et al. (1980) found increased responsiveness to inhaled acetylcholine after a 1-h exposure to 627 and 1,960 $\mu g/m^3$ (0.32 and 1.00 ppm, respectively). Folinsbee and Hazucha (1989) found increased airway responsiveness in 18 females 1 and 18 h after a 70-min exposure to 0.35 ppm O_3 when compared to air. Taken together, these studies suggest that O_3-induced increases in airway responsiveness usually resolve 18 to 24 h after exposure, but may persist in some individuals for longer periods.

Dimeo et al. (1981) were the first to investigate "adaptation" to the increases in airway responsiveness following O_3 exposure. Over 3 days of a 2 h/day exposure to 0.40 ppm O_3, they found progressive attenuation of the increases in airway responsiveness such that, after the third day of O_3 exposure, histamine airway responsiveness was no longer different from the sham exposure levels. Kulle et al. (1982) extended these findings by exposing two groups of healthy volunteers (n = 48) to 0.40 ppm O_3 for 3 h/day for 5 days in a row and found that there was a significantly enhanced response to methacholine after the first 3 days of exposure, but this response slowly normalized by the end of the fifth day. Thus, the attenuation of O_3-induced increases in airway responsiveness followed the same time course as attenuation of other pulmonary function changes.

Gong et al. (1986) demonstrated increased airway responsiveness to histamine at 0.2 ppm O_3 in 17 vigorously exercising elite cyclists who were exposed for 1 h. Folinsbee et al. (1988) found an approximate doubling of the mean methacholine responsiveness in a group of healthy volunteers exposed for 6.6 h to 0.12 ppm O_3. However, on an individual basis, no relationship was found between O_3-induced changes in airway responsiveness and those in FVC and FEV_1, suggesting that changes in airway responsiveness and lung volume occurred by different mechanisms. Horstman et al. (1990) extended Folinsbee's observations by demonstrating significant decreases in the PD_{100} in 22 healthy subjects immediately after a

Table 7-10. Increased Airway Responsiveness Following Ozone Exposures[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.08 0.10 0.12	157 196 235	6.6 h IE at ≈39 L/min	18 °C 40% RH	22 M	Healthy NS, 18 to 32 years old	33, 47, and 55% decreases in cumulative dose of methacholine required to produce a 100% increase in SR_{aw} after exposure to O_3 at 0.08, 0.10, and 0.12 ppm, respectively.	Horstman et al. (1990)
0.10 0.32 1.00	196 627 1,960	2 h	NA	14	Health NS, 24 ± 2 years old	Increased airway responsiveness to methacholine immediately after exposure at the two highest concentrations of O_3.	König et al. (1980)
0.12 0.20	235 392	1 h at $\dot{V}_E$ = 89 L/min followed by 3 to 4 min at ≈150 L/min	31 °C 35% RH	15 M, 2 F	Elite cyclists, 19 to 30 years old	Greater than 20% increase in histamine responsiveness in one subject at 0.12 ppm O_3 and in nine subjects at 0.20 ppm O_3.	Gong et al. (1986)
0.12	235	6.6 h with IE at ≈25 L/min/m^2 BSA	NA	10 M	Healthy NS, 18 to 33 years old	Approximate doubling of mean methacholine responsiveness after exposure. On an individual basis, no relationship between O_3-induced changes in airway responsiveness and FEV_1 or FVC.	Folinsbee et al. (1988)
0.12 ppm O_3-100 ppb SO_2 0.12 ppm O_3-0.12 ppm O_3 Air-100 ppb SO_2		45 min in first atmosphere and 15 min in second IE	75% RH 22 °C	8 M, 5 F	Asthmatic, 12 to 18 years old	Greater declines in FEV_1 and $\dot{V}_{max50\%}$ and greater increase in respiratory resistance after O_3-SO_2 than after O_3-O_3 or air-SO_2.	Koenig et al. (1990)
Air-antigen 0.12 ppm O_3-antigen		1 h at rest	NA	4 M, 3 F	Asthmatic, 21 to 64 years old	Increased bronchoconstrictor response to inhaled ragweed or grass after O_3 exposure compared to air.	Molfino et al. (1991)
0.35	686	70 min with IE at 40 L/min	NA	18 F	Healthy NS, 19 to 28 years old	PD_{100} decreased from 59 CIU after air exposure to 41 CIU and 45 CIU, 1 and 18 h after O_3 exposure, respectively.	Folinsbee and Hazucha (1989)

Table 7-10 (cont'd). Increased Airway Responsiveness Following Ozone Exposures[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics[c]	Observed Effect(s)	Reference
0.20 0.40 0.40	392 784 784	2 h with IE at 2 × resting 2 h with IE at 2 × resting 2 h/day for 3 days	22 °C 55% RH	12 M, 7 F	Healthy NS, 21 to 32 years old	110% increase in ΔSR_{aw} to a 10-breath histamine (1.6%) aerosol challenge after exposure to O_3 at 0.40 ppm, but no change at 0.20 ppm. Progressive adaptation of this effect over 3-day exposure.	Dimeo et al. (1981)
0.40	784	3 h/day for 5 days in a row		13 M, 11 F	Healthy NS, 19 to 46 years old	Enhanced response to methacholine after first 3 days, but this response normalized by Day 5.	Kulle et al. (1982)
0.40	784	2 h with IE at $\dot{V}_E$ = 53 to 55 L/min	22 °C 50% RH	8 M, 10 F	9 asthmatics (5 F, 4 M), 9 healthy (5 F, 4 M), 18 to 34 years old	Decreased $PC_{100SR_{aw}}$ from 33 mg/mL to 8.5 mg/mL in healthy subjects after O_3. $PC_{100SR_{aw}}$ fell from 0.52 mg/mL to 0.19 mg/mL in asthmatic subjects after exposure to O_3 and from 0.48 mg/mL to 0.27 mg/mL after exposure to air.	Kreit et al. (1989)
0.60	1,176	2 h at rest	NA	5 M, 3 F	Healthy NS, 22 to 30 years old	300% increase in histamine-induced ΔR_{aw} 5 min after O_3 exposure; 84 and 50% increases 24 h and 1 week after exposure ($p > 0.05$), respectively. Two subjects had an increased response to histamine 1 week after exposure.	Golden et al. (1978)
0.60	1,176	2 h with IE at 2 × resting	22 °C 55% RH	11 M, 5 F	9 atopic, 7 nonatopic, NS, 21 to 35 years old	Ten-breath methacholine or histamine challenge increased $SR_{aw} \geq 150\%$ in 16 nonasthmatics after O_3. On average, the atopic subjects had greater responses than the nonatopic subjects. The increased responsiveness resolved after 24 h. Atropine premedication blocked the O_3-induced increase in airway responsiveness.	Holtzman et al. (1979)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.

6.6-h exposure to concentrations of O_3 as low as 0.08 ppm. Because methacholine challenges were not conducted at later time points in any of these studies, the duration of the increased airway responsiveness after ambient-level O_3 exposure could not be determined.

No doubt exists that O_3, even at ambient concentrations, produces acute increases in airway responsiveness. Whether O_3 exposure causes protracted increases in airway responsiveness in healthy individuals, induces asthma, or predisposes individuals to asthma is a more difficult question to answer (see Section 7.4.2). However, the increases in airway responsiveness following O_3 exposure, even if short in duration, may have important clinical implications. Several studies have been conducted specifically to determine the significance of acute increases in airway responsiveness after O_3 exposure. These studies, designed to test the hypothesis that an O_3 exposure heightens the response to a subsequent bronchoconstrictor challenge, have exposed asthmatics to O_3 or air and, then, to a known bronchoconstrictor agent to compare the pulmonary function changes after O_3 to those after air. Kreit et al. (1989) were first to investigate the change in airway responsiveness that occurs after O_3 exposure in individuals with asthma. They exposed nine mild asthmatics (baseline $PC_{100SRaw} < 1.5$ mg/mL) for 2 h to 0.40 ppm O_3 with IE and found that the baseline $PC_{100SRaw}$ declined from 0.52 to 0.19 mg/mL after O_3 as compared to 0.48 to 0.27 mg/mL after air. Koenig et al. (1990) demonstrated that a 45-min exposure to 0.12 ppm O_3 followed by a 15-min exposure to 100 ppb SO_2 caused greater changes in FEV_1, respiratory resistance, and $\dot{V}_{max50\%}$ in 14 adolescent asthmatics than did an air-SO_2 exposure combination.

Molfino et al. (1991) examined the effects of a 1-h resting exposure to 0.12 ppm O_3 on the response to a ragweed or grass allergen inhalation challenge. Asthmatic subjects were exposed twice to air and twice to O_3, once per week over a period of 4 weeks. Two allergen challenges were performed, once after air and once after O_3 exposure. The other air and O_3 exposures were followed by a placebo challenge. A ragweed allergen extract was used for six of the seven subjects. The order of experiments was not randomized (in an effort to avoid unexpectedly severe reactions); six of the seven subjects were exposed to the ozone-allergen condition last and five of the seven were exposed to the air-placebo condition first. Allergen responsiveness was expressed as the allergen concentration needed to cause a 15% reduction in FEV_1 or PC_{15}. The PC_{15} was lower after the O_3 exposure than after the air exposure ($p = 0.04$). These observations suggest that allergen-specific airway responsiveness is increased after O_3 exposure. Although it is expected that specific bronchial reactivity will be increased by O_3 exposure based on the marked increases in nonspecific bronchial responsiveness induced by O_3 exposure, such a response would not have been anticipated under these mild exposure conditions where lung function or symptomatic responses have not been observed. The lack of randomization in this study makes it difficult to assess the validity of conclusions based on the statistical analysis. These results are provocative but should be considered preliminary until this experiment can be repeated.

Ozone may be a clinically important co-factor in the response to airborne bronchoconstrictor substances in individuals with asthma. It is plausible that this phenomenon could contribute to increased asthma exacerbations and, even, consequent increased hospital admissions (see Section 7.4.1). Whether the increased airway responsiveness following O_3 exposure produces an accentuated bronchoconstrictor response to inhaled allergens or SO_2 in healthy individuals or those with lung diseases other than asthma is unknown.

Several studies have been undertaken to determine the mechanism of O_3-induced increases in airway responsiveness (also see Chapter 6). Early experiments in dogs (Lee et al., 1977) and humans (Golden et al., 1978) suggested an important role for vagal reflexes because vagal nerve cooling and atropine inhibited the increase in histamine-induced bronchoconstriction caused by O_3. Ozone exposure increased bronchomotor responses to cholinergic stimuli (e.g., acetylcholine and methacholine) in dogs (Holtzman et al., 1983) and humans (Seltzer et al., 1986). Subsequent studies, however, revealed that bilateral vagotomy did not inhibit O_3-induced hyperresponsiveness to subcutaneous histamine in guinea pigs (Gordon et al., 1984). These data provide strong evidence that O_3-induced increased airway responsiveness is mediated, at least in part, by cholinergic receptors on airway smooth muscle cells. Interestingly, Gordon et al. also noted that isometric tension in guinea pig tracheal smooth muscle and lung parenchymal strips in response to histamine and carbachol was not affected by exposure to O_3, suggesting that O_3 affected the in vivo milieu surrounding the smooth muscle rather than produced direct effects on the smooth muscle itself.

It can be hypothesized that the increased epithelial permeability caused by O_3 (see Chapter 6) may allow greater penetration of bronchoconstrictor substances, including methacholine and histamine, and that this would lead to increased airway responsiveness. However, Roum and Murlas (1984) suggested that the increased epithelial permeability after O_3 could not totally explain this phenomenon because parenteral cholinergic challenge after O_3 more reproducibly caused bronchospasm than did inhalation challenge with methacholine. The increased responsiveness to parenteral compared to inhaled cholinergic challenge may, however, have been due to increased bronchial blood flow after O_3 exposure. Therefore, the findings of Roum and Murlas do not exclude increased epithelial permeability as the cause of increased airway responsiveness after O_3 exposure.

Holtzman et al. (1983) first pointed out that O_3-induced acute inflammation may be important in the induction of the increased airway responsiveness. In mongrel dogs exposed to O_3, they found bronchial wall PMN infiltration in those animals that developed increased airway responsiveness to acetylcholine, but not in animals that failed to develop increased airway responsiveness. O'Byrne et al. (1984) later demonstrated that hydroxyurea simultaneously decreased peripheral blood leukocyte counts, decreased PMN influx into bronchial tissue, and prevented increased airway responsiveness in dogs exposed to O_3. Both O_3-induced increased airway responsiveness and bronchial tissue PMN influx returned 6 weeks after treatment was discontinued when peripheral leukocyte counts had normalized. Seltzer et al. (1986) found a larger percentage of PMNs (30.8% versus 8.0%) in BAL fluid after O_3 exposure in their subjects that had a greater than threefold decrease in the provocative concentration, which caused a ≥ 8 L $\times$ cm H_2O/L/s increase in SR_{aw} for methacholine, compared to those subjects that had less than a twofold decrease. These data suggest a possible association between inflammation and increased airway responsiveness after O_3 exposure.

An early study in dogs (O'Byrne et al., 1984) suggested that oxygenation products of arachidonic acid that are sensitive to inhibition by the anti-inflammatory drug indomethacin play a role in O_3-induced hyperresponsiveness without affecting the influx of PMNs. In the first of several human studies with a PG inhibitor, indomethacin did not attenuate the increase in airway responsiveness in subjects exposed to 0.4 ppm O_3 for 2 h (Ying et al., 1990), but did ameliorate the effect of O_3 on spirometric endpoints. Kleeberger and Hudak (1992) observed a marked reduction in PMN influx in O_3-exposed

mice given indomethacin without any change in O_3-induced increases in permeability, as indicated by BAL protein. However, Hazucha et al. (1996) found no effect of ibuprofen on PMN levels or protein in the BAL fluid of O_3-exposed humans (also see Section 7.2.4.5). Seltzer et al. (1986) and Koren et al. (1989a,b) found that O_3 increases a large number of BAL inflammatory mediators (including PGE_2, $PGF_{2\alpha}$, and thromboxane B_2 [TXB_2]), one or more of which may play a role in the increase in airway responsiveness after O_3 exposure.

The role of reactive oxygen metabolites or neuropeptide mediators in the increase in airway responsiveness after O_3 has not been investigated. Furthermore, there has been no direct assessment of alterations in nerve afferents, changes in neurotransmitter concentrations, changes in smooth muscle postsynaptic receptors, or modulation of nerve signal transmission by inflammatory mediators as these pertain to the increase in airway responsiveness after O_3. In conclusion, although the mechanism of O_3-induced increases in airway responsiveness is not completely understood, it appears to be associated with a number of cellular or biochemical changes in the airway (see Section 7.2.4 and Tables 7-11 and 7-12). Because these alterations are part of a complex process, it comes as no surprise that the mechanistic studies on O_3-induced increases in airway responsiveness have not pinpointed an isolated derangement.

7.2.4 Inflammation and Host Defense

7.2.4.1 Introduction

In general, inflammation can be considered as the host response to injury, and the induction of inflammation can be accepted as evidence that injury has occurred. Several outcomes are possible: (1) inflammation can resolve entirely; (2) continued acute inflammation can evolve into a chronic inflammatory state; (3) continued inflammation can alter the structure or function of other pulmonary tissue, leading to diseases such as fibrosis or emphysema; (4) inflammation can alter the body's host defense response to inhaled microorganisms, particularly in potentially vulnerable populations such as the very young and old; and (5) inflammation can alter the lung's response to other agents such as allergens or toxins. It is also possible that the profile of response can be altered in persons with preexisting pulmonary disease (e.g., asthma or COPD) or smokers. At present, it is known that short-term exposure of humans to O_3 can cause acute inflammation and that long-term exposure of laboratory animals results in a chronic inflammatory state (see Chapter 6). However, the relationship between repetitive bouts of acute inflammation in humans caused by O_3 and the development of chronic respiratory disease is unknown.

The previous O_3 criteria document (U.S. Environmental Protection Agency, 1986) contained no studies in which inflammation was measured in humans exposed to O_3. Fiberoptic bronchoscopy since has been used to sample cells and fluids lining the respiratory tract of humans for many markers (Reynolds, 1987). Bronchoalveolar lavage primarily samples the alveolar region of the lung; however, the use of small volume lavages (Rennard et al., 1990) or balloon catheters also allows sampling of the airways. Nasal lavage allows sampling of cells and fluid removed from the nasal passages.

In the past 6 years, several studies have analyzed BAL and NL cells and fluid from humans exposed to O_3 for markers of inflammation and lung damage (see Tables 7-11 and 7-12). The presence of PMNs in the lung has long been accepted as a hallmark of inflammation and has been taken as the major indicator that O_3 causes inflammation in the

Table 7-11. Bronchoalveolar Lavage Studies of Inflammatory Effects from Controlled Human Exposure to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s)	Reference
0.08 0.10	157 196	6.6 h	IE (40 L/min) six 50-min exercise periods + 10 min rest; 35 min lunch	18 M, 18 to 35 years old	BAL fluid 18 h after exposure to 0.1 ppm O_3 had significant increases in PMNs, protein, PGE_2, fibronectin, IL-6, lactate dehydrogenase, and α-1 antitrypsin compared with the same subjects exposed to FA. Similar but smaller increases in all mediators after exposure to 0.08 ppm O_3 except for protein and fibronectin. Decreased phagocytosis of yeast by alveolar macrophages was noted at both concentrations.	Devlin et al. (1990, 1991) Koren et al. (1991)
0.20	392	4 h	IE (50 min at 40 L/min, 10 min rest)	15 M, 13 F, 21 to 39 years old	Bronchial lavage, bronchial biopsies, and BAL done 18 h after exposure. BAL shows changes similar to other studies. Airway lavage shows increased cells, LDH, IL-8. Biopsies show increased number of PMNs.	Aris et al. (1993a)
0.30	588	1 h (mouth-piece)	CE (60 L/min)	5 M	Significantly elevated PMNs in the BAL fluid 1, 6, and 24 h after exposure, with peak increases at 6 h.	Schelegle et al. (1991)
0.40	784	2 h	IE (70 L/min) at 15-min intervals	11 M, 18 to 35 years old	BAL fluid 18 h after exposure had significant increases in PMNs, protein, albumin, IgG, PGE_2, plasminogen activator, elastase, complement C3a, and fibronectin.	Koren et al. (1989a,b)
0.40	784	2 h	IE (70 L/min) at 15-min intervals	11 M, 18 to 35 years old	Macrophages removed 18 h after exposure had changes in the rate of synthesis of 123 different proteins as assayed by computerized densitometry of two-dimensional gel protein profiles.	Devlin and Koren (1990)
0.40	784	2 h	IE (70 L/min) at 15-min intervals	11 M, 18 to 35 years old	BAL fluid 18 h after exposure contained increased levels of the coagulation factors, tissue factor, and factor VII. Macrophages in the BAL fluid had elevated tissue factor mRNA.	McGee et al. (1990)
0.40	784	2 h	IE (70 L/min) at 15-min intervals	10 M, 18 to 35 years old	BAL fluid 1 h after exposure to 0.4 ppm O_3 had significant increases in PMNs, protein, PGE_2, TXB_2, IL-6, LDH, α-1 antitrypsin, and tissue factor compared with the same subjects exposed to FA. Decreased phagocytosis of yeast by alveolar macrophages.	Koren et al. (1991)

Table 7-11 (cont'd). Bronchoalveolar Lavage Studies of Inflammatory Effects from Controlled Human Exposure to Ozone[a]

Ozone Concentration[b]		Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s)	Reference
ppm	$\mu g/m^3$					
0.40	784	2 h/day for 5 days, 2 h either 10 or 20 days later	IE (40 L/min) at 15-min intervals	16 M, 18 to 35 years old	BAL done immediately after fifth day of exposure and again after exposure 10 or 20 days later. Most markers of inflammation (PMNs, IL-6, IL-8, protein, α1-antitrypsin, PGE_2, fibronectin) showed complete attenuation; markers of damage (LDH, elastase) did not. Reversal of attenuation was not complete for some markers, even after 20 days.	Devlin et al. (1995)
0.40	784	2 h	IE (60 L/min) at 15-min intervals	10 M	Subjects given 800 mg ibuprofen or placebo 90 min before exposure. Subjects given ibuprofen had less of a decrease in FEV_1 after O_3 exposure. BAL fluid 1 h after exposure contained similar levels of PMNs, protein, fibronectin, LDH, α-1 antitrypsin, LTB_4, and C3a in both ibuprofen and placebo groups. However, subjects given ibuprofen had decreased levels of IL-6, TXB_2, and PGE_2.	Hazucha et al. (1996)
0.40 0.60	784 1,176	2 h	IE (83 W for women, 100 W for men) at 15-min intervals	7 M, 3 F, 23 to 41 years old	BAL fluid 3 h after exposure had significant increases in PMNs, PGE_2, TXB_2, and $PGF_{2\alpha}$ at both O_3 concentrations.	Seltzer et al. (1986)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.

Table 7-12. Additional Studies of Inflammatory and Host Defense Effects from Controlled Human Exposure to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s)	Reference
Nasal Lavage Studies						
0.12 0.24	235 470	90 min	IE (20 L/min) at 15-min intervals	5M, 5F, asthmatic; 4M, 4F, nonasthmatic; 18 to 41 years old	NL done immediately and 24 h after exposure. Increased number of PMNs at both times in asthmatic subjects exposed to 0.24 ppm O_3; no change in nonasthmatic subjects. No change in lung or nasal function.	McBride et al. (1994)
0.30	588	6 h/day for 5 consecutive days	IE (light treadmill)	24 M (12 O_3, 12 air)	Subjects inoculated with type 39 rhinovirus prior to exposure. NL was performed on the morning of Days 1 to 5, 8, 15, and 30. No difference in virus titers in NL fluid of air and O_3-exposed subjects at any time tested. No difference in PMNs or interferon gamma in NL fluid, or in blood lymphocyte proliferative response to viral antigen.	Henderson et al. (1988)
0.40	784	2 h	IE (70 L/min) at 15-min intervals	11 M, 18 to 35 years old	NL done immediately before, immediately after, and 22 h after exposure. Increased numbers of PMNs at both times after exposure; increased levels of tryptase, a marker of mast cell degranulation, immediately after exposure; increased levels of albumin 22 h after exposure.	Graham and Koren (1990) Koren et al. (1990)
0.50	980	4 h on 2 consecutive days	Resting	41 M (21 O_3, 20 air-exposed), 18 to 35 years old	NL done immediately before and after each exposure and 22 h after the second exposure. Increased levels of PMNs at all times after the first exposure, with peak values occurring immediately prior to the second exposure.	Graham et al. (1988)
0.50	980	4 h	Resting	6 M, 6 F, allergic rhinitics, 31.4 ± 2.0 (SD) years old	NL done immediately after exposure. Increased upper and lower respiratory symptoms and increased levels of PMNs, eosinophils, and albumin in NL fluid.	Bascom et al. (1990)

Table 7-12 (cont'd). Additional Studies of Inflammatory and Host Defense Effects from Controlled Human Exposure to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] $\mu g/m^3$	Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s)	Reference
Clearance Studies						
0.20 0.40	392 784	2 h	IE (light treadmill)	7 M, 27.2 ± 6.0 (SD) years old	Subjects inhaled radiolabeled iron oxide particles immediately before exposure. Concentration-dependent increase in rate of particle clearance 2 h after exposure, although clearance was confined primarily to the peripheral airways at the lower O_3 concentration.	Foster et al. (1987)
0.40	784	1 h	CE (40 L/min)	15 M or F, 18 to 35 years old	Subjects inhaled radiolabeled iron oxide particles 2 h after exposure. No O_3-induced difference in clearance of particles during the next 3 h or the following morning.	Gerrity et al. (1993)
0.40	784	2 h	IE (70 L/min) at 15-min intervals	8 M, 20 to 30 years old	Subjects inhaled ^{99m}Tc-DTPA 75 min after exposure. Significantly increased clearance of ^{99m}Tc-DTPA from the lung in O_3-exposed subjects. Subjects had expected changes in FVC and SR_{aw}.	Kehrl et al. (1987)
0.50	784	2.25 h	IE (70 L/min) at 15-min intervals	16 M, 20 to 30 years old	Similar design and results as earlier study (Kehrl et al., 1987). For the combined studies the average rate of clearance was 60% faster in O_3-exposed subjects.	Kehrl et al. (1989)
In Vitro Studies						
0.25 0.50	490 980	6 h	Human nasal epithelial cells		Increased in ICAM-1, IL-6, IL-1, and TNF expression at 0.5 ppm. No increase in IL-8 expression. No increases at 0.25 ppm.	Beck et al. (1994)
0.25 0.50 1.00	490 980 1,960	1 h	Airway epithelial cell line		Concentration-dependent increased secretion of PGE_2, TXB_2, $PGF_{2\alpha}$, LTB_4, and LTD_4. More secretion basolaterally than apically.	McKinnon et al. (1993)
0.25 0.50 1.00	490 980 1,960	1 h	Airway epithelial cell line and alveolar macrophages		Increased secretion of IL-6, IL-8, and fibronectin by epithelial cells, even at lowest O_3 concentration. No O_3-induced secretion of these compounds by macrophages.	Devlin et al. (1994)

Table 7-12 (cont'd). Additional Studies of Inflammatory and Host Defense Effects from Controlled Human Exposure to Ozone[a]

Ozone Concentration[b]		Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s)	Reference
ppm	$\mu g/m^3$					
In Vitro Studies (cont'd)						
0.30 1.00	588 1,960	1 h	Alveolar macrophages		Concentration-dependent increases in PGE_2 production, and decreases in phagocytosis of sheep erythrocytes. No O_3-induced secretion of IL-1, TNF, or IL-6.	Becker et al. (1991)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.

lungs of humans. Soluble mediators of inflammation (or its resolution) such as cytokines and arachidonic acid metabolites also have been measured in the BAL fluid of humans exposed to O_3. Cytokines that have been reported most often are interleukin (IL)-6 and IL-8, although IL-1 and tumor necrosis factor (TNF) also have been studied. Soluble metabolites of arachidonic acid involved in inflammation and host defense (e.g., PGE_2 and $PGF_{2\alpha}$, thromboxane, and leukotrienes [LTs] such as LTB_4) also have been reported in the BAL fluid of humans exposed to O_3. In addition to their role in inflammation, many of these compounds have bronchoconstrictive properties and may be involved in increased airway hyperreactivity observed following O_3 exposure.

Under normal circumstances, the epithelia lining the large and small airways develop tight junctions and restrict the penetration of exogenous particles and macromolecules from the airway lumen into the interstitium and blood, as well as restrict the flow of plasma components into the airway lumen. However, several studies (see Table 7-12) show that O_3 disrupts the integrity of the epithelial cell barrier in human airways, as measured by increased passage of radiolabeled compounds out of the airways, as well as passage of markers of plasma influx such as albumin, immunoglobulin, and other proteins into the airways. In addition, markers of epithelial cell damage such as lactate dehydrogenase (LDH) activity also have been measured in the BAL fluid of humans exposed to O_3.

Inflammatory cells of the lung such as alveolar macrophages (AMs), monocytes, and PMNs also constitute an important component of the pulmonary host defense system. In their unstimulated state, they present no danger to surrounding pulmonary cells and tissues, but upon activation, they are capable of generating free radicals and enzymes with microbicidal capabilities, but they also have the potential to damage nearby cells. Animal studies have demonstrated that O_3 decreases host defense system function (see Chapter 6, Section 6.2.3).

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

7.2.4.2 Inflammation Assessed by Bronchoalveolar Lavage

Seltzer et al. (1986) were the first to demonstrate that exposure of humans to O_3 resulted in inflammation in the lung. In this study, 10 volunteers were exposed to 0.4 or 0.6 ppm O_3 for 2 h while undergoing exercise, and BAL was performed 3 h later. Bronchoalveolar lavage fluid from subjects exposed to O_3 contained 7.8-fold more PMNs compared with BAL fluid from the same subjects exposed to FA. Additionally, BAL fluid from O_3-exposed subjects contained increased levels of PGE_2, $PGF_{2\alpha}$, and TXB_2 compared to fluid from air-exposed subjects. Koren et al. (1989a,b) also described inflammatory changes in the lungs of 11 subjects exposed to 0.4 ppm O_3 for 2 h while undergoing IE at 70 L/min in a study designed to simulate adults working outdoors or children actively playing. Bronchoalveolar lavage was performed 18 h after O_3 exposure. Subjects exposed to O_3 had an eightfold increase in PMNs in the BAL fluid, confirming the observations of Seltzer et al. In addition, Koren et al. reported a twofold increase in BAL fluid protein, albumin, and immunoglobulin G (IgG) levels, suggestive of increased epithelial cell permeability as a result of O_3 exposure. There was also a 12-fold increase in IL-6 levels in the BAL fluid. Interleukin-1 and TNF were not present in detectable levels in the BAL fluid

of any subject. There was, however, a twofold increase in the proinflammatory eicosanoid PGE_2, as well as a twofold increase in the complement component C3a. This study also provided evidence for stimulation of fibrogenic processes in the lung by demonstrating significant increases in two components of the coagulation pathway, Tissue Factor and Factor VII (McGee et al., 1990), as well as urokinase plasminogen activator and fibronectin (Koren et al., 1989a). Taken together, these two studies demonstrate that exposure of humans to moderate levels of O_3 results in an inflammatory reaction in the lung, as evidenced by substantial increases in PMNs and proinflammatory compounds. Furthermore, these studies demonstrate that both cells and mediators capable of damaging pulmonary tissue are increased after O_3 exposure, as are compounds that play a role in fibrotic and fibrinolytic processes.

Although animal studies have shown that the terminal bronchioles are a major site of O_3-induced inflammation, few human studies have confirmed this finding because BAL primarily samples cells and fluid in the terminal bronchioles and alveoli. However, isolated lavage of the mainstream bronchus using balloon catheters or the more traditional BAL using small volumes of saline have the ability to preferentially measure O_3-induced changes in the large airways. In one study, isolated airway lavage was performed on 14 subjects 18 h after exposure to 0.2 ppm O_3 while undergoing moderate exercise (Aris et al., 1993a). Increases in total lavagable cells, LDH activity, and IL-8 were reported. In contrast, Schelegle et al. (1991), observed no increase in PMNs in the bronchial fluid; however, bronchial biopsies showed increased numbers of PMNs in airway tissue.

The data suggestive of O_3-induced changes in epithelial cell permeability described by Koren et al. (1989a, 1991) and Devlin et al. (1991) support earlier work in which epithelial cell permeability, as measured by increased clearance of radiolabled diethylene triamine pentaacetic acid (^{99m}Tc-DTPA) from the lungs of humans exposed to O_3, was demonstrated (Kehrl et al., 1987). In that study, eight healthy subjects who inhaled ^{99m}Tc-DTPA just prior to exposure to air or 0.4 ppm O_3 for 2 h while undergoing heavy exercise (65 L/min) had increased clearance of the compound. Kehrl et al. (1989) reported similar observations on an additional 16 subjects. For the combined group of 24 subjects exposed for 2 h to 0.4 ppm O_3, the average clearance rate was 60% faster than that observed after air exposure, strongly suggesting increased permeability from the airway lumen and alveolar space to the blood and interstitial spaces. The average O_3-induced decrement in FVC in these subjects was 10%. These changes in permeability most likely are associated with acute inflammation and potentially could allow better access of inhaled antigens and other substances to the submucosa.

Studies in which human AM and airway epithelial cells were exposed to O_3 in vitro suggest that most of the components found in increased levels in the BAL fluid of O_3-exposed humans are produced by epithelial cells. Macrophages exposed to 0.3 and 1.0 ppm (but not 0.1 ppm) O_3 for 1 h showed small increases in PGE_2, but no change in superoxide anion or cytokine production (Becker et al., 1991). In contrast, airway epithelial cells exposed in vitro to 0.1, 0.25, 0.5, and 1.0 ppm O_3 for 1 h showed large concentration-dependent increases in PGE_2, TXB_2, LTB_4, LTC_4, and LTD_4 (McKinnon et al., 1993). These cells also showed increases in IL-6, IL-8, and fibronectin at O_3 concentrations as low as 0.1 ppm (Devlin et al., 1994). Interestingly, macrophages removed 18 h later from subjects exposed to 0.4 ppm O_3 for 2 h while undergoing intermittent heavy exercise (Koren et al., 1989a) showed changes in the rate of synthesis of 123 different proteins as measured by quantitative computerized densitometry of two-dimensional gel protein profiles.

However, AMs exposed to O_3 in vitro showed changes only in the rate of synthesis of six proteins, suggesting that most of the changes seen in the in vivo-exposed AMs were due to actions resulting from mediators released by other cells following O_3 exposure, which then altered macrophage function.

Numerous studies have shown that humans exposed to O_3 for 5 consecutive days experience decrements in pulmonary function on the first and second days, but the decrements diminish with each succeeding day so that by the fifth day, no such effects are observed (see Section 7.2.1). However, these studies did not address the question of whether repeated exposure to O_3 also resulted in attenuation of inflammation or lung damage. Animal studies suggest that although some markers of inflammation may be diminished, underlying damage to lung epithelial cells continues (Tepper et al., 1989). In a recent study (Devlin et al., 1995), humans were exposed to 0.4 ppm O_3 for 5 consecutive days (2 h/day while undergoing IE) and then were exposed to O_3 a single time either 10 or 20 days later. The results show that numerous indicators of inflammation (e.g., PMN influx, IL-6, IL-8, PGE_2, BAL protein, fibronectin, macrophage phagocytosis) show attenuation (i.e., there is a complete disappearance of response, and values are no different from those observed in the same individual after 5 days of exposure to FA). Ten days later, some of these markers regained full susceptibility, but others did not regain susceptibility even after 20 days. In agreement with animal studies, some markers (LDH, elastase) never show attenuation, indicating that tissue damage may continue to occur during repeated exposure.

7.2.4.3 Inflammation Induced by Ambient Levels of Ozone

Devlin et al. (1991) reported an inflammatory response in humans exposed to levels of O_3 at or below 0.12 ppm. In this study, 10 volunteers were exposed to 0.08 and 0.10 ppm O_3 for 6.6 h while undergoing moderate exercise (40 L/min) and underwent BAL 18 h later. An additional eight subjects were exposed to 0.08 ppm O_3. Increased numbers of PMNs and levels of IL-6 were found at both O_3 concentrations. There also were increases in most of the other compounds reported by Koren et al. (1989a,b), including fibronectin and PGE_2. Alveolar macrophage phagocytic capability was also monitored in this study, and it was reported that macrophages removed from humans exposed to both O_3 concentrations had decreased ability to phagocytize *Candida albicans* opsonized with complement. Comparison of the magnitude of inflammatory changes observed in this study and by Koren et al. (1989a,b), when normalized for differences in concentration, duration of exposure, and ventilation, suggest that lung inflammation from O_3 may occur as a consequence of exposure to ambient levels while exercising. Although the mean changes in IL-6, PGE_2, and PMNs reported by Devlin et al. (1991) were small, there was a considerable range of response among the individuals participating in the study. Thus, although some of the study population showed little or no response to O_3, others had increases in IL-6 or PMNs that were as large as or larger than those reported by Koren et al. (1989a,b) when subjects were exposed for 2 h to 0.4 ppm O_3. Interestingly, those individuals who had the largest increases in inflammatory mediators in this study did not necessarily have the largest decrements in pulmonary function, suggesting separate mechanisms underlying these two responses to O_3. These data suggest that, although the population as a whole may have a small inflammatory response to low levels of O_3, there may be a significant subpopulation that is very sensitive to these low levels of O_3. Furthermore, even a small inflammatory response (if it recurs) in the population as a whole should not be discounted.

7.2.4.4 Time Course of Inflammatory Response

The time course of the inflammatory response to O_3 in humans has not been explored fully. Studies in which BAL was performed 1 h (Devlin et al., 1990; Koren et al., 1991) or 3 h (Seltzer et al., 1986) after exposure to 0.4 ppm O_3 demonstrate that the inflammatory response is quickly initiated, and other data (Koren et al., 1989a,b) indicate that, even 18 h after exposure, inflammatory mediators such as IL-6 and PMNs are still substantially elevated. However, a comparison of these studies shows there are differences in the magnitude of response of some indicators, depending on when BAL is performed after O_3 exposure. Ozone-induced increases in PMNs, IL-6, and PGE_2 are greater 1 h after O_3 exposure, whereas BAL levels of fibronectin and plasminogen activator are greater 18 h after exposure. Still other compounds (protein, Tissue Factor) are equally elevated both 1 and 18 h after O_3 exposure. Schelegle et al. (1991) exposed five subjects to FA or 0.3 ppm O_3 for 1 h with a ventilation of 60 L/min. Each subject was exposed to O_3 on three separate occasions, and BAL was performed 1, 6, or 24 h after exposure. In addition, BAL was separated into two fractions: the first 60 mL wash was designated the "proximal airways" fraction (PA), and the remaining three 60 mL washes were pooled and designated the "distal airways and alveolar surface" fraction (DAAS). The percent of PMNs in the PA sample was statistically elevated at 1, 6, and 24 h after O_3 exposure, with a peak response at 6 h. The percent of PMNs in the DAAS sample was elevated at only the 6 and 24 h time points, with equivalent elevations at each time.

7.2.4.5 Effect of Anti-Inflammatory Agents on Ozone-Induced Inflammation

Previous studies (Schelegle et al., 1987; Eschenbacher et al., 1989) have shown that indomethacin, an anti-inflammatory agent that inhibits the production of cyclooxygenase products of arachidonic acid metabolism, is capable of blunting the well-documented decrements in pulmonary function observed in humans exposed to O_3. In a recent study, 10 healthy male volunteers were given 800 mg ibuprofen, another anti-inflammatory agent that blocks cyclooxygenase metabolism, or a placebo 90 min prior to a 2-h exposure to 0.4 ppm O_3. An additional 200 mg was administered following the first hour of exposure. Bronchoalveolar lavage was performed 1 h after the exposure. As expected, subjects given ibuprofen had blunted decrements in lung function following O_3 exposure compared to the same subjects given a placebo (Hazucha et al., 1996). Bronchoalveolar lavage fluid from subjects given ibuprofen also had reduced levels of the cyclooxygenase product PGE_2 as well as IL-6, but no decreases were observed in PMNs, fibronectin, permeability, LDH activity, or macrophage phagocytic function (Hazucha et al., 1995). These data suggest that although anti-inflammatory agents may blunt O_3-induced decrements in FEV_1 and increases in PGE_2, most inflammatory mediators are elevated in the BAL of these subjects.

7.2.4.6 Use of Nasal Lavage To Assess Ozone-Induced Inflammation in the Upper Respiratory Tract

Bronchoalveolar lavage has proven to be a powerful research tool to analyze changes in the lung following exposure of humans to xenobiotics. However, because BAL is expensive, somewhat invasive, and requires specialized personnel and facilities, it usually is done only with small numbers of subjects and in selected medical centers. Therefore, there is increasing interest in the use of NL as a tool in assessing O_3-induced inflammation in the upper respiratory tract, which is the primary portal for inspired air, and therefore the first region of the respiratory tract to come in contact with airborne xenobiotics. Nasal lavage is

simple and rapid to perform, is noninvasive, and allows collection of multiple sequential samples from the same person. Graham et al. (1988) reported increased levels of PMNs in the NL fluid of 21 humans exposed to 0.5 ppm O_3 at rest for 4 h on 2 consecutive days, with NL performed immediately before and immediately after each exposure as well as 22 h after the second exposure. Nasal lavage fluid contained elevated numbers of PMNs at all postexposure times tested, with peak values occurring immediately prior to the second day of exposure. There were no changes in PMN numbers at any time in 20 subjects exposed to clean air for 2 consecutive days. Bascom et al. (1990) exposed 12 subjects with allergic rhinitis to 0.5 ppm O_3 at rest for 4 h, followed immediately by NL. They reported a sevenfold increase in PMNs, a 20-fold increase in eosinophils, and a 10-fold increase in mononuclear cells following O_3 exposure, as well as a 2.5-fold increase in albumin. Graham and Koren (1990) compared inflammatory mediators present in both the NL and BAL fluids of humans exposed to O_3. The same 11 subjects who were exposed to 0.4 ppm O_3 for 2 h with BAL performed 18 h later, as described earlier (Koren et al., 1989a,b), also underwent NL immediately before, immediately after, and 18 h after each exposure (Graham and Koren, 1990). There were significant increases in PMNs in the NL fluid taken both immediately after exposure and on the next day. Increases in NL and BAL PMNs were similar (6.6- and eightfold, respectively), demonstrating a qualitative correlation between changes in the lower airways as assessed by BAL and the upper respiratory tract as assessed by NL. Furthermore, all individuals who had increased PMNs in BAL fluid also had increased PMNs in NL fluid, although the NL PMN increase could not quantitatively predict the BAL PMN increase. Albumin, a marker of epithelial cell permeability, was increased 18 h later, but not immediately after exposure. There were no changes in PGE_2, plasminogen activator, LTC_4, LTD_4, or LTE_4 (Graham and Koren, 1990). However, tryptase, a constituent of mast cells contained in the same granules as histamine, was found in elevated levels immediately after O_3 exposure, but not 18 h later (Koren et al., 1990). McBride et al. (1994) reported that asthmatic subjects are more sensitive to upper airway inflammation at O_3 concentrations that do not affect lung function. Nasal lavage and lung and nasal function were compared in 10 asthmatic and 8 nonasthmatic subjects exposed in a head dome to 0.12 and 0.24 ppm O_3 for 90 min during intermittent moderate exercise ($\dot{V}_E$ = 20 L/min). A significant increase in the number of PMNs in NL fluid was detected in the asthmatic subjects both immediately and 24 h after exposure to 0.24 ppm O_3. Total white blood count, a surrogate for PMN influx, was signficantly correlated with IL-8 in the NL fluid. No significant cellular changes were seen in nonasthmatic subjects, and no changes in lung or nasal function or biochemical mediators were found in either asthmatic or nonasthmatic subjects. These studies suggest that NL may serve as a sensitive and reliable tool to detect inflammation in the upper airways of humans exposed to xenobiotics.

7.2.4.7 Changes in Host Defense Capability Following Ozone Exposure

Concern about the effect of O_3 on human host defense capability derives from numerous animal studies demonstrating that acute exposure to as little as 0.08 ppm O_3 causes decrements in antibacterial host defenses and little, if any, effect on the course of acute viral infection (see Chapter 6, Section 6.2.3). A study of experimental rhinovirus infection in susceptible human volunteers failed to show any effect of 5 consecutive days of O_3 exposure on the clinical evolution or host response to a viral challenge (Henderson et al., 1988). In this study, 24 young males were inoculated with type 39 rhinovirus (1,000 TCID-50) administered as nose drops. Half were then exposed to 0.3 ppm O_3 (6 h/day) for

5 consecutive days while undergoing intermittent light exercise, and half were exposed to clean air under the same regimen. There was no difference in rhinovirus titers in nasal secretions between the O_3-exposed and control groups, nor were there any differences in levels of interferon gamma or PMNs in NL fluid or in blood lymphocyte proliferative response to rhinovirus antigen. However, recent findings that rhinovirus can attach to the intracellular adhesion molecule (ICAM) receptor on respiratory tract epithelial cells (Greve et al., 1989) and that O_3 can up-regulate the ICAM receptor on nasal epithelial cells (Beck et al., 1994) suggest that more studies are needed to explore more fully the potential interaction between O_3 exposure and viral infectivity.

In a single study, human AM host defense capacity was measured in vitro in AMs removed from subjects exposed to 0.08 and 0.10 ppm O_3 for 6.6 h while undergoing moderate exercise. Alveolar macrophages from O_3-exposed subjects had significant decrements in complement-receptor-(but not antigen-antibody [Fc]-receptor)-mediated phagocytosis of *Candida albicans* (Devlin et al., 1991). These data show that acute in vivo exposure of humans to O_3 results in impairment of AM host defense capability, potentially resulting in decreased ability to phagocytose and kill inhaled microorganisms in vivo. Human AMs also have been exposed to O_3 in vitro to investigate whether changes in macrophage host defense functions are due to a direct effect of O_3 on AMs or secondary effects resulting from lung injury and inflammation. Becker et al. (1991) exposed AMs to 0.1 to 1.0 ppm O_3 in vitro for 1 h and showed a concentration-dependent decrease in phagocytosis of antibody-coated sheep erythrocytes; a small increase in PGE_2; and production of significantly lower levels of IL-1, IL-6, and TNF on stimulation with lipopolysaccharide when compared with air-exposed cells (Becker et al., 1991). Although the few studies in which animals have been exposed to virus in conjunction with O_3 exposure provide some evidence to suggest that O_3 impairs the immune system's ability to fight viral infections, there is insufficient human data to know whether O_3 exposure affects viral infectivity. However, there is potential cause for concern that O_3 may render humans and animals more susceptible to a subsequent bacterial challenge.

There are two studies that have investigated the effect of O_3 exposure on mucociliary clearance of inhaled particles, with conflicting results. In one study (Foster et al., 1987), seven male volunteers inhaled radiolabeled ferric oxide ($^{99m}Tc\text{-}Fe_2O_3$) particles and then were exposed to 0.2 and 0.4 ppm O_3 for 2 h while undergoing light IE. The investigators observed a concentration-dependent increase in rate of particle clearance 2 h after exposure, although increased clearance was confined primarily to the peripheral airways in subjects exposed to 0.2 ppm O_3. In the second study (Gerrity et al., 1993), 15 male or female subjects were exposed to 0.4 ppm O_3 for 1 h while undergoing CE (40 L/min); 2 h after exposure, subjects inhaled $^{99m}Tc\text{-}Fe_2O_3$ particles, and clearance was measured with a gamma camera for the next 3 h and on the next morning. There was no difference in the clearance rate of particles in air and O_3-exposed subjects. The discrepancy between these studies may be explained by differences in exposure protocol, time of particle inhalation, or time of clearance measurement, or by the presence of cough immediately following O_3 exposure, which may have accelerated clearance in the first study.

7.2.5 Extrapulmonary Effects of Ozone

It is still believed that O_3 reacts immediately on contact with respiratory system tissue and is not absorbed or transported to extrapulmonary sites to any significant degree

(see Chapter 8). A number of laboratory animal studies presented in Chapter 6, however, suggest that reaction products formed by the interaction of O_3 with respiratory system fluids or tissues may produce effects measured outside the respiratory tract—either in the blood, as changes in circulating blood lymphocytes, erythrocytes, and serum, or as changes in the structure or function of other organs, such as the parathyroid gland, the heart, the liver, and the central nervous system (see Chapter 6, Section 6.3). Very little is known, however, about the mechanisms by which O_3 could cause these extrapulmonary effects.

The results from human exposure studies discussed in the previous criteria document (U.S. Environmental Protection Agency, 1986) failed to demonstrate any consistent extrapulmonary effects (see Chapter 10, Section 10.6 of the 1986 document). Early studies on peripheral blood lymphocytes collected from human volunteers did not find any significant genotoxic or functional changes at O_3 exposures of 0.4 to 0.6 ppm for up to 4 h/day. Limited data on human subjects available at the time the 1986 criteria document was published also indicated that 0.5 ppm O_3 exposure for over 2 h caused transient changes in blood erythrocytes and sera (e.g., erythrocyte fragility and enzyme activities), but the physiological significance of these studies remains questionable. The conclusions drawn from these early studies raise doubt that cellular damage or altered function is occurring to circulating cells at O_3 exposures under 0.5 ppm.

Studies published since the publication of the previous criteria document (U.S. Environmental Protection Agency, 1986) on the potential extrapulmonary effects of in vivo O_3 exposure of human subjects have not been very definitive. Johnson et al. (1986) exposed 11 male nonsmokers to 0.5 ppm O_3 for 4 h on 2 consecutive days. When compared to air controls, O_3 exposure did not result in any significant change in the activity of blood plasma α-1-proteinase inhibitor. Schelegle et al. (1989) exposed 20 O_3-sensitive, healthy young men to 0.20 and 0.35 ppm O_3 with heavy exercise ($\dot{V}_E$ = 50 L/min). Plasma concentrations of $PGF_{2\alpha}$ were elevated after 40 and 80 min of exposure to the higher O_3 level (0.35 ppm). It is likely, however, that the elevation of this ecosanoid in the blood was due either to increased production or to decreased metabolism of $PGF_{2\alpha}$ in the lung.

The demonstration in the previous section (Section 7.2.4) of an array of inflammatory mediators and immune modulators released at the airway surface provides a possible mechanism for effects to occur elsewhere in the body.

7.2.6 Ozone Mixed with Other Pollutants

Although it is well known that polluted air contains a large number of chemical species, the most common approach to evaluating air pollution effects under laboratory conditions has been assessment of responses consequent to exposure to single pollutants. This has been the case for a variety of reasons, not the least of which is the problem inherent in adequately controlling the concentrations of multiple pollutants simultaneously. Further, atmospheric chemistry is very complicated, and it is difficult to adequately assess the exposure mixture as the number of constituent pollutants increases. Observed effects may be related to unknown reaction products, the monitored pollutants being only surrogates. Other problems inherent in mixture studies involve considerations such as whether pollutants are presented simultaneously or in sequential or overlapping patterns. Ideally, the selected pattern should at least approximate one that occurs in the ambient environment. In spite of these difficulties, information from mixture studies is important from the standpoint of attempts to better understand responses of humans to the complex mixture of ambient air.

The previous O_3 criteria document (U.S Environmental Protection Agency, 1986) evaluated the limited database of information available on mixtures of O_3 with one or more pollutants and concluded that pulmonary function changes were no more than additive and, in most cases, were attributable to O_3 alone. Several new studies have since appeared in which human subjects were exposed to mixtures of two or more pollutants or to individual pollutants sequentially (Table 7-13), extending the database for controlled studies. Epidemiological studies also have investigated mixtures of pollutants and have not found evidence suggestive of synergistic effects (see Section 7-4).

7.2.6.1 Ozone and Sulfur-Containing Pollutants

Horvath et al. (1987) compared the pulmonary function responses of male subjects (19 to 29 years of age) with normal baseline pulmonary function to four experimental conditions: (1) FA, (2) 0.25 ppm O_3, (3) 1,200 to 1,600 $\mu g/m^3$ H_2SO_4 aerosol, and (4) 0.25 ppm O_3 + 1,200 to 1,600 $\mu g/m^3$ H_2SO_4 aerosol. Exposures were completed in random sequence, a minimum of 1 week apart, and were conducted at 35 °C and 83% RH. Subjects alternated 20-min rest and exercise ($\dot{V}_E$ = 30 to 32 L/min) periods throughout the 2-h exposures. The results indicated that neither O_3 alone nor O_3 mixed with H_2SO_4 aerosol had significant effects on any pulmonary function, metabolic, or ventilatory parameter.

Koenig et al. (1990) evaluated sequential O_3 (0.12 ppm) and SO_2 (0.10 ppm) exposures in 13 allergic, asthmatic adolescents (12 to 18 years of age). Three subjects used no regular medications, the other 10 used one or more of beta-adrenergic agents, theophylline, and antihistamines. All subjects had a PC_{20} for methacholine of 10 mg/mL or less. Subjects took their morning medication on experiment days if needed, but at least 4 h elapsed between any medication use and the start of the experiment. The subjects participated in three exposures at 22 °C and 75% RH, which were presented in random order and at least 1 week apart. The three exposures were (1) air + SO_2, (2) O_3 + O_3, and (3) O_3 + SO_2. The mouthpiece exposures were 1 h in duration, during which the subjects breathed one test gas for 45 min, followed by a second gas for the final 15 min. Subjects exercised at a $\dot{V}_E$ of about 30 L/min during the second and fourth 15-min segments of the exposure. Pulmonary functions were measured 2 to 3 and 7 to 8 min postexposure. Changes in FEV_1 and R_T were significantly greater following the O_3 + SO_2 exposure than following the other two exposures. Although the subject group was small, the results indicate that O_3 exposure may potentiate responses to SO_2 exposure in asthmatic adolescents. It should be noted that the SO_2 concentration (0.10 ppm) used in this study is a subthreshold level.

Linn et al. (1994) evaluated the pulmonary function and symptom responses of 15 atopic and normal subjects and 30 asthmatic subjects exposed to FA, 0.12 ppm O_3, 100 $\mu g/m^3$ respirable H_2SO_4 aerosol (MMAD = 0.5 μm), and a mixture of the two pollutants. The chamber exposures were 6.5 h in duration, during which the subjects walked on a treadmill ($\dot{V}_E \approx$ 29 L/min) for 50 min of each hour. There was a 30-min lunch period following the third hour. Pulmonary function and symptom responses were measured preexposure and during the hourly 10-min breaks, and a methacholine bronchochallenge test was performed following each exposure. Relative to responses to the FA exposure, H_2SO_4

Table 7-13. Ozone Mixed with Other Pollutants[a]

Concentration[b] ppm	Concentration[b] $\mu g/m^3$	Pollutant	Exposure Duration and Activity	Exposure Conditions[c]	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Peroxyacetyl Nitrate								
0.485 0.27	951 1,337	O_3 PAN	2 h IE $\dot{V}_E \approx 25$ L/min	T = 21 °C WBGT	10 F	Healthy NS, 19 to 36 years old	Exposure to the mixture of PAN + O_3 induced decrements in FVC and FEV_1 averaging 10% greater than observed following exposure to O_3 alone.	Horvath et al. (1986)
0.45 0.30	882 1,485	O_3 PAN	2 h IE $\dot{V}_E \approx 27$ L/min	T ≈ 22 °C RH ≈ 60%	3 M, 5 F	Healthy NS, mean age = 24 years	No differences between responses to exposure to O_3 alone and O_3 + PAN.	Drechsler-Parks et al. (1987b)
0.45 0.60 0.13	882 1,128 644	O_3 NO_2 PAN	2 h IE $\dot{V}_E \approx 25$ L/min	T = 24 °C RH = 55 to 58%	16 M, 16 F	Healthy NS; 16 subjects, 19 to 26 years old; 16 subjects, 51 to 76 years old	No differences between responses to O_3 alone, O_3 + NO_2, O_3 + PAN, or O_3 + NO_2 + PAN.	Drechsler-Parks et al. (1989)
Nitrogen-Containing Pollutants								
0.12 0.30	235 564	O_3 NO_2	1 h (mouthpiece) IE $\dot{V}_E$ = 4 to 5 times resting	T = 22 °C RH = 75%	5 M, 7 F	Healthy NS, 12 to 17 years old	No significant changes in any pulmonary function with O_3 alone or O_3 + NO_2.	Koenig et al. (1988)
0.20	392 500	O_3 HNO_3 H_2O	5 h IE (50 min/h exercise) $\dot{V}_E \approx 40$ L/min 2 h HNO_3 or H_2O fog or air, followed by 1-h break, followed by 3 h O_3	T = 20 °C RH = 5%	6 M, 4 F	Healthy NS, minimum of 10% decrement in FEV_1 after 3 h exposure to 0.20 ppm O_3 with 50 min exercise/h	Exposure to HNO_3 or H_2O fog followed by O_3 induced smaller pulmonary function decrements than air followed by O_3.	Aris et al. (1991)
0.30 0.30	588 564 200	O_3 NO_2 H_2SO_4	2 h CE for 20 min $\dot{V} \approx 25$ L/min	T = 28 to 29 °C RH = 50 to 60%	6 M	Healthy subjects, some smokers	Possible small decrease in SG_{aw}	Kagawa (1986)
0.15 0.15	294 282 200	O_3 NO_2 H_2SO_4	2 h, 60 min total exercise $\dot{V} \approx 25$ L/min		6 M		Possible small decrease in SG_{aw}	
0.15 0.15 0.15	294 282 393 200	O_3 NO_2 SO_2 H_2SO_4	2 h, 60 min total exercise $\dot{V} \approx 25$ L/min		3 M		Possible small decrease in FEV_1	

Table 7-13 (cont'd). Ozone Mixed with Other Pollutants[a]

Concentration[b] ppm	Concentration[b] $\mu g/m^3$	Pollutant	Exposure Duration and Activity	Exposure Conditions[c]	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Nitrogen-Containing Pollutants (cont'd)								
0.30 0.60	588 1,128	O_3 NO_2	1 h (mouthpiece) CE $\dot{V}_E \approx 70$ L/min for men $\dot{V}_E \approx 50$ L/min for women		20 M, 20 F	Healthy NS, 21.4 ± 1.5 (SD) years old for F, 22.7 ± 3.3 (SD) years old for M	No differences between responses to O_3 and $NO_2 + O_3$ for spirometric parameters. Increase in SR_{aw} with $NO_2 + O_3$ was significantly less than for O_3 alone.	Adams et al. (1987)
0.30 0.60	588 1,128	O_3 NO_2	2-h exposure to NO_2 or FA, followed 3 h later by 2-h exposure to O_3 IE $\dot{V}_E$ = 20 L/min/m^2 BSA	T = 21 °C RH = 40%	21 F	Healthy NS, 18 to 34 years old	No significant effect of NO_2 exposures on any measured parameter. Sequential exposure of NO_2 followed by O_3 induced small but significantly larger decrements in FEV_1 and $FEF_{25-75\%}$ than FA/O_3 sequence. Subjects had increased airway responsiveness to methacholine after both exposures, with significantly greater responsiveness after the NO_2/O_3 sequences than after the FA/O_3 sequence.	Hazucha et al. (1994)
Sulfur-Containing Pollutants								
0.12 0.10	235 262	O_3 SO_2	1 h (mouthpiece) IE $\dot{V}_E \approx 30$ L/min 45-min exposure to air or O_3, followed by 15-min exposure to O_3 or SO_2	T = 22 °C RH = 75%	8 M, 5 F	All allergic asthmatics, 12 to 18 years old, medications withheld for at least 4 h before exposures	Prior exposure to O_3 potentiated pulmonary function responses to SO_2; decrements in FEV_1 were −3, −2, and −8% for the air/O_3, O_3/O_3, and O_3/SO_2 exposures, respectively.	Koenig et al. (1990)
0.08 0.12 0.18 100 100	157 235 353 NaCl H_2SO_4	O_3 O_3 O_3	3-h exposure to aerosol, followed 24 h later by a 3-h exposure to O_3. IE (10 min per half hour) $\dot{V}_E$ = 4 times resting (30 to 364 min)	T = 21 °C RH ≈ 40%	Nonasthmatic, 16 M, 14 F Asthmatic 10 M, 20 F	NS, 18 to 45 years old NS, 21 to 42 years old	No significant changes in symptoms or lung function with any aerosol/O_3 combination in the healthy group. In asthmatics, H_2SO_4 preexposure enhanced the small decrements in FVC that occurred following exposure to 0.18 ppm O_3. Asthmatics had no significant changes on FEV_1 with any O_3 exposures, but symptoms were greater.	Utell et al. (1994)
0.12 100	235 H_2SO_4	O_3	6.5 h 2 consecutive days 50 min exercise/h $\dot{V}_E$ = 29 L/min	T = 21 °C RH = 50%	Nonasthmatic, 8 M, 7 F Asthmatic, 13 M, 17 F	NS, 22 to 41 years old NS, 18 to 50 years old	Exposure to O_3 or $O_3 + H_2SO_4$ induced significant decrements in forced expiratory function. Differences between O_3 and $O_3 + H_2SO_4$ were, at best, marginally significant. O_3 is the more important pollutant for inducing respiratory effects. A few asthmatic and nonasthmatic subjects were more responsive to $O_3 + H_2SO_4$ than to O_3 alone.	Linn et al. (1994)

Table 7-13 (cont'd). Ozone Mixed with Other Pollutants[a]

Concentration[b] ppm	Concentration[b] $\mu g/m^3$	Pollutant	Exposure Duration and Activity	Exposure Conditions[c]	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Sulfur-Containing Pollutants (cont'd)								
0.12 0.30 0.05	235 564 70	O_3 NO_2 H_2SO_4 HNO_3	1.5 h with IE for 2 consecutive days; $\dot{V}_E \approx 23.2$ L/min	T = 22 °C RH = 65%	Asthmatic adolescents; 22 completed study; 15 M, 7 F	NS, 12 to 19 years old	No significant pulmonary function changes following any exposure compared to response to clean air. Six additional subjects started the study, but dropped out due to uncomfortable symptoms.	Koenig et al. (1994)
0.25	490 1,200 to 1,600	O_3 H_2SO_4 aerosol	2 h IE $\dot{V}_E$ = 30 to 32 L/min	T = 35 °C RH = 83%	9 M	Healthy NS, 19 to 29 years old	No significant effects of exposure to O_3 alone or combined with H_2SO_4 aerosol.	Horvath et al. (1987)

[a]See Appendix A for abbreviations and acronyms.
[b]Grouped by pollutant mixture.
[c]WBGT = 0.7 $T_{wet\ bult}$ + 0.3 $T_{dry\ bulb\ or\ globe}$.

aerosol exposure alone induced no significant alteration in pulmonary function, symptoms, or bronchial reactivity to methacholine. Exposure to O_3 alone (FEV_1 decrement of about 100 mL compared to the FA response) or mixed with H_2SO_4 aerosol (FEV_1 decrement of about 189 mL compared to the FA exposure) induced significant decrements in forced expiratory function and increased bronchial reactivity. Both effects were greater on the first of 2 consecutive days of exposure. Group mean lung function and methacholine reactivity changes were somewhat larger following O_3 + H_2SO_4 aerosol compared to exposure to O_3 alone, but the differences were, at best, marginally significant and usually nonsignificant, depending on the function tested. However, there were a few individual subjects who showed significantly larger pulmonary function decrements following the exposure to O_3 + H_2SO_4 than following exposure to O_3 alone. The authors concluded that O_3 is more important than H_2SO_4 aerosol in inducing pulmonary dysfunction in normal, atopic, and asthmatic adults. There does, however, appear to be a more sensitive subpopulation that responds to O_3 + H_2SO_4 aerosol more strongly than the average adult.

Utell et al. (1994) reported on the pulmonary function and symptomology responses of 30 healthy adults (18 to 45 years of age) and 30 allergic asthmatics (21 to 42 years of age) who were exposed for 3 h to sodium chloride (NaCl) aerosol (100 $\mu g/m^3$) or H_2SO_4 aerosol (100 $\mu g/m^3$) and, 24 h later, to 0.08, 0.12, or 0.18 ppm O_3 for 3 h. The study was an incomplete block design, in that each subject completed chamber exposures to each of two O_3 concentrations following each of the aerosols (four of the possible six combinations per subject). Out of the total number of subjects, 20 healthy and 20 asthmatic subjects completed each of the possible exposure combinations. Subjects exercised for 10 min out of each half-hour of exposure ($\dot{V}_E$ = 4 times resting; 30 to 36 L/min). Environmental conditions averaged 21 $\pm$ 1 °C and 40 $\pm$ 5% RH. Ozone exposures were separated by at least 2 weeks. Healthy subjects had no significant pulmonary function response (2.1% or less) to O_3 exposure, regardless of the O_3 concentration or the aerosol preexposure. As a group, asthmatics had mean decrements in FVC of 5% or greater in only a few cases: 7.6% following the NaCl/0.08 ppm O_3 combination, 6.3% following the NaCl/0.12 ppm O_3 combination, and 6.5% following the H_2SO_4/0.18 ppm O_3 combination. No combination of aerosol and O_3 concentration induced a decrement in FEV_1 of 5% or greater. Although the statistical analysis indicates that exposure to H_2SO_4 aerosol significantly altered the pattern of response and recovery to O_3 exposure on the next day in asthmatics, the group mean data presented in the report show that functionally there is little difference between the responses to the various exposures or in the time course of recovery. The individual responses of the asthmatic subjects are reported to be more variable than those of the healthy subjects. Asthmatic subjects reported more respiratory symptoms than healthy subjects, but there was no dose-response relationship between O_3 concentration and symptom intensity for healthy or asthmatic subjects. The variability of the responses of the asthmatic subjects makes interpretation of these results difficult. Some of the asthmatic subjects were reported to experience exercise-induced bronchospasm, and, without FA control exposures, it is impossible to determine what, if any, portion of the asthmatic subjects' response is related to exercise-induced bronchospasm, compared to that related to O_3 exposure.

Kagawa (1986) exposed Japanese men to three mixtures: (1) O_3 (0.30 ppm) + NO_2 (0.30 ppm) + H_2SO_4 (200 $\mu g/m^3$), (2) O_3 (0.15 ppm) + NO_2 (0.15 ppm) + H_2SO_4 (200 $\mu g/m^3$), or (3) O_3 (0.15 ppm) + NO_2 (0.15 ppm) + SO_2 (0.15 ppm)+ H_2SO_4 (200 $\mu g/m^3$). Exposures were 2 h in duration, and subjects exercised for a total of 20 min during exposure 1 and for 60 min during exposures 2 and 3. Some of the subjects were

smokers. Reported symptoms were attributed to O_3 exposure, whereas small decrements in airway conductance ($\leq 10\%$) were observed following exposures to mixtures 1 and 2. Although the magnitude of the FEV_1 decrement is not stated, a possible decrease was observed after exposure 3. The responses observed with these mixed exposure conditions were no different than responses reported for exposures to similar concentrations of O_3, indicating no enhanced response due to the presence of the other pollutants in the mixtures.

7.2.6.2 Ozone and Nitrogen-Containing Pollutants

Adams et al. (1987) reported on the responses of 20 males and 20 females (18 to 30 years of age), all healthy nonsmokers, exposed to (1) FA, (2) 0.3 ppm O_3, (3) 0.6 ppm NO_2, and (4) 0.3 ppm O_3 + 0.6 ppm NO_2. Subjects were exposed via mouthpiece for 1 h, during which they exercised continuously at a $\dot{V}_E$ of about 70 L/min for males and 50 L/min for females. The exposures were presented in random order, a minimum of 5 days apart. There were no differences in any pulmonary function (FEV_1 decrement of about 22%) between the O_3 and NO_2 + O_3 exposures, except for SR_{aw}, which was lower following NO_2 + O_3 (+7.3% for females and −9.6% for males) than following O_3 alone (+15.3% for females and +4.0% for males).

Koenig et al. (1988) exposed 14 male and 10 female adolescents to FA, 0.30 ppm NO_2, 0.12 ppm O_3, and 0.30 ppm NO_2 + 0.12 ppm O_3. Twelve of the subjects were healthy normals, and the other 12 were allergic asthmatics. The asthmatics, except for one who took no regular medications, used one or more of beta-adrenergic agents, theophylline, and antihistamines. Asthmatic subjects took their morning medications if needed, but refrained from medication use for at least 4 h prior to the exposures. The mouthpiece exposures were 1 h in duration, during which the subjects exercised in 15-min periods (mean $\dot{V}_E = 32.8 \pm 6.0$ L/min), alternated with 15-min rest periods. No changes in any measure of pulmonary function were observed in normal or asthmatic subjects following O_3 or NO_2 + O_3 exposure.

Sequential exposure to 0.6 ppm NO_2 or FA for 2 h, followed 3 h later by a 2-h exposure to 0.3 ppm O_3 was investigated by Hazucha et al. (1994) in 21 healthy, nonsmoking females (18 to 34 years of age). Subjects alternated 15-min periods of exercise ($\dot{V}_E$ = 20 L/min/m^2 BSA) and 15-min rest periods while in the exposure chamber, and rested in ambient air during the 3-h interexposure period. The 2 exposure days were separated by at least 2 weeks. Ambient conditions in the exposure chamber were 21 °C and 40% RH. Group mean decrements following the FA/O_3 exposure sequence were −10.8, −7.0, −10.2, and −14.9% for PEFR, FVC, FEV_1, and $FEF_{25\text{-}75\%}$, respectively. Following the NO_2/O_3 exposure sequence, the group mean decrements were −14.5, −8.5, −12.0, and −19.5%, respectively, for PEFR, FVC, FEV_1, and $FEF_{25\text{-}75\%}$. Although small, the differences in FEV_1 and $FEF_{25\text{-}75\%}$ between the FA/O_3 and NO_2/O_3 exposure sequences were statistically significant. There were no differences in the changes in SR_{aw} or symptomology between the two exposure sequences. The most striking finding of this study was that, although both exposure sequences increased airway responsiveness to methacholine, responsiveness was potentiated by the NO_2/O_3 exposure sequence compared to the FA/O_3 exposure sequence.

Aris et al. (1991) examined pulmonary function responses to a 3-h exposure to 0.2 ppm O_3 following a 2-h exposure to 0.54 mg/mL nitric acid (HNO_3; volume mean diameter = 6.0 ± 0.2 μm) or 0.55 mg/mL water (H_2O; volume mean diameter = 6.47 ± 0.4 μm) fog. This is a common pattern of pollutant exposure in coastal California areas.

Subjects were 10 healthy adults 21 to 31 years of age; they were prescreened for a decrement of 10% or greater in FEV_1 following 3 h of exposure to 0.2 ppm O_3, during which they exercised for 50 min of each hour ($\dot{V}_E$ = 40 L/min). Decrements in FEV_1 following the screening O_3 exposure ranged from 15 to 49%. The three exposures (FA + O_3, H_2O fog + O_3, and HNO_3 fog + O_3) were presented in random order, and were separated by a minimum of 2 weeks. The authors hypothesized that exposure to acidic fog, followed by O_3 exposure, would induce greater decrements in FVC and FEV_1 than H_2O fog or air followed by O_3 exposure. In fact, both HNO_3 and H_2O fog exposure seemed to ameliorate the effect of subsequent O_3 exposure on FEV_1 and FVC, although only the difference between the FEV_1 responses to FA + O_3 (28.5%) and H_2O fog + O_3 (18.5%) was significant. Group mean comparisons of methacholine responsiveness and O_3 responsiveness (defined as a minimum of a 10% decrement in FEV_1 following the prescreening O_3 exposure) suggest that the subjects classified as O_3 sensitive, on the average, had lower methacholine $PC_{100Sraw}$ doses. The individual data, however, do not always support this conclusion. Two of 10 O_3-sensitive subjects had methacholine $PC_{100SRaw}$ concentrations above the author's cut-off for airway hyperresponsiveness, and 3 of 10 O_3 nonsensitive subjects had hyperreactive airways based on the authors' criteria for methacholine $PC_{100SRaw}$.

Aris et al. (1993b) further examined pulmonary responses to combined O_3 and HNO_3 exposures. Ten healthy, nonsmoking adults, 19 to 41 years of age, were exposed to FA, 500 $\mu g/m^3$ of HNO_3 gas plus 0.2 ppm O_3, or to 0.2 ppm O_3 alone. The exposure protocol was 4 h in duration, with 50 min IE at 40 L/min alternating with 10-min rest periods each hour. Pulmonary function was measured during each rest period, whereas BAL, proximal airway lavage, and bronchial biopsies were performed 18 h after completion of each exposure. Mean FEV_1 and FVC decreased, and mean SR_{aw} and respiratory symptom scores increased across both the HNO_3 + O_3 and the O_3 exposures. The results indicated, however, that HNO_3 combined with O_3 did not exacerbate the pulmonary function decrements or respiratory symptoms caused by O_3 alone. Similarly, there were no statistically significant differences between the HNO_3 + O_3 and the O_3 exposures in the cellular or biochemical constituents in either the BAL or proximal airway lavage fluids or in the bronchial biopsy specimens. The authors concluded that HNO_3 does not potentiate the inflammatory response produced by O_3 in healthy individuals.

The objective of a study by Koenig et al. (1994) was to investigate possible interactions between oxidants (0.12 ppm O_3 + 0.30 ppm NO_2) and an H_2SO_4 aerosol (70 $\mu g/m^3$) with a mass median aerodynamic diameter (MMAD) of 0.6 μm ($\pm\sigma_g$ = 1.5). Twenty-two adolescent allergic asthmatics who also had exercise-induced bronchospasm and a positive response to a standardized methacholine bronchochallenge test completed all exposures. Subjects inhaled FA, O_3 + NO_2, O_3 + NO_2 + H_2SO_4, or O_3 + NO_2 + HNO_3 through a mouthpiece for 90 min on 2 consecutive days. Each pair of exposures was separated by at least 1 week. Subjects exercised ($\dot{V}_E$ about 10 times FVC) and rested in alternating 15-min periods. Pulmonary functions (FVC, FEV_1, $\dot{V}_{max50\%}$, $\dot{V}_{max75\%}$, and R_T) were measured before and after each exposure and on the day following the second consecutive exposure, at which time only pulmonary function was evaluated and a methacholine bronchochallenge was performed. Six additional subjects began the study, but dropped out before completion because of uncomfortable symptoms associated with the exposures; none dropped out following an FA exposure. There were no statistically significant changes in any measured parameter of pulmonary function following the three

pollutant-containing exposures, compared to the FA exposure, contrary to expectations. (Also see Section 7.4 for related epidemiological studies.)

7.2.6.3 Ozone, Peroxyacetyl Nitrate, and More Complex Mixtures

Horvath et al. (1986) exposed 10 healthy young women (19 to 36 years of age) to (1) FA, (2) 0.48 ppm O_3, (3) 0.27 ppm PAN, and (4) 0.48 ppm O_3 + 0.27 ppm PAN. The chamber exposures were 2 h in duration, during which subjects alternated 20-min exercise periods ($\dot{V}_E$ = 25 L/min) and 15-min rest periods. Exposures were completed in random order and were at least 1 week apart. Exposure to PAN alone did not induce any significant changes in pulmonary function. Both O_3 and PAN + O_3 exposure induced significant decrements in FVC, FEV_1, and $FEF_{25-75\%}$; however, the decrements following the PAN + O_3 exposure were significantly larger (average of about 10%), suggesting interaction between PAN and O_3. It should be noted that typical peak ambient PAN concentrations are about 0.05 ppm. Symptom reports indicated that O_3 + PAN exposure induced greater subjective stress than did exposure to O_3 alone.

Drechsler-Parks et al. (1987b) exposed eight healthy young adults (mean age 24 years) to a mixture of 0.30 ppm PAN + 0.45 ppm O_3 on 5 consecutive days to evaluate possible attenuation. Subjects were reexposed to the PAN + O_3 mixture on the third and seventh days following the last consecutive day of exposure. Attenuation occurred with the same pattern and time sequence as has been reported for O_3 alone. The largest group mean decrements occurred following the second exposure, and the subjects became progressively less responsive with subsequent exposures. Two subjects failed to return to baseline values with 5 consecutive days of exposure. Pulmonary function changes after the follow-up exposures indicated that the attenuation response is relatively short lived, in that it began to abate within 3 to 7 days following the fifth consecutive day of exposure. These results are consistent with those of similar studies using exposure to O_3 alone (Horvath et al., 1981; Kulle et al., 1982), suggesting that PAN had no additional effect on attenuation to O_3. A greater number of symptoms was reported following all PAN + O_3 exposures than following exposure to O_3 alone.

Drechsler-Parks et al. (1989) studied 16 older men and women (51 to 76 years of age) and 16 young men and women (19 to 26 years of age) who each completed 2-h chamber exposures to FA, 0.45 ppm O_3, and mixtures of 0.45 ppm O_3 with 0.60 ppm NO_2 and/or 0.13 ppm PAN. Subjects alternated 20-min exercise ($\dot{V}_E$ about 25 L/min) and rest periods. Exposure to O_3 alone and in all combinations induced significant decrements in FVC (14 to 17%), FEV_1 (19 to 22%), and $FEF_{25-75\%}$ (28 to 30%) in the younger group. In the older group, these same three variables were significantly decreased only with NO_2 + O_3 exposure (7.3% for FVC, 8.4% for FEV_1, and 12% for $FEF_{25-75\%}$). Exposure of the older subjects to PAN + O_3 induced significant decrements only in FVC (4.2%) and FEV_1 (8.3%). The PAN + NO_2 + O_3 exposure induced a significant decrement only in FVC (6.4%) in the older subjects. All subjects reported more symptoms following the mixture exposures than following exposure to O_3 alone. These pulmonary function results following the exposure to O_3 + PAN are in contrast to those reported by Drechsler-Parks et al. (1984) and Horvath et al. (1986) on young adults exposed to 0.45 ppm O_3 + 0.30 ppm PAN. The results of both earlier studies suggested an interaction between O_3 and PAN, in that pulmonary function decrements following the mixture exposure were approximately 10% larger than those following exposure to O_3 alone, whereas there were no significant pulmonary function effects with exposure to PAN alone. A likely explanation for this discrepancy is that the

PAN concentration used by Drechsler-Parks et al. (1989) was slightly less than half that used by Drechsler-Parks et al. (1984) and Horvath et al. (1986). Thus, if the additional effect of PAN is linear, an additional effect of PAN + O_3 would be expected to be less than 5%, which probably would not be detected because it is within the variability of the pulmonary function measurements. In any case, ambient PAN concentrations are considerably less than 0.13 ppm. This indicates that, even if PAN and O_3 do interact in some way in their effects on pulmonary function, at typical ambient concentrations of O_3 and PAN, effects can be attributed to O_3 alone.

7.2.6.4 Summary

Information on interactive effects between O_3 and other pollutants remains sparse at this time. However, it is clear that O_3 is responsible for the largest share of observed effects when subjects are exposed to the mixtures of O_3 and other pollutants that have been studied to date. There is no evidence that simultaneous exposure of healthy individuals to ambient concentrations of O_3 plus NO_2, PAN, H_2SO_4, HNO_3, or SO_2 results in significant interaction. However, Aris et al. (1991) have reported that HNO_3 and H_2O fog exposure ameliorates the pulmonary function effects of a subsequent O_3 exposure. Koenig et al. (1990) found that preexposure to O_3 induced significant pulmonary function decrements in allergic asthmatic adolescents following a sequential SO_2 exposure. Both the O_3 and SO_2 concentrations were at subthreshold levels for the experimental design used.

Both studies that reported potentially significant effects have involved sequential exposure protocols, in contrast to the simultaneous exposure protocols, which generally have not shown effects beyond those that would be expected at the O_3 concentration used. It may be that certain preexposures predispose an individual to responses following a subsequent exposure; however, this question remains far from being resolved. Further, these results are related only to spirometry and plethysmography and may not be applicable to other possible endpoints.

7.3 Symptoms and Pulmonary Function in Controlled Studies of Ambient Air Exposures

Controlled O_3 exposure studies under a variety of different experimental conditions have generated a large amount of informative exposure-effects data. However, complete laboratory simulation of the pollutant mix present in ambient air is impossible on practical grounds. Thus, the exposure effects of one or several artificially generated pollutants (i.e., a simple mixture) on symptoms and lung function may not be comparable to those in ambient air where complex mixtures of pollutants likely exist. This section reviews two types of studies that utilize a mobile laboratory or a hypobaric chamber to investigate the acute effects of O_3 during exposures to ambient air or altitude, respectively. These studies can be designed to determine the independent effects of O_3 as well as possible interactions among many pollutants and other conditions present in typical ambient air.

7.3.1 Mobile Laboratory Studies

Quantitatively useful information on the effects of acute exposure to photochemical oxidants on symptoms and pulmonary function originated from field studies using a mobile laboratory, as presented in the previous criteria document (U.S. Environmental Protection

Agency, 1986). These studies offer the advantage of studying the effects of ambient air on a local subject population by combining the experimental methods of both epidemiology and controlled-exposure studies. Field studies using mobile exposure chambers involve subjects exposed to ambient air, FA without pollutants, or FA containing artificially generated concentrations of O_3 that are comparable to those measured in the ambient environment. The exposure air can also be conditioned to a desired temperature and humidity. As a result, measured health responses in ambient air can be compared to those found in more artificial or controlled conditions. The mobile laboratory shares many of the same limitations of stationary exposure laboratories (e.g., limited number of both subjects and artificially generated pollutants for testing). Ambient air studies in the mobile laboratory are dependent on ambient conditions, which can be unpredictable, uncontrollable, and not completely characterizable. Logistical problems (space, power, and locations with local interfering outdoor conditions) limit access to many ambient pollution sites of interest.

As summarized in Table 7-14, investigators at the Rancho Los Amigos Medical Center in California used a mobile laboratory and demonstrated that respiratory effects in Los Angeles residents are related to O_3 concentration and level of exercise (Linn et al., 1980, 1983b; Avol et al., 1983, 1984, 1985a,b,c, 1987). Such effects include pulmonary function decrements at O_3 concentrations of 0.144 ppm in healthy exercising adolescents (Avol et al., 1985a,b) and increased respiratory symptoms and pulmonary function decrements at 0.153 ppm in heavily exercising athletes (Avol et al., 1984, 1985c) and at 0.174 ppm in lightly exercising normal and asthmatic subjects (Linn et al., 1980, 1983b). The observed effects were typically mild, and generally no substantial differences were seen between asthmatic and nonasthmatic subjects. Postexposure pulmonary function decrements appeared to last several hours longer in the asthmatics, but no statistical test was reported for this difference (Avol et al., 1983; Linn et al., 1983b). The medication status of the asthmatic subjects during the studies was not reported, although medications were temporarily withheld prior to exposures. The subjects' clinical severity typically was mild, based on their baseline lung function and exercise capability. Many of the normal subjects with a history of allergy appeared to be more responsive to O_3 than "nonallergic" normal subjects (Linn et al., 1980, 1983b), although a standardized evaluation of atopic status was not performed. Direct comparative studies of exercising athletes (Avol et al., 1984, 1985c) with chamber exposures to oxidant-polluted ambient air (mean O_3 concentration of 0.15 ppm) and purified air containing a controlled concentration of generated O_3 at 0.16 ppm showed no significant differences in lung function and symptoms, suggesting that coexisting ambient pollutants had minimal contribution to the measured responses under the typical summer ambient conditions in Southern California. Effects of copollutants in other regions of the country remain to be investigated with the mobile laboratory. These field studies emphasize the importance of adequate characterization of subjects and the ambient air, exercise levels, duration of exposure, and individual variations in sensitivity in interpreting observed exposure effects. Although these factors need to be investigated over a wider range of experimental conditions, the results from these field studies are, so far, consistent with those from controlled human exposure studies. Short-term respiratory effects of summer ambient oxidant pollution in Southern California are predominantly, if not entirely, caused by ambient O_3 in typical healthy or asthmatic residents, according to mobile laboratory studies (Avol et al., 1984, 1985c). Overall, the symptoms and decrements in lung function were generally modest and, while statistically significant in some cases, were probably not clinically significant.

Table 7-14. Acute Effects of Ozone in Ambient Air in Field Studies with a Mobile Laboratory[a]

Mean Ozone Concentration[b] 6ppm	Mean Ozone Concentration[b] $\mu g/m^3$	Ambient Temperature[c] (°C)	Exposure Duration	Activity Level ($\dot{V}_E$)	Number of Subjects	Observed Effect(s)	Reference
0.113 ± .033	221 ± 65	33 ± 1	1 h	CE (22 L/min)	66 healthy children, 8 to 11 years old	No significant changes in forced expiratory function and respiratory symptoms after exposure to 0.113 ppm O_3 in ambient air.	Avol et al. (1987)
0.144 ± .043	282 ± 84	32 ± 1	1 h	CE (32 L/min)	59 healthy adolescents, 12 to 15 years old	Small significant decreases in FVC (−2.1%), $FEV_{0.75}$ (−4.0%), FEV_1 (−4.2%), and PEFR (−4.4%) relative to control with no recovery during a 1-h postexposure rest; no significant increases in symptoms.	Avol et al. (1985a,b)
0.153 ± .025	300 ± 49	32 ± 2	1 h	CE (53 L/min)	50 healthy adults (competitive bicyclists)	Mild increases in lower respiratory symptom scores and significant decreases in FEV_1 (−5.3%) and FVC; mean changes in ambient air were not statistically different from those in purified air containing 0.16 ppm O_3.	Avol et al. (1984, 1985c)
0.156 ± .055	306 ± 107	33 ± 4	1 h	CE (38 L/min)	48 healthy adults, 50 asthmatic adults	No significant changes for total symptom score or forced expiratory performance in normals or asthmatics; however, FEV_1 remained low or decreased further (−3%) 3 h after ambient air exposure in asthmatics.	Linn et al. (1983b) Avol et al. (1983)
0.165 ± .059	323 ± 115	33 ± 3	1 h	CE (42 L/min)	60 "healthy" adults (7 were asthmatic)	Small significant decreases in FEV_1 (−3.3%) and FVC with no recovery during a 1-h postexposure rest; TLC decreased and ΔN_2 increased slightly.	Linn et al. (1983b) Avol et al. (1983)

Table 7-14 (cont'd). Acute Effects of Ozone in Ambient Air in Field Studies with a Mobile Laboratory[a]

Mean Ozone Concentration[b] ppm	Mean Ozone Concentration[b] $\mu g/m^3$	Ambient Temperature[c] (°C)	Exposure Duration	Activity Level ($\dot{V}_E$)	Number of Subjects	Observed Effect(s)	Reference
0.174 ± .068	341 ± 133	33 ± 2	2 h	IE (2 times resting) at 15-min intervals	34 "healthy" adults, 30 asthmatic adults	Increased symptom scores and small significant decreases in FEV_1 (−2.4%), FVC, PEFR, and TLC in both asthmatic and healthy subjects; however, 25/34 healthy subjects were allergic and "atypically" reactive to polluted ambient air.	Linn et al. (1980, 1983b)

[a]See Appendix A for abbreviations and acronyms.
[b]Ranked by lowest level of O_3 in ambient air, presented as the mean ± SD.
[c]Mean ± SD.

7.3.2 High-Altitude Studies

Symptoms and pulmonary function resulting from exposure to O_3 in commercial aircraft flying at high altitudes and in altitude-simulation studies were reviewed in the previous criteria document (U.S. Environmental Protection Agency, 1986). Attention has focused on the health effects in flight crew, specifically flight attendants because of their physical activities at altitude and exposure patterns to peak levels of cabin O_3. The most quantitatively useful information was based on a series of hypobaric studies of normal nonsmoking subjects who were exposed to 1,829 m (6,000 ft) and O_3 at concentrations of 0.2 and 0.3 ppm for 3 or 4 h (Lategola et al., 1980a,b). Increased symptoms and pulmonary function decrements occurred at 0.3 ppm but not at 0.2 ppm under light exercise conditions. However, the exposure conditions did not reflect higher (peak) O_3 concentrations reported to occur in certain aircraft at high altitudes or the higher cabin altitudes attained by new-generation commercial aircraft.

No reports have appeared subsequently in the literature that specifically study the health effects of aircraft cabin O_3. However, O_3 levels were reported to be very low (average concentration 0.01 to 0.02 ppm) during 92 randomly selected smoking and nonsmoking flights in 1989 (Nagda et al., 1991). None of the flights exceeded the time-weighted average standard of 0.10 ppm (during any 3-h interval) promulgated by the U.S. Federal Aviation Administration, perhaps related to the use of O_3-scrubbing catalytic filters (Melton, 1990). However, in-flight O_3 exposure is possible because catalytic filters are not necessarily in continuous use during flight.

7.4 Field and Epidemiology Studies

7.4.1 Acute Effects of Ozone Exposure

7.4.1.1 Introduction

Field and epidemiology studies addressing the acute effects of O_3 on lung function decrements and increased morbidity and mortality in human populations involve those combinations of environmental conditions and copollutant and activity levels present under real-world conditions of O_3 exposure. This real-world relevance is an advantage over animal or human chamber studies. Thus, results of such studies are essential components of an understanding of overall effects of O_3. However, the conditions under which epidemiologic studies are carried out cannot be controlled in the same way that they can in experimental studies. Parameters that may be difficult or impossible to estimate or control outside the laboratory include actual O_3 exposures, levels of temperature, RH, allergens, correlated pollutants other than O_3, and breathing rates and activity patterns of subjects. Variations in these factors can be important sources of variability in data and results and may, under certain conditions, lead to biases (e.g., confounding) in results. These and other issues of importance in the interpretation of epidemiology study results are discussed in the sections below.

The limitations of epidemiologic studies of O_3 health effects noted above were highlighted in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986), which reached the conclusion that, because of such factors, epidemiologic studies on the acute effects of O_3 on lung function available at that time did not provide information that is quantitatively useful in the standard-setting process. Since publication of the 1986 O_3 criteria document, however, results have become available from a substantial number of

well-conducted, individual-level studies and aggregate-level, time-series studies. New statistical techniques also have become available to deal with confounders that were not appropriately considered when the data were first analyzed. In the following sections, the more recent studies and reanalyses of older studies will be evaluated collectively.

7.4.1.2 Individual-Level Studies

The studies discussed in this section fall into three main categories: (1) summer camp studies, (2) exercise studies, and (3) daily life studies. Summer camp studies involve collection of sequential (usually daily) data on lung function, respiratory symptoms, and environmental conditions over the course of 1 or 2-week attendance at camps. Exercise studies are unique in that lung function and respiratory symptoms are measured before and after each of a series of discrete exercise events in the presence of ambient air pollution. Daily life studies measure lung function, respiratory symptoms, and exacerbation of existing respiratory diseases, along with environmental variables at regular intervals in the course of normal daily activities of a population. These include studies of healthy adults and school-aged children as well as studies of individuals with preexisting disease (e.g., asthma). Medication use also may be monitored in asthmatics. Studies of this kind that focus on exacerbation of asthma symptoms usually have been referred to as panel studies.

The important differences among the three study types relate primarily to issues of exposure assessment. Because subjects usually are out-of-doors or in well-ventilated cabins, exposure estimation errors are minimized in camp and exercise studies. In contrast, larger exposure estimation errors may occur in daily life studies. Camp and daily life studies enable assessment of the effects of cumulative O_3 exposures, whereas exercise studies limit attention to rather brief exposures. Exercise studies offer the potential of assessing individual $\dot{V}_E$ values and O_3 concentrations during the relevant exposure period, whereas such assessments are more difficult in camp and daily life studies.

Although the study designs differ in some ways, the central design feature of all of these study types is the collection of repeated measurements on individuals. This feature is exploited in data analysis by having each subject serve as his or her own control. For continuous outcomes such as lung function, subject-specific linear regressions are usually performed with lung function (or change in lung function) as the outcome variable and O_3 or other environmental factors as the explanatory variable. The regression slope is a measure of individual lung function response to O_3. The mean slope across individuals often is used as a measure of the average population response. A more statistically valid approach involves computing the mean slope with weighting proportional to the inverse variances of the individual slopes. An alternative approach has been to use analysis of covariance methods to fit a population-pooled slope and separate, subject-specific y-intercepts. To date, no studies have used nonlinear (e.g., quadratic) models in relating lung function decrements to O_3 exposures, which in chamber studies have been shown to better describe the functional relationship between O_3 exposures and lung function decrements (see Chapter 9, Section 9.3.4).

Issues in the Interpretation of Individual Level Studies

The most basic question affecting the interpretation of acute O_3 epidemiology studies is whether (and if so, to what extent) the associations observed between O_3 and decreased pulmonary function are causally related to O_3 and not merely due to confounding by some other factor (e.g., temperature, allergens, time trends in spirometry, or other

pollutants). By definition, a confounder is an unmeasured or unaccounted-for variable that has an effect on the measured outcome and also is correlated with O_3 concentrations. Variables that satisfy only one of these two conditions are not confounders. For example, a variable that affects lung function but is independent of O_3 would add variation to lung function measurements but would not confound an O_3/lung function analysis, and a variable that correlates with O_3 but does not directly affect lung function (in the range of measurements) would not confound an analysis of O_3 effects. Other variables might modify the effect of O_3 on lung function, thereby increasing or decreasing the O_3 effect under the conditions of study. Epidemiologists refer to this latter phenomenon as "effect modification". The presence of effect modification does not bias the results of a study, but can provide insights into the range of effect magnitudes (e.g., slope of lung function decrement on O_3 level) that occur under varying environmental conditions.

Ambient air temperature often exhibits a moderate to high correlation over time with O_3 in acute epidemiology studies due,. in part, to the dependence of O_3 formation rate on light intensity. Among the studies reviewed in this section, correlations ranging from −0.06 to 0.90 (mean = 0.51) have been reported. Correlations between O_3 and RH, when reported, have been in the range −0.4 to −0.6. Several human chamber studies have examined the possible direct effects of temperature and RH on lung function independent of O_3, with somewhat mixed results (Stacy et al., 1982; Folinsbee et al., 1985; Eschenbacher et al., 1992). Two studies reported increases in FEV_1 at high temperature (30 and 37 °C) and 60% RH (Stacy et al., 1982; Eschenbacher et al., 1992), whereas the other reported no effect on FEV_1 at 35 °C, and a decrease at 40 °C (Folinsbee et al., 1985). Referring to results of acute O_3 epidemiology studies, Eschenbacher and colleagues (1992) concluded, based on their own results, that "the associations found between ambient O_3 and daily changes in ventilatory function cannot be attributed to the heat and humidity stress often associated with high O_3 concentrations." Temperatures observed in the epidemiology studies reviewed in the present section primarily have been below 30 °C, with occasional peaks as high as 35 °C. It should be noted that subjects studied epidemiologically usually will have had an opportunity to acclimate to ambient temperatures prior to or soon after the start of the study. In any event, given the laboratory findings, a significant confounding role for temperature in these studies seems unlikely. The possibility that changes in ambient temperature may introduce biases in measured lung volumes (e.g., through inaccurate correction of volumes to body temperature) is an issue that deserves further study.

Exposure to specific allergens can influence lung function in individuals who have diseases characterized by IgE-mediated, Fc interactions (i.e., atopy) and may also affect individuals who have an atopic tendency (e.g., as assessed by positive prick skin test or serum levels of total IgE) without diagnosed clinical disease. Raizenne et al. (1989) detected positive reactions to one or more allergens by skin prick in 49% of 96 young nonasthmatic females enrolled in a summer camp study. Few data are available on the correlation between O_3 and allergen levels during acute epidemiology studies. However, because both variables to some are extent influenced by weather patterns, some correlation seems likely. Thus, a possible confounding role of airborne allergens in such studies cannot be ruled out. Because of the specific nature of individual antigen sensitization and uncertainty regarding the full set of relevant allergens in a given setting, attempts to measure and to control statistically for allergen levels on a group level in epidemiology studies may not be very effective.

The potential effects of time trends in spirometry due to training effects are also of concern. There have been several recent studies that have looked at time trends in serial

lung function measurements (mainly FEV_1 and PEFR) independent of air pollution effects (Raizenne et al., 1989; Avol et al., 1990; Hoek and Brunekreef, 1992). In each case, average FEV_1 measurements have been observed to decline steadily over the first few measurements and then to stabilize or recover slightly to a flat pattern. Average FVC measurements follow a similar pattern. In contrast, PEFR often has been observed to increase steadily over successive measurements. Similar patterns have been observed in studies with intervals between lung function measurements ranging from 12 h to 1 week. The consistency of these observations across studies suggests that they represent real phenomena that should be recognized in designing and analyzing studies involving repeated lung function measurements. However, time trends will result in confounding of O_3 effects only if, by chance, the trend correlates with temporal variations in O_3 concentrations. Such chance correlations could be either positive or negative and, if present, would have a larger impact (i.e., produce an undesirable degree of confounding) on studies in which all subjects begin the study simultaneously and have few follow-up measurements. Studies that focus on daily changes in lung function may be less impacted by this phenomenon.

It is also important to consider the roles of other pollutants as possible confounders or effect modifiers. In the studies to be reviewed in this section, most copollutants (e.g., SO_2, NO_2, sulfate, and acid aerosols) were present at levels well below those that have produced lung function decrements in healthy subjects following short-term exposures in chamber studies (see Section 7.2.6). In contrast, an extensive and growing database is available from chamber studies documenting the independent acute effects of ambient-level O_3 on lung function (see Section 7.2.1). Although direct lung function effects of other pollutants at typical ambient concentrations seem unlikely, it has been suggested that the effects of O_3 may be potentiated by coexposure or previous exposure to other pollutants, most notably acid aerosols (Spektor et al., 1988b). Some data from animal studies suggest interactive effects of O_3 and acid exposures for certain pulmonary outcomes (see Chapter 6). However, to date, analyses directed towards this phenomenon in field studies of human lung function (via analysis of the relationship between acid aerosol levels and residuals from regressions of lung function on O_3) have proven negative (Spektor et al., 1988a,b). That is, after controlling for the influence of O_3, no significant association between acid aerosol peaks and lung function decrements has been observed. Acid aerosol episodes, which often occur coincident with high O_3 levels in the summer in the northeastern United States, may extend for several hours or days. However, the possible potentiating effects of prolonged acid peaks on O_3 effects are still poorly understood. Some recent epidemiological studies (Pope et al., 1991; Pope and Dockery, 1992; Koenig et al., 1993; Roemer et al., 1993) have reported significant associations between lung function decrements and ambient particulate matter (PM) concentrations. Although no supporting evidence for lung function effects due to ambient-level particle exposure is yet available from human or laboratory animal controlled studies, the possibility of some confounding by particles in the studies reviewed here cannot be ruled out.

In epidemiologic studies, activity levels are difficult to control and to measure, although this varies with study type (see below). Chamber studies have shown clearly that lung O_3 doses and associated functional effects increase as a function of physical activity level (Hazucha, 1987). Epidemiologic study designs often have been chosen that result in relatively high subject activity levels (exercise studies and camp studies), but generally the studies have been carried out without quantitative information on $\dot{V}_E$ distributions across subjects and time.

Variations in activity levels will introduce variability in the relationship between personal exposure and personal dose. If this variability occurs primarily between subjects, it will result in differing O_3 doses to people exposed to the same O_3 level and yield differences in response that may be misinterpreted as O_3 sensitivity variations. If variability in activity levels occurs over time for a given subject, it will add error to the functional relationship linking lung function and O_3 exposure. In either case, the influence of activity level variability is to add dose estimation error (or misclassification). Estimates of $\dot{V}_E$ based on heart rate measurements can be derived using subject-specific calibrations under representative ranges of exercise levels and types (Samet et al., 1993; Raizenne and Spengler, 1989). However, the utility of such $\dot{V}_E$ estimates for reducing dose-misclassification errors in acute O_3 epidemiology studies has not yet been demonstrated (Kinney, 1986; Spektor et al., 1988b; Raizenne and Spengler, 1989). This is partly due to the logistical difficulties associated with collecting accurate data and also may be due to the fact that, for a given subject, $\dot{V}_E$ variations across days are usually small in comparison to O_3 concentration variations. The same issues arise in the context of O_3 exposure misclassification in "daily life" studies (see below), where outdoor O_3 concentrations are used to estimate exposures of subjects who spend substantial amounts of time indoors during the period over which lung function measurements take place.

Camp Studies of Lung Function in Children

Summer camp studies provide the most extensive and reliable information on the acute pulmonary effects of O_3 under natural conditions. Camp studies involve the collection of sequential (usually daily) data on lung function on each of a large number of children, along with concurrent measurements of O_3 exposures and other environmental factors over the course of a single-week or multiweek summer camp. Data analyses usually consist of estimating the linear association between lung function and environmental variables on an individual basis (allowing each subject to serve as his/her own control) and then testing the mean population association for statistical significance. As noted, summer camps offer the significant advantage that subject exposures are especially well estimated because they are based on on-site, outdoor O_3 monitoring. In addition, these studies assess the pulmonary effects of natural diurnal patterns of O_3 exposures, which often involve broad daytime peaks.

Since the last O_3 criteria document (U.S. Environmental Protection Agency, 1986), eight camp studies have been reported. Design characteristics and results are summarized in Table 7-15. Six of these studies have focused solely on normal (i.e., nonasthmatic) children, one focused on asthmatics exclusively (Thurston et al., 1995), and one used both normal and asthmatic children (Raizenne et al., 1987). Although methods and results varied somewhat across studies, this group of studies collectively provides substantial evidence for associations between ambient O_3 exposures, together with other pollutants, and acute decrements in lung function. Interpretation of these associations as causal is supported by evidence of biological plausibility. For example, the well-documented direct effects of O_3 on lung function in human chamber studies; the evidence, also from chamber studies, indicating a lack of direct effects of other collinear environmental factors (e.g., temperature and acid aerosols) at the levels at which these factors occur in the camp studies; exposure-response relationships; and consistency across studies all provide strong support. Camp studies involving asthmatic children generally have yielded lung function/O_3 associations that are similar in absolute magnitude to those observed in nonasthmatics (Raizenne et al., 1987;

Table 7-15. Acute Effects of Photochemical Oxidant Pollution: Lung Function in Camp Studies[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
Hourly O_3 ranged from ≈10 to 110 ppb. SO_2, NO_x, O_3, $SO_4^=$, H_2SO_4, pH, PM_{10}, $PM_{2.5}$, RH, temperature, barometric pressure, and wind speed and direction also were measured.	Effects of pollutants and other environmental variables on symptoms and lung function were examined in children attending a summer camp at Lake Couchiching, about 100 km north of Toronto, ON. Study was conducted June 30 through July 8, 1983; n = 52, 23 nonasthmatics (11 males, 12 females) and 29 asthmatics (16 males, 13 females), avg. age = 12.1 years. Symptom questionnaire and function tests given twice daily to each child between 7:30 and 9:30 a.m. and 4:30 and 6:30 p.m. Children's activity levels not estimated.	Strongest association between lung function and environmental variables was in nonasthmatics, with FVC decrements significantly correlated ($p < 0.01$) with lagged-avg SO_4, $PM_{2.5}$, and temperature. Unlagged PEFR significantly correlated with 1 h O_3. Also, significant association of temperature with all lung function indices in nonasthmatics, but not in asthmatics. Coefficient of variation stable across morning and evening tests.	Raizenne et al. (1987)[b]
1-h O_3 ranged from <10 to 143 ppb; max 1-h O_3 > 100 ppb on 14 days of total study (6 weeks). For other pollutants and variables measured, see Raizenne et al. (1987) because same protocol used here as in that study.	(a) Effects of pollutants and other environmental variables on lung function were examined in girls attending one of three 2-week Girl Guide camp sessions on the north shore of Lake Erie. Cohort (n = 104) screened by MC and skin-prick tests for 10 common respiratory allergens; five asthmatics withdrawn from the study (n = 99). Lung function tests administered twice daily. Children's activity levels not estimated. (b) Subset of 12 girls (7 MC+, 5 MC-) studied pre- and postexercise on 1 low-pollution (control) day and 1 peak-pollution day (episode, 1 h O_3 > 139 ppb, $SO_4^=$ > 80 $\mu g/m^3$).	(a) Associations between aerometric data and lung function measurements were not reported by pollutant in this reference. Aggregate analysis for full study not reported. Lung function changes reported for 5 episode days only. FEV_1 decrements statistically significant on 2 episode days for methacholine-nonresponsive subjects. (b) Group mean FVC increased postexercise in the n = 12 subset by 40 mL, 71 mL in MC− and 17 mL in MC+. Pollution effect not statistically significant.	Raizenne et al. (1987, 1989)[b]
Continuous 1-h O_3, SO_2, NO_2, and acid aerosols (as H_2SO_4); 1-h O_3 range = 40-143 ppb; max 12-h acid particle concentration = 28 $\mu g/m^3$ in one episode; FP $SO_4^=$ 100 $\mu g/m^3$ for peak hour.	Time-activity model used to evaluate likely cumulative (6-h) O_3 and H_2SO_4 exposures/doses experienced by children in above Lake Erie Girl Guide camp study, summer 1986. See Raizenne et al. (1987, 1989) for protocol and related information. Dosimetry model was developed for relating heart rate (from a 12-min, graded, cycle ergometer test) to ventilation and then to O_3 and H_2SO_4 concentration. Also, five randomly selected children wore portable heart-rate monitors, providing data for use in the dosimetric model.	Application of the dosimetry model used to estimate individual 6-h cumulative doses for O_3 and H_2SO_4 exposures on 1 control and 1 episode day indicated negative trend in lung function (PEFR) as cumulative dose increased for both O_3 and H_2SO_4, although slopes for each did not differ significantly from zero ($p > 0.10$).	Raizenne and Spengler (1989)[b]

Table 7-15 (cont'd). Acute Effects of Photochemical Oxidant Pollution: Lung Function in Camp Studies[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
Max 1 h O_3 ranged from 40 to ≈100 ppb, with max 1 h > 80 ppb on 9 of 27 days of O_3 recorded. O_3, SO_4, H_2SO_4, PM_{15}, $PM_{2.5}$, temperature, humidity, and wind speed and direction measured. Levels not reported for SO_2, pH, NO_3, and NH_4^+.	Effects of pollutants and other environmental variables on respiratory functions in 91 children (53 boys, 38 girls; ages 8-15) attending 2 to 4 weeks of summer camp at Fairview Lake, NJ. Subsets were n = 37 for all 4 weeks, n = 34 for first 2 weeks only, n = 20 for last 2 weeks only. Symptom questionnaire; FVC, FEV_1, MMEF, and PERF (by spirometry) were measured once each test day (most of days in camp) sometime between 11:00 a.m. and 6:30 p.m. All children had validated spirometric data for ≥7 days of their 2- or 4-week camp stay. Activity levels of the children were not estimated. Respiratory health status determined by parental questionnaire only. Children slept in screened-in shelters but otherwise were exposed to ambient air 24 h/day. Average regression slopes for respiratory function vs. max 1-h O_3 concentration reported for the full cohort, for boys and girls separately, and for subsets in attendance for all 4 weeks and for respective 2-week sessions. Regressions also repeated for data below 80 and 60 ppb 1-h O_3, and for data with THI <78 °F.	Average regression slopes (±SE) were −1.03 ± 0.24 and −1.42 ± 0.17 mL/ppb for FVC and FEV_1, respectively; and −6.78 ± 0.73 and −2.48 ± 0.26 mL/s/ppb for PEFR and MMEF, respectively. Most slopes of regression significant at $p < 0.05$ (differences from zero). Not clear if slopes for data subsets significantly different from each other (e.g., function vs. O_3 < 60 ppb and function vs. O_3 < 80 ppb). No formal analysis performed for possible concentration threshold.	Spektor et al. (1988a)[b]

Table 7-15 (cont'd). Acute Effects of Photochemical Oxidant Pollution: Lung Function in Camp Studies[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
Maximal 1 h O_3 concentrations ranged from approximately 40 to 150 ppb over the course of the study. 12-h average aerosol acidity measurements ranged from near 0 to 18.6 $\mu g/m^3$ (H_2SO_4 equivalent). Temperature and RH measured, but levels not reported. THI reached a maximum of 81 °F. All environmental measurements were made on site.	Effects of O_3 and other environmental variables on lung function studied in a group of 46 children (13 girls, 33 boys; ages 8-14) at a 4-week, 1988 summer camp in southwestern New Jersey (Fairview Lake). Same location used in previous camp study by same investigators. Subjects had no history of lung diseases or atopy. Two lung function measurement periods each day (a.m. and p.m.) along with collection of respiratory symptom data. Data collected during or after periods of rain were excluded from analysis. Results for FVC, FEV_1, FEV_1/FVC, $FEF_{25-75\%}$, and PEFR reported. Linear day-of-study trends were examined for lung function. Subject-specific linear regressions were performed relating lung function in a.m., p.m., and p.m. − a.m. differences to O_3 averaged over various periods. Average slopes across subjects were tested for significant differences from zero. Regressions were repeated after excluding days with O_3 at or above 120 ppb. Regression residuals were tested for correlation with THI and H^+ concentrations.	No significant linear day-of-study effect seen for any of the lung function variables tested, but the linear model may not have been optimal for testing this effect. In a subset of 35 subjects with at least 2 consecutive days of lung function measurements, mean regression slopes of a.m. lung function variables on previous-day mean or 1-h maximum O_3 were all significantly negative (e.g., mean slope of FEV_1 on 1-h maximum O_3 was -0.50 ± 0.12 mL/ppb). These results suggest a possible carry-over effect from previous-day O_3 exposures. In the full set of 46 subjects, regressions of p.m. lung function on previous-hour O_3, maximum 1-h O_3 for same day, or average O_3 for day were significantly negative in most cases (e.g., mean slope of FEV_1 on previous-hour O_3 was -1.60 ± 0.30 mL/ppb). All regressions of the p.m. − a.m. lung function differences on intervening O_3 concentrations were significantly negative (e.g., mean slope of FEV_1 on mean O_3 between a.m. and p.m. measurements was -0.63 [± 0.09] mL/ppb). No correlation seen between regression residuals and THI or H^+ concentrations, indicating there was no remaining effect of these variables with lung function after accounting for O_3. However, no models were fit that included O_3 and these variables simultaneously, nor were interaction effects tested for. The strong and consistent associations between lung function decrements and O_3 concentrations in this study contrast with results reported from studies in Canada and California at similar levels of O_3.	Spektor et al. (1991) Spektor and Lippmann (1991)

Table 7-15 (cont'd). Acute Effects of Photochemical Oxidant Pollution: Lung Function in Camp Studies[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
1-h O_3 preceding lung function measurements ranged from 25 to 245 ppb. Pollutants measured on site included O_3; NO_2 (range: 0 to 40 ppb), SO_2 (range: 1 to 8 ppb); and fine (mean = 23.9 $\mu g/m^3$), coarse (mean = 36.6 $\mu g/m^3$), and total (mean = 59 $\mu g/m^3$) PM_{10} mass. Temperature averaged 21.5 °C (range: 13.5 to 25.5 °C) and RH averaged 43.3%.	Effects of O_3 and other environmental factors on lung function examined in 43 children (24 female, 19 male; ages 7-13) attending 1 of 3 sequential weeks (three subjects stayed an additional week) of summer camp in the San Bernardino Mountains of California. Camp was at 5,710 ft above sea level. Lung function measured by spirometry up to three times daily on each subject; analytical measures included FVC, FEV_1, and PEFR. No report of respiratory data derived from questionnaires. Subject activity levels prior to lung function testing were not characterized. Campers slept in well-ventilated cabins. Subjects came mostly from homes in the Los Angeles Basin, and thus were likely to have been exposed to high O_3 levels prior to camp. Simple linear regression models were fit on an individual basis (subject-specific slopes) and by pooling across individuals (common population slope) to determine the linear relations between the three lung function variables and various O_3 metrics (1-h average preceding hour of spirometry, 1-h average 2 h previous to hour of spirometry, or 6-h average preceding spirometry). The common slope model was repeated separately for morning, noon, and evening lung-function measurements, and separately for data with 1-h O_3 levels above and below 120 ppb. Multiple regression models were fit that included O_3 along with temperature, RH, and coarse and fine PM mass.	The population-pooled regression slopes (±SE) of FVC and FEV_1 on previous hour O_3 were −0.40 (±0.10) and −0.38 (±0.09) mL/ppb, respectively ($p < 0.0001$ in both cases); for PEFR, the regression slope was −0.13 (±0.36) mL/s/ppb (not significant). Similar, though slightly more negative, slopes were obtained using 2-h and 6-h average O_3. Interpretation of differences across the three O_3 metrics is substantially hampered by the high correlations among them ($r \geq 0.90$). When temperature, RH, and coarse and fine PM mass were included with O_3 in multiple regression models, the O_3 slopes increased in absolute magnitude to −0.68 (±0.16) and −0.76 (±0.15) mL/ppb for FVC and FEV_1, respectively, and to −1.91 (±0.63) for PEFR. Technical problems with the temperature sensor in the first week of the study did not appear to influence these results. Data were split on the basis of whether or not the maximum 1-h O_3 concentration in the 6 h preceding spirometry was above 120 ppb. Regression slopes relating lung function and previous 1-, 3-, and 6-h average O_3 were more negative in the high concentration stratum. This result is consistent with the nonlinear (e.g., quadratic) relationships between lung function and O_3 exposure observed in chamber studies. Because levels of pollutants other than O_3 were quite low (NO_2 and SO_2), and/or were uncorrelated with O_3 levels (PM), the regression results reported from this well-conducted study are likely to represent real influences of O_3 on lung function.	Higgins et al. (1990); Gross et al. (1991)

Table 7-15 (cont'd). Acute Effects of Photochemical Oxidant Pollution: Lung Function in Camp Studies[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
Daily maximum O_3 concentrations ranged from approximately 60 to 160 ppb (derived visually from figure presented in paper). Other pollutants measured on site included SO_2, NO_2, CO, total hydrocarbons, and size-segregated PM mass. Aside from O_3, all gaseous pollutant levels reported to be very low (data not presented). 24-h TSP concentrations ranged from 18 to 54 $\mu g/m^3$. Airborne allergen data collected. Temperature ranged from 10 to 15 °C at night and from 25 to 35 °C during day. RH ranged from 30 to 45% at night and from 5 to 20% during day.	Effects of O_3 and other environmental variables on lung function examined in 293 children (139 girls, 154 boys; ages 8-17) attending one of six 1-week camp sessions at a summer camp located in the mountains near Idyllwild, CA, 190 km southeast of Los Angeles (altitude: 1,570 m). Lung function measured twice daily on each camper (a.m.: 0730 to 0930; p.m.: 1600 to 1930). Analyses presented for FVC, FEV_1, PEFR, and $FEF_{25-75\%}$. Symptom questionnaires completed prior to each test. Used repeated measures analysis of variance model to test for day-of-study and a.m./p.m. effects on lung function independent of pollution concentrations. Linear regressions of morning, afternoon, and p.m. − a.m. difference of lung function on O_3 were performed with simultaneous control of day and a.m./p.m. effects. Upper and lower quartiles of distribution of individual FEV_1/O_3 regression slopes were examined with respect to subject characteristics. Changes in FEV_1 over several days analyzed in relation to intervening integrated O_3 concentrations.	Significant day-of-study effect observed for FVC and FEV_1 characterized by steady drop over first few days of measurement, followed by partial reversal later in week. For PEFR, p.m. measurements were significantly higher than a.m. measurements. Controlling for day and a.m./p.m. effects, the authors reported that no consistent O_3 effects on lung function were observed. The a.m. lung-function measurements had a significant positive correlation with O_3 averaged over the previous 1, 8, or 24 h. The p.m. measurements reported to have no correlation with O_3. The p.m. − a.m. lung function differences were negatively correlated with previous 8-h average O_3 concentrations, but not with previous 1-h O_3 concentrations. No quantitative results reported for the above lung function/O_3 findings. There were no discernable differences between subjects in the upper and lower quartiles of the distribution of individual regression slopes of a.m. − p.m. FEV_1 difference on previous 1 h O_3. Regressions of change in FEV_1 over several days (four separate intervals ranging from approximately 8 h to approximately 80 h) with integrated O_3 concentrations yielded negative slopes ranging from −0.41 to −1.46 mL/ppb, one of which was statistically significant. The time-trends in FVC and FEV_1 measurements observed in this study are qualitatively consistent with those seen in some other summer camp studies. The lack of consistent negative slopes relating lung function with O_3 concentrations contrasts with other, eastern U.S., summer camp studies at similar O_3 levels.	Avol et al. (1990) Avol et al. (1991)

Table 7-15 (cont'd). Acute Effects of Photochemical Oxidant Pollution: Lung Function in Camp Studies[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
O_3 data collected at a site 8 mi from camps. Daily 1-h O_3 maxima ranged from approximately 40 ppb to approximately 200 ppb. 12-h aerosol acidity concentrations ranged between 14 and 360 neq/m^3. Temperature and RH data obtained from a nearby site.	Report of data collected during two simultaneous summer camps located 2 mi apart in central New Jersey in 1988. 34 subjects were studied, including 20 camp counselors (ages 14-35) and 14 campers (ages 9-13). Study spanned 19 days. Spirometry and respiratory symptom data collected each afternoon. Analysis of FVC, FEV_1, and PEFR in relation to O_3 and temperature using linear regression within camps and subject types (i.e., counselors vs. campers).	Regressions of lung function on 1-h and 8-h average O_3 within several subject subsets yielded inconsistent results, with some mean slopes apparently significantly positive, and one negative mean slope, highlighted by authors, of borderline significance ($p < 0.10$).	Berry et al. (1991)
Daily 1-h maximum O_3 concentrations ranged from 70 to 160 ppb in 1991 and from 10 to 63 ppb in 1992. On-site measurements also made for acid aerosols (approximately 20 to 110 nmoles/m^3 in 1991 and 15 to 55 nmoles/m^3 in 1992) and temperature (between 21 and 32 °C over 2 years).	Effects of O_3 and acid aerosols on peak flow, respiratory symptoms, and medication usage in asthmatic children evaluated at two 1-week summer camps (June of 1991 and 1992) in the Connecticut River Valley. Fifty-two and 55 subjects were studied in 1991 and 1992, respectively, ranging in age from 7-13. Peak flow measured twice daily (approximately 9:00 a.m. and 5:00 p.m.). Combining data from the two studies, individual regressions of daily change in FEV_1 on O_3 or H^+ concentrations were performed.	In subjects without asthma exacerbations during the camps, statistically significant, negative mean slopes were found relating ΔPEFR and O_3 or H^+ concentrations. The correlation between these two pollutants was not reported. The mean slopes were -2.3 (± 0.7) mL/s/ppb for O_3, and -1.2 (± 0.6) mL/s/nmol/m^3 for H^+. In the case of O_3, a scatter-plot with ΔPEFR demonstrated an apparently linear trend. In contrast, the H^+ regression results appeared to be driven entirely by one data point.	Thurston et al. (1995)

[a]See Appendix A for abbreviations and acronyms.
[b]Cited in U.S. Environmental Protection Agency (1992).

Thurston et al., 1995); however, the health significance of a given drop in FEV_1 may be greater for those with preexisting, compromised respiratory function.

Although similar study designs have been employed in most of the camp studies summarized in Table 7-15, differences in analytical methods have made quantitative comparisons between studies difficult to interpret. In particular, it has not been clear to what extent differences in results across studies may be due to differences in study characteristics (e.g., O_3 effect potentiation by other pollutants and activity levels) as opposed to differences in data analysis methods.

For better comparison in this document, data from six of the camp studies summarized in Table 7-15 were reanalyzed using uniform analytical methods. For each study, afternoon lung function data (FEV_1) were regressed on concurrent 1-h O_3 concentrations using an analysis of covariance model that included subject-specific intercepts and a single, pooled O_3 slope. Although intersubject variation in responses to O_3 would be expected on the basis of controlled chamber study results (see Section 7.2), a common-slope model was chosen for this analysis because emphasis was placed on estimating the average response in each study population. The study-specific slopes computed with this model ranged from −0.19 to −1.29 mL/ppb across the six studies (Table 7-16). All but one of these slopes were statistically significant ($p < 0.02$). When data for all six studies were pooled, a slope of −0.5 mL/ppb was observed. The slope from the 1988 Fairview Lake, NJ, study (−1.29 mL/ppb) was greater in absolute magnitude than the slopes from the other studies (which ranged from −0.19 to −0.84 mL/ppb). Overall, however, these pooled results indicate a quantitative consistency among studies that is not as readily apparent in the absence of the combined analysis.

Table 7-16. Slopes from Regressions of Forced Expiratory Volume in One Second on Ozone for Six Camp Studies[a]

Study Name	Slope ± SE (mL/ppb)[b]	p-Value	Reference
Fairview Lake, 1984	−0.50 ± 0.16	0.002	Spektor et al. (1988a)
Fairview Lake, 1988	−1.29 ± 0.27	0.0001	Spektor et al. (1991) Spektor and Lippmann (1991)
Lake Couchiching	−0.19 ± 0.44	0.66	Raizenne et al. (1987)
Lake Erie	−0.29 ± 0.10	0.003	Raizenne et al. (1987, 1989)
San Bernardino Mountains	−0.84 ± 0.20	0.0001	Higgins et al. (1990) Gross et al. (1991)
Pine Springs Ranch	−0.32 ± 0.13	0.013	Avol et al. (1990, 1991)
All studies	−0.50 ± 0.07	<0.0001	

[a]For each study, data were analyzed in one regression model that included a pooled O_3 slope and separate subject-specific intercepts. See Appendix A for abbreviations and acronyms.
[b]Slope is the weighted mean of six study-specific slopes. The SE is the weighted SE of mean slope.

It is not clear why the 1988 New Jersey study yielded a larger slope than the other studies. Possible explanations include greater subject activity levels (resulting in higher O_3 doses at a given exposure level), potentiation of the O_3 effect by other pollutants (such as

acid aerosols), the relative absence of O_3 tolerance in the New Jersey study, or confounding by airborne allergens. There are no firm data on activity levels across the six studies. Thus, whereas this factor surely contributes to the random variability within and between studies, it is not known whether activity levels were substantially and systematically higher in the 1988 New Jersey study. Potentiation of the O_3 effects on lung function in asthmatics by acid aerosols has been demonstrated in a chamber study in which O_3 exposure was administered 1 day following a 3-h exposure to 100 $\mu g/m^3$ H_2SO_4 (Utell et al., 1994). Although the relevance of these data to the nonasthmatic subjects who experienced much lower acid levels at northeastern summer-camps is not clear, they do demonstrate that potentiation can occur between these pollutants. However, this factor alone cannot explain the observed differences across camp results, because a camp study in southern Ontario (Raizenne et al., 1989), which yielded relatively low FEV_1 slopes on O_3, experienced sulfate aerosol levels that were comparable to those seen in New Jersey. Similarly, whereas tolerance due to prior exposures to high O_3 levels has been suggested as an explanation for the smaller slopes seen in the California studies, a lower subject activity level has been suggested to explain the smaller slopes in southern Ontario. Data have not been reported on comparative levels of airborne allergens during the various camp studies. None of the subjects in the 1988 New Jersey study reported a history of asthma or atopy, minimizing the likelihood of confounding by airborne allergens. However, given the lack of allergen data and the potential for substantial numbers of "silent hyper-responders" (Raizenne et al., 1989), this possibility cannot be completely discounted. Thus, no one factor seems adequate to explain the differences in results across studies. Quite possibly, these differences reflect the combined influence of several of the factors discussed above. Indeed, given the many possible sources of camp-to-camp variability, it is surprising that results are as consistent as they are across six studies by three investigative groups.

Several investigators have reported regression results for 1-h average O_3 and for longer averaging times (e.g., 6 to 8 h) (Higgins et al., 1990; Avol et al., 1990, 1991; Spektor et al., 1988a, 1991). In general, similar results have been obtained regardless of the averaging time. Attempts to draw conclusions regarding the relative importance of short-term peaks and longer term averages from such analyses have been hampered by the high degree of correlation between 1-h and multihour averages. Until better analytical methods are found for dealing with this problem, comparative results will remain difficult to interpret.

Lung Function in Exercising Subjects

This subsection discusses studies involving lung function measurement immediately before and after a series of discrete outdoor exercise activities in the presence of air pollution. This design is similar in principle to the ambient chamber studies conducted in the early 1980s (see Section 7.3), in which subjects exercised under a specified protocol in a chamber ventilated with ambient air. Here, however, there is typically less control imposed over exercise duration and intensity, and less assessment of achieved $\dot{V}_E$. Compensating to some extent for this diminished control is the relative ease of collecting numerous repeated measurements at varying ambient O_3 levels for the same subjects, improving the precision of concentration-response estimation. In contrast to camp studies, duration of relevant O_3 exposure is assumed to be known, as it is defined by the length of each exercise event.

Results from five exercise studies (Selwyn et al., 1985; Spektor et al., 1988b; Hoek and Brunekreef, 1992; Hoek et al., 1993a; Braun-Fahrlander et al., 1994; Brunekreef

et al., 1994) are summarized in Table 7-17. One of the studies (Selwyn et al., 1985) was discussed in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986) but is reviewed again here because of its apparent consistency with the more recent study of Spektor et al. (1988b).

Certain design variations across studies are worth noting. In the Houston study, each of 24 recreational runners performed spirometry before and after a series of approximately 28 runs on a track from late spring to early fall (Selwyn et al., 1985). Each run was 3 mi long, and each subject attempted to maintain a similar heart rate across all runs. Minute ventilation was not assessed. In the study carried out in Tuxedo, NY, adult runners and walkers were allowed to choose their own exercise level and duration, but again were encouraged to maintain a steady heart rate for the duration of the study (Spektor et al., 1988b). Minute ventilation of each subject while running was estimated by measurement of $\dot{V}_E$ during a treadmill test that achieved a heart rate typical of that subject's experience while running. In the study of 128 Swiss school children (Braun-Fahrlander et al., 1994), 10-min exercise periods on a cycle ergometer were utilized on four to six occasions over a 6-mo period.

In contrast to these studies, early investigation of O_3 effects in The Netherlands involved lower and more variable exercise levels, without any specific attempt to control exercise intensity (Hoek and Brunekreef, 1992; Hoek et al., 1993a). Here, children engaged in sports training and skills development activities that were characterized by the investigators as low to moderate in intensity. Lung function change after exercise was assessed using peak flow meters. Later studies in The Netherlands investigated the effects of heavy exercise levels of variable duration in amateur cyclists, but lung function was evaluated by spirometry (Brunekreef et al., 1994).

Although the designs varied somewhat, O_3 exposure levels were similar in most of the studies: in Houston, 15-min peaks while running varied from 4 to 135 ppb; in Tuxedo, 1-h O_3 levels ranged from 21 to 124 ppb; and in The Netherlands, 1-h maxima on study days ranged from 10 to 120 ppb. The Swiss study observed O_3 levels between 20 and 80 ppb during the exercise period.

The studies in adults (Selwyn et al., 1985; Spektor et al., 1988b; Brunekreef et al., 1994) involving fairly intense exercise yielded statistically significant mean slopes of ΔFEV_1 (i.e., FEV_1 after exercise minus FEV_1 before exercise) regressed on O_3 levels measured during exercise, whereas the studies in children did not. The mean slope observed in the Tuxedo study across all subjects was -1.35 mL/ppb ($\pm$ 0.35), but was reduced to -0.55 mL/ppb ($\pm$ 0.45) in the group of 10 runners who achieved the highest $\dot{V}_E$ values ($>$100 L/min) during exercise. The mean slope reported from the Houston study was similar to the latter number, -0.4 mL/ppb ($\pm$ 0.16). The large effect level observed in the Tuxedo study led Spektor et al. (1988b) to speculate that O_3 effects may have been potentiated by other pollutants such as acid aerosols; however, this phenomenon was not demonstrated analytically from the available acid monitoring data. In the Houston study, the O_3 effect became small and nonsignificant when temperature and RH were added to the model. Effects of temperature and O_3 on lung function were highly correlated and hard to separate in the Dutch amateur cyclists (Brunekreef et al., 1994), although adjustment for humidity did not change the findings. Given the available knowledge base on the independent effects of O_3 and temperature on lung function, it seems reasonable to interpret the results from these studies as demonstrating acute effects of low concentrations of ambient O_3 on lung function with moderate to heavy exercise. The predominantly negative findings

Table 7-17. Acute Effects of Photochemical Oxidant Pollution: Lung Function in Exercising Subjects[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
1-h O_3 concentration ranged from 21 to 124 ppb, max THI = 78 °C; max acidic aerosol (as H_2SO_4) = 9 $\mu g/m^3$ during study. SO_2, NO_x, PM_{15}, $PM_{2.5}$, $SO_4^{=}$, NO_3, NH_4^+, temperature, and RH measured but not reported.	Effects of O_3 on respiratory function and symptoms examined in 30 nonsmoking adults (2 of 10 non-Caucasian females) exercising almost daily outdoors (Tuxedo, NY) for 15 to 55 min (average ca. 30 min) from July to early August 1985. Pre- and postexercise lung function measured, and questionnaire answered postexercise. Pulse rate, calibrated to $\dot{V}_E$ indoors, taken postexercise. Exercise regimen self-selected but constant for each subject over the course of study. Dosimetry estimated and linear regressions done for pulmonary function changes vs. (1) mean O_3 concentration during exercise and (2) inhaled O_3 dose. Persistence of effects tested by linear regressions of before-exercise lung function on previous-day O_3 during exercise. Subjects screened only by questionnaire; two with previous history of asthma but asymptomatic.	Significant ($p < 0.01$) decrements in FVC, FEV_1, PEFR, $FEF_{25-75\%}$, and FEV_1/FVC associated with O_3. For example, the mean slope of ΔFEV_1 on O_3 across all subjects was −1.35 mL/ppb (±0.35). No persistence of effects seen. No symptoms reported by subjects. Mean decrements showed unexpected inverse relationship with calculated $\dot{V}_E$ levels, as indicated by regressing pulmonary function changes and postexercise function against inhaled O_3 during exercise. $\dot{V}_E$ ranges given, but not group or subset means. Subjects not screened for atopy. Exercise done in Sterling Forest, wooded research park, on paved roads or trails.	Spektor et al. (1988b)
15-min peak O_3 measured during runs averaged 47 ppb (range: 4 to 135 ppb). Ambient T averaged 29.4 °C (range: 18.0 to 37.8 °C). RH averaged 62.6% (range: 37.0 to 88.0%). Levels of other pollutants were low, median values were SO_2, 3 ppb; NO_2, 6 ppb; FP, 10 $\mu g/m^3$. Median of subject-specific correlations of O_3 and RH correlated was -0.42.	Effects of O_3 on lung-function change during running outdoors were examined in 24 conditioned, recreational runners (6 women, 18 men, ages 29-47) at a track 30 mi southeast of Houston, TX, from May to October, 1981. All runs were 3 mi in length, and each subject performed at a near-constant heart rate for the duration of the study. An average of 28 runs completed by each subject during the study. Spirometry carried out before and after each run, with analysis of FVC, FEV_1, $FEF_{25-75\%}$, and $FEF_{0.2-1.2L}$. Change in each lung function variable was regressed, for each subject, on 15-min maximum O_3 measured during the run. The mean slope across subjects was tested for significance. The regression was repeated with temperature and RH in the model.	Mean slope of FEV_1 on O_3 alone was −0.4 mL/ppb ($p = 0.03$). In regressions that included temperature and RH, the O_3 slope dropped to −0.07 (not significant). Although temperature reached high levels during the study, a substantial direct effect of temperature or RH on lung function, relative to that of O_3, seems unlikely. A possible potentiating role of high temperature and RH on $\dot{V}_E$, and corresponding O_3 dose, cannot be ruled out. Lung function effect observed in simple O_3 model seem likely to be a valid reflection of O_3 effects under varying environmental conditions.	Selwyn et al. (1985)

Table 7-17 (cont'd). Acute Effects of Photochemical Oxidant Pollution: Lung Function in Exercising Subjects[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
1-h maximum O_3 concentrations during study ranged from 50 to 240 $\mu g/m^3$ (25 to 120 ppb). The highest 4-h average $PM_{2.5}$ level was 70 $\mu g/m^3$, the highest 4-h average sulfate concentration was 21 $\mu g/m^3$, the highest 24-h average NO_2 concentration was 51 $\mu g/m^3$. Temperature data were collected but levels were not reported.	The relationship between lung function change and O_3 exposures during outdoor exercise examined in a population of 83 children (43 girls, 40 boys; ages not given) in Wageningen, The Netherlands. Study covered the period from late May to mid-July, 1989. Lung function assessed using hand-held peak-flow meters before and after various outdoor, sports-training exercises lasting approximately 1 h. Change in PEFR regressed on O_3, $O_3 \times$ exercise duration, and temperature for each subject; and distribution of slopes were examined. Postexercise PEFR analyzed in relation to same and previous day 1-h O_3 maximum, and temperature. Analyses repeated in subsets of subjects with varying levels of correlation between O_3 and temperature during their series of exercise events.	For 55 children with at least four sets of before and after exercise peak flow measurements, the mean slope of the PEFR change on O_3 during exercise was 0.035 ($\pm$0.030) mL/s/$\mu g/m^3$. For 65 subjects with at least four postexercise measurements, the mean slope of PEFR on previous-hour O_3 was 0.080 ($\pm$0.023), which is statistically significant, but in the nonplausible direction. Adjustment for temperature resulted in negative mean slopes, but these are difficult to interpret because of the high statistical correlation between same-day O_3 and temperature ($r = 0.86$). Exercise events were of low intensity as compared with chamber studies and with the Tuxedo runners study (Spektor et al., 1988b). Significant exposures may have occurred prior to the exercise period. H^+ levels were low (<5 $\mu g/m^3$) as measured simultaneously at three other nonurban sites in The Netherlands. The possibility of a physical effect of temperature on mini-Wright peak flow meter measurements was noted by authors.	Hoek and Brunekreef (1992) Hoek et al. (1993a)
1-h maximum O_3 concentrations during the exercise period ranged from 0.02 to 0.08 ppm. The highest pollutant levels measured during the study period were 0.13 ppm for 1-h mean O_3 and 70 $\mu g/m^3$ for the mean NO_2. No measurements of particulates were available.	The acute effects of ambient O_3 on lung function were examined in 128 Swiss children, aged 9 to 11 years, after 10 min of outdoor exercise on a cycle ergometer (60 W). Study covered the period from May through October 1989. Changes in lung function were regressed on current O_3 concentration, with or without adjustment for temperature, RH, and other factors.	Elevated O_3 levels were significantly associated with decreased peak flows (PEFR), but not FVC or FEV_1, after exercise. The average adjusted regression slope for PEFR was -1.14 mL/s/ppm. This corresponds to an average decrease in PEFR of -7.8 and -11.7 at 0.08 and 0.12 ppm O_3, respectively. The significant association for PEFR, but not FVC or FEV_1, is not consistent with other studies. The low O_3 levels and short exercise period raise a question of plausibility regarding the results.	Braun-Fahrlander et al. (1994)

Table 7-17 (cont'd). Acute Effects of Photochemical Oxidant Pollution: Lung Function in Exercising Subjects[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
During exercise, the maximum hourly O_3 concentration averaged 87 $\mu g/m^3$ (0.04 ppm), with a range of 26 to 195 $\mu g/m^3$ (0.01 to 0.10 ppm). Temperature averaged 17.9 °C, with a range of 7.1 to 30.2 °C. NO_2 and SO_2 concentrations were low; 24-h averages were 26.0 and 7.5 $\mu g/m^3$, respectively. No measurements of PM_{10} were made.	The relationship between lung-function change and O_3 exposure was investigated in 23 amateur cyclists, 18 to 37 years of age, during training sessions and races between June 4 and August 18, 1981, in The Netherlands. Lung function was measured with spirometry 30 min before and between 10 and 60 min after cycling in rural locations. Acute respiratory symptoms were recorded in a diary before and after exercise. The difference between pre- and post-exercise lung function was regressed on the mean O_3 concentration during exercise. Time trend, pollen, ambient temperature, and absolute humidity were taken into account as potential confounders. Regression slopes were pooled, and mean and median slopes were calculated. The effect of O_3 during exercise on mean symptom scores was determined by a logistic regression model; all coefficients were converted to estimated odds ratios.	Lung function was negatively related to O_3 concentration during exercise; effects were stronger in midsummer than in the late summer. Mean regression coefficients were -1.16 ± 0.33, -0.52 ± 0.26, -2.96 ± 1.06, and 0.44 ± 0.46 mL/s/$\mu g/m^3$ for FVC, FEV_1, PEF, and $FEF_{25-75\%}$, respectively. For all but $FEF_{25-75\%}$, the mean coefficients were significantly different from zero. Adjustments for air humidity resulted in slightly more negative coefficients for FEV_1 and PEF. Acute respiratory symptoms of shortness of breath, chest tightness, and wheeze were positively related to O_3.	Brunekreef et al. (1994)

[a]See Appendix A for abbreviations and acronyms.

of the studies in children are more difficult to interpret, but may be related to the low exercise intensities achieved, low exposures, and, perhaps, associated O_3 tolerance that occurred prior to the exercise period under study or to some subtle effect of confounders on the peak flow measurements.

Lung Function in Daily Life Studies

This set of studies is characterized by the assessment of lung function, respiratory symptoms, and environmental factor associations in the course of people's daily lives. This section discusses only the lung function data from these studies. For logistical reasons, studies of this kind usually have involved either spirometry conducted at regular intervals (every 1 to 3 weeks) in schools (Kinney et al., 1989; Castillejos et al., 1992; Hoek et al., 1993b) or self-administered peak flow measurements in subjects of various ages over various periods (Vedal et al., 1987; Krzyzanowski et al., 1989). Although daily life studies have the worthwhile goal of characterizing air pollution effects on respiratory health in the real world, they suffer from significant exposure assessment uncertainties owing to the use of outdoor O_3 monitoring, the incomplete and variable penetration of O_3 indoors, and the preponderance of time spent indoors by study subjects. This problem is probably less severe for the studies involving schoolchildren, who often spend substantial time outdoors after school, when O_3 levels may be elevated. Indeed, three of the school-based studies have found statistically significant associations between lung function and previous-day O_3 levels (Castillejos et al., 1992; Kinney et al., 1989; Hoek et al., 1993b). Another difficulty in interpreting the results of these studies is the possible role of seasonal factors (e.g., pollens, epidemics of respiratory infection, changes in activity patterns) as potential confounders of the analyses.

In addition to these general limitations inherent in the study design, several of the studies summarized in Table 7-18 have other problems that limit their utility for assessing O_3 effects on lung function. The study of Vedal et al. (1987), although well conducted, took place from September through May, a period when O_3 levels generally are low, and other potential respiratory insults may dominate. The statistical significance of results from a study carried out in Tucson, AZ, is difficult to interpret because of the multiple statistical tests performed (Krzyzanowski et al., 1989).

The remaining studies, although subject to the general criticisms noted previously, provide suggestive evidence that ambient O_3 may play a role in short-term lung function declines among children engaged in their normal daily routines (Kinney et al., 1989; Castillejos et al., 1992; Hoek et al., 1993b). The Mexico City study of Castillejos et al. (1992) is especially noteworthy because of the novel observation of FEV_1 and $FEF_{25-75\%}$ decrements that were strongly related to O_3 levels averaged over 24 to 168 h previous to spirometry, but not to previous-hour O_3 levels. The strength of these associations (measured by the ratio of the regression slope to its standard error) increased steadily as averaging time increased. Ozone levels observed throughout this 6-mo study were high by U.S. standards; 1-h average O_3 concentrations in the hour preceding lung function measurements ranged from 14 to 287 ppb, with a mean of 99 ppb. The authors suggested these results may reflect an inflammatory response in the airways rather than the well-known acute physiological response. However, further studies will be necessary to test this hypothesis.

Panel Studies of Symptom Prevalence

Many field and epidemiological studies reviewed both in the last criteria document (U.S. Environmental Protection Agency, 1986) and in the previous section of this document

Table 7-18. Acute Effects of Photochemical Oxidant Pollution: Daily Life Studies of Lung Function and Respiratory Symptoms[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
Max O_3 (1-h) concentrations ranged from 3 to 63 ppb. Other ambient pollutants measured were NO_2, TSP, IP, RSP, FP, SO_2, and FP $SO_4^=$.	Lung function measured by spirometry for 154 children ages 10-12 years (90 males, 64 females) in Kingston and Harriman, TN. Spirometry done between 10 a.m. and 1 p.m. on up to 6 days at least 1 week apart during February to April 1981. Child-specific linear regression models of FVC, $FEV_{0.75}$, MMEF, and $\dot{V}_{max75\%}$ fit on 1-h O_3 max and 24-h FP and FP $SO_4^=$. Means ± SD of distributions of estimated child-specific slopes computed and tested for significance by *t*-test.	Significantly negative mean slopes on O_3 for all lung function variables. For example, mean slope of $FEV_{0.75}$ on O_3 was −0.99 mL/ppb (±0.36). Among regressions on FP and FP $SO_4^=$, only one statistically significant mean slope (i.e., positive mean slope of MMEF on FP). Results insensitive to outlier audits and inconclusive for sensitivity variation. Association between fitted slopes and individual characteristics not significant. Low O_3 levels raise plausibility questions.	Kinney (1986)[b] Kinney et al. (1989)[b]
1-h average O_3 concentrations in hour preceding spirometry ranged from 14 to 287 ppb, with mean of 99 ppb. No other pollutants measured. Temperature ranged from 3.9 to 27.8 °C. RH ranged from 18.9 to 92.3%.	Effects of O_3 on lung function examined during regular school hours in a group of 148 children (65 girls, 83 boys; ages 7-9) from three schools in Mexico City. Spirometry and symptom data (cough/phlegm) collected between 0800 and 1400 hours every 2 weeks over the period January through June 1988. To account for lung growth over the study period, residuals from lung function prediction equations were used in analyses. Analyses limited to 143 subjects with at least seven valid measurements. Schools were not air conditioned, and windows were usually open. Schools and subject residences all within 5 km of O_3 monitoring site. Associations between O_3 and lung function (FVC, FEV_1, and $FEF_{25-75\%}$) examined by computing the weighted mean of subject-specific regression slopes relating these variables. Various O_3 averaging times (from 1 h to 168 h) were tested. After analyzing population as a whole, regressions were repeated in subject subsets defined by sex, report of chronic symptoms, and maternal smoking. Overall regressions repeated with temperature and RH in model with O_3.	Only FVC had a statistically significant negative mean slope in relation to previous-hour O_3 concentration (−0.059 ± 0.23 mL/ppb). This slope is approximately one order of magnitude lower than those observed in some camp studies. Both FEV_1 and $FEF_{25-75\%}$ had significant negative associations with O_3 averaged over the previous 24, 48, and 168 h. For example, the mean slope of FEV_1 on 48-h average O_3 was −0.592 ± 0.109 mL/ppb. The authors speculated that the FVC result reflects the acute, reversible effects of O_3 on one's ability to take a deep breath, whereas the FEV_1 and $FEF_{25-75\%}$ observations may reflect inflammatory effects of more prolonged O_3 exposures. It should be noted that both FVC and FEV_1 had significant negative slopes on 1-h maximum O_3 measured in the previous 24 h. Adjustment for temperature and RH diminished somewhat the associations between lung function and O_3. Associations between lung function decrements and O_3 exposure often appeared larger in children with chronic respiratory symptoms than in those without, and in children of mothers who were current smokers; however, these results were not statistically confirmed.	Castillejos et al. (1992)

Table 7-18 (cont'd). Acute Effects of Photochemical Oxidant Pollution: Daily Life Studies of Lung Function and Respiratory Symptoms[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
Daily maximum 1-h O_3 concentrations on days prior to lung-function testing ranged from 7 to 206 $\mu g/m^3$ (3.5 to 103 ppb). Levels of other pollutants measured (SO_2, NO_2, PM_{10}, and aerosol H^+) were reported to be low during study. Ambient temperature (range: 5 to 31 °C) and some pollen data also were collected.	Associations between morning lung function and previous day O_3 examined during school in 533 children (ages 7-11) from seven schools in three towns in The Netherlands. Towns were selected without local pollution sources and with low levels of pollutants other than O_3. Study spanned the period from March through July, with lung-function measurements collected every 2-3 weeks. An overall time trend was fit to the lung-function data to account for lung growth. Data on FVC, FEV_1, PEFR, and $FEF_{25-75\%}$ analyzed in relation to previous-day 1-h maximum O_3 concentrations using subject-specific linear regressions followed by analysis of mean slopes. Intersubject variations in responsiveness to O_3 were tested via an F-test. The influence of chronic respiratory symptoms and other subject characteristics (e.g., age, sex) on O_3 responsiveness was examined. Models that included other pollutants were also considered.	Negative, usually statistically significant mean slopes seen for lung function regressed on previous-day 1-h maximum O_3 for the seven individual schools. Over all 533 subjects, mean regression slopes for FVC and FEV_1 were -0.20 ± 0.05 and -0.21 ± 0.04 $mL/\mu g/m^3$, respectively; and for PEFR and $FEF_{25-75\%}$ were -0.72 ± 0.22 and -0.45 ± 0.12 $mL/s/\mu g/m^3$, respectively. These coefficients may be doubled to convert to slopes in terms of parts per billion. The authors report that adding SO_2, NO_2, or PM_{10} did not materially change the O_3 slopes. There was evidence for inter-subject variation in O_3 responsiveness, but this variation was not statistically related to available subject characteristics data. Temperature data not included in models, perhaps due to high correlation with O_3. The lung function/O_3 relationships noted above are qualitatively similar to those reported in the 1988 Fairview Lake camp study and the Mexico City school children's study.	Hoek and Brunekreef (1992) Hoek et al. (1993b)
1-h maximum O_3 concentrations on PEFR measurement days ranged from 20 to 103 ppb. No other pollutants assessed, but ambient temperature data included.	Relationship between daily peak flow measurements and ambient O_3 concentrations in a population sample of 732 subjects (both adults and children) over 2-week periods during normal daily activities in Tucson, AZ. Peak flow assessed using hand-held peak-flow meters up to four times per day. PEFR measurements on initial 2 days for each subject dropped to avoid possible learning effects, leaving a series of up to 12 measurement days per subject. Population-pooled regression slopes computed for PEFR on 1- and 8-h average O_3 for children (ages ≤ 15) and adults (ages > 15), controlling for residual auto-correlations. Outcome measures included PEFR diurnal variability and afternoon PEFR levels. Besides O_3, potential explanatory variables included temperature, average time outdoors, acute respiratory infections, asthma, and environmental tobacco smoke exposure.	Significant positive associations observed between O_3 concentrations and PEFR diurnal variability; the effect magnitude was greatest in asthmatic subjects. In children only, noon PEFR was suppressed on days with higher O_3 levels. The uncertain relationship between central site O_3 levels and personal exposures in this southwestern community was not addressed. Although the statistical models employed were appropriate and well chosen, it appears that a substantial amount of exploratory data analysis was performed prior to selection of results to present in the paper, leading to uncertainties regarding the statistical validity of the hypothesis tests presented.	Krzyzanowski et al. (1989)

Table 7-18 (cont'd). Acute Effects of Photochemical Oxidant Pollution: Daily Life Studies of Lung Function and Respiratory Symptoms[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
Means and range of max daily 1-h values: O_3 mean = 32.4 $\mu g/m^3$, range = 0-129 $\mu g/m^3$; SO_2 mean = 51.2 $\mu g/m^3$, range = 18-176 $\mu g/m^3$; NO_2 mean = 40.5 $\mu g/m^3$, range = 12-79 $\mu g/m^3$; CoH mean = 0.38 CoH units, range = 0.1-1.3 CoH units; temperature mean = 1.3 °C, range = −22° to +22 °C.	Follow-up study (September 1980 through April 1981) of pollutant-respiratory symptom relationships in subsets of children from 1979 Chestnut Ridge cross-sectional study of more than 4,000 elementary school children. Subsamples selected from six schools in study area with consistently higher levels of air pollution during previous 4 years. Subsamples (three) stratified by reported symptoms. One or more of following measures taken for 144 children: diaries, symptom questionnaire, spirometry. Telephone follow-up each 2 weeks on diaries, spirometry done at school, pollutants (including O_3) measured at one monitor (data from 17 monitors for SO_2 generally reflected in data at single monitor). Diary panel study covered 8 mo; successive PEFR spirometry studies of 9 weeks each done in respective groups of the three subsamples.	Relationships of maximum hourly SO_2, NO_2, O_3, and CoH and minimum temperature for each 24-h period to daily upper and lower respiratory illness, wheeze, and PEFR were evaluated using multiple regression models adjusted for illness occurrence or levels of PEFR on preceding day. No air pollutant was strongly associated with respiratory illness or with PEFR. Authors concluded that this study can best be interpreted as showing no acute effects of studied pollutants on respiratory symptoms or PEFR in children at levels lower than the current NAAQS, but also noted that conclusion must be tempered by relatively low levels of pollutants encountered and possibility of exposure misclassification.	Vedal et al. (1987)

[a]See Appendix A for abbreviations and acronyms.
[b]Cited in U.S. Environmental Protection Agency (1992).

reported results that indicated associations between ambient oxidant exposures and various measures of respiratory effects (e.g., irritative respiratory symptoms and acute pulmonary function decrements) in children and adults. The aggregation of individual studies provides reasonably good evidence for an association between ambient photochemical oxidants and acute respiratory effects and a database that is generally coherent, consistent, and biologically plausible. In addition, other studies of irritative symptoms in children and adults also were reported in the 1986 document. For example, Hammer et al. (1974) reported qualitative associations between ambient oxidant levels and symptoms such as eye and throat irritation, chest discomfort, cough, and headache at total oxidant levels greater than 0.15 ppm in young adults (nursing students). Wayne et al. (1967) reported a high correlation ($R^2 = 0.89$) between ambient total oxidant levels (1 h prior to competition) and impaired exercise performance (running time) in high school students during cross-country track meets in Los Angeles, CA. Symptoms were not measured, but Wayne speculated that chest discomfort from oxidant inhalation impaired exercise performance. Although results such as these are consistent with evidence from controlled human exposure studies, precise characterization of ambient pollutants and environmental conditions and rigorous statistical analyses were lacking in the studies. Thus, the primarily qualitative data from these and other studies were not satisfactory to provide quantitative conclusions about the relationship of ambient O_3 concentrations and acute respiratory illness.

Schwartz (1992), Schwartz and Zeger (1990), and Schwartz et al. (1988) reanalyzed the original diary data of student nurses reported earlier by Hammer et al. (1974) (Table 7-19). The nurses were told that the diaries were part of a prospective study of viral infections. Logistic regression models including time series analyses were used to control for autocorrelation effects that are frequently present in time series data. The reanalysis for daily prevalence rates of symptoms (Schwartz et al., 1988) confirmed that ambient oxidants were significantly associated with cough and eye discomfort. However, earlier reported associations between oxidants and headache or chest discomfort were not confirmed. Cough was the one symptom that showed an apparent threshold near 0.20 ppm total oxidants, which approximates the threshold value reported by Hammer et al. (1974). Further reanalysis of the diary data (Hammer et al., 1974) by Schwartz (1992) and by Schwartz and Zeger (1990) for the effects of air pollutants on the risk of new episodes of respiratory and other symptoms and on their durations revealed interesting findings. The mean plus or minus standard deviation (SD) level of oxidants was 0.102 ± 0.074 ppm. In logistic regression models, an increase in oxidant concentration by one SD (0.074 ppm) was associated with a 17% increased risk of chest discomfort and a 20% increased risk of eye irritation. These associations were highly significant ($p < 0.001$). In addition, photochemical oxidants were significantly ($p < 0.0001$) associated with the duration of episodes of cough, phlegm, and sore throat.

Krupnick et al. (1990) and Ostro et al. (1993) reanalyzed daily health data from over 5,000 children and adults living in the Los Angeles area during a 6-mo period (September 1978 to March 1979) (Table 7-19). The original study was reported by Flesh et al. (1982). The presence or absence of daily respiratory symptoms associated with daily exposure to ambient O_3 and other air pollutants was analyzed in a pooled, cross-sectional, time-series model. Krupnick et al. (1990) reported statistically significant effects of O_3 levels on daily reported respiratory symptoms in healthy nonsmoking adults, but not among smokers, children, and patients with chronic respiratory disease. Ostro et al. (1993) evaluated the daily reports of 321 nonsmoking adults and, using a logistic regression model,

Table 7-19. Acute Effects of Photochemical Oxidant Pollution: Symptom Prevalence[a]

Pollutants and Environmental Variables	Study Description	Results and Comments	Reference
Total oxidant, CO, SO_2, NO, and NO_2 measured; total oxidant concentrations reached episodic levels (maximum 1-h/day <0.4 to 0.5 ppm); mean daily temperature was 71.8 °C.	Reanalysis of daily diary study of student nurses working and living at schools in Los Angeles (see U.S. Environmental Protection Agency, 1986, for details of Hammer et al., 1974). This series of papers reexamines the nurses' data using logistic regression models and time-series methods to account for serial correlation (autocorrelation) of symptoms on successive days. The effects of total oxidants on daily prevalence rates of symptoms, risks of developing new symptoms (episodes), and duration of episodes were analyzed.	Associations found between total oxidants and prevalence of cough and eye irritation, confirming part of findings of original study. Association with cough only at oxidant concentrations above approximately 0.20 ppm. Previously reported associations between oxidants and chest discomfort and headache (Hammer et al., 1974) not confirmed. Oxidants associated with increased risk (incidence) of chest discomfort and eye irritation and duration of episodes of cough, phlegm, and sore throat. Duration of symptoms showed concentration-response relationships even below 0.12 ppm. Findings suggest different effects of oxidants on symptom characteristics. Lack of daily particulate measurements, small number of subjects, and heterogeneous individual responses restrict quantitative interpretation of results. Lung function was not measured.	Schwartz (1992) Schwartz and Zeger (1990) Schwartz et al. (1988)
Daily O_3, NO_2, SO_2, and CoH and every sixth-day sulfates measured at one site (Azusa). 1-h daily maximum O_3 0.1 ppm, 7-h average O_3 0.07 ppm; sulfates 8.43 $\mu g/m^3$; maximum temperature was 22.4 °C.	Reanalysis of daily diaries completed during 181-day survey period (September 1978 to March 1979) by 756 children and 572 adults (Krupnick et al., 1990) and 321 nonsmoking adults (Ostro et al., 1993) living in Glendora, Covina, or Azusa, CA (see Flesh et al., 1982 for details). Presence or absence of 19 (upper and lower) respiratory and two nonrespiratory symptoms recorded daily. Presence or absence of symptoms analyzed in a pooled cross-sectional time-series model. Nonpollution factors, including sex, gas stove use, day of study, and a chronic disease indicator were included in final regression models used to measure effects of ambient air pollution. Logistic regression analyses for entire sample to determine effect of each pollutant on health endpoints. Lagged effects of each pollutant and effects in individuals (n = 74) without air conditioners and those with preexisting respiratory infection were analyzed.	Logistic regression model indicated significant associations between incidence of lower respiratory symptoms and healthy nonsmoking adults (but not among smokers, children, or patients with chronic respiratory disease); 1-h daily maximum O_3 levels (OR = 1.22, 95% CI of 1.11-1.34, for a 0.1 ppm change), 7-h average O_3 level (OR = 1.32, 95% CI of 1.14-1.52), and ambient sulfates (OR = 1.30, 95% CI of 1.09-1.54, for a 10 $\mu g/m^3$ change). CoH was significantly related to daily symptoms in children. Gas stove in the home was associated with lower respiratory tract symptoms (OR = 1.23, 95% CI of 1.03-1.47), as were the effects of O_3 in subgroups without residential air conditioner (OR = 1.24) and with preexisting respiratory infection (OR = 1.24). All the above increased risks were statistically significant ($p < 0.05$). Interpretation of results limited by selection of sample; undersampling of young adults; aggregation of symptoms of all severity levels into one measure; possible reporting bias; and absence of indoor exposure, aeroallergen, and lung function data.	Krupnick et al. (1990) Ostro et al. (1993)

[a]See Appendix A for abbreviations and acronyms.

found a statistically significant association between the incidence of lower respiratory tract symptoms and 1-h daily maximum and 7-h average O_3 levels (22 and 32% increased risk, respectively, with 0.1-ppm increase in O_3) and ambient sulfates (30% increased risk with a 10-$\mu g/m^3$ change). The lower respiratory tract effects of O_3 were greater in the subgroups with gas stoves, without residential air conditioners, and with preexisting respiratory infection. Interpretation of the results is limited by the selection of the sample for analysis; undersampling of young adults; aggregation of symptoms of all severity levels into one measure; possible reporting bias; and absence of indoor exposure, outdoor aeroallergen, and lung function data.

The results from the above panel studies suggest a modest but biologically plausible relationship between short-term exposure to ambient oxidants/O_3 and respiratory symptoms. The interpretation of these recent reanalyses is limited by several factors. Heterogeneous individual responses occur, and analyses of grouped data possibly may miss susceptible subgroups. The lack of specific measurements of O_3 and other pollutants (especially particles) and of personal exposure or risk variables (e.g., time-activity data) weaken the assessment of confounders and effect modifiers. In addition, the overall data analysis pertains to small and very selected samples that have uncertain representativeness to the general population.

Aggravation of Existing Respiratory Diseases

Prior epidemiological data on the effects of ambient O_3 levels in subjects with existing respiratory disease have been difficult to interpret due to methodological limitations (U.S. Environmental Protection Agency, 1986). Exacerbation of asthma and other health endpoints subsequently has been evaluated, and more recent studies have observed possible increases in symptom aggravation or changes in lung function of asthmatic subjects in relation to increased O_3 or total oxidant levels, as well as interactions between O_3 concentrations and temperature. However, no consistent pattern of findings for aggravation of symptoms or lung function changes has been reported for patients with other types of chronic lung disease. Some of the major issues in interpreting results from studies of respiratory exacerbations have been inadequate sample size and characterization of the study subjects, lack of information on the possible effects of medications, the absence of records for all days on which symptoms could have occurred, inadequate interpretation of the clinical significance of measured changes, the role of confounders and effect modifiers (e.g., temperature, humidity, particles, aeroallergens), and personal or group characterization of indoor-outdoor exposures. For example, Whittemore and Korn (1980) and Holguin et al. (1985) found small increases in the probability of asthma attacks associated with previous attacks, decreased temperature, and incremental increases in oxidant and O_3 concentrations. Lebowitz et al. (1982, 1983, 1985) and Lebowitz (1984) reported effects in asthmatics, such as decreased PEFR and increased respiratory symptoms, that were related to the interaction of O_3 and temperature. None of these studies adequately assessed possible effect modification by other pollutants, particularly inhalable particles, which may have independent effects.

Epidemiological studies published since the 1986 criteria document (U.S. Environmental Protection Agency, 1986) have attempted to control for many methodological issues (e.g., with [1] better estimates of exposure to pollutants [as well as O_3] and environmental variables that can confound or modify responses, [2] serial measurements of pulmonary function for determining correlations with pollutants and other environmental

variables, [3] better biomedical characterization of cohorts, and [4] more robust analytical approaches that control for autocorrelation of environmental variables and health responses). Recent studies generally have provided further evidence that supports a relationship between ambient O_3/oxidant concentrations and respiratory morbidity in asthmatic subjects (Table 7-20).

Gong (1987) studied the relationship between air quality and respiratory status of 83 asthmatic subjects living in a high-oxidant area of Los Angeles County. The study covered February to December 1983, but data analyses were limited to a 230-day period (April 15 through November 30) because of staggered entry of subjects into the study and the high frequency of missing or incomplete data encountered in the earlier part of the study period. Regression and correlation analyses between O_3 and average symptom scores, asthma medication index (AMI), and day and night PEFR across subjects showed weak, nonsignificant relationships. These daily outcome variables were compared across days with maximum 1-h-average O_3 in three ranges: <0.12 ppm, 0.12 to 0.19 ppm, and >0.20 ppm; "no statistical or clinical significance was detected." Individual exposures and activity patterns were not estimated in these two analyses. Multiple regression analyses also indicated the lack of a significant overall relationship between O_3 (and their independent variables) and respiratory status, despite the use of lagged variables and the inclusion of other pollutants, meteorological variables, aeroallergens, and AMI. Total suspended particles directly affected PEFR, but the relationship was not consistent in the analysis. Aeroallergens showed significantly negative relationships to respiratory variables, but only the effect of certain molds was considered clinically relevant. Temperature and humidity showed no significant effect on the respiratory variables on this study.

Although there was no significant overall effect of O_3 on respiratory variables in the 83 asthmatic subjects, multiple regression analysis of subjects whose O_3 coefficients on various days were in the top quartile for dependent variables (respiratory measures) showed significant and consistent effects of O_3 on Day t and the previous day (Day $t - 1$). Multiple regression testing of subsets for associations of symptom score or day or night PEFR on the same-day O_3 and previous-day values of the same responses showed highly significant O_3 coefficients for all three respiratory measures.

The clinical significance of responses in symptom scores and day and night peak flow was evaluated for all subjects by individual regression analyses. No subject had evidence of significant worsening of symptoms attributable to O_3 during the study. Adult subjects with high scores in fatigue, hyperventilation, dyspnea, congestion, and rapid breathing in the Asthma Symptom Checklist had more negative slope coefficients for O_3 than subjects with low-to-moderate scores on the checklist. "Responders" (statistically identified by multiple regression analysis) scored consistently higher in the factors representing fatigue, hyperventilation, and rapid breathing. The higher scores of these responders, however, "were apparently not associated with differences in ambient O_3 concentrations since the test scores were similar during relatively low (first test) and high (second test) O_3 days. The significance of the psychological results is unclear at this time" (Gong, 1987).

Lebowitz et al. (1987) performed a time series analysis to evaluate daily respiratory responses to outdoor and indoor air pollutant and aeroallergen exposures in potentially sensitive adults living in a dry climate (Tucson, AZ). Daily symptoms and PEFR were recorded in well-characterized groups of asthmatics, allergic subjects, patients with chronic airways obstruction, and asymptomatic healthy controls (total sample size of 204) over 2 years. Daily diaries included acute symptoms, medication use, and doctors' visits.

Table 7-20. Aggravation of Existing Respiratory Diseases by Photochemical Oxidant Pollution[a]

Pollutants and Environmental Variables	Study Description	Results and Comments	Reference
Air pollutant measurements for April to November 1983 used in statistical analyses. Daily maximum of NO_x, SO_2, CO, THC less than California standards or NAAQS. $SO_4^{=}$ > 25 $\mu g/m^3$ on 4 days; TSP > 100 $\mu g/m^3$ on 78% of days with data. Daily maximum 1-h average O_3 concentrations (from continuous monitoring) = 0.01-0.11 ppm on 103 days, 0.12-0.19 ppm on 65 days, 0.2-0.34 ppm on 60 days, and 0.35-0.38 ppm on 3 days. Outdoor aeroallergens sampled with Roto-Rod: spores, pollens, grasses, molds, miscellaneous debris; all generally low except for group of common molds (rusts, smuts, mushroom) present in thousands per square centimeter on sampler. Mean (±SD) daily temperature at 1 p.m. during 200 days: 26 ± 11 °C, range 13-41 °C; 128 days with ≥ 24 °C.	Effects of pollutants and other environmental variables on respiratory symptoms and PEFR evaluated in 11-mo population study of asthmatics living in high-O_3 area (Glendora) of Los Angeles County, CA. Detailed questionnaires given at outset on medical/occupational histories and personal factors, including general activity patterns; psychological testing (Asthma Symptom Checklist, State-Trait Anxiety Inventory, etc.) also given, once during good air period and once during smoggy period. Lung function (spirometry) and bronchodilator responses measured at outset in all subjects. Daily diaries (checked 2×/week), mini-Wright peak flow meters, and Nebulizer Chronolog attached to metered-dose inhaler used to record symptoms, day and night PEFR, and medication use, respectively. Multiple regression analyses for overall group; then subsets (two groups of "responders") analyzed separately and compared with rest of cohort.	Eight of 91 subjects completing study (of 109 recruited) showed no variability in asthma status during the 230-day study. Respiratory status of final study population (n = 83 with generally mild or stable asthma), as a whole, not related, either clinically or statistically, to maximum 1-h average O_3 on Days t, t-1, t-2, or t-3 for any respiratory variable even when adjusting for medication use, symptoms, and PEFR on Day t-1. Subset analyses showed association of O_3 with symptoms and with day and night PEFR in subjects in top quartile for respiratory measures, but association did not follow a consistent relationship with ambient O_3 concentrations. $\dot{V}_E$ levels during outdoor time not estimated. Outcomes not related to time outdoors vs. indoors or to outdoor time on "clean" vs. "smoggy" days. Subsets ("responders") differed from rest of cohort mainly in scores of Asthma Symptom Checklist for factors representing fatigue, hyperventilation, and rapid breathing, but there was no difference in responders between clean and smoggy periods. Aeroallergens from maple, oak, beech, and elm trees showed significant (and clinically relevant) relationships to respiratory variables. Exposure assessment limited by outdoors-only monitoring and lack of time-activity data.	Gong (1987) Gong et al. (1985)
Hourly outdoor O_3, CO, NO_2, and TSP measured from three stations. Hourly maxima used for O_3 and CO; daily CO and NO_2 derived as weighted measures for each cluster sampling site, and daily values were used. Sample of homes monitored inside and outside for particulates and gases and evaluated for housing characteristics (e.g., gas stove usage). Meteorological variables measured daily. Temperature data not reported.	Effects of outdoor and indoor air pollutants and aeroallergens evaluated in a 2-year study of 22 subjects with asthma, 33 with airway obstructive disease, 30 atopics, and 14 normals living in an arid environment (Tucson, AZ). Subjects part of a community population sample of 117 families (see U.S. Environmental Protection Agency, 1986, for details of Lebowitz et al., 1982, 1983, 1985; Lebowitz, 1984) and had well-characterized symptoms, medication use, lung function, methacholine response (in a subsample), and immunological status. Daily diaries (acute symptoms, medication use, and doctors' visits) and daily PEFR (2×/day) performed for 3 mo, 2-4×/study period. Duration of time spent outdoors recorded. Spectral time series analyses used to evaluate each respiratory response variable for periodic tendencies and covariance (dependent and independent) functions as processes in time in the different groups.	Asthmatics had greatest number of respiratory complaints, which were related to the presence of gas stoves, active smoking, humidity, and temperature. O_3 was associated with peak flow and temperature (late spring), wheeze (Day t-3 with humidity), and productive cough (Day t-2). O_3 (Day t-3) was related to productive cough during the summer in allergic subjects. Outdoor gases and meteorological variables significantly related to symptoms and PEFR, both independently and as effect modifiers. No significant O_3 effect in patients with obstructive disease or in normals. Small number of subjects and study days and lack of indoor NO_2 and PM_{10} measurements, measured pollutant values, and effect estimates limit quantitative interpretation of study.	Lebowitz et al. (1987)

Table 7-20 (cont'd). Aggravation of Existing Respiratory Diseases by Photochemical Oxidant Pollution[a]

Pollutants and Environmental Variables	Study Description	Results and Comments	Reference
Outdoor O_3 levels measured hourly by three stations and maximum 1- and 8-h average values were used to represent O_3 levels for all subjects on a given day. For each day of the study, the mean of the maximum 8-h O_3 average for the 4 preceding days was calculated to be an index of cumulative exposure. PM_{10} was measured daily at one station. Mean ± SD of maximum 1-h O_3 concentrations was 0.055 ± 0.014 ppm (range: 0.015-0.092 ppm), moving average maximum 8-h O_3 levels were 0.046 ± 0.013 ppm (0.09-0.082 ppm). Maximum daily outdoor temperature was 87 °F (30 °C) per person-day, maximum PM_{10} was 187 $\mu g/m^3$ (mean 42 $\mu g/m^3$).	Temporal effect of ambient O_3 concentration on PEFR during 30-mo study period in 287 children (13% physician-diagnosed asthmatics) and 523 nonsmoking adults (9% asthmatics) in the Tucson community population sample. Mini-Wright peak flow meters used four or fewer times per day but only for 2-week periods, and only one meter was assigned/household. Children's tests were supervised by adult, and initial 2 days of observation were eliminated from analysis. Symptoms from daily diaries were also used in analysis. Random-effects longitudinal model was used for analyses to account for autocorrelation of PEFR values. Multifactorial ANCOVA was used to analyze day-to-day changes in daily average PEFR and symptom prevalence rates (the dependent variables) in relation to 8-h O_3 values on the same day and previous days (lags of 0 and 1).	Analyzed PEFR data limited to at least 12 measurements for at least 6 days in 78% of children and 74% of adults. Noon PEFR in nonasthmatic and asthmatic children was lower with higher 1-h maximum O_3 levels: −11.9 L/min/0.1 ppm O_3 ($p < 0.05$) and −31.0 L/min/0.1 ppm O_3 ($p < 0.1$), respectively. Effect of 8-h O_3 mean on evening PEFR seen only in asthmatic children, possibly reflecting a cumulative O_3 response during course of day. Among adults, evening PEFR was decreased in asthmatics who spent more time outdoors on days with higher O_3 concentrations (C × T effect). The ANCOVA model showed significant interactive effects of O_3 × temperature × PM_{10} on daily average PEFR. Daily rates of allergic-irritant symptoms increased with the maximum 8-h O_3 average (>0.056 ppm) on the previous day and increased more with interactions of O_3 × temperature × PM_{10}. Missing PEFR data, possible overestimation of outdoor O_3 exposure, large variability of responses in asthmatics, medication use on days with high O_3 levels, relatively low O_3 levels, and uncertain effects of indoor and outdoor allergens and respiratory infections limit interpretation.	Lebowitz et al. (1991) Krzyzanowski et al. (1992)

Table 7-20 (cont'd). Aggravation of Existing Respiratory Diseases by Photochemical Oxidant Pollution[a]

Pollutants and Environmental Variables	Study Description	Results and Comments	Reference
Hourly O_3, twice daily (9:00 a.m. and 9:00 p.m.) acidic aerosols (sulfates, SO_4, and H^+), and pollen counts were measured on site. Hourly temperature, RH, and O_3 measured from nearby monitors. In 1991, pollution levels increased daily until Day 5, when maximum 1-h O_3 reached 0.154 ppm and daytime H^+ and sulfate levels were 245 nm/m^3 and 26.7 μg/m^3, respectively. In 1992, air quality was better (e.g., the highest daily 1-h maximum O_3 was 0.063 ppm). Temperature data not reported.	Effects of ambient summertime haze air pollution on asthmatic children (ages 7-13) attending 1-week asthma camp in Connecticut River Valley were evaluated during June 1991 (n = 50) and 1992 (n = 55). PEFR and symptoms (2×/day) and number of as-needed (p.r.n.) inhaled bronchodilator treatments given by on-site physician during each study day were recorded. Correlations between health outcomes and air pollutants were performed.	In 1991, daily total number of p.r.n. treatments highly correlated ($r > 0.80$) with maximum O_3, SO_4, daytime H^+, and maximum temperature, but only SO_4 ($r = 0.97$) and H^+ ($r = 0.985$) were significant ($p < 0.05$) and remained so after temperature was included in the analysis. Daily pollen counts were not associated with treatments p.r.n. Afternoon chest symptoms (cough, phlegm, and wheeze) and changes in morning-afternoon PEFR values (excluding children given medication) were significantly correlated ($p < 0.05$) with O_3 and H^+, respectively. Scheduled medications did not apparently provide a protective effect ($X^2 = 3.25$, $p = 0.067$), although the failure to achieve statistical significance is not unexpected given the small sample size. In 1992, change in PEFR (magnitude not reported), chest symptoms, and the fewer daily exacerbations (maximum 27 vs. 37 in 1991) were not significantly correlated with pollution, pollen, or temperature. Only sore throat, runny nose, and eye irritation were correlated with pollen counts. Although the data are only in preliminary form, the 1991 results appear consistent with an effect of summertime haze air pollution on PEFR, chest symptoms, and asthma exacerbations. The 1992 results are consistent with less health effects owing to cleaner ambient conditions. Small number of subjects and study days and lack of results for other pollutants limit the interpretation of the studies.	Thurston et al. (1995)

[a]See Appendix A for abbreviations and acronyms.

A sample of homes was evaluated for environmental characteristics and was monitored indoors and outdoors at the home for gases and particles, in addition to regional stationary outdoor monitors. Asthmatics showed the most respiratory responses. Outdoor O_3 levels were significantly ($p < 0.05$) related to wheeze, productive cough, and peak flow (late spring) in the asthmatic group. Statistical interactions between O_3 and smoking, presence of a gas stove, maximum temperature, and minimum humidity ($R^2 = 0.49$) were found. The other groups did not demonstrate an O_3 effect, except for the atopic group, which had increased summertime productive cough related to O_3 levels. Thus, these results indicate an O_3 effect on asthmatics and that statistical interactions between O_3 and other environmental factors are significantly related to symptoms and peak flow. On the other hand, the results are largely descriptive and qualitative without adequate effect estimators.

A subsequent analysis of the same community population sample in Tucson (Lebowitz et al., 1991; Krzyzanowski et al., 1992) evaluated the temporal relationship between PEFR and ambient O_3 in 287 children and 523 nonsmoking adults. During part of the study period, ambient particles with a MMAD of 10 μm or less (PM_{10}) were collected daily at one monitoring station. A random-effects longitudinal model and multifactorial analysis of covariance were used for analyses. During the study period, the maximum ambient O_3 concentrations were relatively low (i.e., the 1-h maximum never exceeded 0.092 ppm). In children, noon peak flows were decreased on days when there was a high O_3 concentration. Children with physician-confirmed asthma experienced the greatest decrease in noon peak flow. Evening peak flow also was significantly related to O_3 in children, especially asthmatic children, suggesting a cumulative O_3 response during the course of the day. Among adults, evening peak flows were decreased in asthmatics who spent more time outdoors on days when O_3 levels were high. After adjustment for covariates, significant statistical interactions of 8-h O_3 levels with PM_{10} and temperature on daily PEFR were found. There was a significant increase in allergic-irritant symptom rates related to prolonged exposure to O_3 (maximum 8-h average on the previous day and the interactions of O_3, temperature, and humidity). The study had some methodologic problems (e.g., missing daily PEFR data in many subjects, lack of information about specific hours spent outdoors, medication usage, and relatively low O_3 levels during the study period). Nonetheless, the data analyses, control of confounders, and overall exposure assessment strengthen the conclusions of the study: the respiratory response to O_3 is acute, occurs more often in asthmatics, and increases as temperature and PM_{10} increase.

The respiratory effects of ambient O_3 and other coexisting pollutants were evaluated during a 1-week asthma camp in the Connecticut River Valley in June of 1991 and 1992 (Thurston et al., 1995). Each child (age 7 to 13 years) participated in the same daily activities all week. Peak flow and symptoms were recorded twice a day, as well as the number of as-needed (p.r.n.) treatments of inhaled bronchodilator administered by an on-site physician during each day (each representing an exacerbation of asthma). Hourly measurements of O_3 and twice daily samples of acidic aerosols (sulfates [SO_4] and hydrogen ions [H^+]) were collected. The results indicate a strong association between the ambient air pollution mix and the occurrence of asthmatic exacerbations in children. During 1991, pollution levels progressively increased until Day 5, when the 1-h maximum O_3 concentration reached 0.154 ppm, and the daytime (9:00 a.m. to 9:00 p.m.) H^+ and SO_4 concentrations were 254 nm/m^3 and 26.7 μg/m^3, respectively. The correlations of the daily total number of p.r.n. treatments required with daily maximum O_3, daytime SO_4 and H^+, and maximum temperature were all high ($r > 0.8$), but only SO_4 ($r = 0.97$) and

H^+ ($r = 0.98$) were significant ($p < 0.05$) given the small number of days involved. Afternoon symptoms (cough, phlegm, and wheeze) and morning-afternoon change in PEFR (without medication) were significantly correlated ($p < 0.05$) with O_3 and H^+, respectively. During 1992, the local air quality was better (e.g., the daily 1-h maximum O_3 concentration was only 0.063 ppm). There were fewer asthmatic exacerbations (maximum of 27 versus 37 in 1991), and they were not significantly correlated with pollution, pollen, or temperature. Pollutants were not significantly correlated with symptoms or PEFR. Overall, the 1991 data indicate a coherence in the associations of summertime haze air pollution with peak flow, chest symptoms, and asthma exacerbations in children. The lack of correlation during 1992 likely was due to the improved air quality and indirectly supports the results of the previous year. An adequate interpretation of these preliminary results is limited by the small number of subjects and study days and the lack of results for other pollutants. These camp studies remain to be reported in more detail.

The above epidemiological studies have generally supported a direct association between ambient O_3/oxidant concentrations and acute respiratory morbidity in asthmatics. The recent studies have strengthened their conclusions by improvements or new approaches in the estimations of O_3 exposure, confounders, and effect modifiers; characterization of the subjects and serial measurements of their responses; and analytical approaches. Thus, the aggregate results can be viewed as biologically and temporally plausible, consistent, and coherent to some extent; however, some methodological problems persist. The studies share certain deficiencies such as small numbers of subjects (which may reduce statistical power) and the lack of time-activity measurements and significant data about individual responses and their distribution. The independent effect of ambient O_3, as estimated by statistical models in epidemiological studies, is difficult, at best, to clearly differentiate from those of copollutants because O_3 (or another pollutant such as H^+) may be acting only as an indicator of the toxic potency of the ambient mixture of pollutants. This, in combination with measurement error and uncontrolled associations with other factors, complicates analytical findings about the relationships among components of an ambient mixture and may not accurately disentangle the effects of O_3 in a biologically appropriate fashion.

7.4.1.3 Aggregate Population Time Series Studies

Aggregate population, or "ecological", time series studies are epidemiological investigations in which the associations between air pollution and human health outcomes are evaluated over time in the population as a whole (e.g., with respect to deaths per day in a given city) and for which outcomes and exposures are not matched for the individuals within the population. Indeed, aggregate population time series studies of extreme air pollution episodes have provided some of the clearest evidence of the adverse effects of air pollution on humans. For example, during the historic December 1952 London Fog episode, in which extremely high sulfur oxide and PM air pollution levels were experienced, total mortality in Greater London rose from roughly 300 to 900 deaths/day, and acute respiratory hospital admissions rose from 175 to 460/day (United Kingdom Ministry of Health, 1954). At more routine levels of air pollution, any effects of air pollution are necessarily less obvious, and, as shall be discussed below, methodological issues exist as to the proper analysis and interpretation of such aggregate population time series data.

The previous criteria document (U.S. Environmental Protection Agency, 1986) discussed several methodological issues with regard to the epidemiological studies of O_3 and photochemical oxidants available at that time. Limitations identified included interferences

by or interactions with other pollutants and meteorological factors in the ambient environment; lack of comprehensive exposure issue assessments, such as individual activity patterns and evaluation of pollutant monitor appropriateness; difficulty in identifying the responsible oxidant species; and inadequate characterization of the study population. However, most of these criticisms are not relevant to time series studies. For example, because the same population is being followed from day to day, the study population acts as its own control, obviating the need for a detailed population characterization. Also, central site monitoring data can be useful in these studies for two reasons: (1) although O_3 concentrations can vary spatially within an airshed, they usually are highly correlated across sites over time, so that correlational time series studies are not as dependent on detailed exposure assessments as are, for example, cross-sectional studies; and (2) if the ultimate use of these studies is to be included as criteria for ambient standards, the attainment of which is evaluated at central monitoring stations, then these are the data most relevant for analysis. However, the usually high correlation of the 1-h daily maximum O_3 concentration with other averaging times (e.g., an 8-h average daily maximum) inhibits the ability of such time series studies to discriminate the most biologically relevant O_3 averaging time.

Of the concerns raised by the previous criteria document regarding epidemiological studies in general, the most relevant to time-series studies is the potential for other serially correlated environmental factors (e.g., temperature or other pollutants) to confound the unique identification of O_3 as a critical causal factor in any environmental health effects identified via time series analyses of aggregate population data. As discussed by Thurston and Kinney (1995), either upward or downward bias in the O_3 effect estimate can result if the model is misspecified. In the case of underspecification, if another environmental factor that is both serially correlated with O_3 over time and also may be causally related with the effect under consideration (e.g., temperature stress effects on mortality) is excluded from the analysis, then O_3 may "pick up" that environmental factor's effect in the model, biasing the O_3 coefficient upward. Conversely, the inclusion of variables in the model that are correlated with O_3 concentrations but are unlikely to be causally related to the health outcome (e.g., the inverse of wind speed) results in model overspecification, which may bias the O_3 coefficient downward. Only variables that are biologically plausible should be included in a time-series model, and intercorrelations of the model coefficients should be low if model specification bias is to be minimized.

One aspect of evaluating time series epidemiologic studies of the health effects of air pollution that was not raised directly by the previous criteria document but which can be crucial to proper interpretation is the statistical question of how each study has addressed the potentially confounding influences of long-wave (e.g., seasonal) variations in the health outcome data. The seasonality of morbidity and mortality was mentioned explicitly in Hippocrates' treatise on "Airs, Waters, and Places" and has been studied over the years (Hechter and Goldsmith, 1961). In respiratory diseases such as asthma, this seasonality of admissions is very common, due in part to the multifactorial nature of these diseases. For example, spring and fall increases in pollen and winter influenza epidemics superimpose long-wave cycles on the day-to-day variations in respiratory hospital admission rates. Such long-wave cycles need to be addressed as part of any time series analysis for two reasons: (1) they result in strong autocorrelations that violate the underlying assumptions of most statistical approaches used to analyze such data; and (2) their inclusion can lead to misleading conclusions (i.e., confounding), in that the long-wave relationships would likely obscure the acute (i.e., short-wave) effects being evaluated. The need to address seasonal cycles in

respiratory disease time series data in order to avoid spurious long-wave dominated correlations has long been recognized (e.g., Ipsen et al., 1969) but too often has been ignored or inadequately addressed in the published literature. Autocorrelation, although often contributed to by seasonal cycles in the data, can be introduced by other causes as well. For example, Lipfert (1993) noted the need for hospitalization studies to take into account both weekly and seasonal temporal patterns in the data. Thus, an important criterion for the evaluation of aggregate population time series studies of the acute morbidity and mortality effects of O_3 is whether or not the authors have appropriately addressed all long-wave periodicities in the data as part of their analysis.

There are a variety of statistical approaches available to address all long-wave confounding in time series analyses, each having advantages and disadvantages. The primary goal in invoking such procedures is to eliminate the long-wave autocorrelation "noise" in the data without inadvertently removing any O_3-related health effects "signal" at the same time. In particular, steps that address autocorrelation in the model but also remove or explain short-wave variance in the health outcome variable of interest (e.g., by applying prefilters to the series that affect periodicities down to a few days or by analyzing the residuals from prior regressions of the outcome variable on "control" variables that are correlated with O_3, such as temperature) carry with them the risk of also removing short-wave associations of interest before the actual analysis has begun. Furthermore, although there are standard regression diagnostics available to determine whether autocorrelation remains a significant problem (e.g., the Durbin-Watson statistic), no such check exists to determine whether the autocorrelation removal methods also have inadvertently removed an O_3-health effects association of interest. Thus, although steps must be taken in time series analyses to address the potentially large biases resulting from long-wave (e.g., seasonal) autocorrelations, care must be taken not to also remove the signal of interest when dealing with the autocorrelation problem.

Emergency Room Visits and Hospital Admissions

Many investigators have evaluated the associations between hospital emergency room visits or hospital admissions and air pollution. Hospital admissions are far more common (as counts per day) than, for example, mortality, thereby providing greater statistical reliability and avoiding the distributional complications that may be presented by low counts. Also, admission to the hospital is a well defined endpoint, having the desirable feature that every patient must have been seen by a physician and deemed sick enough to require hospitalization. Emergency room (ER) visits provide larger daily counts, but are not necessarily as severe an endpoint. In a well-designed study in Quebec, Canada, hospital admission diagnosis at discharge was found to be very reliable, with the study confirming the classification of respiratory admissions in general 92% of the time, and asthma admissions 95% of the time (Delfino et al., 1993). Similarly, a study by Martinez et al. (1993) of respiratory emergency room admissions in Barcelona, Spain, during 1985 to 1989 concluded that identification of asthma admissions was highly reliable, as was the discrimination of asthma and COPD diagnoses. Daily series of hospital admissions thus represent an especially useful research resource for the investigation of the human health consequences of O_3 exposure.

Hospital admission and ER visit studies that have considered O_3 associations are summarized in Table 7-21. In the previous criteria documents (U.S. Environmental Protection Agency, 1978, 1986), such studies were found to give inconsistent results for

Table 7-21. Hospital Admissions/Visits in Relation to Photochemical Oxidant Pollution: Time Series Studies[a]

Concentration(s) (ppm)	Pollutant	Study Description	Results and Comments	Reference
0.11 to 0.28 avg max 1 h during low and high periods, respectively	Oxidant	Comparison of admissions to Los Angeles County Hospital for respiratory and cardiac conditions during smog and smog-free periods from August to November 1954.	No consistent relationship between admissions and high-smog periods; however, statistical analyses were not reported. Clear seasonal trends in admissions (increasing from summer to winter) not addressed.	California Dept. of Public Health (1955[b], 1956[b], 1957[b])
0.12 avg concentration 6 a.m. - 1 p.m.	Oxidant	Respiratory and cardiovascular admissions to Los Angeles County Hospital for residents living within 8 mi of downtown Los Angeles between August and December 1954.	Inconclusive results; partial correlation coefficients between total oxidants and admissions were variable. Method of patient selection was not given. Other pollutants were not considered. Seasonal trend not addressed.	Brant and Hill (1964)[b] Brant (1965)
(Not reported)	Oxidant	Admissions of Blue Cross patients to Los Angeles hospitals with >100 beds between March and October 1961; daily average concentrations of oxidant, O_3, CO, SO_2, NO_2, NO, and PM by Los Angeles air pollution control districts.	Correlation coefficients between admissions for allergies, eye inflammation, and acute upper and lower respiratory infections and all pollutants were statistically significant; correlations between cardiovascular and other respiratory diseases were significant for oxidant, O_3, and SO_2; significant positive correlations were noted with length of hospital stay for SO_2, NO_2, and NO_x. Correlations were not significant for temperature and RH or for pollutants with other disease categories. Reported seasonal variations in admissions and pollution not addressed.	Sterling et al. (1966[b], 1967[b])
(Not reported)	Oxidant	Admissions for all adults and children with acute respiratory illness in four Hamilton, Ontario hospitals during the 12 mo from July 1, 1970, to June 30, 1971; city-avg pollution monitoring for O_x (KI), SO_2, PM, CoH, CO, NO_x, HC, temperature, wind direction and velocity, RH, and pollen.	Correlation found between admissions and an air pollution index for SO_2 and CoH; negative correlation between temperature and admissions; and nonsignificant negative correlations found with concentrations of O_x (KI). However, clear, long-wave trends (e.g., seasonality) not addressed.	Levy et al. (1977)[c]
(Not reported)	O_3	Emergency room visits for cardiac and respiratory disease in two major hospitals in the city of Chicago from April 1977 to April 1978; 1-h concentrations of O_3, SO_2, NO_2, NO, and CO from an EPA site close to the hospital, 24-h concentrations of TSP, SO_2, and NO_2 from the Chicago Air Sampling Network.	No significant association between admissions for any disease groups and O_3, CO, or TSP; SO_2 and NO accounted for part of the variation of ER visits for respiratory and cardiovascular admissions. However, the analysis has a lack of control for confounding, possible unaddressed seasonality in admissions (time series not shown), and model overspecification (e.g., use of wind speed).	Namekata et al. (1979)[c]
0.07 and 0.39 avg max 1 h during low and high periods, respectively	O_3	ER visits and hospital admissions for children with asthma symptoms during periods of high and low air pollution in Los Angeles from August 1979 to January 1980; daily maximum hourly concentrations of O_3, SO_2, NO, NO_2, HC, and CoH; weekly maximum hourly concentrations of $SO_4^=$ and TSP; biweekly allergens and daily meteorological variables from regional monitoring stations.	Asthma positively correlated with CoH, HC, NO_2, and allergens on same day and negatively correlated with O_3 and SO_2; asthma positively correlated with NO_2 on Days 2 and 3 after exposure; correlations were stronger on Day 2 for most variables; nonsignificant correlation for $SO_4^=$ and TSP. Monthly admissions and pollution data indicate strong seasonality, which is not accounted for. This results in (seasonally driven) positive correlations with CoH, HC, and NO_2 and negative correlations with O_3.	Richards et al. (1981)[c]

Table 7-21 (cont'd). Hospital Admissions/Visits in Relation to Photochemical Oxidant Pollution: Time Series Studies[a]

Concentration(s) (ppm)	Pollutant	Study Description	Results and Comments	Reference
0.03 and 0.11 avg max 1 h for low and high areas, respectively	Oxidant	Daily hospital ER admissions in four Southern California communities during 1974 and 1975. Max hourly average concentrations of oxidant, NO_2, NO, CO, SO_2, CoH; 24-h average concentrations of PM and $SO_4^=$; and daily meteorological conditions from monitoring sites 8 km from the hospitals.	Admissions significantly associated with oxidant and temperature in all locations. Long-term trends and day-of-week effects appropriately controlled, but not seasonality. Path-analysis-guided regression used to discriminate among the intercorrelated pollutant and meteorological factors, indicating O_3 to be most important only at the highest O_3 site. However, lack of catchment area population figures and inadequate seasonality adjustments prevent quantitative use of results.	Goldsmith et al. (1983)[c]
0.03 to 0.12 avg of max 1-h/day for 15 stations	O_3	Admissions to 79 acute-care hospitals in Southern Ontario for the months of January, February, July, and August in 1974 and 1976 to 1983. Hourly average concentrations of particulate (CoH), O_3, SO_2, NO_2, and daily temperature from 15 air sampling stations within the region.	Excess respiratory admissions most strongly associated ($p \leq 0.001$) with O_3, sulfate, and temperature during July and August with 24- and 48-h lag. No such associations exist for nonrespiratory (control) diseases. Seasonality minimized by selection of narrow study period, and day-of-week effects controlled. $SO_4^=$ and O_3 highly intercorrelated ($r = 0.65$), making effect discrimination difficult. A lack of independent regression coefficients prevents quantitative application of results.	Bates and Sizto (1983, 1987, 1989) Bates (1985)
0.025 to 0.075 3-mo avg of monthly means from all city sites	O_3	Analysis of quarterly hospital admission rates for childhood asthma in Hong Kong during 1983-1987 ($n = 19$). Quarterly means of SO_2, NO_2, NO, O_3, TSP, and RSP considered.	Concludes that asthma is negatively correlated with SO_2, but not with O_3. However, analysis uses quarterly means and lacks seasonality controls.	Tseng and Li (1990)
0.001 to 0.085 mean 1-h max O_3 (avg of 11 sites)	O_3	Analysis of emergency room visits, by cause, to acute care hospitals in the Vancouver, BC, area July 1984 to October 1986. SO_2, NO_2, O_3, SO_4, and temperature considered.	Summer (May to October) total emergency (but not respiratory) visits significantly correlated with temperature and O_3. Day-of-week effects addressed. Seasonality reduced by study period selection, but opposing within season cycles in asthma visits and O_3 not addressed, which may have weakened reported O_3-respiratory visit relationship. Also, O_3 levels much lower than in previously studied in Southern Ontario.	Bates et al. (1990)
0 to 0.13 avg of max 1 h/day for two stations	O_3	ER admissions for COPD in Barcelona, Spain, during 1985 to 1986. 24-h avg SO_2 and BS city-wide averages. 1-h max SO_2, CO, NO_2, and O_3 obtained from two stations.	A weak but statistically significant association found between COPD admissions and levels of SO_2, BS, and CO, after accounting for seasonality and autocorrelation and during season-specific analyses. However, O_3 was eliminated from the analysis based on its seasonally driven negative correlation with admissions (prior to long-wave controls). Thus, no conclusions regarding O_3 can be made from this work.	Sunyer et al. (1991)
0 to 0.04 daily mean	O_3	Hospital admissions for asthma in Helsinki, Finland, from 1987 to 1989; 24 h average SO_2, NO_2, TSP, and O_3 city-wide averages.	After accounting for daily minimum temperature, NO_2 and O_3 were significantly correlated on the same day as admissions, whereas O_3 was most significant on the prior day ($p = 0.006$). However, long-wave peaks (e.g., in April for asthma) were not addressed and autocorrelation was not assessed.	Ponka (1991)

Table 7-21 (cont'd). Hospital Admissions/Visits in Relation to Photochemical Oxidant Pollution: Time Series Studies[a]

Concentration(s) (ppm)	Pollutant	Study Description	Results and Comments	Reference
0.06 to 0.13 mean of 1000 to 1500 hours O_3 (0.12 1-h max was exceeded on 42 of 226 total study days, whereas 0.08 was exceeded on 102 days)	O_3	ER visits for asthma, bronchitis, and finger wounds (a nonrespiratory control) at nine hospitals in central New Jersey were analyzed for the period May to August 1988 and 1989. Daily values of O_3 and SO_2 obtained from nearest of five monitoring sites. Barometric pressure, temperature, RH, and visibility (as an index of sulfate) obtained from a Newark measurement station.	Bivariate correlations indicated asthma visits to be strongly negatively correlated with temperature and weakly negatively correlated with O_3, suggesting a seasonality influence, despite limitation to the O_3 season. However, simultaneous regression of asthma visits on all environmental variables yielded significant (positive) O_3 and (negative) temperature coefficients only, suggesting that temperature acted as a long-wave control variable, revealing the short-wave O_3 relationship with asthma. Day-of-week effects on visits found unimportant. No environmental associations seen with bronchitis or control cases (finger cuts).	Cody et al. (1992)
0.00 to 0.05 avg of daily means from 22 stations	O_3	Admissions to 79 acute-care hospitals in Southern Ontario for the months of January, February, July, and August in 1979 to 1985. Hourly average O_3, SO_2, NO_2, temperature, RH, wind speed, barometric pressure, and daily average TSP and $SO_4^{=}$.	An elaborate reanalysis of the Bates and Sizto (1989) data set augmented to 1985. Long-wave influences controlled using time period subsets and AR modeling. Despite possible overspecification of models (e.g., use of wind speed) and AR filtering of the short wave, results confirm Bates and Sizto's overall conclusions regarding significant O_3 associations. Response to air pollution estimated to be 19 to 24% of summer respiratory admissions, although the exact contribution by O_3 to the total was not estimated.	Lipfert and Hammerstrom (1992)
0.01 to 0.05 3-mo avg of daily means from all city sites	O_3	Age-specific quarterly asthmatic hospital discharge rates in Hong Kong from 1983 to 1989 examined in relation to quarterly mean levels of TSP, RSP, NO_2, NO_x, and O_3 (n = 27).	Concludes that asthma morbidity is correlated with particles, but not O_3. However, analysis uses quarterly means and lacks seasonality controls.	Tseng et al. (1992)
0.03 to 0.21 1-h daily max at central site in each area	O_3	Daily emergency admissions to acute care hospitals for asthma, total respiratory, and control disease categories in the New York City, Albany, and Buffalo, NY, metropolitan areas from June to August 1988 and 1989; daily 1-h maximum O_3 and temperature and daily average sulfate and acid aerosols (H^+) considered.	Significant positive associations found for O_3, $SO_4^{=}$, and H^+ with asthma and total respiratory admissions, but not for control categories. Long-wave and day-of-week effects removed, and temperature effects controlled. Strongest O_3 associations in higher pollution year (1988) and in more urban population centers (Buffalo and New York, NY).	Thurston et al. (1992)
0.01 to 0.16 1-h daily max at central site	O_3	Daily admissions to 22 acute care hospitals in Toronto, Ontario, for asthma, total respiratory, and control disease categories during July and August 1986, 1987, and 1988; daily 1-h maximum O_3, SO_2, NO_2, temperature, and daytime (9:00 a.m. to 5:00 p.m.) $SO_4^{=}$ and H^+ considered.	Significant positive correlations found for O_3, H^+, $SO_4^{=}$, PM_{10}, and TSP with asthma and for total respiratory admissions, but not for SO_2 or NO_2, and not with control admissions. Long-wave and day-of-week effects removed. Multivariate regressions and sensitivity analyses suggested that O_3 was the pollutant of primary importance, but H^+ may potentiate O_3 effects. Except for H^+, all PM metrics considered became nonsignificant when entered into regressions simultaneously with O_3. Ozone significant even after dropping days >0.12 ppm.	Thurston et al. (1994)

Table 7-21 (cont'd). Hospital Admissions/Visits in Relation to Photochemical Oxidant Pollution: Time Series Studies[a]

Concentration(s) (ppm)	Pollutant	Study Description	Results and Comments	Reference
0.01 to 0.15 1-h daily max	O_3	Daily emergency respiratory admissions to 168 acute care hospitals in Ontario, Canada, during May to August 1983 to 1988 were related to daily levels of O_3 and SO_4 at the nearest of 22 and 9 monitoring sites, respectively. Admissions broken into 0 to 1, 2 to 34, 35 to 64, and 65+ age groups, and by geographical subregion.	Ozone and $SO_4^{=}$ positively and significantly associated with admissions for asthma and COPD in all age groups. Associations consistent across regions. Seasonal and day of week effects addressed prior to analysis. Analyses also controlled for individual hospital influences. No pollutant associations found for nonrespiratory control admissions. Simultaneous regressions suggest O_3 to be more important than $SO_4^{=}$.	Burnett et al. (1994)
0.01 to 0.11 1-h daily max averaged over seven sites in Montreal	O_3	Daily urgent hospital admissions to 31 hospitals in Montreal, Canada, during May-October and August-July from 1984-1988 related to daily levels of O_3, SO_4, PM_{10}, temperature, and humidity. Admissions broken into asthma, nonasthma respiratory, total respiratory, and a nonrespiratory "control" group of admissions categories.	Ozone and temperature positively and significantly correlated with total respiratory admissions during the July-August period, but not with control admissions categories. However, O_3 and T are both nonsignificant when entered simultaneously.	Delfino et al. (1994a)
0.02 to 0.16 1-h avg daily max 0.01 to 0.12 8-h avg daily max	O_3	Daily numbers of emergency asthma visits by patients 1 to 16 years old to an inner city hospital in Atlanta, GA, from June to August 1990 were related to daily levels of O_3, SO_2, PM_{10}, pollen, and T.	Hospital visits were found to be significantly higher on days when the previous day's 1-h max O_3 exceeded 0.11. No relationship was found below 0.11, or with 8-h avg daily maximum O_3. Day-of-week effects were accounted for. Seasonality effect reduced by study period selection, but probable long-wave seasonal cycles superimposed on the day-to-day fluctuations were not directly addressed, which probably weakened the reported O_3-admissions associations.	White et al. (1994)
0.01 to 0.04 (10th to 90th percentile) 24-h daily avg	O_3	Daily respiratory admissions by patients ≥ 65 years of age in Birmingham, AL, from 1986 to 1989 were related to daily levels of O_3, PM_{10}, temperature, and dew point. COPD and pneumonia admissions examined. Multiple O_3 monitoring sites averaged, but the numbers of sites varied over time.	COPD and pneumonia admissions positively correlated with O_3 and PM_{10} over time. A 50 ppb increase in 24 h average O_3 was associated with RR = 1.14 for pneumonia (95% CI = 0.94 to 1.38) and RR = 1.17 for COPD (95% CI = 0.86 to 1.60). Seasonal fluctuations addressed using 48 monthly dummy variables. Auto-regression methods employed to reduce autocorrelation. Day-of-week effects not addressed.	Schwartz (1994a)
0.01 to 0.04 24-h daily avg (10th to 90th percentile) 0.02 to 0.09 1-h avg daily max (10th to 90th percentile)	O_3	Daily respiratory admissions for patients ≥ 65 years of age in Detroit, MI, from 1986 to 1989 were related to daily levels of O_3, PM_{10}, temperature, and dew point.	Pneumonia and COPD respiratory admissions were found to be significantly associated with both PM_{10} and O_3, even after eliminating noncompliance days. Monthly dummy variables were employed to account for seasonal variations, but day of week effects were not addressed. Asthma admissions were not associated with pollution, but this was attributed to the very low counts in this category for the elderly.	Schwartz (1994b)

Table 7-21 (cont'd). Hospital Admissions/Visits in Relation to Photochemical Oxidant Pollution: Time Series Studies[a]

Concentration(s) (ppm)	Pollutant	Study Description	Results and Comments	Reference
0.01 to 0.04 (10th to 90th percentile) 24-h daily avg	O_3	Daily respiratory admissions for patients ≥65 years of age in Minneapolis-St. Paul, MN, from 1986 to 1989 were related to daily levels of O_3, PM_{10}, temperature, and dew point.	Pneumonia respiratory admissions were significantly associated with O_3 and PM_{10}. No O_3-COPD association was found. The pneumonia RR associated with a 50-ppb increase in 24-h average O_3 was RR = 1.22 (95% CI = 1.02 to 1.47). Excluding days with 1-h max O_3 above 120 ppb did not alter results. Various methods, including the use of monthly dummy variables, were used to control for seasonality effects, all yielding similar results.	Schwartz (1994c)
0.053 (±0.005) Mean (±SD) 10 a.m. to 3 p.m. avg	O_3	ER visits for asthma at central New Jersey hospitals from May to August 1986 to 1989 related to daily levels of O_3 and temperature. Other environmental variables considered include RH, sulfates, NO_2, SO_2, and visibility.	Asthma visits were significantly associated with O_3 and a.m. temperature, but not with other environmental variables considered. O_3 coefficient implies a 44% mean effect, but unaddressed temperature effects may be a confounding factor. An analysis limited to July and August reduces this concern, yielding a 16% mean effect by O_3.	Weisel et al. (1995) Weisel (1994)

[a]See Appendix A for abbreviations and acronyms.
[b]Reviewed in U.S. Environmental Protection Agency (1978).
[c]Reviewed in U.S. Environmental Protection Agency (1986).

reasons that were not apparent. A common weakness of many of those studies, however, was a failure to control adequately for seasonal differences in hospital usage and O_3 concentration. Therefore, each of the updated critiques in this table now includes an evaluation of how the data were (or were not) controlled for long-wave influences (e.g., seasonality). With this factor taken into account, the older studies' varying results are now more understandable. In a number of these studies, documented long-wave periodicities in the data were ignored, resulting in nonsignificant associations (i.e., California Department of Public Health, 1955, 1956, 1957; Brant and Hill, 1964; Brant, 1965; Levy et al., 1977; Namekata et al., 1979) or even significant negative correlations between O_3 and hospital visits and admissions (Richards et al., 1981) as a result of the generally higher respiratory admission rates in the colder months, when O_3 levels are at their lowest. Two studies that did not control for seasonality still reported significant positive correlations between hospital admissions and oxidants (Sterling et al., 1966, 1967; Goldsmith et al., 1983), although Sterling et al. excluded the winter months from the analysis and Goldsmith et al. did detrend the data. Also, unlike any of the previously cited studies, both of these analyses controlled for day-of-week effects on hospital admission rates (e.g., due to consistently lower admissions on weekends), an important factor in hospital admissions variations that also must be addressed (see Sterling et al., 1966). Moreover, the one previously reviewed study that adequately controlled for both long-wave and day-of-week influences (Bates and Sizto, 1983, 1987, 1989) reported very significant associations ($p < 0.001$) between O_3 levels and summertime (July and August) respiratory hospital admissions. However, other intercorrelated environmental variables (e.g., acidic sulfates) also may have been cofactors in this association (Bates and Sizto, 1987). Overall, a review of these older studies suggests that, if the data are analyzed using newer statistical techniques, a significant association may be found between elevated ambient O_3 concentrations and acute increases in daily respiratory hospital admissions.

Since the last criteria document (U.S. Environmental Protection Agency, 1986), a number of new ER visit and hospital admissions studies have been completed, a few of which share some of the same statistical flaws found in many of the older studies. For example, Tseng and Li (1990) and Tseng et al. (1992) failed to control for the seasonality of admissions and pollutants in their statistical analyses of quarterly hospital admissions in Hong Kong, causing them to report no associations with O_3, but a significant (and likely spurious) negative correlation of age-specific asthma admissions with quarterly mean SO_2 in the first of these papers and a significant (and also likely spurious) positive association with TSP in the second paper, but no association with O_3. Sunyer et al. (1991) failed to consider seasonality in their initial evaluation of an O_3 relationship with COPD hospital admissions in Barcelona, Spain; causing them to eliminate O_3 from consideration in the study and any evaluation of possible health effects. Also, Bates et al. (1990), using a largely descriptive approach, characterized the seasonal periodicities of Vancouver, BC, respiratory ER visits. Their subanalysis of the warm season (May through October) included a dominant fall asthma peak, which would obscure any summertime O_3 associations, and therefore, little can be inferred from this data analysis about the existence or nonexistence of an acute relationship between O_3 and Vancouver hospital visits for respiratory causes. Ponka (1991) showed significant O_3 associations with asthma hospital admissions in Helsinki, Finland, over a 3-year period. The model also included temperature, but did not address directly the noted long-wave variations in both admissions and pollution. Thus, whether a study has adequately addressed statistical confounding by the prominent long-wave cycles in respiratory hospital

admissions series, which are clearly dominated by other causes (e.g., spring pollen, fall respiratory infection, winter influenza seasons), continues to be a crucial criterion in evaluating the usefulness of a study's results.

Fortunately, there are also a number of new studies that have addressed both long-wave and day-of-week influences in their analyses. Cody et al. (1992) did not control directly for seasonality, but they did narrow their analysis of central New Jersey hospital ER visits to the high O_3 season (May through August). Even so, their initial correlational analysis yielded negative associations between hospital visits and both temperature and O_3, which suggests that within-season long-wave effects existed (e.g., generally higher asthma visits in May, at the end of the pollen season, when O_3 and temperature are lower on average than in July or August). However, the authors did conduct subsequent regressions of respiratory visits on both temperature and O_3 simultaneously, yielding a significant positive coefficient for O_3 and a negative coefficient for temperature, which suggests that the inclusion of temperature may have indirectly accounted for the long-wave cycle, allowing the positive short wave O_3-visit relationship to be seen. Day-of-week influences were considered, but found to be unimportant for these ER visit data. No such pollution-hospital visit relationship was found for finger cut (i.e., control disease) visits.

Weisel et al. (1995) examined central New Jersey hospital ER visits for asthma (mean = 5.4/day) during the high O_3 season (May through August) for 1986 through 1990. Using a stepwise regression analysis, a significant positive coefficient for O_3 and a negative coefficient for morning temperature was found. Other environmental factors considered, including rate of temperature change, RH, every-sixth-day sulfates, NO_2, SO_2, and visibility (an index of fine particles [FPs]), were not found to be correlated with asthma visits. This study did not directly address long-wave confounding, instead following the same approach as Cody et al. (1992) in using temperature to indirectly control for such seasonal confounding and diminishing autocorrelation to nonsignificance (DW = 2). However, it is not clear to what extent O_3 may be inadvertently picking up short-wave temperature effects not modeled by this specification. These are likely to be opposite to the seasonal effect apparently being captured by the temperature variable (as indicated by its negative coefficient). The highest O_3 coefficient was found on the lowest O_3 year, which is consistent with unaddressed confounding. Thus, the O_3 effects reported (which imply an overall O_3 mean effect equal to 44% of all asthma visits) should be viewed as maximum effects estimates, possibly contributed to by colinear high temperature influences. Indeed, limiting the analysis to July and August of each year (thereby reducing long-wave confounding) resulted in a less negative temperature coefficient and an overall O_3 coefficient one-third of that for May through August (Weisel, 1994), implying approximately a 16% mean effect, which is more consistent with published hospital admissions study results. A covariance analysis presented indicates an average 28% increase in the number of hospital ER visits for asthma on high-O_3 days (above 0.06 ppm) versus low-O_3 days (below 0.06 ppm) after controlling for temperature, but seasonal cycles were again not directly accounted for in the analysis. Overall, these results are consistent with an O_3 effect on asthma morbidity.

Thurston et al. (1992) analyzed unscheduled (emergency) admissions to acute care hospitals in three New York State metropolitan areas during the summers of 1988 and 1989. Environmental variables considered included daily 1-h maximum O_3 and 24-h average SO_4 and acid aerosol (H^+) concentrations, as well as daily maximum temperature recorded at central sites in each community. Long-wave periodicities in the data were reduced by selecting a June through August study period. However, because of remaining within-season

long-wave cycles in the data series (i.e., day-to-day fluctuations superimposed on an annual cycle in admissions), data were prefiltered using sine and cosine waves with annual periodicities. Day-of-week effects also were controlled via regression. These adjustments resulted in nonsignificant autocorrelations in the data series and also improved the pollution correlations with admissions. For example, in New York City, the same-day O_3-asthma correlation rose from a nonsignificant $r = 0.04$ in the raw data to a significant $r = 0.24$ after prefiltering. This shows the importance of addressing long-wave cycles in such data, even when these data come from a single season. In contrast, correlations between the pollution data and hospital admissions for nonrespiratory control diseases were nonsignificant both before and after prefiltering. The strongest O_3-respiratory admissions associations were found during the period of high pollution in the summer of 1988 and in the most urbanized communities considered (i.e., Buffalo and New York City). After controlling for temperature effects via simultaneous regression, the summer haze pollutants (i.e., $SO_4^{=}$, H^+, O_3) remained significantly related to total respiratory and asthma admissions. However, these pollutants' high intercorrelation prevented the clear discrimination of a single pollutant as the causal agent. Depending on the index pollutant, the admission category, and the city considered, it was found that summer haze pollutants accounted for approximately 5 to 20% of June through August total respiratory and asthma admissions, on average, and that these admissions increased approximately 30% above average on the highest pollution days.

Lipfert and Hammerstrom (1992) reanalyzed the Bates and Sizto (1989) hospital admissions data set for 79 acute-care hospitals in southern Ontario, incorporating more elaborate statistical methods and extending the data set through 1985. Long-wave influences were once again reduced by using the short study periods previously employed by Bates and Sizto (e.g., July and August only for summer), as well as by employing prewhitening and autoregressive procedures to the data. Day-of-week effects also were controlled. In addition, the models were specified much more extensively, to include a variety of new meteorological variables that may have caused some confounding with the pollutant variables (e.g., wind speed correlated at $r = -0.55$ with NO_2). Despite possible model overspecification (e.g., the inclusion of wind speed), summer haze pollutants (i.e., O_3, SO_4, SO_2) were still found to have significant effects on hospital admissions in southern Ontario. In contrast, pollution associations with hospital admissions for accidental causes became nonsignificant in these models. Although air pollution concentrations were generally within U.S. air quality standards, the pollutant mean effect accounted for 19 to 24% of all summer respiratory admissions, although the "responsible" pollutants could not be selected by the authors with certainty.

Burnett et al. (1994) also employed the Ontario acute care hospital database to analyze the effects of air pollution on hospital admissions, but their analysis considered all of Ontario and analyzed the data from each individual hospital, rather than aggregating the counts by region. Slow moving temporal cycles, including seasonal and yearly effects, were removed (via an 19-day, moving-average-equivalent, high-pass filter), and day-of-week effects were controlled prior to the analysis. Poisson regression techniques were employed because of the low daily admission counts at individual hospitals. Ozone displayed a positive association with respiratory admissions in 91% of the 168 hospitals, and 5% of summertime (May through August) respiratory admissions (mean = 107/day) were attributed to O_3 (mean = 50 ppb). Positive associations were found in all age groups (0 to 1, 2 to 34, 35 to 64, and 65+). A parallel analysis of nonrespiratory admissions showed no such associations,

which indicates the association specificity. Ozone was found to be a stronger predictor of admissions than SO_4, which accounted for an additional 1% of summertime respiratory admissions. Temperature had no effect on the pollution-respiratory admission relationship.

Thurston et al. (1994) focused their analysis of respiratory hospital admissions in the Toronto metropolitan area during the summers (July through August) of 1986 to 1988, when they directly monitored for strong particulate acidity (H^+) pollution on a daily basis at several sites in that city. Long-wave cycles, and their associated autocorrelations, were removed by first fitting sine and cosine series having annual periodicity (as well as day-of-week dummy variables) to the data via regression, and analyzing the resulting residuals. Strong and significant positive associations with asthma and respiratory admissions were found for both O_3 and H^+, and somewhat weaker significant associations with $SO_4^=$, $PM_{2.5}$, PM_{10}, and TSP, as measured at a central site in downtown Toronto. No such associations were found for SO_2 or NO_2, nor for any pollutant with nonrespiratory control admissions. Temperature was only weakly correlated with respiratory admissions and became nonsignificant when entered in regressions with air pollution indices. Simultaneous regressions and sensitivity analyses indicated that O_3 was the summertime haze constituent of greatest importance to respiratory and asthma admissions, although elevated H^+ was suggested as a possible potentiator of this effect. During multipollutant, simultaneous regressions on admissions, O_3 was consistently the most significant. Of the particle metrics, only H^+ remained statistically significant when entered into the admissions regressions simultaneously with O_3. Sensitivity analyses also showed that dropping all days above the current U.S. O_3 standard of 0.12 ppm (2 of a total 117 days) did not significantly change the O_3 coefficients. The simultaneous O_3, H^+, and temperature model indicated that 21 $\pm$ 8% of all respiratory admissions during the three summers were associated with O_3 air pollution, on average, and that admissions rose an estimated 37 $\pm$ 15% above that otherwise expected on the highest O_3 day (0.159 ppm). Moreover, despite differing health care systems, the Toronto regression results for the summer of 1988 were remarkably consistent with previously reported results for that same summer in Buffalo, NY, (see Table 7-22).

Delfino et al. (1994a) studied daily urgent hospital admissions for respiratory and other illnesses at 31 hospitals in Montreal, Canada during the warm periods of the year between 1984 and 1988. Respiratory admissions were considered as a whole and split into asthma and nonasthma categories, using definitions compatible with those previously used by Bates and Sitzo (1987) and by Thurston et al. (1994). Both 1-h and 8-h maximum O_3 concentrations were considered in the analyses, as well as weather variables (temperature and relative humidity) and PM measurements, although 83% (five out of every six) PM measurements were not directly measured but, instead, were estimated from other environmental variables including visibility, temperature, and O_3 concentration on those missing PM days (Delfino et al., 1994b). Seasonal cycles were addressed by applying a 19-day moving average high-pass filter to the health and environmental data before analysis for associations. Day-of-the-week and autocorrelation effects also were addressed, when present. For the months of July and August, during the study period, a significant association was found between all respiratory admissions and both 8-h daily maximum O_3 ($p \leq 0.01$) and 1-h daily maximum O_3 ($p \leq 0.03$) 4 days prior to admission, despite the fact that no day exceeded 0.12-ppm 1-h daily maximum O_3 (90th percentile = 118 $\mu g/m^3$ or 0.06 ppm O_3). Of the significant bivariate environmental-admission associations found, the association with 8-h maximum O_3 was the highest reported ($r = 0.15$), tied only by the 4-day lag in temperature. However, the addition into the regression of temperature on the

Table 7-22. Comparison of Regressions of Daily Summertime Respiratory Admissions on Ozone and Temperature in Toronto, Ontario, and Buffalo, New York, for the Summer of 1988[a]

City and Year	Respiratory Admissions Category	Temperature, Pollutant Model Specification	Pollutant Regression Coefficient (Admissions/ppb/10^6 persons ± SE)	Pollutant Mean Effect (% ± SE)	Max/Mean Pollutant Relative Risk ± SE
Toronto (pop. = 2.4×10^6) 1988 summer	Total respiratory (mean = 14.1/day)	T(LG2), O_3 (LG1)[b]	0.022 ± 0.010[c]	26.4 ± 11.8	1.34 ± 0.15
Toronto (pop. = 2.4×10^6) 1988 summer	Total asthma (mean = 9.5/day)	T(LG2), O_3 (LG1)	0.014 ± 0.008[c]	25.3 ± 14.9	1.32 ± 0.19
Buffalo (pop. = 2.0×10^6) 1988 summer	Total respiratory (mean = 25.0/day)	T(LG2), O_3 (LG2)	0.030 ± 0.016[c]	18.4 ± 9.9	1.25 ± 0.09
Buffalo (pop. = 2.0×10^6) 1988 summer	Total asthma (mean = 7.1/day)	T(LG2), O_3 (LG3)	0.012 ± 0.004[d]	23.9 ± 10.1	1.25 ± 0.14

[a]See Appendix A for abbreviations and acronyms.
[b]LG = lag between exposure and admission, in days.
[c]$p < 0.05$ (one-way test).
[d]$p < 0.01$ (one-way test).

Source: Thurston et al. (1994).

same day as the O_3 reduced both the O_3 and the temperature associations to nonsignificance. It should be noted that the authors also found a significant association between asthma admissions and their estimated PM_{10} variable during May through October; however, because both temperature and O_3 were used to estimate these observations, it is difficult to interpret this association separately from that for O_3 and temperature. No significant correlations were found between O_3 and nonrespiratory, control admissions (e.g., appendicitis). The authors conclude that their findings "should be regarded as a reflection of the potential public health burden of respiratory disease attributable to photochemical air pollutants."

White et al. (1994) reported daily emergency room visit records from June through August 1990 at a large inner city hospital in Atlanta, GA. Daily counts of visits for asthma or reactive airway disease by patients 1 to 16 years of age (mean = 6.6/day) were related to daily levels of O_3, SO_2, PM_{10}, pollen, and temperature. Seasonality likely was reduced by the study period selection, although no effort was made to address possible within-season long-wave cycles in the data. Day-of-week and temperature effects were controlled as part of a Poisson model employed to address the small admission numbers at a single hospital. This model yielded a 1.42 admissions rate ratio ($p = 0.057$, 95% CI = 0.99 to 2.0) for the number of asthma visits following days with O_3 levels equal to or exceeding a 1-h maximum of 0.11 ppm, which is consistent with the relative risk values reported by Thurston et al. (1992, 1994). No admissions relationship with O_3 was seen below 0.11 ppm or with 8-h average O_3.

In a study of Birmingham, AL, data, Schwartz (1994a) separately examined O_3 and PM_{10} influences on hospital admissions by the elderly for pneumonia (mean = 5.9/day) and COPD (mean = 2.2/day) causes from 1986 to 1989. Other potentially confounding pollutants (e.g., SO_2 and NO_2) were not considered, nor was any control admission category analyzed. Poisson regression analyses were employed controlling for time trends, seasonal fluctuations, and weather, but day-of-week effects (which can be a large influence on such admissions) were not addressed. Weather was controlled by including dummy variables for seven (unspecified) temperature and dew point range categories in the regression. Seasonal fluctuations were controlled through the use of 48 monthly dummy variables, which raises the concern that within-month long-wave confounding may have remained. However, autoregressive models were reportedly used whenever serial correlation was found in model residuals. Base model results (excluding winter months) yielded a 2-day lag relative risk (RR) estimate of 1.14 for pneumonia admissions from a 50 ppb increase in 24-h average O_3 (95% confidence interval, CI = 0.94 to 1.38). Excluding days exceeding 120 ppb yielded similar results (RR = 1.12, CI = 0.92 to 1.37). For COPD, the basic model yielded a RR = 1.17 (CI = 0.86 to 1.60), whereas excluding days above 120 ppb similarly gave RR = 1.18 (CI = 0.86 to 1.62). No models considered O_3 and PM_{10} simultaneously. Two other comparative models (the inclusion of sine/cosine cycles of various periodicities up to 2 years in the regression and the analysis of deviations from a nonparametric smoothing of admission counts) were tested for PM_{10}, but not for O_3, so the model sensitivity of the O_3 effect was not tested. Overall, even after excluding days exceeding the standard, this work indicated a fairly consistent O_3 effect across respiratory categories that approached, but did not reach, statistical significance.

Schwartz (1994b) analyzed O_3 and PM_{10} air pollution relationships with daily hospital admissions of persons 65 years or older in the Detroit, MI, metropolitan statistical area from 1986 to 1989. Daily counts for pneumonia (mean = 15.7/day), asthma

(mean = 0.75/day), and all other COPDs (mean = 5.8/day) were regressed on the pollution variables and various seasonal, trend, and temperature dummy variables, using Poisson modeling. However, day-of-week effects were not addressed. Ozone was analyzed with respect to both its daily 24-h average and 1-h maximum. Autoregressive analyses and residuals plots indicated no remaining autocorrelation in the model. Both O_3 and PM_{10} were significant in simultaneous pollutant models for pneumonia and COPD but not for asthma (which was ascribed to the low daily counts for this category). These simultaneous coefficients were reportedly similar to those from the single pollutant models, although the correlations of the coefficients were not provided. Dropping all days exceeding the 1-h maximum O_3 standard did not change the size of the O_3 coefficients, which remained significant ($p < 0.01$). Based on the regression coefficients and data presented, it can be estimated that the mean effect for O_3 (11.6%) was double that for PM_{10} (5.7%) in the pneumonia model, but comparable for COPD (12.2% for O_3 versus 10.2% for PM_{10}). On an absolute scale, these results imply that O_3 was associated with 1.7 ($\pm$0.2) respiratory admissions by the elderly/day/100 ppb (as a 1-h maximum) per million persons in the Detroit metropolitan area. This estimate does not include admissions by persons less than 65 years of age (which likely would have included higher asthma admissions, for example), so that the total respiratory admissions associated with O_3 in the entire population likely would be higher than estimated from this work.

Schwartz (1994c) evaluated the associations of both PM_{10} and O_3 with respiratory hospital admissions by the elderly in Minneapolis-St. Paul, MN, from 1986 to 1989. Due to small counts, Poisson modeling methods were employed. Various modeling approaches were employed to address weather influences, including (1) the use of annual, monthly, temperature, and dew point dummy variables; (2) a stepwise spline approach to fit data dependence on time, temperature, and dew point (an indicator of the water content of the air); and (3) a generalized additive model using nonparametric smooth functions of time, temperature, and dew point temperature. Autoregressive methods were employed to eliminate autocorrelations, when significant. However, these various complex statistical manipulations were not sufficiently documented to permit critical review of these methods or replication of results (e.g., dummy variable ranges were not provided and statistical packages were not referenced). Although no association was found for COPD in the elderly, O_3 did make a significant independent contribution to hospital admissions by the elderly for pneumonia (mean = 6.0/day), even after controlling for weather and PM_{10}. Although all models gave similar results, the best data fit (as measured by analysis of deviance) and strongest O_3 association was reported for the stepwise spline model, which yielded a pneumonia admissions relative risk of 1.22 (95% CI = 1.02 to 1.47) for a 50 ppb increase in the 1-day lag of the 24-h average of O_3. The use of 1-h daily maximum O_3 in these analyses reportedly yielded less significant associations with admissions. However, eliminating days with either PM_{10} above 150 $\mu g/m^3$ or a 1-h maximum O_3 above 120 ppb from the analysis did not alter results significantly.

Table 7-23 intercompares the O_3-respiratory hospital admissions effect estimates for the various studies providing sufficient information to allow the derivation of such pollutant-specific estimates. The estimates are presented in two ways: (1) as an absolute number of daily admissions per 100-ppb increase in 1-h O_3 concentration per million persons, total population, and (2) as a percent increase in the daily admission rate of the relevant admissions category, presented as a relative risk per 100-ppb increase in 1-h O_3 concentration. A reference increment of 100-ppb O_3 is employed here because this is

Table 7-23. Summary of Effect Estimates for Ozone in Recent Studies of Respiratory Hospital Admissions[a]

Location	Reference	Respiratory Admission Category	Effect Size (± SE) [Admissions/100 ppb O_3/day/10^6 persons]	Relative Risk (95% CI)[b] [RR of 100 ppb O_3, 1-h max]
New York City, NY[c]	Thurston et al. (1992)	All	1.4 (± 0.5)	1.14 (1.06 to 1.22)
Buffalo, NY[c]	Thurston et al. (1992)	All	3.1 (± 1.6)	1.25 (1.04 to 1.46)
Ontairo, Canada[c]	Burnett et al. (1994)	All	1.4 (± 0.3)	1.10 (1.06 to 1.14)
Toronto, Canada[c]	Thurston et al. (1994)	All	2.1 (± 0.8)	1.36 (1.13 to 1.59)
Montreal, Canada[d]	Delfino et al. (1994a)	All	1.4 (± 0.5)	1.22 (1.09 to 1.35)
Birmingham, AL[e]	Schwartz (1994a)	Pneumonia in elderly	0.73 (± 0.54)	1.11 (0.97 to 1.26)
Birmingham, AL[e]	Schwartz (1994a)	COPD in elderly	0.83 (± 0.33)	1.13 (0.92 to 1.39)
Detroit, MI[e]	Schwartz (1994b)	Pneumonia in elderly	0.82 (± 0.26)	1.22 (1.12 to 1.35)
Detroit, MI[e]	Schwartz (1994b)	COPD in elderly	0.90 (± 0.41)	1.25 (1.07 to 1.45)
Minneapolis, MN[e]	Schwartz (1994c)	Pneumonia in elderly	0.41 (± 0.19)	1.117 (1.03 to 1.39)
Minneapolis, MN[e]	Schwartz (1994c)	COPD in elderly	[f]	[f]

[a]See Appendix A for abbreviations and acronyms.
[b]One-way ($\beta \pm 1.65$ SE).
[c]1-h daily maximum ozone data employed in analysis.
[d]8-h daily maximum ozone data employed in analysis.
[e]24-h daily average ozone data employed in analysis. (1 h/24 h avg ratio = 2.5 assumed to compute effects and RR estimates).
[f]Not reported (nonsignificant).

approximately the difference between the maximum and the mean 1-h daily maximum O_3 in these studies (e.g., in Toronto, the 1988 mean = 69 ppb; maximum = 159 ppb). The absolute effect estimates relative to total population have the advantages that the total effect can be readily "partitioned" into subcategories (e.g., by age group or disease subcategory), and it also can be applied easily to other situations (i.e., only the population and O_3 levels are required), but this may not be appropriate if the other population makeup is very different from the study populations (e.g., in age distribution). The relative risk estimates are intuitively interpretable but are not as readily applied elsewhere (i.e., the respiratory disease prevalence rates must be known), and the effect will vary depending on the prevalence, which differs widely between populations and even throughout the year within a single population (as respiratory morbidity is generally higher in winter than summer). For example, this accounts for much of the apparent inconsistency between the Burnett et al. (1994) and Thurston et al. (1994) relative risks, in that the Thurston et al. (1994) Toronto values are for July and August only (when the prevailing number of respiratory admissions per day are generally at an annual minimum), whereas the Burnett et al. (1994) Ontario values are relative to respiratory admissions averages over more months of the year, yielding one-fourth the effect as a relative risk, even though the absolute effect estimate is two-thirds of the Thurston et al. (1994) estimate. In the case of the Schwartz studies of the elderly, the assumption has been made, based on data presented by Schwartz (1994b), that the 1-h daily maximum O_3 is 2.5 times the 24-h average, and the 100-ppb 1-h maximum estimates provided for these studies therefore are derived from a 40-ppb increase in 24-h average O_3. The absolute effect size results from these particular studies suggest that a large portion of the O_3 effects noted in the previous total respiratory admissions studies are contributed by COPD and pneumonia cases in the elderly. Based on results presented by Thurston et al. (1992, 1994), the other major contributor is asthma admissions, which are usually more prevalent in younger age groups. Overall, the results presented in Table 7-23 collectively indicate that ambient O_3 often has a significant effect on hospital admissions for respiratory causes, ranging in these studies from 1 to 3 total respiratory admissions/day/100 ppb $O_3/10^6$ persons, or from a 1.1 to 1.36 relative risk/100 ppb O_3.

Daily Mortality

Past studies of the possible association of O_3 (oxidants) with human mortality summarized in prior O_3 criteria documents (U.S. Environmental Protection Agency, 1978, 1986) were sometimes suggestive of an association, but each study was flawed in some way. These studies are included in Table 7-24, with annotation as to the document in which they were reported. Most of these studies considered daily mortality in Los Angeles, CA, during the 1950s and 1960s. Unlike most historical hospital admissions studies, many of these studies did recognize and attempt to control for seasonality in the data series. Notable exceptions are the California Department of Public Health studies (1955, 1956, 1957), which were further weakened by their qualitative treatment of the air pollution data. The Mills (1957a,b) analyses also employed a questionable exposure assessment method (the Standard Research Institute smog index), which diminishes its usefulness. Massey et al. (1961) reported no significant correlations between community differences in mortality and differences in oxidant levels over time, but the investigators compared two communities with very different populations (e.g., age distributions), a likely confounder in such cross-sectional comparisons. Mills (1960), while reporting mortality-oxidant associations and effects (370 respiratory and cardiovascular deaths/year), did not control for potential temperature

Table 7-24. Daily Mortality Associated with Exposure to Photochemical Oxidant Pollution[a]

Concentration(s) (ppm)	Pollutant	Study Description	Results and Comments	Reference
≤1.0 peak (undefined)	Oxidant	Relationship between daily concentrations of photochemical oxidants and daily mortality among residents of Los Angeles County aged 65 years and older during the periods August through November 1954 and July through November 1955.	Heat had a significant effect on mortality; no consistent association between mortality and high oxidant concentrations in the absence of high temperature. However, seasonal trends were not addressed, and pollution data treatment was qualitative.	California Department of Public Health (1955[b], 1956[b], 1957[b])
≤0.38 max 1 h/day	Oxidant	Data extended to include the period from 1956 through the end of 1959.		Tucker (1962)
(Not reported)	Oxidant	Relationship between daily maximum oxidant concentrations and daily cardiac and respiratory mortality in Los Angeles for the periods 1947 to 1949; August 1953 through December 1954; and January through September 1955.	Positive relationship between daily maximum oxidant concentrations and mean daily death rates on high-smog days vs. low-smog days. Questionable exposure analysis, including use of the SRI smog index.	Mills (1957a[b],b[b])
0.10 to 0.42 (undefined) for 148 days of 1949	O_3			
(Not reported)	Oxidant	Comparison of daily mortality in two Los Angeles County areas similar in temperature, but with different levels of daily maximum and mean oxidant levels (KI); SO_2 and CO concentrations were also measured.	No significant correlations between differences in mortality and differences in pollutant levels. However, the populations differed in socioeconomic and age distribution characteristics.	Massey et al. (1961)[b]
0.02 to 0.37 average of 1-h daily max from all Los Angeles sites	Oxidant	Daily respiratory and cardiac death counts for Los Angeles County, 1956 to 1958, related to daily maximum oxidant concentrations. All days above 96 °F daily maximum temperature eliminated from analysis. Each day's average of daily oxidant maxima was related to that day's deviation from monthly mean mortality.	A stratification of the mortality deviations vs. oxidant concentration revealed increasing mortality with increasing oxidant concentration, even in the cooler months. The use of deviations addresses data seasonality. It is estimated that over 300 deaths/year in Los Angeles are associated with oxidants. However, the lack of temperature controls below 96 °F is a major weakness.	Mills (1960)

Table 7-24 (cont'd). Daily Mortality Associated with Exposure to Photochemical Oxidant Pollution[a]

Concentration(s) (ppm)	Pollutant	Study Description	Results and Comments	Reference
0.05 to 0.21 monthly avg	Oxidant	Reanalysis of the relationship between KI and daily mortality from cardiac and respiratory diseases in Los Angeles for the years 1956 through 1958.	Used deviations from sine wave fit to reduce seasonality of pollution and mortality, but fit of monthly variations was inadequate. Significant correlations found between pollutants and mortality for cardiorespiratory diseases, but autocorrelation adjustments by authors reportedly reduced these associations to nonsignificance.	Hechter and Goldsmith (1961)[b]
0.003 to 0.128 max 1 h/day	O_3	Relationship between daily mortality and daily 1-h maximum concentrations of O_3 in Rotterdam, The Netherlands, during the months of July and August 1974 and 1975.	Mortality significantly higher during relatively high pollution ($0.05 < O_3 < 0.125$) and heat episodes in 1975. However, no significant mortality difference due to moderate O_3 episodes ($0.05 < O_3 < 0.08$) in 1974, in the absence of high temperature. Such aggregated analyses of serial data makes interpretation difficult.	Biersteker and Evendijk (1976)[c]
0.02 to 0.29 six-site mean of daily 1-h max	O_3	Total, respiratory, and cardiovascular mortality in Los Angeles County, 1970 to 1979, related to O_3, CO, SO_2, NO_2, HC, PM, daily max temperature, and RH. Low-pass filter used to eliminate short-wave associations so that only seasonal associations could be studied.	Frequency domain analysis indicated a significant short-wave O_3-mortality association, but this was not investigated further. The filtered (i.e., long-wave) data analysis indicated O_3 to be a nonsignificant contributor to seasonal variations in mortality.	Shumway et al. (1988)
0.02 to 0.29 six-site mean of daily 1-h max	O_3	Shumway et al. (1988) 1970 to 1979 Los Angeles mortality dataset reanalyzed using a high-pass filter to allow investigation of short-wave (acute) associations with environmental variables, after removing seasonality effects. Environmental variables considered included temperature, RH, extinction coefficient, carbonaceous PM, SO_2, NO_2, CO, and O_3.	Filtered environmental and mortality data analyses demonstrated significant associations between short-term variations in total mortality and pollution, controlling for temperature. Day-of-week effects found not to affect the relationships. Of the pollutants considered, O_3 had the strongest association with total mortality. Similar results found for cardiovascular deaths, but not for respiratory deaths (for which only temperature was significant).	Kinney and Ozkaynak (1991)

Table 7-24 (cont'd). Daily Mortality Associated with Exposure to Photochemical Oxidant Pollution[a]

Concentration(s) (ppm)	Pollutant	Study Description	Results and Comments	Reference
Not reported 1-h daily max	O_3	Total daily deaths in Detroit, MI, 1973 to 1982, analyzed using Poisson methods. Environmental variables considered included TSP, SO_2, temperature, dew point, and O_3.	Significant associations found between mortality and PM, but not O_3. However, O_3 data and results not presented, so it is difficult to evaluate reported conclusion. Seasonality controlled via multiple dummy weather and time variables, and autocorrelation addressed using autoregressive techniques. Possible overspecification of weather controls may be a factor in the nonsignificance of O_3 in this analysis.	Schwartz (1991)
0.000 to 0.064 24-h avg (in TN, no exceedances of 0.12 ppm 1-h max; in MO, five exceedances with max = 0.15 ppm)	O_3	Associations between total daily mortality and air pollution were investigated in St. Louis, MO, and Kingston-Harriman, TN, during September 1985 through August 1986. Environmental variables considered include temperature, RH, PM_{10}, $PM_{2.5}$, sulfate, aerosol acidity, SO_2, NO_2, and O_3.	Statistically significant daily mortality associations were found with PM_{10}, but not with O_3. Autocorrelation removed via season indicators, multiple temperature/climate variables, and AR modeling. The nonsignificant O_3 coefficient may have been contributed to by the more conservative autocorrelation removal measures taken, lower O_3 concentrations, and shorter study period, relative to other recent mortality studies.	Dockery et al. (1992)

[a]See Appendix A for acronyms and abbreviations.
[b]Reviewed in U.S. Environmental Protection Agency (1978).
[c]Reviewed in U.S. Environmental Protection Agency (1986).

influences on mortality below 96 °F daily maximum. Hechter and Goldsmith (1961) reanalyzed the Mills (1960) data using a simple annual sine wave seasonality correction and obtained significant oxidant correlations until an autocorrelation adjustment was applied; this reportedly caused the pollutant-mortality correlations to drop to nonsignificance (results not presented). Biersteker and Evendijk (1976) conducted a t-test of difference analysis of two summers of time series data from Rotterdam for 1974 and 1975. Although significant mortality differences could be seen during 1975 heat-pollution episodes ($0.05 < O_3 < 0.125$ ppm), no significant mortality increase could be seen during the cleaner and cooler summer episodes ($0.05 < O_3 < 0.08$ ppm). Statistical time series methods were needed to address probable confounding by temperature effects. Overall, the various exposure assessment and statistical analyses weaknesses in the studies reported in previous O_3 criteria documents have prevented the drawing of definitive conclusions in those past documents as to whether or not there is a significant association between O_3 and human mortality.

Although relatively few O_3 mortality studies have been conducted and published since the last criteria document (U.S. Environmental Protection Agency, 1986), the statistical methods and pollution data employed in these studies have improved, compared with the older studies discussed above. Shumway et al. (1988) focused on long-wave variations in mortality, finding that O_3 was a nonsignificant contributor to seasonal variations in Los Angeles mortality during 1970 to 1979. As might have been expected, temperature was found to be the principal environmental factor influencing seasonal mortality fluctuations. This paper's exploratory frequency domain analysis did indicate a significant short-wave (i.e., cycles on the order of a few days in period) association between O_3 and mortality, but this result was not pursued in the subsequent regression analyses.

Kinney and Ozkaynak (1991) reanalyzed the 1970 to 1979 Los Angeles County mortality and environmental data set for short-wave pollution-mortality associations using seasonal and day-of-week controls. After prefiltering the environmental and mortality time series using a high-pass filter, significant associations were demonstrated between air pollution and short-wave (acute) variations in total mortality, even after controlling for temperature influences. Day-of-week effects also were accounted for but were found not to affect pollutant-mortality associations. In the regression models considered, the 1-day lag of O_3 concentration gave the strongest pollutant associations with total mortality. This O_3 coefficient was statistically separable from the other significant pollutants in the analysis (CO, NO_2, and PM), although these other three pollutants were too intercorrelated to separate from each other. Expressed as an elasticity, the O_3 regression coefficient (0.03 ± 0.01 [SE] deaths/ppb) over all years indicated that a 1% increase in O_3 concentration was associated with a 0.015% increase in total mortality. This result would imply an O_3 mean effect on the order of 1.5% of total mortality throughout the year (i.e., 830 total deaths/year). Results for individual years varied widely in terms of the O_3 coefficient size and significance, which indicates the need for multiple years of data to discern an effect of such a small size, relative to other mortality causes. Ozone regression results for cardiovascular deaths (average = 87/day) were qualitatively similar to those for total mortality (average = 152/day), but only temperature was significant for respiratory deaths (average = 8/day), probably due to low count number effects for this category (i.e., Poisson models may have been required). Overall, although the Shumway et al. (1988) analysis of these 1970 to 1979 Los Angeles data indicates that disease factors and other pollutants dominate the overall seasonal cycles in mortality in Los Angeles, the Kinney and Ozkaynak (1991) short-wave analysis documents that O_3 explained a small but statistically

significant portion of day-to-day variations in total mortality in that city over a 10-year period.

Schwartz (1991) analyzed total daily human mortality in Detroit, MI, during the 10-year period from 1973 to 1982, primarily investigating the effects of PM using Poisson methods. Although actual results are not presented for O_3, it is stated in the discussion of results that O_3 was "highly insignificant as a predictor of daily mortality." Weather is controlled for extensively in the model specification before the introduction of the air pollution variables. The fact that O_3 is usually correlated over time with meteorology raises the concern that the model may be overspecified, but no diagnostics (e.g., correlations of the coefficients) are presented to allow for an evaluation. Although previous-day temperature was included in the model, the only direct seasonality control attempted was to limit the analysis to nonwinter months. Thus, it is not clear to what extent within-season long-wave confounding also may be influencing the results. If present, such long-wave confounding would be expected to bias the O_3 coefficient downward towards nonsignificance in this case (because O_3 is usually highest, and mortality lowest, in summer) and would result in autocorrelation in the model. No model residual diagnostics are reported (e.g., DW statistics or plots of the model residuals), so the extent of this problem, if present, cannot be evaluated directly. However, autoregressive methods were employed, which should have addressed any autocorrelation problems. Overall, the poor documentation of the mortality-O_3 modeling, especially regarding the lack of model specification details or model coefficient intercorrelations, makes the author's statement regarding O_3 and mortality difficult to evaluate.

Dockery et al. (1992) conducted an analysis of total daily human mortality in St. Louis, MO, and Kingston-Harriman, TN, during the 1-year period from September 1985 through August 1986 aimed primarily at assessing the effects of PM on mortality. One of the strengths of this study is the fact that multiple air pollutants were measured and considered. Thus, as part of the analysis, O_3 and other gaseous pollutants also were considered and found to have nonsignificant associations with mortality in these cities. The statistical analysis addressed autocorrelation in the mortality data through the use of multiple climate indices (i.e., daily mean temperature, hot day, cold day, humid day, hot and humid day, season, and interactive terms) and through the incorporation of autoregressive modeling. This approach is possibly more conservative than that employed by Kinney and Ozkaynak (1991), and the lack of a significant O_3 coefficient in this analysis may be due in part to the statistical modeling approach, which may or may not have affected an O_3 mortality relationship in the data in the process of addressing autocorrelation and so extensively controlling for temperature (which is usually correlated with O_3 over time). Also, the lack of any O_3 associations with total mortality may be due in part to the relatively low O_3 levels found in these particular communities (especially in Kingston-Harriman, where no O_3 exceedances occurred) during the study year (maximum 24-h mean O_3 <0.065 ppm). Overall, this study did not show an association between O_3 and mortality, but this may in part be a product of the particular methodological and exposure characteristics of this study vis-à-vis the identification of O_3 health effects.

7.4.1.4 Summary and Conclusions

Recent epidemiology studies addressing the acute effects of ambient O_3 have yielded significant associations with a wide range of health outcomes, including lung function decrements, aggravation of preexisting respiratory disease, and increases in daily hospital

admissions and mortality. Individual-level camp and exercise studies clearly indicate that lung function can decrease in a concentration-related manner in response to O_3 exposures occurring in ambient air. The combined results of these studies provide useful, quantitative information on the pulmonary effects of ambient O_3 exposures. Results from daily life studies, although more difficult to interpret quantitatively due to exposure assessment uncertainties, are qualitatively consistent with camp and exercise studies. There is limited evidence from several studies suggesting that ambient O_3-induced lung function decrements may persist for up to 24 h. Results from lung function epidemiology studies generally are consistent with those of human chamber studies. An O_3-related worsening of symptoms in selected groups of healthy individuals and detrimental changes in symptoms, lung function, and medication use in asthmatics have been observed qualitatively and, to a lesser extent, quantitatively. The relationship is consistent, temporally plausible, and moderately coherent.

Emergency room visit and hospital admission studies considered in this document collectively indicate that, when the major confounders to such analyses are addressed (e.g., seasonality, day-of-week effects), consistent associations are seen between acute occurrences of respiratory morbidity and O_3 exposure. The evidence is especially strong for hospital admissions, as the association has been seen by numerous researchers at a variety of localities using a wide range of appropriate statistical approaches. Although the absolute size of the effect varied somewhat across localities and statistical approaches, these analyses suggest that, in the summertime (when many other respiratory illness causes have abated), O_3 air pollution is associated with a substantial portion (on the order of 10 to 20%) of all respiratory hospital visits and admissions. Moreover, certain of these analyses also indicate that, on the highest O_3 days, this pollutant's estimated contribution can increase to the point where it is associated with nearly half of all respiratory hospital admissions. Moreover, significant associations also are seen between O_3 and hospital visits and admissions at exposures below 0.12 ppm 1-h daily maximum O_3.

As was also the case for the O_3-hospital admissions time series studies, many of the older O_3-mortality studies had methodological or statistical weaknesses that prevented clear conclusions. However, since the release of the previous criteria documents, one of the two most useful new studies (Kinney and Ozkaynak, 1991) indicated statistically significant effects by O_3 on short-term (acute) human mortality. The one relevant new study that did not show any O_3 association (Dockery et al., 1992) employed much more extensive climate and autocorrelation control methods and was conducted over a much shorter time period than the other study. Also, the study that showed an O_3-mortality association considered an urban area experiencing 1-h maximum O_3 concentrations above 0.15 ppm, whereas the other study areas (eastern Tennessee and St. Louis, MO) did not. Thus, although the analysis of daily series of human mortality and air pollution has yielded small but statistically significant associations with O_3 in one study, the sensitivity of this association to statistical modeling methods and to O_3 concentration level needs further investigation.

7.4.2 Chronic Effects of Ozone Exposure

7.4.2.1 Introduction

At the time of the publication of the previous EPA air quality criteria document (U.S. Environmental Protection Agency, 1986), little useful data were available on the chronic effects of O_3 exposure. Table 11-10 of that document summarized the limited number of studies available at that time and concluded "...it is unlikely that any of these

studies can be used to develop quantitative exposure-response relationships for ambient oxidant exposures. Further study of well-defined populations over long periods of time is required before any relationship between photochemical oxidants and the progression of chronic diseases can be conclusively demonstrated from population studies" (U.S. Environmental Protection Agency, 1986). The document noted that existing studies failed to demonstrate any consistent relationship between chronic oxidant exposure and changes in pulmonary function, chronic symptoms, chromosomal abnormalities, or chronic disease mortality.

The largest study that had been performed at the time of the 1986 criteria document was that of Detels et al. at the University of California at Los Angeles (UCLA) (Detels et al., 1979, 1981; Rokaw et al., 1980). This study employed a population-based sample of households in selected communities in the Los Angeles South Coast Air Basin. A standardized interview was administered, and individuals underwent various tests of lung function. Air pollution data were derived from a network of monitoring stations maintained by the South Coast Air Quality Management District of the California Air Resources Board (ARB). The usefulness of the findings of this study was considered to be limited due to a number of factors: (1) variable timing of testing in the several study communities over a 4-year period, (2) paucity of data on self-selection (completion rates between 70 to 79%) and migration in and out of the study communities, (3) inconsistent demonstration of reproducibility of the pulmonary function measurements, (4) mixed ethnicity of the study population, (5) inadequate data on individual exposure and failure to adjust exposure estimates for migration in and out of the study areas, and (6) methods employed for comparisons of health effects.

The 1986 criteria document also summarized the first of the Adventist Health Smog (AHSMOG) studies (Hodgkin et al., 1984) on the occurrence of COPD in relation to chronic air pollution exposures. However, the data from this first publication were felt to be of limited value because only symptom data were reported and the exposure assessment was insufficient.

7.4.2.2 Recent Epidemiological Studies of Effects of Chronic Exposure

By the very nature of the problem of the establishment of a link between chronic exposure to O_3 and the occurrence of chronic health effects, epidemiological studies remain the only approach for obtaining human data. As has been noted in the 1986 document, principal problems for such studies relate to (1) the specification of individual exposures over the relevant periods of life of the study subjects; (2) the coincident effects of other oxidant species (e.g., NO_x, derivative acid species) and other air pollutants (acid aerosols, particulate species); (3) seasonal effects that relate to pollutant and meteorologic factors, which affect specific pulmonary function measurements relevant during the course of longitudinal studies or over studies that utilize multiple cross-sectional samples; and (4) control for effects of factors such as occupational exposures, cigarette smoking, etc. In addition, past epidemiologic studies have not had access to any human histologic specimens in relation to the exposure groups under study nor have specific mechanisms been investigated to explain any of the symptom or functional outcomes observed.

Histologic and Immunologic Effects

Sherwin has presented some provocative preliminary, histologic data and uses it to offer a hypothesis on the importance of pathologic changes in the centriacinar region (CAR)

of the lung in relation to chronic pulmonary effects of oxidant air pollution (Sherwin, 1991; Sherwin and Richters, 1991). Only the publication that presents the primary data (Sherwin, 1991) is reviewed here, because there is some redundancy in the two available publications. Sherwin (1991) obtained lungs from 107 subjects, 15 to 25 years of age, who died of a sudden death without evidence of overt disease, lived in Los Angeles County, had no autopsy evidence or history of drug use, and had no lung trauma. Abnormalities of the CAR were evaluated by a pathologist who was "blinded" to basic demographic data. Centriacinar region disease was defined as the extension of a respiratory bronchiolitis into the proximal acinar structures (i.e., chronic inflammatory cells and histiocytes into alveolar ducts, sacs, and alveoli immediately adjacent to a respiratory bronchiole). The odds ratio for severe CAR disease in subjects who lived in metropolitan Los Angeles versus those who lived in other cities in Los Angeles County was 4.0 (95% confidence limit [CL], 1.4 to 11.3; a calculation based on data in Sherwin [1991] Tables 2 and 3).

Unfortunately, no exposure data (or lifetime residence data) were available for the subjects in the Sherwin study, nor were smoking histories, cotinine results, or occupational histories available. The smoking history data is of critical importance because respiratory bronchiolitis has been shown to be an early pathologic change found in the pulmonary airways of young smokers (Niewoehner et al., 1974). Additional problems for this study were the fact that most subjects were of low socioeconomic status, and only 10 of the subjects were female. Furthermore, the study is limited by a lack of quantitative morphometry on the lung specimens and by the lack of a control group from an ambient environment with low oxidant pollution. Therefore, although Sherwin's data are of considerable interest, particularly in relation to the primate O_3 exposure data that show similar effects (see Chapter 6, Section 6.2.4), they currently are not of value in the determination of appropriate human exposure levels for O_3, nor do they even establish the fact that the oxidant environment found in metropolitan Los Angeles, indeed, is responsible for the observed pathologic changes.

Zwick et al. (1991) carried out a study of allergic sensitization and cellular immune responses in children (median age of 11 years) from four schools in two Austrian cities. Two years of meteorologic data and continuously measured levels of SO_2, NO_2, and O_3 were available for both cities. Monitors were within 2 km of the study schools, except for one O_3 monitor that was 13 km from a school in the "high"-O_3 area. "Allergic diseases" (rhinitis, conjunctivitis, and asthma), response to prick test antigens, total IgE concentration, number of subjects with IgE >100 kU/L, and total IgG concentration did not differ between the subjects in the two cities. Adjustment for sex, age, active and passive smoking, and types of cooking and home heating did not alter the results. Children from the high-O_3 environment had small, but statistically significant, decreases in the absolute and relative numbers of OKT4+ (helper/inducer) T cells and OKNK+ (natural killer) cells and increases in OKT8+ (suppressor) T cells. Adjustment for active and passive smoking and recent respiratory illness did not alter the results. The frequency of subjects with a measurable PD_{20} to histamine also was increased in the high-O_3 area. No relationship between the T-cell findings and PD_{20} or any of the other immunologic markers are provided.

The Zwick et al. (1991) results are limited by lack of any exposure data and by lack of detail for the O_3 and other ambient air pollution data. Except for data on the average percentage time above specific levels of O_3, there are no useful data that can be applied to the observations reported. Moreover, the differences observed in the various T-cell subsets were relatively small and of questionable biological significance. There are no analyses that

relate the T-cell findings to the clinical and functional data (see Table 7-25) that are reported. Finally, although the communities were said to be similar on all meteorologic and other ambient pollution data, inspection of the author's Table 1 (Zwick et al., 1991) indicates that the mean (averaging time not given) NO_2 levels in the low-O_3 community were fourfold greater than those in the high-O_3 community (42 $\mu g/m^3$ versus 11 $\mu g/m^3$). No data on acid species or particles are provided, although both study cities were free of heavy industry and heavy traffic.

Calderon-Garciduenâs et al. (1992) have studied chronic exposure to the ambient air of southwestern metropolitan Mexico City in relation to histologic abnormalities of the nasal mucosa. The exposed group consisted of subjects who spent at least 8 h/day while working at a naval hospital in southwestern metropolitan Mexico City. Ninety-two percent of the group lived in the same area as the hospital, and all had lived in southwestern metropolitan Mexico City for >2 mo (n = 47). Controls consisted of (1) subjects who lived in Veracruz and who had not left this area over a period of at least 5 years before the onset of the study (n = 12) and (2) new arrivals (<30-day residence in southwestern metropolitan Mexico City) at the naval hospital who came from low-O_3, "non-polluted" ports (n = 17). Nasal biopsies were obtained for all subjects in May through June, 1990, as were histories on residence, smoking, occupation, allergies, etc. All three groups were matched for age, sex, and occupation. There were no differences in familial allergy history or personal smoking (specific data not given in paper). There was a progressive increase in both nasal symptoms and nasal histologic abnormalities in relation to presumed O_3 exposure (Veracruz < new arrivals < long-term residents of southwestern metropolitan Mexico City). The principal histologic change was basal cell hyperplasia, with squamous cell metaplasia and mucosal atrophy occurring less frequently. Only 11% of those with >60-day residence in southwestern metropolitan Mexico City showed normal mucosa.

Unfortunately, no ambient air data were presented for SO_2 or particles, which are said to be low relative to other parts of the city, or other pollutants that could be present. In addition, because the monthly average maximal O_3 concentrations are (and have been since late 1986) well above the current U.S. 1-h standard of 120 ppb, the Calderon-Garciduenâs et al. (1992) data are of limited value to understanding low ambient O_3 exposures. (This conclusion probably applies even if one considers the different concentrations represented by a given parts-per-billion value at different altitudes.) Subjects in southwestern metropolitan Mexico City are subjected to O_3 levels of between 100 and 400 ppb for several hours per day in the winter and spring. Despite the lack of data on other air pollutants and specific exposure data for individual subjects, this study does provide useful evidence to suggest upper respiratory damage as a consequence of prolonged exposure to ambient air mixtures.

Pulmonary Function, Respiratory Symptoms, and Chronic Respiratory Disease

The Adventist Health Smog Study. Since the publication of the 1986 criteria document (U.S. Environmental Protection Agency, 1986), a number of studies have been published that attempt to define chronic respiratory system health effects in relationship to ambient O_3 concentrations (see Table 7-26). Among these, the series of publications from the AHSMOG study (Hodgkin et al., 1984; Euler et al., 1987, 1988; Abbey et al., 1991a,b) will be discussed first and as a set.

Table 7-25. Pathologic and Immunologic Changes Associated with Chronic Ozone Exposure[a]

Concentrations(s)		Pollutants and Environmental Variables	Study Description	Results and Comments	Reference
ppm	$\mu g/m^3$				
Not provided		Not provided, Los Angeles County, not further specified	Autopsy study of lungs from sudden death victims 15 to 25 years old whose residence was Los Angeles County; examination of lungs for inflammatory changes in CAR of lungs.	Most severe CAR disease in residents of metropolitan Los Angeles County versus other county areas; data limited by lack of smoking history, personal exposure and occupational data; interesting hypothesis, but role of O_3 unknown.	Sherwin and Richters (1991)
0.095 to 0.188, time metric not given	186 to 368	O_3	Study of allergic sensitization and cellular immune responses in children (median age, 11 years) in two Austrian cities, 1989.	Small increases in OKT4+ (helper/inducer) and OKT8+ (suppressor) T-cells and small decrease in natural killer cells in "high" ozone community; increase in number of subjects with measurable PD_{20} histamine in "high" ozone area; no relationship between T-cell findings and any clinical immunologic measure, lung function, or PD_{20}; meaning of results unclear.	Zwick et al. (1991)
0.150 to 0.275 monthly average	Approx. 294 to 539	O_3	Study of nasal histology in persons living in southwestern Mexico City and Veracruz; subjects matched on age, sex, occupation; similar allergy and smoking histories.	Increased occurrence of nasal dysplasia in southwestern Mexico City residents, especially those with more than 5 years residence; no data on other air pollutants; data not directly relatable to U.S. conditions because Mexico City residents are exposed to O_3 levels between 0.1 and 0.4 ppm for several hours each day, all year long, with relatively few days below 0.1 ppm.	Calderon-Garciduenâs et al. (1992)

[a]See Appendix A for acronyms and abbreviations.

Table 7-26. Effects of Chronic Ozone Exposure on Pulmonary Function, Respiratory Symptoms, and Chronic Respiratory Disease[a]

Concentrations(s) ppm	Concentrations(s) $\mu g/m^3$	Pollutants and Environmental Variables	Study Description	Results and Comments	Reference
0.033 median average annual hourly value	65	O_3	Study of relationship of air pollution to levels of FVC, FEV_1, and PEFR based on 1976 to 1980 supplement to NHANES and data from EPA SAROAD monitoring system; subjects 6 to 24 years of age; exposure values based on hourly O_3 values for previous 365 days; data for TSP, NO_2, and SO_2; and data for important demographic, smoking, and health covariates.	Nonlinear relationship between annual average O_3 and function measurements with threshold at approximately 0.040 ppm; findings limited by inability to control for multiple pollutant effects, relatively crude assignment of exposure; data consistent with effect on forced flow at O_3 levels at or below 0.12 ppm.	Schwartz (1989)
0.034 to 0.050 90th percentile annual mean 1-h daily max	67 to 98	O_3	1983 to 1984 cross-sectional study of 2nd- to 6th-grade students in Ontario and Manitoba, Canada; data on SO_2, NO_2, nitrates, and sulfates; respiratory health, demographic, smoking, and home cooking fuel data; and spirometry.	Ontario town had more O_3 days >0.080 ppm; small decrements (≤2%) in FVC and FEV_1 were found in the Ontario town compared to the Manitoba town; any O_3 possible effects were completely confounded with SO_4 effects.	Stern et al. (1989)
0.024 to 0.031 annual mean 1-h daily max	47 to 61	O_3	1985 to 1986 cross-sectional study of 7- to 11-year-old children from (n = 3,945) five rural towns in Ontario and five towns in Saskatchewan, Canada; data on SO_2, sulfates, NO_3, NO_2, and PM_{10}; respiratory health multiple covariates; spirometry including flow at mid-lung volumes.	Ontario towns had higher levels of O_3 and SO_4 in summer months and for 90th and 99th-percentiles of distributions; 90th percentile mean 1-h maxima were 80 ppb vs. 47 ppb for O_3 and 11.5 $\mu g/m^3$ vs. 3.1 $\mu g/m^3$ for SO_4; magnitude of FEV_1 and FVC effects was similar to Stern et al. (1989); no effect for mid-volume flows, except for subjects with asthma; coincidence of increased O_3 and SO_4 precludes definite statements concerning O_3 effects.	Stern et al. (1994)
0.008 to 0.118 average hourly concen-trations, 1974 to 1979	16 to 231	O_3	Study of chronic respiratory symptoms in adults with use of 1979 National Health Interview Survey data and 1974 to 1979 EPA SAROAD data; data on respiratory health, demography, and smoking; and data for TSP.	Data for only 29% of those eligible could be used; average hourly O_3 concentration over period 1973 to 1979 associated with report of sinusitis and hay fever after control for covariates and TSP; no association with asthma or emphysema; large amount of data reduction, lack of adequate exposure assignment, lack of occupational exposure histories, and lack of adequate data on other pollutants make results very difficult to interpret.	Portney and Mullahy (1990)

Table 7-26 (cont'd). Effects of Chronic Ozone Exposure on Pulmonary Function, Respiratory Symptoms, and Chronic Respiratory Disease[a]

Concentrations(s) ppm	Concentrations(s) $\mu g/m^3$	Pollutants and Environmental Variables	Study Description	Results and Comments	Reference
0.015 to 0.052 average HMV	29 to 102	O_3	Cross-sectional study of children ages 6 to 15 years in a community in Austrian alps divided into three zones based on SO_2, NO_2, and O_3; respiratory health, demographic, and spirometry data.	Only difference in respiratory history was increased adjusted prevalence of asthma in zone with highest O_3 (6.4%; 0.052 ppm HMV) vs. the zones with lower O_3 concentrations (4.8%; 0.015 ppm HMV; 2.7%, 0.026 ppm HMV); no meaningful differences in spirometry indices; data limited by use of single monitoring site for 1,200 km^2 area; effects of SO_2 and NO_2 on asthma prevalence not well studied.	Schmitzberger et al. (1993)
0.10 to 0.20 3-mo mean daily peak hourly values for Lancaster and Glendora, respectively	196 to 392	Oxidants	5-year follow-up of Lancaster and Glendora, CA, cohorts; from UCLA population study of CORD restricted to nonsmoking, non-Hispanic whites, 7 to 59 years old.	No difference in respiratory symptoms over follow-up for either community; across all age groups, slope of Phase III of N_2 washout deteriorated more rapidly in Glendora; in subjects $\geq$14 years of age, more rapid decrease in spirometric indices in Glendora; interpretation hampered by large losses to follow-up, inability to disentangle multiple pollutant effects.	Detels et al. (1987)
0.04 to 0.07 mean peak daily peak hourly values 1972 to 1981; Long Beach and Lancester, respectively	78 to 137	Oxidants	5- to 6-year follow-up of Lancaster and Long Beach, CA, cohorts from UCLA CORD study; Long Beach with higher NO_2, SO_4, and TSP than Lancaster.	All reported excess functional decline for Long Beach likely due to bias in decline estimates between locations; data not useful with regard to possible O_3 effects.	Detels et al. (1991)
Not reported		Oxidant	Prevalence of respiratory symptoms in nonsmoking Seventh Day Adventists residing for at least 11 years in high- (South Coast) and low- (San Francisco, San Diego) photochemical air pollution areas of California; ARB regional air basin monitoring data for oxidants, NO_2, SO_2, CO, TSP, and SO_4 from 1973 to 1976.	Slightly increased prevalence of respiratory symptoms in high pollution area; after adjusting for covariables, 15% greater risk for COPD due to air pollution (not specific to oxidants); past smokers had greater risk than never-smokers; when past smokers were excluded, risk factors were similar. In addition, insufficient exposure assessment and confounding by environmental conditions limit the quantitative use of this study.	Hodgkin et al. (1984)

Table 7-26 (cont'd). Effects of Chronic Ozone Exposure on Pulmonary Function, Respiratory Symptoms, and Chronic Respiratory Disease[a]

Concentrations(s) ppm	Concentrations(s) $\mu g/m^3$	Pollutants and Environmental Variables	Study Description	Results and Comments	Reference
Not reported		Oxidant	Cross-sectional analysis of above populations; uses hours above various "threshold" values for oxidants, TSP, SO_2 based upon California, EPA, and World Health Organization max levels; period covered, 1966 to 1976; data available for important covariates (sex, occupation, environmental tobacco smoke, race, age, education, past smoking).	OX (10) most significantly associated with COPD after adjustment for covariates; number of hours above higher thresholds less significant; when TSP, SO_2, and OX (10) entered in same regression, TSP (200) only pollutant associated with COPD; high correlation between OX (10), TSP (200), and SO_2 (hours more than 4 pphm). Improved exposure assessment over previous paper; however, no clear statement possible about effects of oxidants due to colinearity with TSP and SO_2.	Euler et al. (1988)
Not reported		O_3	Same as Abbey et al. (1991a) but analysis applied to COPD severity and a "multi-pollutant" analysis performed; also evaluated effect of using data for different time periods of ambient air monitoring.	Cumulative incidence of COPD symptoms when each pollutant entered separately, similar to above study; joint effects of OZ (10) and TSP (200) and mean concentrations of each pollutant evaluated only for cumulative asthma incidence; TSP (200) entered logistic regression in preference to OZ (10) but mean O_3 concentration entered in preference to mean TSP; change in asthma severity associated with mean O_3 concentration (1977 to 1987) and with exceedance frequency for OZ (10), OZ (12), and TSP (200) considered separately; findings for asthma severity similar to cumulative incidence when TSP and O_3 evaluated together; in no analysis did TSP and O_3 both remain jointly significant, nor were there any interactions; data unable to unequivocally disentangle effects of individual pollutants.	Abbey et al. (1993)

[a]See Appendix A for acronyms and abbreviations.

The basic population for these studies represents California-resident, Seventh-Day Adventists aged ≥25 years of age who had lived 11 years or longer (as of August 1976) in either a high-oxidant-polluted area (South Coast Air Basin [Los Angeles and vicinity] and a portion of the nearby Southeast Desert Air Basin) or a low-pollution area (San Francisco or San Diego). This sample was supplemented by an additional group of subjects who met the 11-year residence requirement but who came from low-exposure rural areas in California. The total, baseline sample (March 1977) comprised 8,572 individuals, of whom 7,267 enrolled. From this group, 109 current smokers and 492 subjects who had lived outside of the designated areas for a portion of the previous 11 years were excluded. Detailed respiratory illness and occupational histories were obtained. In these studies, "COPD" refers to "definite chronic bronchitis", "definite emphysema", and "definite asthma" as defined by the study questionnaire. Measures of pulmonary function are not included.

Air monitoring data were obtained from the California ARB monitoring system. Ninety-nine percent of the subjects (excluding the rural supplement) lived at a distance from the nearest ARB monitoring site that was considered to provide relatively reliable concentration estimates for the outdoor, ambient environment at their residence. Concentrations at the monitors were interpolated to the centroid of each residential zip code from the three nearest monitoring sites with the use of a $1/R^2$ interpolation. Subsequent development of exposure indices took account of the improvements in ARB data after 1973. Data were available for total oxidants, O_3, TSP, SO_2, NO_2, CO, and SO_4 (excluding 1973 to 1975).

The initial report from this study was summarized in the 1986 criteria document (U.S. Environmental Protection Agency, 1986). Based upon a multiple logistic regression that adjusted for smoking, occupation, race, sex, age, and education, it was estimated that residence in the South Coast Air Basin conferred a 15% increase in risk for prevalent COPD. No estimates of exposure were provided, and the data were considered to be of limited utility.

In their 1988 publication, Euler et al. provided exposure estimates based on the cumulative number of hours, over 11 years prior to the baseline, that individuals lived in environments at various oxidant thresholds, beginning at 10 pphm [OX (10)] (196 $\mu g/m^3$) and the total dosage to which they would be exposed. The estimates in this report did not correct for time spent indoors. When the OX (10) was the only pollutant considered, each 750 h/year increment in exposure was associated with a 20% increase in risk for COPD in a multiple logistic regression analysis that adjusted for effects of occupation, passive exposure to tobacco smoke, personal smoking, sex, age, race, and education (baseline data only). Moreover, the data were compatible with a threshold effect at 10 pphm. However, when hours above a TSP concentration of 200 $\mu g/m^3$ [TSP (200)] and SO_2 concentration of 4 pphm were included in the logistic regression model, only TSP (200) was associated with the occurrence of COPD. No significant interactions were found between the various pollutant thresholds. The authors noted that their failure to control for time spent indoors may have led to an underestimation of the oxidant effect. Moreover, the fact that 74% of the variance of OX (10) was explained by the other pollutants certainly reduced the power of this study to detect an independent effect of oxidants on the occurrence of COPD. The authors also noted the limitations imposed by the cross-sectional nature of the data that were used in this analysis. Thus, on the basis of this study, no clear statement could be made about the chronic respiratory system effects of oxidant exposure.

A major improvement in the methods for assessment of exposure was presented in Abbey et al (1991a). Previous exposure estimates were refined by the computation of "excess concentrations" (concentration minus cutoff, summed over all relevant time periods and corrected for missing data). Exposures also were corrected for time spent at work and time away from residence, with estimates provided for the environments where work occurred and for geographic areas away from residence. The quality of the interpolations (in terms of distance of monitor from residence zip codes) also was evaluated and incorporated into the estimates. Adjustments were made for the time spent indoors by individuals. New indices were developed that were based on O_3, rather than on total oxidants. The investigators demonstrated correlation coefficients of 0.98 between monthly mean total oxidants and O_3 at concentrations ≤ 12 pphm. (It should be noted that a more appropriate comparison would have been between the mean and the differences of the two measurements.)

The above estimates were applied to data that included 6 years of follow-up of the study population (Abbey et al., 1991b). This analysis focused on incident occurrence of obstructive airways disease (AOD—same definition as for COPD above). Incident symptoms of AOD were significantly associated with hours above several TSP thresholds, but not with hours above any O_3 threshold. There was a suggestion of an association between hours above 10 pphm O_3 [OZ (10)] and the 6-year cumulative incidence of asthma [RR for 500 h/year above OZ (10) = 1.40 (95% CL = 0.90 to 2.34)] and definite bronchitis (RR = 1.20 [95% CL = 0.97 to 1.53]). Approximately 43% of the study population experienced at least 500 h in excess of the OZ (10) criterion. Cumulative incidence estimates were adjusted with the use of Cox proportional hazard models for the same variables noted in the original publication of Hodgkin et al. (1984), as well as the presence of possible symptoms in 1977 and childhood respiratory illness history. None of the analyses included both O_3 and TSP thresholds. No data were provided on the details of the subjects available for the prospective analysis and their representativeness versus the entire base population. Therefore, assuming no bias due to selective loss to follow-up, these data are consistent with a small O_3 effect and are limited by the same considerations of colinearity and subsequent reduction of power noted above.

Another analysis by Abbey et al. (1993) evaluated changes in respiratory symptom severity with the TSP and O_3 thresholds noted above. In this analysis, logistic regression, rather than Cox proportional hazard modeling, was used to assess cumulative incidence of components of the COPD/AOD complex; and multiple, linear regression was used to evaluate changes in symptom severity. When O_3 was considered by itself, there was a trend toward an increased risk of asthma for a 1,000-h average annual increment in the OZ (10) criterion (RR = 2.07, 95% CL = 0.98 to 4.89). In this analysis, there was a suggestion that recent ambient O_3 concentrations were more related to cumulative incidence than past concentrations. Change in asthma severity score was associated significantly with the 1977 to 1987 average annual exceedance frequency for O_3 thresholds of 10 and 12 pphm. No significant effects were found for COPD or bronchitis alone. In contrast to the above study of cumulative incidence, the investigators carried out an analysis in which TSP (200) and OZ (10) were allowed to compete for entry into a model to evaluate asthma cumulative incidence and changes in severity. In the cumulative incidence model that employed exceedance frequencies (number of hours above threshold), TSP (200) entered before OZ (10); when average annual mean concentrations were used, O_3 entered before TSP. From this, the authors concluded that both TSP and O_3 were relevant to asthma cumulative

incidence. In no case did both pollutants remain significant simultaneously in the same regression. No interactions were observed between TSP and O_3 for either metric. A similar result was observed for change in asthma severity. As in previous analyses, there was a high correlation between TSP (200) and OZ (10) exceedance frequencies (0.72) and their respective average annual mean concentrations (0.74).

The AHSMOG study represents the most extensive effort to date to provide realistic exposure estimates within the constraints of a large, population-based study. Moreover, the exposure estimates for photochemical oxidants have been tied to current O_3 levels and have taken into account many of the sources of inaccuracy and imprecision in the assignment of exposures to individuals (short of detailed personal monitoring). As such, they do represent a considerable improvement over all other studies to date. Nonetheless, it is not possible from these data to determine if there is an effect of O_3 on the outcomes that were studied. This largely is due to the difficulty of partitioning effects between O_3 and particles.

Other Studies. Subsequent to the publication of the 1986 criteria document, two additional publications have emerged from the UCLA study (Detels et al., 1987, 1991). The data presented are derived from the same population bases that were used in previous publications; and, therefore, they are subject to the same limitations that were cited in the introduction to this section.

In 1987, Detels et al. reported a 5-year follow-up study of white, non-Hispanic subjects from the Lancaster and Glendora study areas. The 12- and 3-mo mean peak hourly total oxidant values from 1972 to 1982 for Lancaster and Glendora were 7 and 10 pphm and 11 and 20 pphm, respectively. Only 47 and 58% of subjects, respectively, were retested with both the questionnaire and measures of lung function. Effects of air pollution on the days of testing were evaluated by comparing lung function test results in a subgroup of individuals who were tested three times at 3- to 4-mo intervals. No effect was observed, but the power to find differences was low. Over the follow-up period, there were no changes in reported respiratory symptoms for either community. In adults ($\geq$19 years of age) who never smoked, all spirometric and nitrogen-washout results showed more rapid deterioration in Glendora. Differences were significant only for mid-expiratory flows and for slope of Phase III from the nitrogen-washout curve. The effects were greater in females, in whom changes in FEV_1 also were significant. In subjects less than 19 years of age, only changes in slope of Phase III were significant, although FVC in Glendora females was lower than that observed in Lancaster.

The results of this study remain limited by the lack of adequate exposure data and the failure to control for the possible effects of other ambient pollutant differences between the communities. Problems with loss to follow-up represent a significant issue, especially for the pulmonary function measurements, given that approximately 50% of the original subjects were not available for repeated testing. Baseline comparability is also of concern because subjects who were retested in Lancaster had a better slope of Phase III than those not retested. Because this measure most consistently differed between the two study communities, the possibility of selection bias is very real. Overall, these results do not strengthen the usefulness of this study for the attribution of an effect of oxidant exposure on respiratory health.

The 1991 report from the UCLA group compared Lancaster with Long Beach, the latter area with relatively high levels of SO_2, sulfates, NO_x, and hydrocarbons, as well as increased total oxidant levels (mean 1-h daily peak values, 1972 to 1982, 30 pphm versus

110 pphm, respectively) (Detels et al., 1991). As above, the analysis was restricted to non-Hispanic whites who never smoked cigarettes and with 5 years of follow-up. Only 47% of the Lancaster cohort and 44% of the Long Beach cohort had pulmonary function retested on two occasions. Over the age range of 25 through 59 years, changes in slope of Phase III of the nitrogen-washout curve and most spirometric indices were significantly worse in Long Beach, compared to Lancaster. In subjects under 25 years of age, there were significant differences in slope of Phase III, especially in subjects 7 to 10 years of age.

All of the limitations identified for the 1989 report apply to this report as well. Moreover, comparison between the two communities of the interlaboratory differences (mobile laboratory versus UCLA reference laboratory; 3% sample) indicated that average annual decrements in FEV_1 were exaggerated by -13 mL/year (standard error ± 7 mL/year) in Long Beach versus -2 mL/year (± 7 mL/year) in Lancaster. Application of this difference to the data in their Table 6A would suggest that the "significant" difference in FEV_1 for both males and females may be largely, if not entirely, due to bias. Thus, all of the functional differences reported in this study are suspect on this basis alone. This, of course, ignores any additional biases that may have been due to the large losses to follow-up in both communities.

A number of additional studies have addressed data relevant to the chronic effects of O_3 on respiratory health (see Table 7-26).

Schwartz (1989) evaluated the effect of air pollution on children and young adults ages 6 to 24 years with the use of data derived from NHANES II (Second National Health and Nutrition Examination Survey, February 1976 to 1980). All individuals in each census tract were assigned average pollutant values derived from monitors located within 10 miles of the centroid of the census tract. Average hourly values for the 365 days preceding spirometry were used, and an annual average was created for O_3 (EPA Storage and Retrieval of Aerometric Data [SAROAD] database). For O_3 (chemiluminescence and ultraviolet [UV] spectroscopy), six of the seven hourly readings between 11:00 a.m. and 5:00 p.m. were required to include a day's data. Only 1,005 of the 3,922 (25.6%) of the subjects lived close enough to a monitor to have O_3 exposures assigned to them. Data for TSP, NO_2, and SO_2 were assigned to 47.1, 13.6, and 21.2% of the subjects, respectively. Analyses were restricted to consideration of single pollutants, because the author reported that there was insufficient overlap between the locations where data were available for all or any combination of pollutants. Data for a variety of relevant personal and demographic covariates were available. Statistical analyses appropriate to the correlation structure of the data (induced by the sampling design of NHANES) were utilized. There was a nonlinear relationship between the annual hourly average O_3 concentration and FVC, FEV_1, and PEFR. A threshold of effect around 0.04 ppm was observed, above which there appeared to be a linear decline in FVC (only data shown graphically). The effect persisted after control for sex, race, age, family income, educational level, chronic respiratory symptoms, and smoking history. Results were little affected by region or use of a 2-year averaging time. Ozone levels above the threshold were significantly associated with an FVC $<70\%$, a result not seen for TSP but observed for NO_2.

The major limitation of the Schwartz (1989) analysis is the inability to distinguish between the effects of O_3, TSP, and NO_2 and the choice of only a single metric for O_3 (hourly average). Support for the former concern can be seen in the similarity of the effects of NO_2 and O_3 in the logistic regression analyses, which suggests that the results could reflect the joint effect of a number of species in a complex oxidant environment. The

operating assumption that near-term (1- to 2-year) exposure reasonably reflects a "lifetime" of exposure is highly suspect in the mobile U.S. population. In fact, restriction of the analysis to subjects who still resided in the state in which they were born led to slight reductions of O_3 effect, especially for FEV_1 and PEFR. Despite these limitations, the data do suggest that, for children and young adults, if there is a chronic O_3 effect (or, more accurately for these data, a subchronic effect) on lung function, it could occur at levels at or below 120 ppb. However, the particular pattern of exposure (peaks, season, etc.) that may be relevant cannot be discerned from these data.

In 1989, Stern et al. reported a cross-sectional study, conducted in 1983 and 1984, of the relationship between respiratory health effects of second- through sixth-grade children in two Canadian communities (one in southern Ontario and one in southern Manitoba). The Ontario region was characterized by low levels of gaseous pollutants (SO_2 and NO_2) and moderately elevated levels of particulate sulfate, FPs, and O_3. Frequent episodes of elevated sulfate and O_3 concentrations occurred in the summer and early fall. The Manitoba community was not subject to the same pattern of air pollutants. Gases and O_3 (measured by chemiluminescence) were sampled continuously, and TSP, sulfates, and total nitrates were sampled every sixth day. Fixed monitoring stations were established at the center of each community, and monitoring was carried out from October 1983 to April 1984. Ozone measurements in Ontario were derived from sites between 35 to 45 km from the study area. Average annual maximum O_3 concentrations were similar in the two communities (0.136 and 0.130 ppm in Ontario and Manitoba, respectively), but the frequency of elevated O_3 events (>0.080 ppm, Canadian standard for 1-h maximum) was more frequent in Ontario (30 days) than in Manitoba (3 days) in 1983. Ninety-two percent of the subjects (n = 1,317) provided data from detailed questionnaires, but only 70% (1,010) provided spirometric data (tested in fall and winter months). There were no meaningful differences in the prevalence of all of the respiratory health outcomes studied after adjustment for parental smoking, gas cooking, sex, length of residence, parental education, and past respiratory illness history. Ontario children had a 2% lower FVC (adjusted for age, sex, height, and parental smoking) and a 1.7% lower FEV_1; both differences were statistically significant. The differences were somewhat greater when children with underlying respiratory illness or symptoms were excluded from the analyses. These data are very difficult to interpret in relation to O_3 due to the marked colinearity between the O_3 and sulfate levels in Ontario. Moreover, the differences observed in lung function are very small (an average of 50 mL and 40 mL for FVC and FEV_1, respectively) and of questionable importance without further follow-up data on the subjects. Such follow-up data would need to attempt to identify whether the small decrements observed are "across the board" with respect to the overall population or the result of decrements in a susceptible subset of the population, particularly a set of children at the lower end of the pulmonary function distribution. In these children, small decrements might be associated with adverse respiratory effects as a result of their already lowered (absolute or relative) levels of lung function.

Stern et al. (1994) extended the 1983 and 1984 (Stern et al., 1989) study to 10 rural Canadian communities. Five towns in southwestern Ontario and five towns in Saskatchewan were selected and studied between September 1985 and March 1986. Children 7 to 11 years of age were studied (n = 3,945) with techniques similar to the previous study. In 1986, SO_2, NO_2, and O_3 were monitored continuously through a 10-site network (one site in each town). Particles were sampled every 3 days for 24 h in Saskatchewan and every 6 days in Ontario. Annual mean 1-h maximum O_3 concentrations were slightly higher in

Ontario, but the 90th and 99th percentile values were much greater (90th: 80 ppb versus 47 ppb; 99th: 115 ppb versus 57 ppb). This was particularly true for the months of June to August. The levels of PM_{10} and nitrate did not vary between the areas and were well within the Canadian Ambient Air Quality Objectives. Annual mean SO_4 levels were three to four times greater in Ontario communities (6.6 $\mu g/m^3$ versus 1.9 $\mu g/m^3$).

The adjusted (age, sex, parental education, gas cooking, and parental smoking) prevalence of respiratory symptoms did not differ among the 10 communities. Adjusted (height, weight, plus the above adjustment factors) FVC and FEV_1 averaged 1.7 and 1.3% less, respectively, in the five Ontario towns. No differences were observed for PEFR, $FEF_{25-75\%}$, or $\dot{V}_{max50\%}$. The results did not change when the analysis was restricted to life-long residents or to children without respiratory symptoms. Although not statistically significant, Ontario children with doctor-diagnosed current asthma had $FEF_{25-75\%}$ and $\dot{V}_{max50\%}$ levels that were 6.6 and 6.5%, respectively, lower than similar children in Saskatchewan. Overall, the prevalence of asthma was 4% for the entire sample.

These results are consistent, in terms of the magnitude of the FEV_1 and FVC effects, with those of the previous Stern study (Stern et al., 1989). In addition, these data provide suggestive evidence of enhanced effects for children with current asthma. The two major limitations of the study are recognized by the authors: (1) the effects observed cannot be attributed to O_3 or to SO_4 (or acid) aerosols and could be due to either part of the pollutant mixture or attributed to the combination of the component, and (2) the differences in the mean values reported do not take into account the variability in the pulmonary function distribution and the variability of responses across the distribution (see above).

Portney and Mullahy (1990) used the 1979 U.S. National Health Interview Survey and SAROAD data to explore the relationship between O_3 and TSP and chronic respiratory disease. Average hourly O_3 concentrations from 1974 to 1979 were used; data from 1974 to 1979 and data from 1979 alone were evaluated. Individuals were matched to the nearest centroid of the census tract in which they lived in 1979. Individuals were excluded if they lived >20 miles from the nearest monitor. Only 29.3% of the 4,500 adults surveyed who participated in the smoking and respiratory disease supplemental interview and for whom residential data were available could be included. Seven different model specifications (probit analysis) evaluated cumulative (5-year) and 1-year effects of O_3 on various respiratory diseases. Hourly average O_3 concentrations, but not TSP concentrations, over 1974 to 1979 were significantly associated with the report of sinusitis and hay fever after control for smoking, sex, income, race, education, temperature, and stability of residence. In contrast, neither O_3 nor TSP were associated with reported asthma and emphysema. An enormous amount of data reduction, the lack of individual exposure data, lack of specification of the age and sex distribution of the study population, lack of data on occupational exposures, the use of a single O_3 metric, and the restricted formulation of the particulate data all severely limited the usefulness of these data.

Kilburn et al. (1992) studied the effect of "air pollution" on expiratory flows and vital capacity in Mexican-American children in Los Angeles. In 1984, 556 second- and fifth-grade students were studied, and 251 of these were studied again in 1987. The analytical strategy, the losses to follow-up, and the lack of reasonable exposure data make the data from this study virtually uninterpretable.

A study by Castillejos et al. (1992) evaluated the effects of acute exposures to ambient O_3 concentrations on pulmonary function and respiratory symptoms. One-hundred and forty-eight 9-year-old children in the southwest part of Mexico City were studied

between January and June 1988. Weekly spirometric measurements were made over 10 weeks. Ambient air data were obtained from the monitoring system maintained by the Mexican government and included hourly values for temperature, RH, and O_3 concentration. Ozone concentration exceeded 120 ppb on 74% of days and "frequently" exceeded 240 ppb. No data are presented for SO_2 or particles. All subjects had to live within 5 km of a monitoring station. The study demonstrated that levels of FEV_1 and $FEF_{25-75\%}$ were associated with mean hourly O_3 levels in the preceding 24, 48, and 168 h. The authors interpreted their data as consistent with a subchronic effect of O_3 on measures derived from spirometry that may be due to an "inflammatory process". However, this interpretation seems at odds with the statement in the paper that the initial FEV_1 measurements for the group did not differ from those observed in a comparable age group in the Harvard Six Cities Study who were not exposed to O_3 concentrations as high as those reported in this study. If the overall level of pulmonary function of this group does not differ from those children who live in ambient environments with far lower O_3 concentrations, the data would suggest that the subchronic effects observed are not translated into persistent abnormalities, at least as can be observed with spirometry.

Austrian investigators (Schmitzberger et al., 1992) described a cross-sectional study of the effects of O_3 on the respiratory health of 1,156 children, ages 6 to 15 years. Pulmonary function in two different areas with differing "annual" O_3 concentrations (actual metric on which "annual" based not given) were compared (52 ppb versus 26 ppb). No differences were observed for FVC. All flow measures (FEV_1, $FEF_{50\%}$, and $FEF_{75\%}$) were significantly lower in the children in the "high"-O_3 area. These data are of limited value for a variety of reasons, the most important being lack of individual exposure data, lack of data on other pollutants, the uninterpretable specification of "annual" O_3 concentration, lack of data on chronic respiratory illness (especially asthma), and the lack of data on smoking for the teenage members of the subject group.

Schmitzberger et al. (1993), following up on their preliminary data (Schmitzberger et al., 1992), studied additional subjects in the Austrian Tyrolian Alps. Three zones were identified based upon ambient air conditions: (1) Zone 1 was characterized by annual mean SO_2 (UV fluorescence) of 20 $\mu g/m^3$, monthly mean NO_2 (Palmes tubes) of 17 ppb, and annual mean O_3 (chemiluminescence) of 15 ppb (maximum half-hour mean = 102 ppb); (2) Zone 2 was characterized by values of 14 $\mu g/m^3$, 13 ppb, and 26 ppb (112 ppb), respectively; and (3) Zone 3 was characterized by 12 $\mu g/m^3$, 8 ppb, and 52 ppb (146 ppb), respectively. Children ages 6 to 15 years who lived in the study areas for ≥ 3 years were enrolled. Respiratory health questionnaire data and forced expiratory flows were obtained. Full data were available from 81% of the enrolled subjects. Adjusted (age, sex, environmental tobacco smoke, socioeconomic status, and home heating) levels of FVC and forced flows did not follow the gradient in O_3 concentrations. Although Zone 3 differed significantly from Zone 2 on several measures, there were no meaningful differences with Zone 1. Adjusted asthma prevalence was highest in Zone 3 (6.4% versus 4.8 and 2.7% for Zones 1 and 2, respectively). There were no differences for other respiratory symptoms. Although the authors conclude that "residence in the area of elevated O_3 increases the risk...of low small airway-related lung function," careful inspection of the data does not support this conclusion. This conclusion is based on the supposed increased frequency of FEV_1 of less than 70% in Zone 3 relative to the other zones, although the specific data are not provided. Moreover, the mean levels for all functional measurements are lowest in Zone 1, the zone with the lowest O_3 concentrations and the highest SO_2 and NO_2

concentrations. This report is handicapped by the lack of any information that could be used to access individual exposures. Moreover, only a single monitoring station was employed that was placed at the center of Zone 1, which itself was at the center of the study area (1,200 km^2). No information is provided as to how the concentrations of the various pollutants were estimated for Zones 2 and 3. Therefore, these data virtually are of no quantitative value.

Other Chronic Disease Morbidity and Mortality

Only AHSMOG study has provided any data on possible O_3-related health effects other than those related to the respiratory system or malignant disease of the respiratory system (Abbey et al., 1991b; Mills et al., 1991) (see Table 7-27). The population studied and the assignment of exposures has been presented previously (Hodgkin et al., 1984; Euler et al., 1988; Abbey et al., 1991a).

In their initial study based on 6 years of follow-up, Mills et al. (1991) found that for 500 h in excess of the OZ (10) threshold, there was a relative risk of 2.24 for respiratory cancer incidence after adjustment for a number of factors listed previously. When the TSP (200) and OZ (10) thresholds were allowed to compete for entry into a Cox proportional hazards model for respiratory cancer incidence, the O_3 threshold entered in preference to TSP. Ozone exposure was not associated with excess respiratory cancer mortality or incidence of nonrespiratory cancer over the 6-year follow-up period.

A second chronic disease study from the AHSMOG population extended the above observations to include myocardial infarction and all-cause mortality (Abbey et al., 1993). Incident chronic respiratory disease also was included in this analysis. Ambient levels of O_3 were not associated with incidence of myocardial infarction at any of the threshold indices that were tested. Neither the mean concentration of O_3 nor any of the thresholds were associated with incidence of chronic respiratory diseases, as previously defined. However, there was a trend toward an association between 6-year cumulative incidence of asthma and 500-h exceedance of the OZ (10) threshold (RR = 1.40, 95% CL = 0.99 to 2.34).

7.4.2.3 Conclusions

The body of data that has accumulated since publication of the previous air quality criteria document for O_3 (U.S. Environmental Protection Agency, 1986) provides only suggestive evidence for health effects of chronic O_3 exposure. Most of the studies suffer from one or another of the following limitations: (1) simplistic assignment of exposure in terms of choice of O_3 metrics or adequate adjustment for relevant covariates and (2) lack of ability to isolate effects related to O_3 from those of other pollutants, especially the particulate fraction. The AHSMOG study has made substantive progress in the problem of the assignment of individual exposures (Abbey et al., 1991a). Unfortunately, the results from this study cannot disentangle the effects of chronic O_3 exposure from those due to chronic exposure to the particulate fraction of ambient pollution. The study also lacks sufficient power to evaluate the possibility of interactions between O_3 and particulate pollution in relation to health effects. Thus, the overall data are not conclusive, but current evidence is suggestive of possible health effects from chronic exposure to O_3.

Table 7-27. Effects of Chronic Ozone Exposure on the Incidence of Cardiovascular and Malignant Diseases[a]

Concentrations(s) ppm	Concentrations(s) $\mu g/m^3$	Pollutants and Environmental Variables	Study Description	Results and Comments	Reference
Not reported		Hodgkin et al. (1984)	Hodgkin et al. (1984) and Abbey et al. (1991a); analysis based upon exceedance frequencies, 1973 to 1977, and cancer cumulative incidence, 1977 to 1982.	Exceedance of OZ (10) threshold borderline associated with respiratory cancer; no association with mean concentration; multipollutant analysis with TSP (200) and OZ (10), only OZ (10) entered the logistic regression for respiratory malignancy; TSP (200) was significant for females for all malignancy; no association between O_3 and any measure of cancer mortality; overall results suggestive of O_3 effect on respiratory cancer morbidity at level of exposure within range experienced by large percentage of study population.	Mills et al. (1991)
Not reported		See above	See above	No association between any O_3 threshold and all-cause mortality or incidence of myocardial infarction.	Abbey et al. (1991b)

[a]See Appendix A for acronyms and abbreviations.

7.5 Summary and Conclusions

7.5.1 Controlled Human Studies of Ozone Exposure

7.5.1.1 Effects on Pulmonary Function

Controlled human O_3 exposure studies have provided the strongest and most quantifiable exposure-response data on the health effects of O_3. This chapter reviews the results of studies involving subjects exposed to O_3 concentrations ranging from 0.08 to 0.75 ppm O_3 while at rest or during CE or IE of varying intensity for periods of up to 8 h. In many of these studies, small sample size and suboptimal experimental design limit the ability to generalize to the larger population. Of particular concern in considering studies with small sample sizes is the risk of making a beta (Type II) error: the incorrect conclusion that no difference exists between treatments when comparisons are not significantly different. The likelihood of making a Type II error greatly limits the ability to determine the minimum O_3 concentration that results in a significant pulmonary response in the larger population. As a result, the conclusions drawn from many of the studies cited in this chapter may underestimate the presence of responses at low O_3 concentrations in healthy, young adults.

Healthy Subjects

Results from studies of at-rest exposures to O_3 for 2 h in healthy adult subjects have demonstrated decrements in forced expiratory volumes and flows occurring at and above 0.5 ppm O_3 (Folinsbee et al., 1978; Horvath et al., 1979). Airway resistance is not clearly affected during at-rest exposure to these O_3 concentrations.

With moderate IE for 2 h, eliciting a $\dot{V}_E$ of 30 to 50 L/min, decrements in forced expiratory volumes and flows, secondary to decreases in IC, have been observed in healthy adult subjects at and above 0.3 ppm O_3 (Folinsbee et al., 1978; Seal et al., 1993). With IE ($\dot{V}_E \geq 65$ L/min), pulmonary symptoms and decrements in forced expiratory volumes and flows are present following 2-h exposures to 0.12 ppm O_3 (McDonnell et al., 1983). Symptoms are present and decrements in forced expiratory volumes and flows occur at 0.16 to 0.24 ppm O_3 following 1 h of continuous heavy exercise ($\dot{V}_E \approx 55$ to 90 L/min) (Adams and Schelegle, 1983; Folinsbee et al., 1984; Avol et al., 1984; Gong et al., 1986) and following 2 h of intermittent heavy exercise ($\dot{V}_E \approx 65$ to 68 L/min) (McDonnell et al., 1983; Kulle et al., 1985; Linn et al., 1986). With longer exposures of 4- to 8-h duration, responses have been observed at lower O_3 concentrations and lower ventilation rates. In the range of concentrations between 0.08 and 0.16 ppm, a number of studies using moderate IE and durations between 4 and 8 h have shown significant responses under the following conditions: 0.16 ppm for 4 h of IE at $\dot{V}_E \approx 40$ L/min (Folinsbee et al., 1994), 0.08 to 0.12 ppm for 6.6 h of IE at $\dot{V}_E \approx 35$ to 40 L/min (Folinsbee et al., 1988; Horstman et al., 1990), and 0.12 ppm for 8 h of IE at $\dot{V}_E \approx 40$ L/min (Hazucha et al., 1992). Symptom and spirometry responses were increased, with increased duration of exposure, O_3 concentration, and total ventilation. Airway resistance is only modestly affected with moderate or even heavy exercise combined with O_3 exposure to concentrations as high as 0.5 ppm O_3 (Folinsbee et al., 1978; McDonnell et al., 1983; Seal et al., 1993). Increased breathing frequency (f) and decreased V_T, while maintaining $\dot{V}_E$, occur with exposure to 0.20 to 0.24 ppm O_3 when combined with heavy exercise for 1 to 2.5 h (McDonnell et al., 1983; Adams and Schelegle, 1983). Differences in response to a given O_3 concentration among individuals have been shown to be reproducible (Gliner et al., 1983; McDonnell et al., 1985b), indicating some individuals are consistently more responsive to O_3 than others.

Group mean decrements in pulmonary function can be estimated roughly when expressed as a nonlinear function of effective (i.e., exposure) dose of O_3, the simple product of O_3 concentration, mean ventilation, and exposure duration (Silverman et al., 1976; Folinsbee et al., 1978; Adams et al., 1981). The O_3 concentration appears to make a greater impact on the pulmonary function response than does $\dot{V}_E$ or exposure duration (Folinsbee et al., 1978; Adams et al., 1981), and, indeed, Larsen et al. (1991) suggest an exponent of approximately 4/3 for the O_3 concentration. Another way of expressing this relationship is that doubling the O_3 concentration under any given exposure scenario will have a greater impact on spirometry responses than doubling either $\dot{V}_E$ or exposure duration. However, at any given O_3 concentration, the major external determinants of response are $\dot{V}_E$ and exposure duration. Because of the broad range of intersubject variability, and the inability to identify characteristics that influence this variability (other than age), efforts to estimate or model individual responses have so far been fruitless (McDonnell et al., 1993). Nevertheless, prediction of group mean FEV_1 responses using the variables of the O_3 concentration, $\dot{V}_E$, and exposure duration can be successful (Adams et al., 1981; Folinsbee et al., 1978, 1988; Hazucha, 1987; Hazucha et al., 1992; Larsen et al., 1991; McDonnell et al., 1993).

In acute O_3 exposure studies of 3 h or less in duration, the responses observed during and following acute exposure to O_3 at concentrations between 0.12 and 0.50 ppm in normal, healthy human subjects include decreases in TLC, IC, FVC, FEV_1, $FEF_{25-75\%}$, and V_T and increases in SR_{aw}, f, and airway responsiveness. Ozone exposure also has been shown to result in the symptoms of cough, PDI, SB, throat irritation, and wheezing. Similar responses are seen with 4- to 8-h exposures in the O_3 concentration range between 0.08 and 0.16 ppm.

When viewed collectively, these physiological and symptom responses may be separated into four general categories, including (1) symptoms, (2) changes in lung volume or spirometry, (3) changes in R_{aw}, and (4) changes in airway responsiveness. These categories are based on the absence of correlation between spirometry responses and change in R_{aw} or airway responsiveness. The attenuation by atropine of R_{aw} but not spirometry responses supports the notion of independent mechanisms. The attenuation by indomethacin or ibuprofen of spirometry responses, but not changes in R_{aw} or airway responsiveness, also supports this categorization. A bronchodilator, albuterol, given to healthy subjects prior to O_3 exposure did not prevent changes in spirometry, symptoms, or airway responsiveness. Symptoms ratings represent reflex responses (e.g., cough) or a perceptual evaluation of consciously appreciated afferent information (e.g., chest tightness, PDI), and it is therefore somewhat difficult to separate these responses from the more objective physiological responses. However, cough and pain on deep inspiration are related temporally to spirometry and breathing pattern responses (i.e., volume-related changes). In repeated exposure studies, changes in spirometry and breathing pattern become attenuated with the same time course as the changes in symptom responses.

Recent multihour O_3 exposure studies indicate that spirometry and symptom responses to concentrations as low as 0.08 ppm occur in healthy subjects with exposures lasting 6 to 8 h. Prolonged exposures (8 h) at lower O_3 concentrations (0.12 ppm) also indicate that there is a plateau of response to O_3 (Hazucha et al., 1992). Although suggested in previous studies (Gliner et al., 1983), such a plateau is difficult to verify with the typical duration of less than 2 h and the large responses seen with higher concentrations. The level of the response plateau (i.e., the spirometry decrement at which the response no longer

changes) must be dependent on the dose rate of exposure (i.e., the product of the O_3 concentration and $\dot{V}_E$) because the magnitude of response at a higher dose rate may greatly exceed the response plateau seen at a lower dose rate. Prolonged exposure studies also suggest that O_3-induced spirometry responses depend on the immediate exposure history. With relatively low dose rates (e.g., Hazucha et al., 1992), responses to exposure that occurred 2 to 4 h previously may influence the current response. The cumulative effect of exposures has not been studied at higher dose rates, but greater persistence of effects may be expected based on the longer recovery period at higher doses rates.

Recovery from O_3 exposure has not been systematically investigated in a large group of subjects, but available information indicates that an initial phase of recovery proceeds relatively rapidly, and some 40 to 65% of the acute response appears to be recovered within about 2 h (Folinsbee and Hazucha, 1989). However, there is some indication that the spirometric responses, at least to higher O_3 concentrations, are not fully recovered within 24 h (Folinsbee and Horvath, 1986; Folinsbee et al., 1994). Collectively, these observations suggest that there is a rapid recovery of O_3-induced spirometry and symptom responses, which may occur during resting exposure to O_3 (Folinsbee et al., 1977) or as O_3 concentration is reduced during exposure (Hazucha et al., 1992), and a slower phase, which, in some cases, may take at least 24 h to complete. Repeated exposure studies at higher concentrations typically show that the response to O_3 is enhanced on the second of several days of exposure. This enhanced response suggests a residual effect of the previous exposure, about 22 h earlier, even though the preexposure spirometry may be the same as on the previous day. The absence of the enhanced response with repeated exposure at lower O_3 concentrations may be the result of a more complete recovery or less damage to pulmonary tissues.

Studies of repeated daily exposure to O_3 have shown that O_3-induced changes in spirometry, symptoms, R_{aw}, airway responsiveness, and airway inflammation are attenuated with repetitive exposure. At higher dose rates, symptom and spirometry responses may be enhanced on the second exposure. Attenuation of response within 3 to 5 days is a consistent finding in repeated exposure studies, regardless of O_3 exposure dose rate, although attenuation of response occurs after fewer exposures at the lower dose rates. The attenuation of response appears to occur more rapidly in less responsive individuals (Horvath et al., 1981) or in responsive subjects exposed to lower O_3 dose rates (Folinsbee et al., 1978, 1994). Loss of attenuation is relatively rapid, with O_3 responsiveness being partially restored within 4 to 7 days (Kulle et al., 1982; Linn et al., 1982b), and normal responsiveness restored within 1 to 2 weeks after a series of 4 or 5 daily O_3 exposures. The attenuation of airway responsiveness may occur somewhat more slowly than that of symptom and spirometry responses. Airway inflammation also appears to attenuate, but less completely than the spirometry responses and with a more gradual recovery (Devlin et al., 1995; Folinsbee et al., 1995). Some markers of inflammation (e.g., LDH and elastase) have not demonstrated attenuation.

The mechanisms leading to the observed pulmonary responses induced by O_3 are beginning to be better understood. The available descriptive data suggest several possible mechanisms, some leading to alterations in lung volumes, symptoms, and exercise breathing patterns, and others leading to increases in central and peripheral R_{aw}. These mechanisms appear to involve (1) O_3 reactions with the airway lining fluid and epithelial cell membranes; (2) local tissue responses, including injury and inflammation; and (3) stimulation of neural afferents (bronchial C fibers) and the resulting reflex responses and symptoms. Much

remains to be understood in order to determine how each event in this cascade contributes to the pulmonary responses induced by acute O_3 inhalation in human subjects.

Subjects with Preexisting Disease

Of the subpopulations studied, those with preexisting impediments in pulmonary function and exercise capacity are of primary concern in evaluating the health effects of O_3 because even a small change in function is likely to have more impact on a person with reduced reserve. Inherent in these studies are several limitations that, at present, hamper the ability to make definitive conclusions regarding the relative O_3 responsiveness of the various groups of subjects studied. Furthermore, it is ultimately necessary to determine whether their responses are representative of the larger population with preexisting disease. These limitations include subject selection (in controlled studies, typically only people with milder disease are selected or volunteer for study), standardized methods for the characterization of some responses, and limited range of exposure doses utilized to examine some endpoints.

These limitations are evident in studies on subjects with COPD, chronic bronchitis, and ischemic heart disease. For patients with COPD performing light to moderate IE, no decrements in pulmonary function were observed after 1- and 2-h exposures to $\leq$0.30 ppm O_3 (Linn et al., 1982a, 1983a; Solic et al., 1982; Kehrl et al., 1985) and only small decreases in forced expiratory volume were observed for 3-h exposures of chronic bronchitics to 0.41 ppm O_3 (Kulle et al., 1984). Small decreases in arterial blood oxygen saturation also have been observed in some of these studies, but the interpretation of these results and their clinical significance is uncertain.

Similar limitations also apply to the early studies examining O_3 effects in adult and adolescent asthmatics. Decrements in pulmonary function were not observed for adult asthmatics exposed for 2 h at rest (Silverman, 1979) or with intermittent light exercise (Linn et al., 1978) to O_3 concentrations of 0.25 ppm and less. Similarly, no significant changes in pulmonary function or symptoms were found in adolescent asthmatics exposed for 1 h at rest to 0.12 ppm O_3 (Koenig et al., 1985) and in adolescent asthmatics and nonasthmatics exposed to 0.12 and 0.18 ppm O_3 with intermittent moderate exercise up to 1 h (Koenig et al., 1987, 1988), although a small decrease in forced expiratory flow at 50% of FVC was observed in asthmatics after exposure to 0.12 ppm O_3. More recent observations by Kreit et al. (1989), Eschenbacher et al. (1989), and Linn et al. (1994) suggest that mild to moderate asthmatics are at least as sensitive to the acute effects of O_3 inhalation as healthy subjects when the asthmatics are exposed to O_3 under conditions that elicit a significant response in healthy subjects. Kreit et al. (1989) and Eschenbacher et al. (1989) exposed adult asthmatic and nonasthmatic subjects to 0.4 ppm O_3 with intermittent moderate exercise for 2 h and observed a greater response in R_{aw}, FEV_1, and $FEF_{25\text{-}75\%}$ in the asthmatic subjects, although changes in FVC and symptoms were similar in both groups. Ozone exposure also resulted in a marked increase in airway responsiveness to methacholine in both the asthmatic and nonasthmatic subjects. These responses take on greater importance when it is considered that the observed O_3-induced pulmonary effects were superimposed on preexisting impairment of pulmonary function and airway responsiveness. In addition, the observations of Koenig et al. (1990) and Molfino et al. (1991) suggest the possibility that acute exposure to O_3 at doses that do not produce measurable pulmonary function decrements may increase the responsiveness of asthmatics to inhaled SO_2 or antigens.

7.5.1.2 Symptom Responses to Ozone

Following exposure to O_3 many subjects report respiratory symptoms, the most common of which are cough, shortness of breath, and PDI. There is a broad range of severity in rating symptom responses among subjects in these studies. A number of 2-h O_3 exposure studies that have examined the exposure dose-pulmonary function and symptom response relationships have included a semiquantitative analysis of symptom responses (Avol et al., 1983; Kulle et al., 1985; McDonnell et al., 1983; Seal et al., 1993). In each of these studies, as O_3 concentration increased, the pulmonary function response became more negative (a decrease in FEV_1), and the level of the respiratory symptoms (cough, shortness of breath, PDI) increased. The mean decrement in FEV_1 in each of these studies was highly correlated ($r > 0.98$ in all cases) with the mean change in symptom rating or symptom score (the determination of symptoms varied between studies). This high correlation results primarily because each of these variables is highly correlated with O_3 concentration. Correlation of individual changes in symptoms and changes in pulmonary function seldom exceed $r = 0.6$ (Horstman et al., 1990). Contributing to this low individual correlation is the fact that symptoms scores have lower test-retest reliability than tests of lung function. For example, McDonnell et al. (1985b) report test-retest coefficients of about 0.9 for spirometry responses but only about 0.8 for symptoms. Thus individual symptom responses are not good predictors of individual pulmonary function responses. However, group mean symptom responses still provide a good marker of the average FEV_1 response to O_3 exposure. In two of the very heavy exercise studies (McDonnell et al., 1983; Avol et al., 1983) symptoms of cough or total respiratory symptom scores were increased significantly at 0.12 and 0.16 ppm O_3, respectively. In the heavy exercise study (Seal et al., 1993), cough symptoms increased significantly at 0.18 ppm O_3. Other studies that support this relationship of symptoms and pulmonary function have been conducted with various exposure durations and exercise intensities (Gong et al., 1986; Horstman et al., 1990) ranging from 1 h of severe exercise to 6.6 h of moderate exercise at O_3 concentrations from 0.08 to 0.20 ppm.

In comparing the spirometry and symptom responses of older adults and young adults exposed to O_3 under the same conditions, Drechsler-Parks et al. (1989) found significantly lower spirometry responses (-19% versus -6% FEV_1) in the older adults. In addition, the incidence of respiratory symptom responses for the three symptoms most commonly reported with O_3 exposure were almost twice as high in the young adults, whereas the incidence of symptoms unrelated to O_3 exposure (e.g., eye irritation, muscle soreness) typically was greater in the older adults. The comparable or greater incidence of nonrespiratory symptoms in the older adults clearly indicates that they felt less respiratory discomfort in conjunction with their smaller spirometry responses. Asthmatics, when compared with nonasthmatics, tend to have greater changes in R_{aw} and expiratory flow with O_3 exposure (Kreit et al., 1989; Horstman et al., 1995) but similar changes in lung volume (i.e., FVC). Asthmatics also have similar symptom responses for cough, PDI, and shortness of breath, although, in one study (Horstman et al., 1995), asthmatics reported a higher incidence of wheezing.

In repeated exposure studies (Folinsbee et al., 1994; Linn et al., 1982b), the changes in symptoms track the changes in spirometry. With repeated exposure to high O_3 concentrations, the change in FEV_1 is typically greatest on the second exposure day. Correspondingly, symptoms are increased on the second exposure day and diminish to near baseline levels by the fourth or fifth exposure when the spirometry responses become negligible. With repeated exposure to lower O_3 concentrations, the largest spirometry

response is seen on the first day and attenuates by the third or fourth day. Symptom responses also are largest on the first day and are attenuated with the same time course. In a single 2-h study in which symptom and spirometry responses were measured during exposure and recovery, the mean changes in symptoms and spirometry responses followed similar time courses (McDonnell et al., 1987).

Intervention studies examining the effects of various drug treatments on the responses to O_3 also report parallel changes in symptoms and spirometry. Although atropine blocked the increase in R_{aw} in response to O_3 exposure, it did not alter the spirometry or symptom responses (Beckett et al., 1985). Similarly, albuterol and salbutamol, which had no effect on O_3-induced changes in spirometry, also had no effect of symptom responses (McKenzie et al., 1987; Gong et al., 1988). The anti-inflammatory medications indomethacin and ibuprofen, which partially inhibit the spirometry responses to O_3 exposure, also cause a reduction in respiratory symptoms (Schelegle et al., 1987; Hazucha et al., 1994).

The individual correlations between symptoms and spirometry responses are relatively low (<0.6) and are of little predictive value. However, the group mean responses have similar exposure-response characteristics and follow a similar time course of response to exposure and recovery. Symptom and spirometry responses also follow a similar time course of attenuation to repeated exposure and they are affected similarly by a number of medication interventions.

7.5.1.3 Effects on Exercise Performance

Endurance exercise performance and $\dot{V}O_{2max}$ may be limited by acute exposure to O_3 (Adams and Schelegle, 1983; Schelegle and Adams, 1986; Gong et al., 1986; Foxcroft and Adams, 1986; Folinsbee et al., 1977; Linder et al., 1988). Gong et al. (1986) and Schelegle and Adams (1986) found that significant reductions in maximal endurance exercise performance may occur in well-conditioned athletes while they perform CE ($\dot{V}_E > 80$ L/min) for 1 h at O_3 concentrations ≥ 0.18 ppm. Data from Linder et al. (1988) suggest that small decrements in maximal exercise performance may occur at O_3 concentrations less than 0.18 ppm. The mechanisms that lead to these responses and the minimum O_3 concentration at which these effects occur have not yet been defined clearly. Reports from studies of exposure to O_3 during high-intensity exercise indicate that breathing discomfort associated with maximal ventilation may be an important factor in limiting exercise performance. However, these studies do not exclude the possibility that some as yet undefined physiological mechanism may limit exercise performance.

7.5.1.4 Effects on Airway Responsiveness

Ozone exposure causes an increase in nonspecific airway responsiveness as indicated by a reduction in the concentration of methacholine or histamine required to produce a given reduction in FEV_1 or increase in SR_{aw}. Increased airway responsiveness is an important consequence of exposure to O_3 because its presence means that the airways are predisposed to narrowing on inhalation of a variety of stimuli (e.g., specific allergens, SO_2, cold air). Markedly increased airway responsiveness is a classical feature of asthma and also may be present with other respiratory diseases (e.g., chronic bronchitis, acute viral infections) and even in a sizeable percentage of the healthy asymptomatic population. Many studies have demonstrated O_3-induced increases in nonspecific airway responsiveness in healthy subjects after a 1- to 2-h exposure with exercise to concentrations in the range of

0.20 to 0.60 ppm (Golden et al., 1978; Holtzman et al., 1979; König et al., 1980; Dimeo et al., 1981; Gong et al., 1986; Folinsbee and Hazucha, 1989) and after 6.6 h of exposure to concentrations in the range of 0.08 to 0.12 ppm (Folinsbee et al., 1988; Horstman et al., 1990). Ozone-induced increases in airway responsiveness tend to resolve within 24 h after exposure but may persist in selected individuals for longer periods (Golden et al., 1978).

Ozone exposure of asthmatic subjects, who characteristically have increased airway responsiveness at baseline, can cause further increases in responsiveness (Kreit et al., 1989). The difference in baseline airway responsiveness between healthy and mild asthmatic subjects may be as much as 100-fold, whereas the changes in airway responsiveness induced by O_3 are typically two- to fourfold. Similar relative changes in airway responsiveness are seen in asthmatics exposed to O_3 despite their markedly different baseline airway responsiveness. One study (Molfino et al., 1991) has been published suggesting an increase in specific (i.e., allergen-induced) airway reactivity. This response was observed after a 1-h resting exposure of atopic asthmatics to 0.12 ppm O_3. One of the important aspects of this observation of increased airway responsiveness after O_3 exposure is that this represents a plausible link between ambient O_3 exposure and increased hospital admissions for asthma. However, experimental design flaws preclude the use of this study in the determination of a lowest-observed-effect level.

Changes in airway responsiveness after O_3 exposure appear to be resolved more slowly than changes in FEV_1 or respiratory symptoms. Furthermore, in studies of repeated exposure to O_3, changes in airway responsiveness tend to be somewhat less susceptible to attenuation with consecutive exposures than changes in FEV_1 (Dimeo et al., 1981; Kulle et al., 1982; Folinsbee et al., 1994). The question of whether chronic O_3 exposure can induce a persistent increase (or decrease) in airways responsiveness has not been studied adequately.

Increases in airway responsiveness do not appear to be strongly associated with decrements in lung function or increases in symptoms. This conclusion is based on studies in healthy subjects; however, asthmatics who have widely different baseline airway responsiveness exhibit FEV_1 changes after O_3 exposure that are similar to those seen in healthy subjects (Kreit et al., 1989).

The mechanism of O_3-induced increases in airway responsiveness is only partially understood, but it appears to be associated with a number of cellular and biochemical changes in airway tissue. Airway inflammation may be temporally associated with the presence of increased airway responsiveness (Holtzman et al., 1983; O'Byrne et al., 1984; Seltzer et al., 1986), but many animal models of induced neutrophilia report a conflicting role of these cells in eliciting nonspecific bronchial hyperresponsiveness. Several animal species, for example, have shown an increased airway responsiveness induced by O_3 exposure in the absence of an influx of PMNs into the airway mucosa (Evans et al., 1988; Okazawa et al., 1989; Li et al., 1992). In one human study (Ying et al., 1990), preexposure treatment with the anti-inflammatory drug indomethacin blocked the effect of O_3 on FEV_1 and FVC but not on airway responsiveness; however, cyclooxygenase inhibitors have not been effective at blocking the O_3-induced influx of PMNs into BAL fluid (Hazucha et al., 1996; Kleeberger and Hudak, 1992). Therefore, O_3-induced airway responsiveness may not be due to the presence of PMNs in the airway or to the release of arachidonic acid metabolites. Rather, it seems likely that the mechanism for this response is multifactorial, possibly involving the presence of cytokines, prostanoids, or neuropeptides; activation of macrophages, eosinophils, or mast cells; and epithelial damage that increases direct access of

mediators to the smooth muscle or receptors in the airways that are responsible for reflex bronchoconstriction.

7.5.1.5 Inflammation and Host Defense Effects

A number of studies clearly show that a single acute exposure (1 to 4 h) of humans to moderate concentrations of O_3 (0.2 to 0.6 ppm) while exercising at moderate to heavy levels results in a number of cellular and biochemical changes in the lung, as assessed by measurement of BAL constituents (Seltzer et al., 1986; Kehrl et al., 1987; Koren et al., 1989a,b, 1991; Schelegle et al., 1991; McGee et al., 1990; Aris et al., 1993a; Devlin et al., 1995). These exposures result in an inflammatory response characterized by increased numbers of PMNs, increased permeability of the epithelial cells lining the respiratory tract, cell damage, and production of proinflammatory cytokines and prostaglandins. This response can be detected as early as 1 h after exposure (Koren et al., 1991; Schelegle et al., 1991) and persists for at least 18 h (Koren et al., 1989a; Aris et al., 1993a). The response profile of these mediators is not defined adequately, although it is clear that the time course of response varies for different mediators and cells (Schelegle et al., 1991, Koren et al., 1989a, 1991).

A single study (Devlin et al., 1991) provides evidence that many of these changes also occur in humans exposed to 0.08 and 0.10 ppm O_3 with moderate exercise for 6.6 h. Decrements in the ability of AMs to phagocytose microorganisms also were reported in this study.

Ozone also causes inflammatory changes in the nose, as indicated by increased levels of PMNs and albumin, a marker for increased epithelial cell permeability. Increases in tryptase levels immediately after O_3 exposure suggested the release of mast cell products.

There appears to be no strong correlation between any of the measured cellular and biochemical changes and changes in lung function measurements, suggesting that different mechanisms may be responsible for these processes. Alternatively, the absence of a correlation may reflect either the temporal misalignment of these measurements, the fact that changes detected in the lavage fluid do not quantitatively reflect events occurring in tissues where functional or symptomatic events originate, or that BAL fluid may not be collected from the same lung region primarily implicated in pulmonary function responses. The idea of different mechanisms is supported by a study in which ibuprofen, a cyclooxygenase inhibitor, blunted the O_3-induced decrements in lung function without altering the O_3-induced increase in PMNs or epithelial cell permeability, although ibuprofen did change the concentration of a number of mediators, some of which may be related to changes in function (Hazucha et al., 1994).

In vitro studies suggest that epithelial cells are the primary target of O_3 in the lung and that O_3 induces them to produce many of the mediators found in the BAL fluid of humans exposed to O_3. Although O_3 does not induce AMs to produce these compounds in large quantities, it does directly impair the ability of AMs to phagocytose and kill microorganisms.

7.5.1.6 Factors Modifying Responsiveness to Ozone

Many variables that at least have potential for influencing response to O_3 remain inadequately addressed in the available clinical data. Factors such as smoking status, age, gender, race or ethnic group, season, and mode of breathing during exposure have been evaluated inadequately for their potential influence on responses to O_3 exposure.

Information derived from O_3 exposure of smokers is limited. Some degree of attenuation appears to occur in active smokers and may be reversed following smoking cessation (Emmons and Foster, 1991), but available results should be interpreted with caution. The possibility of age-related differences in response to O_3 has been explored to some extent since the publication of the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986). Young adults historically have provided the subject population for air pollutant exposure studies. Pulmonary function responsiveness appears to decrease with age, although symptom rates remain similar to those of young adults (Drechsler-Parks et al., 1987b, 1989, 1990; Bedi et al., 1988; Reisenauer et al., 1988; McDonnell et al., 1993). The limited information available on the responses of children and adolescents to O_3 (McDonnell et al., 1985a; Avol et al., 1985a, 1987; Koenig et al., 1987, 1988) does not indicate that children and adolescents are either more or less responsive than young adults. Of the studies that have investigated gender differences in responsiveness to O_3, some (Lauritzen and Adams, 1985; Horvath et al., 1986; Adams et al., 1987; Drechsler-Parks et al., 1987a,b; Messineo and Adams, 1990) have suggested that women are more responsive to O_3 than men. However, the absence of consistent findings with respect to gender differences indicates that it cannot be concluded that men and women respond differently to O_3. Comparison of responses across gender, racial, ethnic, and age groups is complicated by the determination of equivalent exposures. For example, women and children have smaller lungs than adult men. Thus, with a given exposure concentration, duration, and ventilation, humans with smaller lungs will presumably receive a large relative intrapulmonary exposure. Some attempts have been made to normalize responses according to BSA or lung capacity (e.g., FVC). The only study in which this factor has been investigated systematically (Messineo and Adams, 1990) found no influence of lung size on the spirometry responses under identical exposure (O_3 concentration, $\dot{V}_E$, and T) conditions. Three studies (Fox et al., 1993; Seal et al., 1995; Weinmann et al. (1995) have compared pulmonary function responses of women during different phases of the menstrual cycle, but the results are conflicting. The responses of black and white young adults to various concentrations of O_3 have been compared in one study (Seal et al., 1993). The data suggested that black males experienced significant decrements in pulmonary function at a lower concentration of O_3 than white males, but that there were no differences among the responses of white males and black and white females. Thus, the question of ethnic or racial differences in responsiveness to O_3 is answered inadequately, and the available results should be interpreted with caution. No new studies are available on the effects of heat stress (i.e., increased temperature or RH) on O_3 responses. One study (Linn et al., 1988) suggests that sensitivity to O_3 may be related to seasonal variations in ambient O_3 concentrations; this finding needs to be confirmed. Two studies (Hynes et al., 1988; Adams et al., 1989) have reported that differences in the inhalation route (e.g., oral versus nasal or oronasal) appear to be of negligible importance in the responses of exercising adults to O_3 exposure. Studies of O_3 uptake in the upper airway (Gerrity et al., 1988) confirm the negligible differences between oral and nasal inhalation (also see Chapter 8). None of these potential influences on O_3 responsiveness (age, gender, race, hormonal fluctuations, smoking, seasonal variations in responsiveness, and ambient environmental factors) has been investigated thoroughly. However, the observation that healthy older adults appear to be less responsive to O_3 exposure than young adults has been confirmed to the point that it can be considered in risk assessment. Nevertheless, this does not address fully the question of age differences because children and adolescents remain inadequately studied.

7.5.1.7 Extrapulmonary Effects of Ozone

It still is believed that O_3 reacts immediately on contact with respiratory systems fluids and tissues and is not absorbed or transported to extrapulmonary sites to any significant degree. A number of laboratory animal studies reported in the previous chapter (Chapter 6) and early studies on human subjects reported in this chapter suggest that reaction products formed by the interaction of O_3 with respiratory system fluids or tissues may produce effects measured outside the respiratory tract—either in the blood, as changes in circulating blood lymphocytes, erythrocytes, or serum, or as changes in the structure or function of other organs, such as the parathyroid, the heart, the liver, and the central nervous system. No extrapulmonary effects have been reported to date in other organ systems of O_3-exposed human subjects, except for limited data indicating that acute (1- to 2-h) exposures with exercise at concentrations ≥ 0.35 ppm O_3 caused transient changes in blood cells and plasma. The interpretation of all these effects in regards to potential human health effects at ambient levels of exposure (<0.35 ppm O_3) is not clear. However, the demonstration in this chapter of an array of inflammatory mediators and immune modulators released at the airway surface in response to O_3 provides a possible mechanism for effects to occur outside of the lung. Additional studies are needed, therefore, in order to determine if there are any significant extrapulmonary effects of O_3 exposure and at what levels of exposure they might occur.

7.5.1.8 Effects of Ozone Mixed with Other Pollutants

No significant enhancement of respiratory effects (i.e., more than additive) has been consistently demonstrated for mixtures of O_3 with SO_2, NO_2, H_2SO_4, HNO_3, or particulate aerosols, or with multiple combinations of these pollutants. There is general agreement among studies of simultaneous exposure of healthy adults and asthmatic adolescents to mixtures of O_3 and NO_2, SO_2, H_2SO_4, or HNO_3 that pulmonary function responses are not significantly different from those following exposure to O_3 alone when compared to studies conducted at the same O_3 concentration. Exposure to high PAN concentrations (i.e., 0.3 ppm) combined with O_3 has been reported to induce greater pulmonary function responses than exposure to O_3 alone (Horvath et al., 1986), but when the PAN concentration is reduced to the ambient range, any additional effect of PAN in the mixture appears to be negligible (Drechsler-Parks et al., 1989).

In addition to simultaneous exposures to pollutant mixtures, studies of the responses to O_3 exposure either preceded or followed by another pollutant have been performed. To the extent that these exposure sequences mimic real ambient conditions, the results could be useful in the risk assessment process. Koenig et al. (1990) demonstrated that exposure of allergic (and probably asthmatic) adolescents to O_3 and then to SO_2 resulted in significant pulmonary function decrements not seen with an O_3-O_3 sequence or FA-SO_2 sequence. These results also can be interpreted in light of the fact that O_3 increases nonspecific bronchial responsiveness and that the increased SO_2 responses may simply reflect this increased responsiveness. Such responses would be unlikely in nonatopic healthy adolescents. Other studies (Aris et al., 1991; Hazucha et al., 1994; Linn et al., 1994; Utell et al., 1994) have assessed the responses to O_3 after previous exposure to another pollutant. Aris et al. (1991) found that preexposure to water or HNO_3 fog appeared to attenuate responses to O_3, whereas Hazucha et al. (1994) observed an increased airway responsiveness after O_3 exposure preceded by NO_2 exposure relative to O_3 exposure alone. Two studies of combined or sequential exposure to H_2SO_4 aerosol and O_3 suggest a possibly enhanced

response to O_3 in asthmatics when the exposure is combined with or preceded by exposure to H_2SO_4 aerosol (Linn et al., 1994; Utell et al., 1994). These findings are intriguing, but must be replicated before they can be useful for quantitative health assessment. Much is unknown about responses to air pollutant mixtures. Only a limited number of pollutant combinations and exposure protocols have been investigated, and subject groups are small and may not be representative of the general population. Few studies have included more than two pollutants, and most combinations have been evaluated in single studies. Furthermore, only rarely are endpoints other than pulmonary function and plethysmography measured.

7.5.2 Field and Epidemiology Studies of Ozone Exposure

Individual-level camp and exercise studies provide useful, quantitative information on the exposure-response relationships linking human lung function declines with O_3 exposure occurring in ambient air. Their utility derives largely from the reliability with which individual exposures can be estimated using outdoor measurements in studies of these kind. Although it usually has not been possible to isolate O_3 exposures from other copollutants (e.g., acid aerosols) and environmental factors (e.g., temperature) in the design of such studies, the available body of evidence now strongly supports a dominant role of O_3 in the observed lung function decrements.

The most extensive epidemiologic database on pulmonary function responses to ambient O_3 comes from camp studies. Six recent key studies from three separate research groups provide a combined database on individual exposure-response relationships for 616 children ranging in age from 7 to 17 years, each with at least six sequential measurements of FEV_1 and previous-hour O_3 exposures while attending summer camps (Avol et al., 1990; Higgins et al., 1990; Raizenne et al., 1987, 1989; Spektor et al., 1988a, 1991). When analyzed together using consistent methods, these data yielded an average relationship between FEV_1 and previous-hour O_3 concentration of -0.50 mL/ppb. The highest 1-h O_3 levels measured in five of the six studies ranged from 100 to 160 ppb, with one study reporting concentrations as high as 245 ppb. Minimum O_3 values ranged from 10 to 60 ppb. Although the regression results noted above were based on 1-h O_3 levels, exposure in camp studies usually extended for multiple hours. Because of the high level of correlation between single- and multiple-hour averages in the studies, these results may therefore, represent, to some extent, the influence of multihour exposures. In addition to the camp study results, two key studies involving lung function measurements before and after well-defined exercise events in adults have yielded exposure-response slopes of -0.40 and -1.35 mL/ppb (Spektor et al., 1988b; Selwyn et al., 1985). Ozone concentrations during exercise events of approximately 0.5 h duration ranged from 4 to 135 ppb in these studies. Consistent with chamber studies, there is no clear evidence from individual-level studies for a response threshold for the average population effects of O_3 on pulmonary function decline. However, as with chamber studies, there is evidence that responsivity varies across individuals. Thus, pulmonary function decline as a function of ambient O_3 exposure for an individual may be either greater than or less than the mean responses noted above.

Recent results of daily-life studies also support a consistent relationship between ambient O_3/oxidant exposure and acute respiratory morbidity in the population. Respiratory symptoms (or exacerbation of asthma) and decrements in PEFR occur with increasing ambient O_3, especially in asthmatic children (Lebowitz et al., 1991; Krzyzanowski et al.,

1992; Thurston et al., 1995). Concurrent temperature, particles, H^+, aeroallergens, and asthma severity or medication status also may contribute as independent or modifying factors. The aggregate results show greater responses in asthmatic individuals than in nonasthmatics (Lebowitz et al., 1991; Krzyzanowski et al., 1992), indicating that asthmatics constitute a sensitive group in epidemiologic studies of oxidant air pollution.

Recent aggregate population time series studies of O_3-related health effects provide relevant evidence of acute responses, even below a 1-h maximum of 0.12 ppm O_3. Emergency room visits, hospital admissions, and mortality all have been examined as possible outcomes of exposure to O_3. In the case of ER visits, the evidence is limited (e.g., Bates et al., 1990; Cody et al., 1992; White et al., 1994; Weisel et al., 1995), but results generally are consistent with an effect of O_3 on morbidity. Mortality studies vis-à-vis O_3 also are rather limited, but are more mixed in their results. One of two new, well-designed studies indicate a significant association between O_3 and total mortality in Los Angeles, CA, even after controlling for the potentially confounding effects of temperature and PM (Kinney and Ozkaynak, 1991). Los Angeles experienced peak 1-h maximum O_3 concentrations above 0.2 ppm during this study period. However, at lower concentrations, over a shorter time span, and with different statistical methods, a second study (Dockery et al., 1992) did not detect a significant O_3 association with mortality. The strongest and most consistent evidence of O_3 effects, both above and below 0.12 ppm O_3, then, is provided by the multiple studies that have been conducted over the last decade on summertime daily hospital admission for respiratory causes in various locales in eastern North America (Bates and Sizto, 1983, 1987, 1989; Thurston et al., 1992, 1994; Lipfert and Hammerstrom, 1992; Burnett et al., 1994). These studies consistently have shown that O_3 air pollution is associated with an increased incidence of admissions, accounting for roughly one to three excess respiratory hospital admissions per 100 ppb O_3 per million persons. This association has been shown to remain even after statistically controlling for the possible confounding effects of temperature and copollutants (e.g., H^+, SO_4, and PM_{10}), as well as when considering only days having 1-h maximum O_3 concentrations below 0.12 ppm. Furthermore, these results imply that O_3 air pollution can account for a substantial portion of summertime hospital admissions for respiratory causes on the most polluted days. Overall, the aggregate population time series studies considered in this chapter provide strong evidence that ambient exposures to O_3 can cause significant exacerbations of preexisting respiratory disease in the general public at concentrations below 0.12 ppm O_3.

Studies of chronic health effects that may relate to long-term exposure to ambient pollutants still have not provided enough data to determine if there are respiratory or other health effects that result directly from chronic O_3 exposure. However, the aggregate evidence to date suggests that chronic O_3 exposure, along with other environmental factors, could be responsible for health effects in exposed populations.

The most useful set of data has been provided by the AHSMOG studies (Hodgkin et al., 1984; Euler et al., 1987, 1988; Abbey et al., 1991a,b, 1993). These studies have provided the most refined measures of chronic exposure to date (including adjustment for quality of the monitoring data as determined by distance of monitoring sites from subject residences, topography, time spent indoors, and time spent at work). The most consistent effects that can be attributable, in part, to O_3 relate to an increase in 10-year cumulative incidence of asthma (RR = 2.07 for each 1,000 h above 10 pphm) and an increase in asthma severity. Unfortunately, for the entire set of studies, the colinearity between O_3 and TSP

reduces the confidence that effects can be attributed to O_3 alone, O_3 in combination with the particulate fraction of ambient pollution, or the combination of the two. Some support for an effect on persons with asthma also can be derived from a recent Canadian study (Stern et al., 1994) that demonstrated nonstatistically significant 6.6 and 6.5% reductions in $FEF_{25-75\%}$ and $\dot{V}_{max50\%}$ for people living in Ontario relative to those in Saskatchewan. Again, however, the effects of O_3 are impossible to disentangle from the other contributors such as the acid summer haze that characterizes the United States east of the Mississippi River.

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8

Extrapolation of Animal Toxicological Data to Humans

8.1 Introduction

A full evaluation of the health effects of ozone (O_3) requires an integrated interpretation of human clinical, epidemiological, and animal toxicological studies. Each of these three research approaches has inherent strengths and limitations. Animal toxicological data are valuable because they provide concentration- and duration-response information on a fuller array of effects and exposures than can be studied in humans. However, historically, use of animal toxicological data has been limited because of difficulties in quantitative extrapolation to humans. Recent advances in the state-of-the-art of extrapolation have reduced several uncertainties, which will be discussed in this chapter.

Qualitative animal-to-human extrapolation generally is accepted because O_3 causes similar types of effects in several animal species, from mouse to nonhuman primate (Chapter 6). Also, when similar endpoints (e.g., inflammation and pulmonary function) have been examined in O_3-exposed animals and humans, similar effects are observed. However, quantitative extrapolation (i.e., if a certain exposure causes a specific effect in animals, what exposure is likely to cause that same effect in humans?) is the goal but is controversial. Such an extrapolation requires an integration of dosimetry and species sensitivity. *Dosimetry* is defined as the dose delivered to a site in the respiratory tract (RT). As can be seen in Section 8.2, substantial information is available on dosimetry in several species, including humans. Dosimetric studies that are referenced in the earlier O_3 criteria document (U.S. Environmental Protection Agency, 1986) are summarized only briefly here; newer research is the focus. Species sensitivity, discussed in Section 8.3, refers to the sensitivity of a specific species to the delivered dose. For example, even if the same dose of O_3 were delivered to a specific respiratory tract site in rats and humans, differences in species sensitivity to that dose are likely because of variations in defense mechanisms and perhaps other factors. Section 8.3 also provides a more holistic approach to extrapolation by quantitatively comparing exposure-response data obtained in animals and humans. Sections 8.4 and 8.5 are intended to draw the forgoing information together, reaching conclusions about the potential for acute and chronic human health effects based on animal studies. Lastly, Section 8.6 presents the summary and major conclusions from the chapter.

Although this chapter focuses on animal-to-human extrapolation, dosimetric studies also can be used to elucidate interpretations of the human studies described in Chapter 7. For example, knowledge of dosimetry in humans as related to age and exercise can enhance understanding of human susceptibility factors.

8.2 Ozone Dosimetry

8.2.1 Introduction

Dosimetry refers to measuring or estimating the quantity or rate of a chemical absorbed by target sites within the RT. The compound most directly responsible for toxic effects may be the inhaled gas O_3 or its chemical reaction products. Complete identification of the actual toxic agents and their integration into dosimetry are complex issues that have not been resolved. Thus, most dosimetry investigations are concerned with the dose of the primary inhaled chemical. In this context, a further confounding aspect can be the units of dose (e.g., mass retained per breath, mass retained per breath per body weight, mass retained per breath per respiratory tract surface area). That is, when comparing dose between species, what is the relevant measure of dose? This question has not been answered; units are often dictated by the type of experiment or by a choice made by the investigators.

Experimental and theoretical (dosimetry modeling) studies are used to obtain information on dose. Experiments have been carried out to obtain direct measurements of absorbed O_3 in the RT, the upper RT (URT; region proximal to the tracheal entrance), and the lower RT (LRT; region distal to tracheal entrance); however, experimentally obtaining dosimetry data is extremely difficult in smaller regions or locations, such as specific airways or the centriacinar region (CAR; junction of conducting airways and gas exchange region), where lesions caused by O_3 occur (see Chapter 6, Section 6.2.4). Nevertheless, experimentation is important for determining dose, making dose comparisons between subpopulations and between different species, assessing hypotheses and concepts, and validating mathematical models that can be used to predict dose at specific respiratory tract sites and under more general conditions.

Theoretical studies are based on the use of mathematical models developed for the purposes of simulating the uptake and distribution of absorbed gases in the tissues and fluids of the RT. Because the factors affecting the transport and absorption of gases are applicable to all mammals, a model that uses appropriate species or disease-specific anatomical and ventilatory parameters can be used to describe absorption in the species and in different-sized, aged, or diseased members of the same species. Importantly, models also may be used to make interspecies and intraspecies dose comparisons, to compare and reconcile data from different experiments, to predict dose in conditions not possible or feasible experimentally, and to better understand the processes involved in toxicity.

8.2.2 Summary of 1986 Review of Experimental and Theoretical Dosimetry

A summary of the more relevant experimental and theoretical dosimetry studies contained in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986) is presented. The reader is referred to the earlier document for completeness.

Experiments on the nasopharyngeal removal of O_3 in laboratory animals suggested that the fraction of O_3 uptake depends inversely on flow rate (Yokoyoma and Frank, 1972), uptake was greater for nose than mouth breathing (Yokoyoma and Frank, 1972), and tracheal and chamber concentrations were related linearly (Yokoyoma and Frank, 1972; Miller et al., 1979). Only one investigation measured uptake by the LRT, finding 80 to 87% uptake by the LRT of dogs (Yokoyoma and Frank, 1972). At the time, there were no reported results for human URT or LRT uptake. With the exception of two relatively crude studies by

Clamann and Bancroft (1959) and Hallett (1965), there were no data on O_3 uptake in humans at the time of the earlier criteria document (U.S. Environmental Protection Agency, 1986).

Several mathematical dosimetry models were developed to simulate the processes involved in O_3 uptake and to predict O_3 uptake by various regions and sites within the RT. The model of Aharonson et al. (1974) was used to analyze nasopharyngeal uptake data. Applied to O_3 data, the model indicated that the average mass transfer coefficient of this region and the mass retained increased with increasing air flow, but the percent uptake decreased.

Models were developed to simulate LRT uptake (Miller et al., 1978, 1985). The models were very similar in their treatment of O_3 in the airways and airspaces and in their use of morphometric data to define the dimensions of the air compartments and liquid lining. Both the 1978 and the 1985 models of Miller and co-workers took into account reactions of O_3 with constituents of the liquid lining. However, these models differed in their treatment of chemical reactions in the liquid lining, and the later model included transport and chemical reactions within tissue and blood, whereas the first model did not (an instantaneous reaction at the liquid-tissue interface was assumed, so the O_3 concentration was defined as zero). In both models, tissue dose was defined as the O_3 flux to the liquid-tissue interface. Both models predicted O_3 tissue dose to be relatively low in the trachea, to increase to a maximum in or near the CAR, and then to decrease distally; this was characteristic for both the animal and the human simulations (Miller et al., 1978, 1985).

Prior to 1986, there were no experimental results that were useful in judging the validity of the modeling efforts. However, a comparison of the results of Miller and co-workers with morphological data (showing the CAR to be most affected by O_3; see Chapter 6, Section 6.2.4) indicated qualitative agreement between the site of predicted maximum tissue dose and the site of observed maximum morphological damage in the pulmonary region.

8.2.3 Experimental Ozone Dosimetry Data

8.2.3.1 Introduction

Models of O_3 uptake in the RT have reached a scale of sophistication that provide some highly specific predictions regarding the location and magnitude of O_3 dose. However, before these models can be exploited to their fullest degree in extrapolating dose within and between species, validation of the models with experimental data is essential. This section will review the experimental database on which the modeling of O_3 dosimetry is both based and validated. This will help facilitate discussion of the models themselves in subsequent sections. Table 8-1 provides a summary of all post-1986 experimental O_3 dosimetry studies.

8.2.3.2 In Vivo Ozone Dosimetry Studies

The model predictions of Overton et al. (1987), based on the original model of Miller et al. (1985), provided specific predictions about the regional and total uptake efficiencies of O_3 in laboratory rats. It was therefore necessary to test these predictions with actual data. The first data on total RT uptake of O_3 in rats were obtained by Wiester et al. (1987). Ozone uptake was measured in 30 awake, unanesthetized Sprague-Dawley (S-D) rats receiving a nose-only exposure. Each rat was situated within a plethysmograph that continuously monitored the animal's breathing pattern. Air with O_3 flowed by rats' noses at 1,200 mL/min for 1 h at a concentration of 0.3, 0.6, or 1.0 ppm O_3. Determination of RT

Table 8-1. Experimental Studies on Ozone Dosimetry[a]

Type of Study	Species (Strain)	Uptake	Breathing Patterns	Results	Reference
In vivo, nose only	Rat (S-D)	Total RT	V_T = 2.8 mL f = 150 bpm $\dot{V}_E$ = 400 mL/min	Uptake measured in 30 rats exposed to 0.3, 0.6, or 1.0 ppm O_3 for 1-h. Uptake measured using mass balance. Total RT uptake efficiency measured at 40%. Uptake efficiency was independent of O_3 concentration.	Wiester et al. (1987)
In vivo	Rat (F344) Rat (S-D) Rat (Long-Evans) Guinea pig (Hartley)	Total RT	For rats: V_T = 2.6 mL f = 120 bpm $\dot{V}_E$ = 330 mL/min For guinea pigs: V_T = 2.4 mL f = 77 bpm $\dot{V}_E$ = 188 mL/min	All uptake measurements at 0.3 ppm O_3. In addition F344 rats were measured at 0.6 ppm. Uptake measurements made with system of Wiester et al. (1987). Total RT uptake efficiency averaged 47% and was strain and species independent. Uptake efficiency again was shown to be independent of O_3 concentration in F344 rats.	Wiester et al. (1988)
In vivo	Rat (F344)	Total RT, head, larynx/trachea, lung	V_T = 2.05 mL f = 150 bpm $\dot{V}_E$ = 290 mL/min	Regional uptake measured by assaying for recoverable ^{18}O from respiratory tract tissue after animal inhaled ^{18}O-enriched 1 ppm O_3 for 2 h. Fifty-four percent of inspired O_3 was taken up by total RT. Of the O_3 taken up, 49.6% taken up by the head, 6.7% by the larynx/trachea, and 43.6% by the lungs.	Hatch et al. (1989)
In vivo	Human	Total RT, URT, LRT	V_T = 800 mL f = 12 and 24 bpm $\dot{V}_I$ = 350 and 634 mL/s oral, nasal, and oronasal breathing	Uptake efficiencies of URT, LRT, and total RT measured by sampling inspired and expired air from catheter inserted through nose to posterior oropharynx. Uptake efficiencies computed from peak plateau concentrations on inspiration and expiration. Inspiratory URT uptake efficiencies averaged 40%; inspiratory plus expiratory LRT uptake efficiencies averaged 92%. Small but significant decreases in URT and LRT uptake efficiencies with increasing f. No effect of concentration on uptake. Uptake efficiency of mouth relatively greater than nose by about 10%.	Gerrity et al. (1988)
In vivo	Human	Total RT, URT, LRT	V_T = 1,239-1,650 mL f = 25-35 bpm $\dot{V}_E$ = 41 L/min	Twenty healthy male subjects exposed to 0.4 ppm O_3 while exercising at $\dot{V}_E$ of 41 L/min for 1 h. Uptake efficiencies of URT, LRT, and total RT measured at beginning and at end of exposure by method of Gerrity et al. (1988). Subjects mouth-breathed only. Uptake efficiencies computed as mass fractions. URT inspiratory efficiency was 40% and did not change during exposure. LRT efficiency dropped from 68% to 62% during exposure. LRT decrease correlated with drop in V_T. Cumulative dose of O_3 to LRT was predictive of V_T drop.	Gerrity and McDonnell (1989); Gerrity et al. (1989); Gerrity et al. (1994)
In vitro	Pig Sheep	Trachea	$\dot{V}_I$ = 50-200 mL/s	Unidirectional O_3 uptake efficiencies of trachea decreased with increasing flow from 0.5 to 0.15 for the sheep and 0.12 for the pig. Mass transfer coefficients generally were independent of flow.	Ben-Jebria et al. (1991)

Table 8-1 (cont'd). Experimental Studies on Ozone Dosimetry[a]

Type of Study	Species (Strain)	Uptake	Breathing Patterns	Results	Reference
In vitro	N/A	N/A	N/A	Ozonolysis studies on various unsaturated fatty acids, rat erythrocyte ghost membranes and rat BAL. Dominant processes are the production of aldehydes and peroxides due to reactions between O_3 and olefins.	Pryor et al. (1991)
In vivo	Human	20-200 mL depth into total RT	V_T = 500 mL $\dot{V}_I$ = 250 mL/s	Uptake efficiencies by measuring recovery of O_3 boluses delivered at 20 mL increments into lung to depth of 200 mL. At deepest depth, only 6% of O_3 could be recovered. Ozone uptake by conducting airways larger than predicted by Miller et al. (1985).	Hu et al. (1992b)
In vivo	Human	20-200 mL depth into total RT	V_T = 500 mL $\dot{V}_I$ = 150, 250, 500, 750, 1,000 mL/s	Same technique as Hu et al. (1992b), but investigating flow effects. Increasing flow caused marked shift of delivered O_3 toward the periphery of the conducting airways (i.e., the greater the inspiratory flow, the greater the amount of O_3 delivered to the lung periphery), where it is available for absorption. Mass transfer coefficients in upper airways independent of flow, but in conducting airways they increase proportional to flow. Lung liquid lining mass transfer coefficient computed to be 1.4 cm/s in the URT, falling to 0.17 cm/s in the respiratory airways. Reaction rate constant between O_3 and the lung liquid lining was computed as 7.3×10^6/s in the URT, falling to 8.2×10^5/s in the distal conducting airways.	Hu et al. (1994)
In vivo	Human	20-200 mL depth into total RT	V_T = 500 mL $\dot{V}_I$ = 250 mL/s	Comparison of O_3 bolus uptake between oral and nasal routes. Nose was found to be 30% more efficient at removing O_3 from the air stream than the mouth.	Kabel et al. (1994)
In vitro	Rat (S-D)	Lung	V_T = 2.71 mL f = 50-103 bpm FRC = 4 and 8 mL	Perfused and nonperfused rat lungs ventilated with 1 ppm O_3. Uptake efficiency of lungs dropped from 95% at 50 bpm to about 50% at 103 bpm. No change in uptake efficiency when lungs inflated from FRC = 4 to 8 mL.	Postlethwait et al. (1994)
In vivo	Human	Total RT, URT, trachea, mainstem bronchi	V_T = 810 mL f = 12 bpm $\dot{V}_I$ = 320 mL/s	O_3 uptake efficiencies in conducting airway structures determined by sampling air from anatomical sites ranging from the vocal cords to bronchus intermedius in 10 subjects undergoing transnasal bronchoscopy while being exposed to 0.4 ppm O_3. Total RT uptake efficiency was 91%. Uptake efficiencies of mouth-vocal cords: 17.6%; vocal cords-upper trachea: 12.8%; upper trachea-main bifurcation carina: 11.5%; main bifurcation carina-bronchus intermedius: 0%.	Gerrity et al. (1995)
In vivo	Human	Total RT	V_T = 593-642 mL f = 16 bpm $\dot{V}_E$ = 9.2-9.8 L/min Nasal and oral breathing	Total RT uptake efficiency measured using the same technique (Wiester et al., 1987; Wiester et al., 1988) applied to rats. During nasal breathing, total RT uptake efficiency was 73%. During oral breathing, total RT uptake efficiency was 76% and significantly higher than with nasal breathing.	Wiester et al. (1996)

[a]See Appendix A for abbreviations and acronyms.

O_3 uptake was determined by mass balance. The uptake was the difference in mass in the upstream air and in the air downstream of the rat. Total RT O_3 uptake efficiency of approximately 40% was measured and was independent of O_3 concentration. During data acquisition, the animals had an average tidal volume (V_T) of about 2.8 mL, an average breathing frequency (f) of about 150 breaths per minute (bpm), and an average minute ventilation ($\dot{V}_E$) of about 400 mL/min. This study was followed by another (Wiester et al., 1988) in which total RT uptake was measured in three strains of rats and in the guinea pig. Specifically, Fischer 344 (F344), S-D, and Long-Evans rats and Hartley guinea pigs were exposed for 1 h to 0.3 ppm O_3; F344 rats also received a 0.6-ppm exposure. All rats in the Wiester et al. (1988) study had f's between 112 and 132 bpm, V_Ts between 2.4 and 2.8 mL, and $\dot{V}_E$s between 299 and 364 mL/min. The guinea pigs had a V_T of 2.4 mL (not different from rats), an f of 77 bpm, and a $\dot{V}_E$ of 188 mL/min. Uptake was measured as in the previous experiment (Wiester et al., 1987). Total RT uptake of O_3 was species-independent and averaged 47%; this was higher than in the previous study because a different calculation method for fractional uptake was used. As in the first experiment, exposure concentration did not affect uptake. Wiester et al. (1987) corrected all flows used in uptake calculations for body temperature and relative humidity. In their later work, however, they found that this correction was not warranted, resulting in a slightly higher computed O_3 uptake efficiency (Wiester et al., 1988).

These data are in disagreement with the model predictions of Overton et al. (1987), who made predictions of O_3 uptake in two different rat anatomical lung models (Kliment, 1973; Yeh et al., 1979). Simulations were conducted in both anatomical models, varying f and V_T at fixed $\dot{V}_E$; functional residual capacity (FRC) also was varied as a fraction of total lung capacity (TLC). The Kliment (1973) anatomical lung model gave consistently high predictions for uptake when compared with actual data. The predictions using the Yeh et al. (1979) model came closer. Total RT uptakes (not including the head) for the Yeh et al. (1979) model were predicted to range between 46 and 60% at f = 154 bpm and V_T = 1.25 mL and between 70 and 80% for f = 81 bpm and V_T = 2.4 mL. However, when the fact that these predictions do not include the head of the animal is considered, it is evident that the model predictions overestimate the total RT uptake in rats. The question is whether the measurements are accurate or whether there is a problem with the model formulation. The data of Postlethwait et al. (1994), presented below, suggested that the data of Wiester et al. (1987, 1988) may be reasonable. The Postlethwait et al. (1994) data in the excised rat lung suggested a clear inverse dependence of lung uptake on f. At a V_T of 2.5 mL, the O_3 uptake efficiency of the excised lung fell from nearly unity at f = 50 bpm to almost 50% at f = 100 bpm. For extrapolation purposes, a key question here is what should be considered the normal resting f of a rat. Although Wiester et al. (1987, 1988) allowed their rats to acclimate to the plethysmograph by monitoring f and began uptake measurements only after f had plateaued at a minimum, it is still uncertain whether f's of 120 to 150 bpm are reasonable. In a summary of studies of the pulmonary function of rats in response to O_3, Tepper et al. (1993) found typical f's of 100 bpm. Although the models appear to overestimate the O_3 uptake efficiency of the rat RT, the discrepancy is not large, and the near agreement indicates that the O_3 dosimetry models have predictive capability.

In addition to data on the total RT O_3 uptake efficiency, in vivo data on regional O_3 dosimetry in animal models have begun to emerge. Hatch and Aissa (1987) and Aissa and Hatch (1988) first described a method to measure O_3 uptake in animals by exposing them

nose-only, while in a plethysmograph, to O_3 enriched with ^{18}O, a stable isotope of oxygen. After exposure, bronchoalveolar lavage (BAL) fluid and respiratory tract tissue were assayed for excess ^{18}O using isotope ratio mass spectrometry. One problem with this technique is that all of the absorbed ^{18}O cannot be accounted for, thus possibly leading to an underestimation of dose. The technique used by Hatch and Aissa (1987) involves the detection of excess ^{18}O in $^{18}O_3$ reaction products after tissue pyrolysis. Thus, $^{18}O_3$ that is degraded to $H_2{}^{18}O$ or $^{18}O_2$ is lost and cannot be detected in dry tissue. Eight male F344 rats (four previously exposed chronically to O_3 for 1 year to an urban pattern generally consisting of a 0.06-ppm baseline with 1-h daily spikes rising to 0.25 ppm) were exposed for 2 h to 1 ppm of ^{18}O-enriched O_3 (Hatch et al., 1989). During exposure, the rats breathed at 150 bpm and had a V_T of 2.05 mL and a $\dot{V}_E$ of 290 mL/min. After exposure, the lung, trachea, and head were analyzed separately for ^{18}O. Overall, the animals took up 54.3% of inspired O_3. Although this value of O_3 uptake efficiency is higher than that found by Wiester et al. (1987, 1988), considering the fact that the coefficient of variation for O_3 uptake efficiency measurements is around 20% in all studies, the result of Hatch and Aissa (1987) is consistent with the data of Wiester et al. (1987, 1988). Of the O_3 taken up by the animals, 49.6% was taken up by the head, 6.7% by the larynx/trachea, and 43.6% by the lungs. By assuming equal uptake efficiencies by compartments on inspiration and expiration, inspiratory uptake of O_3 by these regions was computed. It was determined that the rat nasopharynx (NP) had an inspiratory efficiency of 17.4%, and that the larynx/trachea removed 2.7% of the remaining O_3.

This technique recently has been extended to humans. Hatch et al. (1994) showed that when human subjects were exposed to 0.4 ppm $^{18}O_3$ while exercising intermittently at $\dot{V}_E$ = 60 L/min for 2 h, the amount of recovered ^{18}O in lavagable cells indicated that the human cells incorporated 4 to 5 times the O_3 dose (i.e., concentration of $^{18}O_3$) that was incorported by the BAL cells from rats exposed to 0.4 ppm O_3 for 2 h at rest. Consequently, to compare absorbed $^{18}O_3$ doses between rats and humans using BAL requires the assumption that the amount of lavagable cell membrane available to react with $^{18}O_3$ is comparable between the two species. The difference between rats and humans could be accounted for by the fact that the humans were exercising, whereas the rats were not. However, as was noted above, not all absorbed $^{18}O_3$ can be accounted for.

8.2.3.3 In Vitro Ozone Dosimetry Studies

The use of whole, intact animals to study O_3 uptake is needed to ascertain the actual amounts of O_3 absorbed. However, it is also important to understand some of the more fundamental processes governing O_3 uptake, such as the biochemistry of O_3/liquid and O_3/tissue interactions to determine chemical reaction rates essential to O_3 dosimetry models. Furthermore, the use of intact animals does not allow more precise determinations of the role of physiological parameters on O_3 uptake. For this reason, there have been some limited attempts at utilizing animal tissue explants and whole lungs to study O_3 uptake.

Ben-Jebria et al. (1991) studied O_3 uptake by the trachea of sheep and pigs to investigate mass transfer coefficients. Ozone boluses of 1 ppm were passed through excised tracheae. Tracheae were obtained from a slaughter house 0.5 to 2 h after slaughter, and, although they were kept coated with physiologic saline, they were not maintained at body temperature, possibly resulting in underestimation of in vivo uptake. The lengths and diameters of the pig tracheae were not too different from human tracheal dimensions. The flow dependence of mass uptake and the mass transfer coefficient (K) were determined for

flows between 50 and 200 mL/s. Uptake efficiencies in the pig decreased with increasing flow from about 0.5 to 0.12 and in the sheep decreased from about 0.5 to 0.15. Mass transfer coefficients generally were independent of flow (K = 0.5 cm/s in pigs and 0.35 cm/s in sheep), indicating the lack of dependence of uptake on gas-phase diffusion processes. This contrasts with the conclusion of Aharonson et al. (1974) for the NP of dogs where the investigators observed that the slight inverse dependence of uptake on flow observed by Yokoyama and Frank (1972) leads to the conclusion that the mass transfer coefficient for the NP of the dog should increase with flow, suggesting a role of the boundary layer in limiting diffusion of O_3 to the wall of the NP. The different geometries of a trachea and an NP may account for the differing observations.

A significant feature of the Ben-Jebria et al. (1991) study was the use of a rapidly responding O_3 analyzer. In order to conduct their O_3 uptake studies, Ben-Jebria and Ultman (1989) and Ben-Jebria et al. (1990) developed a rapidly responding O_3 analyzer. The analyzer relies on the reaction of O_3 with alkenes such as ethylene, propylene, cyclohexane, etc. Ten alkenes were tested. Ninety percent step-response times of 130 to 540 ms were achieved with varying degrees of linear response with O_3 concentration. The authors concluded that the best alkene was 2-methyl-2-butene, with optimum 10 to 90% responses of 110 ms and minimum detectable limits of O_3 of 18 ppb. Interference with CO_2, however, was found, requiring measurements of CO_2 to correct the analyzer response.

Postlethwait et al. (1994) used an isolated rat lung preparation to investigate the effects of vascular perfusion, inspired dose, temperature, and distal lung surface area on O_3 absorption by the LRT. Vascular perfusion had little or no effect on uptake efficiency of O_3. When the lung was exposed to 1 ppm O_3 and ventilated with a V_T of 2.5 mL, a FRC of 4 mL, and an f of 50 bpm, uptake efficiency was 95%. As f increased with fixed V_T, uptake efficiency began to drop, reaching nearly 50% at an f of 100 bpm. When the lung temperature was dropped from 37 to 25 °C, uptake efficiency dropped from 95 to 85% at 50 bpm. This drop was exploited to investigate other factors (such as flow, volume, and lung surface area) governing uptake because it moved respiratory tract uptake further below 100%. The observation of a dependence of uptake on temperature indicates that uptake efficiency is chemical-reaction dependent, thus possibly coupling uptake to reaction product formation. Another interesting result from this study was the lack of dependence of uptake on FRC. When FRC was doubled from 4 to 8 mL at 25 °C, fractional O_3 uptake was unchanged. This latter result suggests that O_3 uptake is virtually complete by the time O_3 reaches the alveolar spaces of the lung. Otherwise it would have been expected that the uptake efficiency would have risen with increased FRC.

To further investigate the reactions of O_3 with the lung, Pryor et al. (1991) performed ozonolysis studies of various unsaturated fatty acids (UFAs), rat erythrocyte ghost membranes, and rat BAL. These studies demonstrated significant production of hydrogen peroxide and aldehydes and that production of hydrogen peroxide was due primarily to reactions between O_3 and olefins. The authors concluded that the reaction of O_3 with UFAs in the lung fluid lining and cell membranes produce hydrogen peroxide and aldehydes that may be important mediators in the toxicity of O_3. The quantitative results of these studies led Pryor (1992) to hypothesize about the degree to which O_3 reacts with the liquid lining of the lung and with lung tissue. A simple model calculation was performed using the Einstein-Smoluchowski equation to estimate the half-life of O_3 in bilayers and cell membranes. Pryor (1992) concluded that a substantial fraction of O_3 reacts in the bilayer, and that only in regions of the lung where the lung lining fluid layer is less than 0.1 μm thick will

O_3 penetrate to tissue, and only then will O_3 react in cell membranes before penetrating further. The overall conclusion is that the toxic effects of O_3 may be mediated not just by O_3 directly but by reactive intermediates such as aldehydes and hydrogen peroxide. This raises the question as to the relevant dose of O_3: is it the total dose, the dose to the liquid lining, the tissue dose, or the dose of reactive intermediates delivered to tissue?

8.2.3.4 Human Ozone Dosimetry Studies

Significant progress has been made in the area of human O_3 dosimetry since the previous criteria document (U.S. Environmental Protection Agency, 1986). Studies have been conducted defining total and regional respiratory tract uptake, the dependence of uptake on physiological parameters, and the role of uptake in modulating response.

Gerrity et al. (1988) reported on measurements of O_3 uptake by the extrathoracic airways (airways proximal to the posterior oropharynx) and intrathoracic airways (airways distal to the posterior oropharynx) in 18 healthy, young male volunteers. Ozone uptake was measured by placing a small polyethylene catheter through the nose and positioning the distal tip in the posterior oropharynx. Breath-by-breath samples of O_3 were collected, and the peak plateau concentrations were compared with chamber concentrations. The effects on uptake of O_3 concentration (0.1, 0.2, 0.4 ppm), f (12 and 24 bpm at fixed V_T), and mode of breathing (oral, nasal, and oronasal) were tested. The O_3 analyzer had a moderately rapid response with a 90% response time of 700 ms. Inspiratory V_T ranged between 754 and 848 mL; mean inspiratory flow at 12 bpm was 350 mL/s; at 24 bpm it was 634 mL/s. The authors measured extrathoracic uptake efficiency of O_3 on inspiration at approximately 40% and intrathoracic uptake efficiency (inspiration plus expiration) at approximately 92%. They essentially found no effect of O_3 concentration on uptake (intrathoracic uptake was significantly higher at 0.4 ppm, but the difference was very small). They did find that both intrathoracic and extrathoracic uptake decreased with increasing f (at fixed V_T), falling by about 7% for extrathoracic uptake and by about 3% for intrathoracic uptake when f increased from 12 to 24 bpm. The finite response time of the analyzer may have affected the results at the 24 bpm f by overestimating extrathoracic uptake and underestimating intrathoracic uptake. However, because uptake was defined relative to plateaus of concentration, the response time of the analyzer was adequate to reach a plateau in the 1.2-s inspiratory time at 24 bpm. It is important to note here that, when utilizing the data from this study to compare with other studies and models, the uptake efficiencies measured are comparable to steady-state unidirectional measurements of uptake. Another feature is that Gerrity et al. (1988) consistently measured a small, non-zero plateau of O_3 on expiration. This plateau is not consistent with the suggestion from models of O_3 uptake and the data of Postlethwait et al. (1994) (nor with the later work of Gerrity et al. [1995] that is presented below) that no O_3 should be washed out from lung volumes beyond the conducting airways. This observation of Gerrity et al. (1988) may have been an artifact of the manner in which O_3 was measured by sampling from the posterior oropharynx. There may have been entrainment of O_3 in the pharyngeal airspaces that was washed out after expiration of dead-space air. Regardless, the concentration of O_3 exhaled from the alveolar phase of washout was very low.

One of the most startling results from the work of Gerrity et al. (1988) was the finding that there was only a small, but statistically significant, difference between uptake by the nose and by the mouth. The mouth had approximately 10% greater uptake efficiency than the nose. The combined oronasal passage had an uptake efficiency greater than the nose

by another 8%. This suggests that persons who breathe nasally are at no less risk than persons who breathe oronasally. Adams et al. (1989) investigated this possibility by comparing functional responses in subjects acutely exposed to O_3 while breathing either orally or oronasally. Healthy subjects were exposed on five separate days to 0.4 ppm O_3. In the first four exposures, subjects were exposed by face mask (with or without nose clip) for 30 min at an exercise level of 75 L/min or for 75 min at exercise level of 30 L/min. The fifth exposure was for 30 min at 75 L/min, with exposure through a mouthpiece. There were no differences in pulmonary function response (forced expiratory volume in 1 s [FEV_1], forced vital capacity [FVC], or forced expiratory flow) with face-mask exposure among all experimental groups (i.e., no nose clip, $\dot{V}_E$, or time effect). Pulmonary function response was, however, greater with a mouthpiece. Adams et al. (1989) speculated that the greater response with the mouthpiece was due to O_3 scrubbing by the face mask or by facial hair. It also may have been due to different oral configurations imposed by a mouthpiece. Hynes et al. (1988) also investigated whether functional responses were affected by the mode of breathing. Healthy subjects were exposed to 0.4 ppm O_3 for 30 min in an exposure chamber. On two different occasions, each subject breathed either through the nose or the mouth exclusively. There was no difference in pulmonary function response between these two routes of exposure. Taken together, the studies of Adams et al. (1989) and Hynes et al. (1988) are consistent with the observations of Gerrity et al. (1988) on the equal efficiency of all routes of breathing for extrathoracic O_3 scrubbing.

This study was followed by another study (Gerrity and McDonnell, 1989; Gerrity et al., 1989, 1994) in which the relationship between O_3 uptake and functional response was investigated. Healthy subjects were exposed to 0.4 ppm O_3 for 1 h while exercising continuously at 40 L/min. Ozone uptake was measured at the beginning and at the end of exposure while the subjects were still exercising, using the technique of Gerrity et al. (1988). In contrast to the work of Gerrity et al. (1988), uptake was computed in this study by integrating concentration times flow instead of using peak plateau measurements. Also, in this work, the 90% response time of the analyzer was 1.2 s (compared with 0.7 s in the previous work). The authors found that about 40% of the inspired O_3 was taken up by the URT (i.e., the same as the extrathoracic airways described in Gerrity et al., 1988) during inspiration, and that this did not change during exposure. Total RT uptake efficiency was approximately 80%, and it did not change during exposure. However, LRT (i.e., the intrathoracic airways described in Gerrity et al., 1988) uptake efficiency fell during exposure from 68 to 62% and was correlated with the O_3-induced fall in V_T (V_T fell from 1,650 to 1,239 mL; f increased from 25.2 to 34.8 bpm; inspiratory flow fell from 1,506 to 1,357 mL/s; $\dot{V}_E$ increased slightly from 40.8 to 40.9 L/min), suggesting that the V_T reduction may have a protective effect on dose delivered to the periphery of the lung. It is not likely that the finite analyzer response time affected uptake measurements. Evidence of this is the lack of dependence of URT uptake on changes in V_T or f. The low values for uptake in the LRT may have been due to an artifact from the relatively slow response time of the analyzer, which was approximately equal to inspiratory and expiratory times. As a check on their results, the authors compared their data with the previous work of Gerrity et al. (1988) by computing uptake by the original technique using peak plateau concentrations. When that was done, the URT uptake efficiencies were 17 and 22% at the beginning and end of exercise, respectively, and the LRT efficiencies were 96 and 92% at the beginning and end of exercise, respectively. The URT change computed this way was not significant, but

the LRT efficiency drop was. When viewed at in this manner, the data from this experiment are consistent with those from the previous experiment.

Because there is a need to compare human O_3 uptake data with rat O_3 uptake data, it is essential that there be confidence in the reliability of the different approaches. To help establish the comparability of techniques, Wiester et al. (1996) measured total RT uptake in humans using a similar, although obviously scaled-up, system to that used for rats (Wiester et al., 1987, 1988). Healthy subjects breathed 0.3 ppm O_3 while seated in an exposure chamber; their faces were placed in a sealed face mask. The face mask was attached to a large tube through which chamber air was circulated with a pump at a rate of $\approx$40 L/min. Upstream and downstream O_3 concentrations were measured continuously, as was ventilation with an induction plethysmograph. Subjects breathed at rest, either through nose or mouth (average f = 16 bpm, V_T = 598 to 642 mL, $\dot{V}_E$ = 9.2 to 9.8 L/min). While nose breathing, 73% of inspired O_3 was taken up by the total RT, and, while mouth breathing, 76% of inspired O_3 was taken up, which was significantly higher than that found with nose breathing. This difference is probably not, however, biologically significant. Significant negative correlations between f and uptake in both mouth- and nose-breathers were found, similar correlations were found with $\dot{V}_E$, but no correlations were found between uptake and V_T or any other measure of breathing pattern or pulmonary function.

The observations in Wiester et al. (1996) of a slight increase of total RT uptake efficiency with oral breathing and the inverse correlation of total RT uptake efficiency with f are consistent with those of Gerrity et al. (1988). Furthermore, the data on total RT uptake are consistent overall with that of Gerrity et al. (1988, 1994). The data from Gerrity et al. (1988) reporting total RT uptake efficiencies of about 95% were based on minimum plateau measurements, thus reflecting uptake during steady-state flow conditions, as opposed to the cyclical conditions of actual breathing. The data of Gerrity et al. (1994), on the other hand (in which total RT efficiencies of 80% were reported), were obtained by integrating the product of concentration and flow, thus more accurately reflecting the actual mass uptake of O_3 during cyclical breathing when Gerrity et al. (1994) computed uptake using the methodology from Gerrity et al. (1988), they found that the total RT uptake measurements were comparable. Thus total RT mass uptake efficiencies at rest of 80% are not unreasonable.

Hu (1991), Hu et al. (1992b, 1994), and Ultman et al. (1993) took a different approach to measuring respiratory tract uptake of O_3. They exploited the development of a rapid responding O_3 analyzer (Ben-Jebria and Ultman, 1989; Ben-Jebria et al., 1990) to measure the recovery of small boluses of O_3 delivered to different volumetric depths of the RT. Ozone uptake was measured in a set of four experiments. In the baseline experiments, absorption of O_3 boluses was measured in healthy male subjects at rest. The O_3 boluses were 10 mL in size, with a peak concentration of 3 ppm. The O_3 analyzer characteristics were sample flow of 400 mL/min, 2-methyl-2-butene as reactive alkene, 10 to 90% step-response time of 110 ms, and lower detection limit (18 ppb). In the baseline experiments, the V_T was 500 mL, and the inspiratory and expiratory flow rates were 250 mL/s. In a complete set of measurements, bolus recovery was examined for penetrations of 10 to 200 mL, in 10 mL increments. In the second set of experiments, the effects of flow were measured by measuring bolus recovery as a function of penetration depth for flows of 150, 250, 500, 750, and 1,000 mL/s at a fixed V_T of 500 mL. In a third set of experiments, bolus recovery was measured as a function of penetration depth at a flow of 250 mL/s and a V_T of 500 mL; the bolus delivered to a rubber mouthpiece or to a nasal cannula was

compared, thereby examining potential uptake differences between the two pathways. In the fourth set of experiments, the effects of O_3 concentration on uptake were determined by delivering boluses with peak concentrations of 0.5, 1.0, 2.0 and 4.0 ppm. The latter experiments were conducted because acute studies in isolated dog airways showed that absorption efficiency was inversely related to inhaled concentration between 0.1 and 20 ppm (Vaughan et al., 1969; Yokoyama and Frank, 1972). However, later experiments in guinea pigs, rabbits (Miller et al., 1979), and humans (Gerrity et al., 1988) showed a lack of concentration dependence, implying a linear relationship between concentration and dose. The dependence or lack of dependence of uptake efficiency on O_3 concentration provides information on the order of reactions of O_3 with lung fluid lining and tissue. Under steady-state conditions, concentration independence of uptake efficiency suggests that first-order processes play a role. However, because O_3 absorption is coupled to both interfacial transfer (gas-phase to solute O_3) and subsequent reaction, at face value, the conclusion cannot be reached that saturated absorption rates are solely due to saturation of the reaction components.

In all four experiments, Hu and colleagues computed the first three moments of the inspired and expired bolus distributions with respect to volume. The zeroth moment of a bolus is the O_3 mass contained in the bolus. Thus the zeroth moments of the inspired and expired boluses were used to compute O_3 uptake efficiency (Λ; or absorbed fraction), breakthrough volume (the mean volume of the exhaled bolus), and bolus dispersion. The first moment of a bolus is its mean volumetric position. The first moment on inspiration gives the penetration volume (V_p), and the first moment on expiration gives the breakthrough volume (V_B). In the absence of any O_3 uptake, a longitudinally mixed bolus should have $V_B = V_p$. The second moment of a bolus is its variance. The difference in variance between the expired and inspired bolus (σ^2) is a measure of gas mixing, or dispersion, in the lung.

In the baseline experiments, the breathing pattern was a resting pattern with a V_T of 500 mL and an average inspiratory flow of 250 mL/s. These experiments were performed on nine male subjects and showed that almost all O_3 was absorbed beyond a penetration depth of 180 mL. Only about 6% of inhaled O_3 was recovered at the 180 mL penetration depth, and, beyond that depth, it was very difficult to obtain an accurate measurement of recovery. The investigators also found that V_B was greater than V_p at penetration depths less than 100 mL, after which V_B leveled out at a constant value. Dispersion was insensitive to penetration depth. An important finding of the baseline experiment was that at quiet resting ventilation, about 50% of the O_3 mass in a bolus inhaled through the upper airways is taken up by the upper airways. To compare these data with results of Gerrity et al. (1988), it is necessary to assume that inspiratory and expiratory uptake efficiencies are equal. Then the unidirectional uptake efficiency of the upper airways to a depth comparable to that at which Gerrity et al. (1988) positioned their sampling catheter is about 30%, which is approximately 25% less than the 40% results of Gerrity et al. (1988). This difference might be due to the presence of a mouthpiece in the experiments of Hu and colleagues, which could reduce the uptake efficiency of the oral pathway. The functional response data of Adams et al. (1989) suggest that this might be the case.

The flow experiments showed that there was a general right shifting of the $\Lambda - V_p$ curves with increasing flow (i.e., increasing flow causes a deeper penetration of O_3 into the lung with lower fractional uptake by the conducting airways). Eventually, all of the O_3 is still absorbed. Breakthrough volume showed a similar pattern at all flows (i.e.,

greater than penetration volume at small V_p but flattening out at larger V_p). As flow increased, the level of the plateau increased. Dispersion, although constant as a function of V_p at all flows, increased linearly with increasing flow.

The studies of Hu and colleagues investigating the role of exposure route in modulating O_3 uptake efficiencies reported that the nose absorbed approximately 30% more than the mouth. This result is at variance with the findings of Gerrity et al. (1988), which indicate that there was only a slightly higher uptake by the oral pathway when compared with the nasal pathway. Gerrity et al. (1988) studied uptake by the two pathways without the use of a mouthpiece or any other delivery system. Subjects were free to breathe naturally. It is possible that the use of mouthpieces and nasal canulas in the studies of Hu and colleagues caused artifacts, resulting in their findings for nasal and oral uptake efficiencies. The study of Adams et al. (1989) supports this, showing enhanced pulmonary function response to O_3 during a mouthpiece exposure compared to face-mask/oral exposure. Finally, the concentration-dependence studies showed that uptake efficiency was not affected by the concentration of inspired O_3 between 0.3 to 4 ppm, implying that O_3 uptake is governed by linear processes.

One of the very unique features of the approach to measuring uptake efficiency taken by Hu and colleagues is that the O_3 bolus recovery data can be used to derive local mass transfer coefficients for the conducting airways. Regional mass transfer coefficients derived experimentally in this way can then be used as input into mathematical model simulations, thereby potentially leading to more accurate models of O_3 dose to the RT.

Hu and colleagues define the parameter Ka (per second) as one that is suitable to characterize local O_3 absorption. It is the product of the overall K (centimeters per second), which reflects the combined contribution of diffusion and chemical reaction to uptake, and the local surface/volume ratio (a; per centimeter). From the $\Lambda - V_p$ curves, these investigators derived values for Ka. Thus, the experimentally derived mass transfer coefficients depended on assumptions about airway anatomy and morphology. As a result of the various experiments, these investigators found a number of important properties of Ka:

- The proximal subcompartment of the nose has a Ka that is 70% larger than the Ka for the proximal mouth compartment (see, however, the comment made above regarding the nose/mouth differences).
- Ka's in the upper airway compartment were between 1.20 and 2.24/s and relatively insensitive to flow, indicating that diffusion resistance of O_3 through the gas boundary layer is much less than through the mucus film. These data also are consistent with the pig and sheep tracheae experiments of Ben-Jebria et al. (1991).
- In the proximal and distal conducting airway subcompartments, $(Ka)^{-1}$ was linearly related to $(flow)^{-1}$, suggesting that the gas-phase absorption rate constant is directly proportional to flow. In lower airways, therefore, diffusion resistance of the gas boundary layer is important. Hu and colleagues concluded that the gas boundary layer contributes 80 to 90% of the overall diffusion resistance in the central airway compartment.
- The mass transfer coefficient in the lung liquid lining is estimated to fall from 1.4 cm/s in the URT to 0.17 cm/s in the respiratory airways.
- The chemical reaction rate between O_3 and the lung liquid lining was estimated to be 7.3×10^6/s, 2.3×10^6/s, and 8.2×10^5/s in the upper airways, proximal conducting airways, and distal conducting airways, respectively.

- The overall mass uptake coefficients determined in the work of Hu (1991) are significantly higher than those used in the model of Miller et al. (1985). If the mass transfer coefficients in the model are adjusted upward, the total uptake efficiency would be higher than measurements have shown, requiring a downward adjustment of pulmonary region mass transfer coefficients.

Gerrity et al. (1995) took a somewhat more conventional approach in an attempt to acquire regional information on O_3 absorption in the human RT. Healthy subjects underwent transnasal bronchoscopy while in an exposure chamber in which 0.4 ppm O_3 was present. Subjects were asked to breathe at 12 bpm. Inspired and expired air was sampled through a Teflon catheter that had been passed through the biopsy channel of the bronchoscope and positioned approximately in the center of the airway lumen. The air was drawn into a rapid response O_3 analyzer (Ben-Jebria and Ultman, 1989; Ben-Jebria et al., 1990) with a 90% response time of 250 mL/s while using ethylene as the reactive alkene. Average V_T was 810 mL, and average inspiratory flow was 320 mL/s. Air was sampled for five breaths from above the vocal cords, at the entrance to the trachea, above the main bifurcation carina, and midway through the bronchus intermedius. Flow was measured simultaneously by a pneumotach attached to a simple cylindrical mouthpiece. Before and after each measurement, a set of samples from the mouth was collected for reference. Uptake was defined as the fraction of O_3 mass lost across any anatomical segment; mass was determined by integrating the product of O_3 concentration and flow. By way of comparison with the other human O_3 uptake studies, Gerrity et al. (1994) found that total RT uptake of O_3 measured in this fashion was 91%. This is higher than the resting data of Wiester et al. (1996); however, the average V_T in this study of 810 mL, compared with the 600 mL VT reported by Wiester et al. (1996) may account for this difference. When Gerrity et al. (1995) computed the unidirectional uptake efficiencies between the mouth and the various sampling sites, they found that 17.6, 27.0, 35.5 and 32.5% of the O_3 passing into the mouth is taken up by structures up to the vocal cords, the upper trachea, the main bifurcation carina, and the bronchus intermedius, respectively. They also computed the unidirectional uptake efficiencies across individual airway segments: 17.6% between the mouth and just above the vocal cords, 12.8% from above the vocal cords to the upper trachea, 11.5% from the upper trachea to the main bifurcation carina, and essentially zero between the carina and bronchus intermedius. The uptake between the mouth and just above the vocal cords is considerably lower than that measured earlier by Gerrity et al. (1988), even considering the fact that, in the earlier study, peak plateaus were used. As has been noted earlier, it is possible that the mouthpiece played a role in reducing the uptake efficiency of the mouth. The uptake efficiency of O_3 across the trachea is in line with the data from sheep and porcine tracheae at the higher flow rates (Ben-Jebria et al., 1991). The present data are also consistent with the bolus uptake data of Hu (1991) and colleagues, which also were acquired with a mouthpiece. When the O_3 bolus data are used to compute unidirectional uptake efficiencies (assuming that the segmental efficiencies are the same on inspiration and expiration), the Hu et al. (1992b) data yield uptake efficiencies of 21, 36, 44, and 46% between the mouth and the vocal cords, the upper trachea, the main bifurcation carina, and the bronchus intermedius, respectively. The O_3 bolus data are, therefore, in good accord with the data of Gerrity et al. (1995). The measured uptake efficiencies across airway segments clearly appear to be higher than those predicted by the model of Miller et al. (1985). If higher uptake coefficients in the conducting airways were used in the model of Miller et al. (1985), the model would overestimate total RT O_3 uptake. To adjust for this,

pulmonary uptake coefficients would have to be reduced. Unfortunately, the data of Hu and associates cannot provide information beyond the conducting airways.

Gerrity et al. (1995) also measured O_3 washout volumes (i.e., the expired volumes required to cause a specified drop in O_3 concentration). This type of data provides insight into the location of major sites of O_3 uptake. At the mouth, the 90% washout volume was 142 mL, and, at the upper trachea, the 90% washout volume was 62 mL. By the time the entire anatomical dead space of the lungs was washed out, the O_3 concentration had fallen to zero (Gerrity et al., 1995). It is unclear whether the absence of recovered O_3 after washout of the conducting airways was due to O_3 not penetrating beyond the conducting airways or to all of the O_3 that penetrated beyond the conducting airways being absorbed. The latter possibility is more likely based on the observations of Hu (1991).

In assessing the work of Gerrity et al. (1995), it is significant to note that these investigators measured expired plateaus of O_3 concentration that were zero. This contrasts with the earlier work of Gerrity et al. (1988, 1994) in which a non-zero expiratory plateau was observed. The non-zero expiratory plateau may have been due to a number of factors that are unclear. Because ethylene was the reacting alkene in all cases, it is unlikely that interference with other gases such as carbon dioxide (CO_2) was responsible. Another possibility is that O_3 in the early expiratory phase became entrained in the posterior oropharynx and persisted for the duration of expiration.

8.2.3.5 Intercomparison of Ozone Dosimetry Studies

The previous sections emphasized the methods and results of individual experimental studies on O_3 dosimetry. This section will focus on comparisons of the in vivo studies with each other and will draw on these comparisons to arrive at conclusions regarding the utility of these data for extrapolation purposes. The discussion will be divided into three sections, focusing on total RT uptake efficiency, unidirectional URT uptake efficiency, and LRT uptake efficiency.

There are two categories that distinguish various data sets among each other. The first category is the mouth/nose category listed in the Tables 8-2 to 8-4. Studies indicated by "M" or "N" were performed with unencumbered breathing by either oral (M) or nasal (N) breathing. *Unencumbered* indicates the absence of a mouthpiece or canula. Data listed as "M/N" are pooled from data encompassing oral and nasal breathing. Data shown as "Mouthpiece" or "Nasal canula" are acquired using these devices to deliver the O_3 to the animal or human subject, or to measure flow.

The second category is the method used to compute O_3 uptake efficiency. There are essentially two methods. One method, referred to as the steady-state method, relies on measuring the loss of O_3 from a steady air flow moving across an anatomical structure. An example is the data of Yokoyama and Frank (1972) in which a constant flow of ozonated air through the URT of a dog was maintained by a tracheal canula attached to a pump. Uptake efficiency was measured by changes in equilibrium O_3 concentration. Another example is the study of Gerrity et al. (1988), which used the steady-state method by measuring the peak inspiratory and minimum expiratory O_3 concentrations through a catheter in the posterior pharynx. These measurements were compared to the ambient chamber concentration to obtain uptake efficiencies of the URT and LRT. The second method is referred to as the non-steady state method. This method uses the integration of the product of flow and O_3 concentration to compute O_3 masses that, in turn, are used to compute

Table 8-2. Total Respiratory Tract Uptake Data[a]

Species	Mouth/Nose	Method	V_T(mL)	Inspiratory Flow (mL/s)	f (bpm)[b]	F_t	Reference
Human	M	Steady	832	509	18	0.97	Gerrity et al. (1988)[c]
Human	N	Steady	754	456	18	0.96	Gerrity et al. (1988)
Human	M/N	Steady	832	350	12	0.97	Gerrity et al. (1988)
Human	M/N	Steady	778	634	24	0.96	Gerrity et al. (1988)
Human	M	Non-steady	1,650	1,360	25	0.88	Gerrity et al. (1994)[c]
Human	M	Non-steady	1,239	1,360	35	0.87	Gerrity et al. (1994)
Human	M	Steady	1,650	1,350	25	0.97	Gerrity et al. (1994)
Human	M	Steady	1,239	1,360	35	0.95	Gerrity et al. (1994)
Human	Mouthpiece	Non-steady	825	330	12	0.91	Gerrity et al. (1995)
Human	M	Non-steady	631	539	16	0.76	Wiester et al. (1996)
Human	N	Non-steady	642	514	16	0.73	Wiester et al. (1996)
Human	Mouthpiece	Non-steady	500	250	15	0.86	Hu et al. (1992b)
Human	Mouthpiece	Non-steady	1,000	250	7.5	0.93	Hu et al. (1992b)
Rat (F344)	N	Non-steady	2.8	12.2	118	0.44	Wiester et al. (1988)
Rat (S-D)	N	Non-steady	2.4	9.6	123	0.46	Wiester et al. (1988)
Rat (Long-Evans)	N	Non-steady	2.7	12.3	132	0.48	Wiester et al. (1988)
Rat (F344)	N	Non-steady	2.6	11.3	113	0.54	Hatch et al. (1989)
Guinea pig	N	Non-steady	2.4	7.5	77	0.53	Wiester et al. (1988)

[a]See Appendix A for abbreviations and acronyms. M = mouth exposure by natural breathing; N = nasal exposure by natural breathing; M/N = pooled data from mouth and nasal exposure; Mouthpiece = exposure by mouthpiece; Steady = uptake computed during constant unidirectional flow; Non-steady = uptake computed by integration during cyclic breathing; F_t = total RT uptake.
[b]f is either measured or is computed from reported flows and volumes.
[c]Total RT uptake reported by Gerrity et al. (1988) and Gerrity et al. (1994) did not include the contribution from URT uptake efficiency during expiration. The data include an expiratory URT contribution, assuming it equals inspiratory URT uptake efficiency.

uptake. The studies of Wiester et al. (1988) in rodents, and Wiester et al. (1996) in humans are examples of this technique, as is the study of Gerrity et al. (1994).

Total Respiratory Tract Uptake Efficiency

Table 8-2 provides a summary of in vivo data in all animal species of respiratory tract O_3 uptake efficiency (F_t). The data reported for the studies of Gerrity et al. (1988, 1994) have been adjusted from the published values to account for the fact that the F_t cited in those papers did not include uptake in the URT on expiration. To make the adjustment, the URT uptake efficiency on expiration was assumed to equal the inspiratory uptake efficiency. The F_t data listed for the study of Hu et al. (1992b) were derived from their bolus recovery data by integrating the data over the desired V_T. Because Hu et al. (1992b) could not recover boluses from a depth greater than 220 mL, it was assumed that any bolus delivered

Table 8-3. Unidirectional Upper Respiratory Tract Uptake Efficiency Data[a]

Species	Mouth/Nose	Method	Inspiratory Flow (mL/s)	f (bpm)[b]	F_{urt}	Reference
Human	M	Steady	509	18	0.40	Gerrity et al. (1988)
Human	N	Steady	456	18	0.43	Gerrity et al. (1988)
Human	M/N	Steady	350	12	0.41	Gerrity et al. (1988)
Human	M/N	Steady	634	24	0.38	Gerrity et al. (1988)
Human	M	Non-steady	1,360	25	0.37	Gerrity et al. (1994)
Human	M	Non-steady	1,360	35	0.41	Gerrity et al. (1994)
Human	M	Steady	1,360	25	0.16	Gerrity et al. (1994)
Human	M	Steady	1,360	35	0.22	Gerrity et al. (1994)
Human	Mouthpiece	Non-steady	337	12	0.18	Gerrity et al. (1995)
Human	Mouthpiece	Non-steady	250	15	0.30	Ultman et al. (1993)
Human	Mouthpiece	Non-steady	250	15	0.47	Ultman et al. (1993)
Dog (beagle)	Nasal canula	Steady	83.3	N/A[c]	0.72	Yokoyama and Frank (1972)
Dog (beagle)	Nasal canula	Steady	667	N/A	0.37	Yokoyama and Frank (1972)
Dog (beagle)	Mouthpiece	Steady	83.3	N/A	0.34	Yokoyama and Frank (1972)
Dog (beagle)	Mouthpiece	Steady	667	N/A	0.12	Yokoyama and Frank (1972)
Rat (F344)	N	Non-steady	11.3	113	0.17	Hatch et al. (1989)
Guinea pig	N	Steady	2.7	N/A	0.62	Miller et al. (1979)
Rabbit	N	Steady	16.7	N/A	0.41	Miller et al. (1979)

[a]See Appendix A for abbreviations and acronyms. M = mouth exposure by natural breathing; N = nasal exposure by natural breathing; M/N = pooled data from mouth and nasal exposure; Mouthpiece = exposure by mouthpiece; Nasal canula = exposure by nasal canula; Steady = uptake computed during constant unidirectional flow; Non-steady = uptake computed by integration during cyclic breathing; F_t = total RT uptake.
[b]f is either measured or is computed from flows and V_T.
[c]N/A = not applicable.

to a depth greater than 220 mL was absorbed completely. The derivation of F_t from the bolus data was done for V_Ts of 500 and 1,000 mL.

To assess the consistency of the data, it is useful to examine it as a function of flow. Figure 8-1 shows F_t as a function of inspiratory flow for all human studies. The F_t from the bolus recovery data of Hu et al. (1994) are shown for V_Ts of 500, 1,000, and 1,500 mL. An overview of the data suggests that, with respect to F_t, there is good agreement among the various experimental methods for humans. The data clearly show that F_t decreases with increasing flow and increases with increasing V_T, both of which are qualitatively consistent with model predictions.

One observation is quite prominent: the rat data of Wiester et al. (1988) and Hatch et al. (1989) (not shown in Figure 8-1) are considerably lower than the human data. Even if it is assumed that the rats were breathing up to three times resting ventilation

Table 8-4. Lower Respiratory Tract Uptake Efficiency Data[a]

Species	Mouth/Nose	Method	V_T (mL/s)	Inspiratory Flow (mL/s)	f (bpm)[b]	F_{lrt}	Reference
Human	M/N	Steady	832	350	12	0.93	Gerrity et al. (1988)
Human	M/N	Steady	778	634	24	0.89	Gerrity et al. (1988)
Human	M	Non-steady	1,650	1,360	25	0.68	Gerrity et al. (1994)
Human	M	Non-steady	1,239	1,360	35	0.62	Gerrity et al. (1994)
Human	M	Steady	1,650	1,360	25	0.96	Gerrity et al. (1994)
Human	M	Steady	1,239	1,360	35	0.92	Gerrity et al. (1994)
Human	Mouthpiece	Non-steady	844	337	12	0.95	Gerrity et al. (1995)
Human	Mouthpiece	Non-steady	500	250	15	0.78	Hu et al. (1992b)
Human	Mouthpiece	Non-steady	1,000	250	7.5	0.89	Hu et al. (1992b)
Dog (beagle)	N/A[c]	Non-steady	168	112	20	0.87	Yokoyama and Frank (1972)
Dog (beagle)	N/A	Non-steady	168	168	30	0.83	Yokoyama and Frank (1972)
Rat (F344)	N	Non-steady	2.6	11.3	113	0.33	Hatch et al. (1989)

[a]See Appendix A for abbreviations and acronyms. M = mouth exposure by natural breathing; N = nasal exposure by natural breathing; M/N = pooled data from mouth and nasal exposure; Mouthpiece = exposure by mouthpiece; Nasal canula = exposure by nasal canula; Steady = uptake computed during constant unidirectional flow; Non-steady = uptake computed by integration during cyclic breathing; F_t = total RT uptake.
[b]f is either measured or is computed from flows and V_T.
[c]N/A = not applicable.

(equivalent to an inspiratory flow in humans of approximately 1,000 mL/s), the rat data would still be significantly lower than what was measured in humans. The consistency of the human data of Wiester et al. (1996) (in which the same methodology was used to measure F_t in humans as was used for rats) with the other human data strongly suggests that the low F_t in rats is not a function of the methodology employed. Overall, the evidence reasonably points to the conclusion that F_t in a rat is smaller than in a human.

Unidirectional Upper Respiratory Tract Uptake

Table 8-3 summarizes the data on the unidirectional O_3 uptake efficiency of the URT (F_{urt}). In general, F_{urt} describes the uptake efficiency of anatomical structures proximal to the larynx. A possible exception to this are the data of Hu et al. (1992b). In that study, and other bolus studies, it was assumed that the URT is the volume of the RT 50 mL distal to the lips. This may or may not include the larynx.

The wide variety of URT uptake data in different species and under different flow conditions allows some intra- and interspecies comparisons. To make comparisons among different species, however, requires assumptions about the scaling of breathing patterns among species. Gerrity (1989) examined nasopharyngeal uptake data available in different species at that time by examining the data as a function of the ratio of predicted resting flow to measured flow (scaled flow). The predicted flows were obtained using the allometric

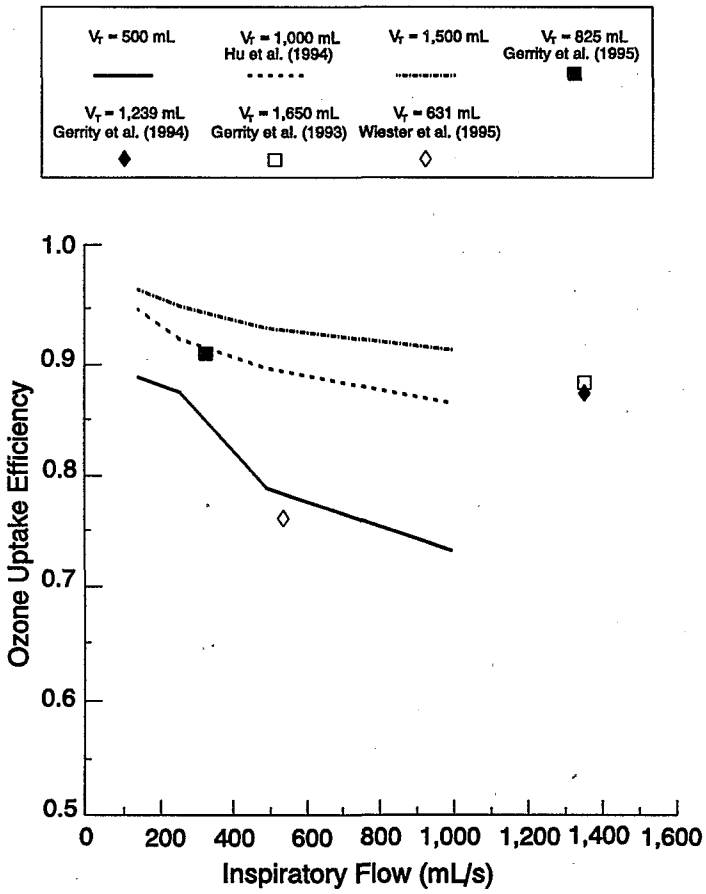


Figure 8-1. Total respiratory tract uptake as a function of inspiratory flow in humans.

equations of Guyton (1947). Ozone uptake efficiency of the URT was then described as a single function of scaled flow and species weight. When newer data become available, this approach can be extended to URT data acquired by both mouth and nasal breathing.

Figure 8-2 shows $1 - F_{urt}$ for nasal breathing plotted as a function of the ratio between predicted and measured flow. As with the previous work of Gerrity (1989), all of the data, except the beagle dog data, are roughly consistent with each other. The body weight dependence of the Gerrity (1989) analysis is illustrated by the two dashed lines representing the predicted range of flow dependencies between rats (lower line) and humans (upper line). All species, except the beagle dogs, fall within these boundaries.

Figure 8-3 shows the data for $1 - F_{urt}$ by the oral route plotted in the same manner. All of the data are for humans except for the two beagle dog data points. Several observations are worth noting. First, the data for the beagle dog appear to be consistent with the human data. The analysis of Gerrity (1989) predicts that the human data generally would lie above the dog data, although not to a large degree. This suggests that the oral passage of the dog may have O_3 scrubbing properties similar to the human oral passage. Second, within the human data, use of a mouthpiece appears to reduce the uptake efficiency of the oral passageway. The closed diamonds in Figure 8-3 are the data from the study of Gerrity et al.

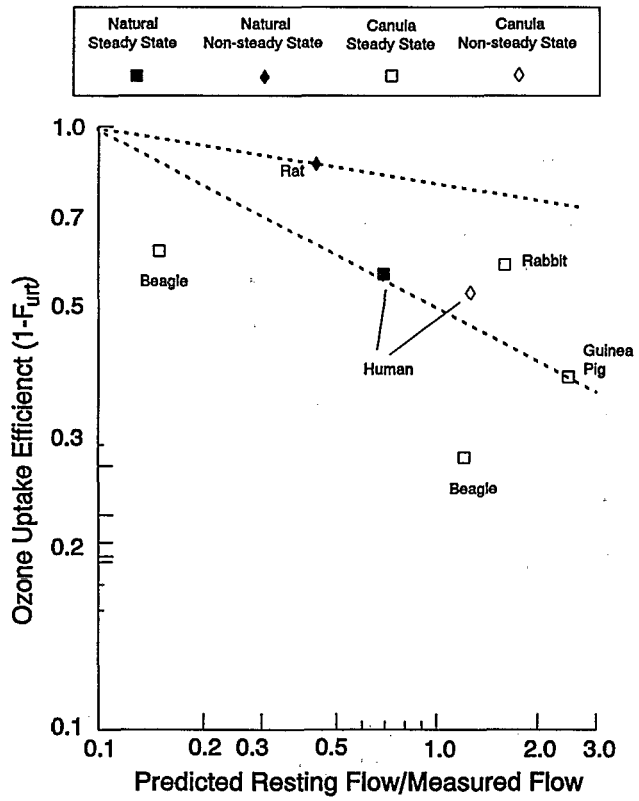


Figure 8-2. Unidirectional uptake efficiency in the upper respiratory tract by the nasal pathway. The ratio of predicted resting flow to measured flow is a way to scale flow to allow for interspecies comparisons. The beagle dog data are from Yokoyama and Frank (1972), the rat data are from Hatch et al. (1989), the rabbit and guinea pig data are from Miller et al. (1979), and the human data are from Gerrity et al. (1988) (closed square) and Ultman et al. (1993) (open diamond). Lines representing predictions for uptake efficiency are from Gerrity (1989).

(1994), which involved unencumbered breathing. These data generally are lower than the data of Hu et al. (1994) and of Gerrity et al. (1995). The fact that the Hu et al. (1994) and Gerrity et al. (1995) data are consistent with each other supports this speculation. This observation may account for the result of Ultman et al. (1993) that the uptake efficiency of the URT is greater by the nasal pathway than by the oral pathway, which is counter to the observations of Gerrity et al. (1988).

Lower Respiratory Tract Uptake

Table 8-4 summarizes the data on the uptake efficiency of the LRT tract (F_{lrt}). In this discussion, F_{lrt} is the uptake efficiency of the LRT relative to the concentration of O_3 entering the LRT. The human data of Gerrity et al. (1988, 1994, 1995) and the rat data of Hatch et al. (1989) include the larynx in the LRT. The beagle dog data of Yokoyama and

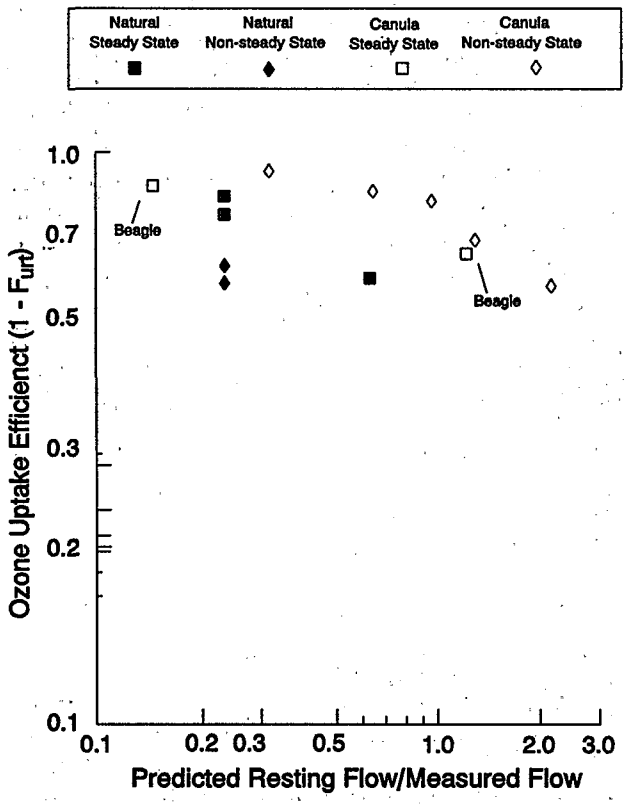


Figure 8-3. Unidirectional uptake efficiency in the upper respiratory tract by the oral pathway. The ratio of predicted resting flow to measured flow scales the flow to allow for interspecies comparison. The closed squares and diamonds at a scaled flow of 0.25 are from Gerrity et al. (1994), the closed square at a scaled flow of 0.65 is from Gerrity et al. (1988), the open diamond at a scaled flow of 0.97 is from Gerrity et al. (1995), the remaining open diamonds are from Hu et al. (1994).

Frank (1972) does not include the larynx. The influence of the larynx in the human data of Hu et al. (1992b) is uncertain because of the volumetric definition of the URT (see discussion above).

Human data obtained using non-steady state methods by the oral pathway are plotted in Figure 8-4 as a function of inspiratory flow. As in Figure 8-1, the data of Hu et al. (1994) are plotted for three different V_Ts: 500 mL, 1,000 mL, and 1,500 mL. The first observation is that the single data point of Gerrity et al. (1995) is approximately 20% higher than the data of Hu et al. (1994) at a comparable flow and V_T. Second, the data of Gerrity et al. (1994) are markedly lower than that suggested for comparable flows and V_Ts by the data of Hu et al. (1994). In assessing this discrepancy, it is important to keep in mind that the URT is defined in the work of Hu et al. (1994) as a fixed volume of 50 mL distal to the lips. This is an inaccurate definition that could influence greatly the estimation of LRT uptake efficiency. The coherence of all of the data on F_t and F_{urt} by the oral pathway

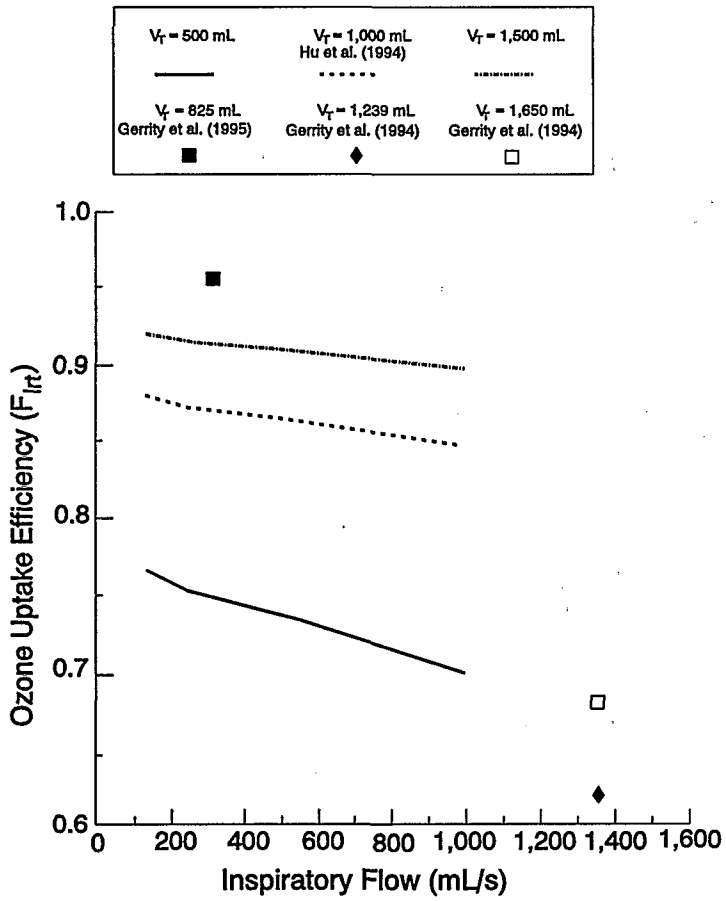


Figure 8-4. Uptake efficiency of the lower respiratory tract as a function of inspiratory flow in humans.

suggests that F_{lrt} also should be consistent among studies. If it is assumed that the data represented by the Gerrity et al. (1994, 1995) studies more accurately reflect human LRT uptake efficiency, then the flow dependence for F_{lrt} would be considerably steeper than suggested by the data of Hu et al. (1994).

Finally, the F_{lrt} data of Yokoyama and Frank (1972) in beagle dogs were acquired with flows that were about 100 and 150% of resting flow rates and V_Ts approximately 150% of those predicted (Guyton, 1947). When flow is scaled in the dogs, the F_{lrt}s of 0.87 and 0.83 at the two flow rates are consistent with the human data of Gerrity et al. (1995) but higher than the Hu et al. (1994) data.

8.2.4 Dosimetry Modeling

8.2.4.1 Background

Table 8-5 presents a summary of theoretical studies of the uptake of O_3 by the RTs (or regions) of humans and laboratory animals that have become available since the 1986 review. Although there are 10 investigations listed, there are only five distinct

Table 8-5. Theoretical Ozone Dosimetry Investigations[a]

Species and Region Modeled/Anatomical Model	Liquid Lining and Tissue Transport and Chemical Reactions	Dosimetry Model[b]/Subject of Investigation	Results/Predictions	Reference
Guinea pig LRT/Kliment (1973), Schreider and Hutchens (1980); rat LRT/ Kliment (1973), Yeh et al. (1979)	Miller et al. (1985)	Enhanced Miller et al. (1985). Investigates the effect on predictions of anatomical models, FRC, ventilation, and TB liquid lining rate constant. Simulates O_3 uptake in anatomical models of rat lobes.	With respect to different anatomical models for the same and different species: qualitative similarity in the shape of net and tissue dose versus airway number curves, but significant differences in regional fractional uptakes. Maximum tissue dose in vicinity of PAR. PAR dose decreases with increasing time of flight to this region. Maximum tissue dose in the vicinity of the first pulmonary region segment of anatomical models.	Overton et al. (1987)
Human, rat, rabbit, and guinea pig LRT/not specified	Miller et al. (1985) and Overton et al. (1987)	Miller et al. (1985) and Overton et al. (1987). Compares O_3 dose profiles of human, rat, guinea pig, and rabbit. Uses model and experimental data to estimate O_3 dose-response curves for decrements in FEV_1 (humans) and for BAL proteins in rat, guinea pig, and rabbit. Compares LRT uptake predictions to the human experimental data of Gerrity et al. (1988).	Similarity among species in the shape of the airway segment curve: tissue dose increases distally in the TB region, reaches a maximum in the first pulmonary region segment for human, rat, and guinea pig and in the last TB segment of the rabbit, and then decreases distally in the pulmonary region. Predictions of uptake distal to the oropharynx are in agreement with Gerrity et al. (1988).	Miller et al. (1988)
Rat total RT/URT: Schreider and Raabe (1981); TB: Uses rat cast data (Raabe et al., 1976) to define TB region paths; PUL: Yeh et al. (1979) for model of the acinus	TB and pulmonary region mass transfer coefficients based on Overton et al. (1987)	Enhanced Overton et al. (1987). Illustrates dose distribution along the longest and shortest (as defined by time of flight) paths from the trachea to the most distal alveoli.	Threefold difference in PAR doses of the shortest and longest paths from trachea to PAR. Dose distributions along the longest or shortest path were qualitatively similar to Overton et al. (1987), with maximum tissue dose in the first pulmonary region generation.	Overton et al. (1989)
Human LRT (newborn to adult)/TB: based on Yeh and Schum (1980); PUL: based on Hansen and Ampaya (1975)	Miller et al. (1985)	Enhanced Overton et al. (1987). From various sources, develops age-dependent LRT anatomical models. For quiet and maximal exercise breathing, applies the dosimetry model of Miller et al. (1985) to several ages from birth to adult, illustrating the LRT distribution of absorbed O_3.	For quiet breathing, the LRT distribution of dose, the percent uptake, and the PAR dose are not very sensitive to age; but are more sensitive during exercise. Regardless of age and breathing state, the largest O_3 dose occurs in the PAR. No uptake in the URT.	Overton and Graham (1989); Miller and Overton (1989)

Table 8-5 (cont'd). Theoretical Ozone Dosimetry Investigations[a]

Species and Region Modeled/Anatomical Model	Liquid Lining and Tissue Transport and Chemical Reactions	Dosimetry Model[b]/Subject of Investigation	Results/Predictions	Reference
Human total RT/URT: Hanna and Scherer (1986); LRT: Weibel (1963)	Time-dependent molecular diffusion and first-order reactions in liquid lining and interstitium; transfer through epithelium modeled as a permeability process—no reactions in this layer. URT and LRT liquid lining rate constants: 50 and 1 times that of Miller et al. (1985), respectively	Model development. Lung dimensions scaled to those of a young male and a young female. Contrasts LRT air-phase concentrations during exercise and rest. Compares male and female air-phase O_3 concentrations and male and female subepithelial concentrations.	URT uptake may be greater in cold than in warmer air. For the ventilatory parameters used, (1) subepithelial O_3 concentrations are a maximum in the terminal bronchioles or the first respiratory bronchioles, and (2) these concentrations are greater in the female than in the male for most of the RT.	Hanna et al. (1989)
Human, distal segment of a lobe/ based on Horsfield et al. (1971)	Similar to Miller et al. (1985)	Monte Carlo simulation; transport processes defined in terms of probabilities based on physical and chemical principles. The effect of lung asymmetries on the distribution of uptake in the pulmonary region.	Tissue dose in the PAR along the shortest path is approximately 50% larger than that along the longest path.	Ultman and Anjilvel (1990)
Human LRT/Weibel (1963)	Investigates two formulations: (1) Miller et al. (1978) and (2) Miller et al. (1985)	Model development; assumes quasi-steady conditions. Air-phase concentration and tissue dose profiles for the two reaction schemes, various ventilatory parameters, and various liquid lining transport and chemical parameters. Compares predictions of first-order reaction scheme to results of Miller et al. (1985).	This model does not conserve mass. Predictions should only be considered qualitatively. Maximum tissue dose in respiratory bronchioles for both chemical reaction schemes.	Grotberg et al. (1990) (Grotberg [1990])
Rat LRT/based on serial reconstruction of a set of intrapulmonary airways and their ventilatory units combined with a single path from the larynx to the reconstructed set based on Yeh et al. (1979)	TB and pulmonary region mass transfer coefficients based Overton et al. (1987)	Model development. Illustrates the influence of ventilatory unit size and proximal anatomic dead space and on the uptake and distribution of inhaled O_3 in ventilatory units. (Illustrates the influence of ventilatory unit volume on the distribution of inhaled O_3 within ventilatory units.)	Ventilatory unit uptake is significantly influenced by both proximal airway dead space and ventilatory unit volume. Flux of O_3 to air-liquid interface in the proximal portions of larger ventilatory units are significantly greater than in smaller units.	Mercer et al. (1991) (Mercer and Crapo [1993])

Table 8-5 (cont'd). Theoretical Ozone Dosimetry Investigations[a]

Species and Region Modeled/Anatomical Model	Liquid Lining and Tissue Transport and Chemical Reactions	Dosimetry Model[b]/Subject of Investigation	Results/Predictions	Reference
Rat ventilatory unit/ Mercer et al. (1991)	Mercer et al. (1991)	Mercer et al. (1991). Along a path distally from a bronchiolar-alveolar duct junction; compares experimentally determined changes in ventilatory unit wall thickness due to an O_3 exposure to dosimetry model predictions of flux to the air-liquid interface.	As a function of distance from the BADJ; experimentally determined relative changes in ventilatory unit wall thickness due to O_3 exposure are very similar to predicted relative fluxes to the air-liquid interface.	Pinkerton et al. (1992); Miller and Conolly (1995)
Human total RT/URT: Fredberg et al. (1980); LRT: Weibel (1963)	Pseudo steady-state diffusion and first order reactions combined with biochemical data of Miller et al. (1985)	Model development. Illustrates LRT distribution of (1) air-phase concentration at various times during the breathing cycle and (2) O_3 flux (dose) to liquid lining and to tissue.	Flux of O_3 to air-liquid interface decreases distally; flux to tissue increases along the conducting airways, reaches a maximum in the terminal bronchioles, then decreases rapidly in the gas exchange region.	Hu et al. (1992a)

[a]See Appendix A for abbreviations and acronyms. Generally, for modeling purposes, PAR is the first pulmonary region, generation, or model segment; PUL = pulmonary region.
[b]Refers to the theoretical or mathematical formulation aspects of gas transport and reactions without the specification of morphometric and physiological parameter values.

dosimetry models (with respect to groups of co-workers and independent model formulation): the models of (1) Overton et al. (1987) and Miller et al. (1985); (2) Hanna et al. (1989); (3) Grotberg et al. (1990), although they considered two reaction schemes; (4) Mercer et al. (1991); and (5) Hu et al. (1992a). In some cases, several references have been grouped into one investigation. This is because the multiple studies came from the same co-workers or laboratory and added to or were complementary to the original or common dosimetry modeling theme.

Major factors affecting the local uptake of reactive gases in the RT were respiratory tract morphology and anatomy; the route of breathing (nose or mouth); the depth and rate of breathing (V_T and f); the physicochemical properties of the gases; the processes of gas transport; and the physicochemical properties of the liquid lining of the RT, respiratory tract tissue, and capillary blood. A detailed discussion of these factors can be found in Overton (1984), Ultman (1988), and Overton and Miller (1988).

Because all of the dosimetry models listed in Table 8-5 were developed to simulate uptake in the LRT or the total RT, these models have some common aspects. These include the formulation of O_3 transport and wall loss in the air compartments of the RT, the use of species-dependent morphometric models or data to define air and liquid lining compartment dimensions, and a description of the transport and loss of O_3 in the liquid lining and tissue.

In all the dosimetry models that have become available since 1986, except for Ultman and Anjilvel (1990) and Grotberg et al. (1990), which are discussed later, O_3 transport and loss processes in air compartments were approximated in terms of a one-dimensional, time-dependent, partial differential equation of continuity. This type of equation accounts for axial convection and dispersion or diffusion and the loss of O_3 by absorption at the gas-liquid interface. The use of this approximation is very common in modeling the transport in the LRT of gases such as oxygen, nitrogen, helium, and CO_2 (e.g., Scherer et al., 1972; Paiva, 1973; Chang and Farhi, 1973; Yu, 1975; Pack et al., 1977) and has been assumed to be applicable to O_3. Ultman and Anjilvel (1990) used a Monte Carlo method to simulate O_3 uptake. Based on the physical and chemical principles of mass transport in the RT, probabilities were assigned to the fate of a molecule in a way so as to account for convection, dispersion, and loss to the liquid lining.

Dosimetry models published since 1986 can be grouped according to how transport and chemical reactions are modeled in respiratory tract fluids and tissues: those based on the formulation of (1) Miller (1977) and Miller et al. (1978), who used an instantaneous reaction scheme, and (2) Miller et al. (1985), who used a quasi-steady, first-order reaction scheme. These two approaches are discussed in the earlier criteria document (U.S. Environmental Protection Agency, 1986). In addition to the use of similar formulations for liquid and tissue transport/reactions, all of the post-1986 studies used essentially the same biochemical data of either Miller et al. (1985) for humans or Overton et al. (1987) for laboratory animals. The implication is that most of the studies are expected to predict qualitatively similar results.

There are minor variations on the second chemical reaction formulation. Hanna et al. (1989) used a time-dependent diffusion-reaction equation, instead of the time-independent (quasi-steady) equation used by Miller et al. (1985). Based on the rate constants used by Hanna et al. (1989) and on discussions in Miller et al. (1985) and in Grotberg et al. (1990), the modeled transport processes in the liquids and tissues can be inferred as essentially quasi-steady, which is equivalent to the second formulation. Another variation

uses mass transfer coefficients determined by the second formulation and the biochemical assumptions of Miller et al. (1985) or Overton et al. (1987). The liquid and tissue transport/reaction formulation for specific investigations is indicated in column 3 of Table 8-5.

In addition to the assumptions and the formulation of equations that describe the transport and loss of O_3 in the RTs of humans or laboratory animals, it is important to evaluate whether simulation results reflect accurate solutions to the mathematical dosimetry model formulation. Of the five distinct model formulations listed in Table 8-5, Overton et al. (1987), Mercer et al. (1991), and Hu et al. (1992a) discuss most or all of the relevant issues of stability, solution convergence, and mass conservation. In addition, using steady unidirectional flow in a straight tube as a test case, they report successfully simulating analytical solutions to their equations of transport and uptake. Neither Hanna et al. (1989), Ultman and Anjilvel (1990), nor Grotberg et al. (1990) address the issue of accuracy. There is no reasonable way to judge whether the solutions of Hanna et al. (1989) or of Ultman and Anjilvel (1990) accurately represent solutions to their dosimetry model assumptions and formulations; however, with the exception of Grotberg et al. (1990), there are no reasons to assume that the solutions of these models are not accurate.

Because the Grotberg et al. (1990) model formulation is different than the others, an explanation is needed. Based on the smallness of relevant parameters, Grotberg et al. (1990) assume quasi-steady conditions for O_3 concentration and air velocity in the air compartments and obtain approximate analytical solutions to the time-independent, three-dimensional equation of continuity for a model airway and apply the results to the morphometric model of Weibel (1963). One advantage of analytical solutions is that they account naturally for parameters (such as dispersion and gas-phase, mass-transfer coefficients) or local processes (e.g., possibility of high uptake at airway entrances) that must be known and estimated for, or incorporated into, the one-dimensional approach. Grotberg et al. (1990) carried out simulations using anatomical and physiological conditions based on Miller et al. (1985) and compared their results. Although qualitatively similar to Miller et al. (1985), Grotberg and co-workers predicted significantly larger pulmonary tissue doses (up to 10-fold). A comparison of the pulmonary region doses predicted by Grotberg et al. (1990) to those predicted by Miller et al. (1985) indicates that the Grotberg et al. (1990) model does not conserve mass (it predicts that the pulmonary region absorbs over 3.4 times the amount of O_3 inhaled). The overprediction may be an artifact of the quasi-steady approximation, because effects due to differences in the time of flights from the trachea to different LRT locations are not taken into account. In any case, the quantitative predictions reported by Grotberg et al. (1990) are questionable.

Chemical data more recent than that used for the dosimetry models in Table 8-5 show that compounds other than unsaturated fatty acids (the only compound with which O_3 is assumed to react in the models using the second chemical reaction formulation) are as reactive or more reactive with O_3 (Pryor, 1992). Using these data, estimates of O_3 diffusion coefficients in the liquid lining and bilayers, layer thicknesses, and data on the concentrations of biocompounds in these layers, Pryor (1992) estimates that most of any O_3 that penetrates into a cell bilayer reacts within the layer (very little if any penetrates to the cell interior), and O_3 will not penetrate the liquid lining where it is greater than 0.1 μm thick. Several relevant comments are given by Pryor (1992): the calculations are considered a crude first approximation; the possibility that a small fraction of O_3 may penetrate the bilayer and reach

the cell interior can not be excluded; and surfactant layers can be very thin, and some cells may not be protected very well or at all.

If the conclusions of Pryor (1992) are essentially correct, they have implications for past and future dosimetry modeling studies because past investigations have underestimated the reactivity of O_3 with biocompounds; with respect to cellular damage, products of O_3 reactions in the liquid lining may be the main toxic compound; increasing the value of rate constants would have no effect on predictions of dosimetry models using the instantaneous reaction scheme because rate constants in this approach are assumed to be infinite, but increasing the concentration of reacting biocompounds would increase uptake; (4) use of unsaturated fatty acid data only (with a first-order reaction scheme) results in an underestimate of the reactivity of O_3 in the liquid lining and an overestimate of the O_3 tissue dose, and a possible underestimation of the toxic dose due to reaction products; and (5) with higher O_3 reaction rates, the first-order chemical-reaction formulation would result in larger predicted uptakes.

8.2.4.2 Dosimetry Model Predictions

Similarity of Model Predictions

A survey of dosimetry modeling results shows that, in some areas of investigation, there is a qualitative similarity in predictions by models of different groups of investigators for different species or subpopulations.

(1) *Distribution of LRT dose (dose profiles or dose versus generation).* As shown in Figure 8-5, beginning at the trachea, net dose (O_3 flux to air-liquid interface) slowly decreases distally in the tracheobronchial region (TB) and rapidly decreases distally in the pulmonary region. Tissue dose (O_3 flux to liquid-tissue interface) is very low in the trachea, increases to a maximum in the terminal bronchioles or first airway generation in the pulmonary region, and rapidly decreases distally from this location (e.g., Miller et al., 1978, 1985, 1988; Overton et al., 1987, 1989; Overton and Graham, 1989; Grotberg et al., 1990; Hu et al., 1992a).

If O_3 were the only toxic agent and all the tissues of the LRT were equally sensitive to the same dose, the models predict that the greatest morphological damage would occur in the vicinity of the junction of the conducting airways and the pulmonary region and decrease rapidly (distally) from this area, which is consistent with observations in laboratory animals (see Chapter 6, Section 6.2.4). On the other hand, using the best estimates of morphometric and physiologically based biochemical parameters of Miller et al. (1978, 1985) and Overton et al. (1987), the models predict extremely (relatively) low tissue doses in the trachea and large bronchi; this suggests very little or no tissue damage should occur there, which is contrary to observations (see Chapter 6, Section 6.2.4). However, this is moot, if, as suggested by Pryor (1992), the toxic substances are primarily reaction products of O_3 and not O_3 itself. In this case, the O_3 net local dose, not the local O_3 tissue dose, may be a better estimator of local toxic tissue dose, because the rate of production of products would be related to the rate of O_3 uptake.

(2) *Effect of exercise or increased ventilation.* The effect of exercise is to slightly increase the TB dose and to significantly increase the pulmonary region total dose (mass of O_3) and the CAR dose (mass per unit surface area) (e.g., Miller et al., 1979, 1985; Overton et al., 1987, 1989; Overton and Graham, 1989; Hanna et al., 1989; Grotberg et al., 1990).

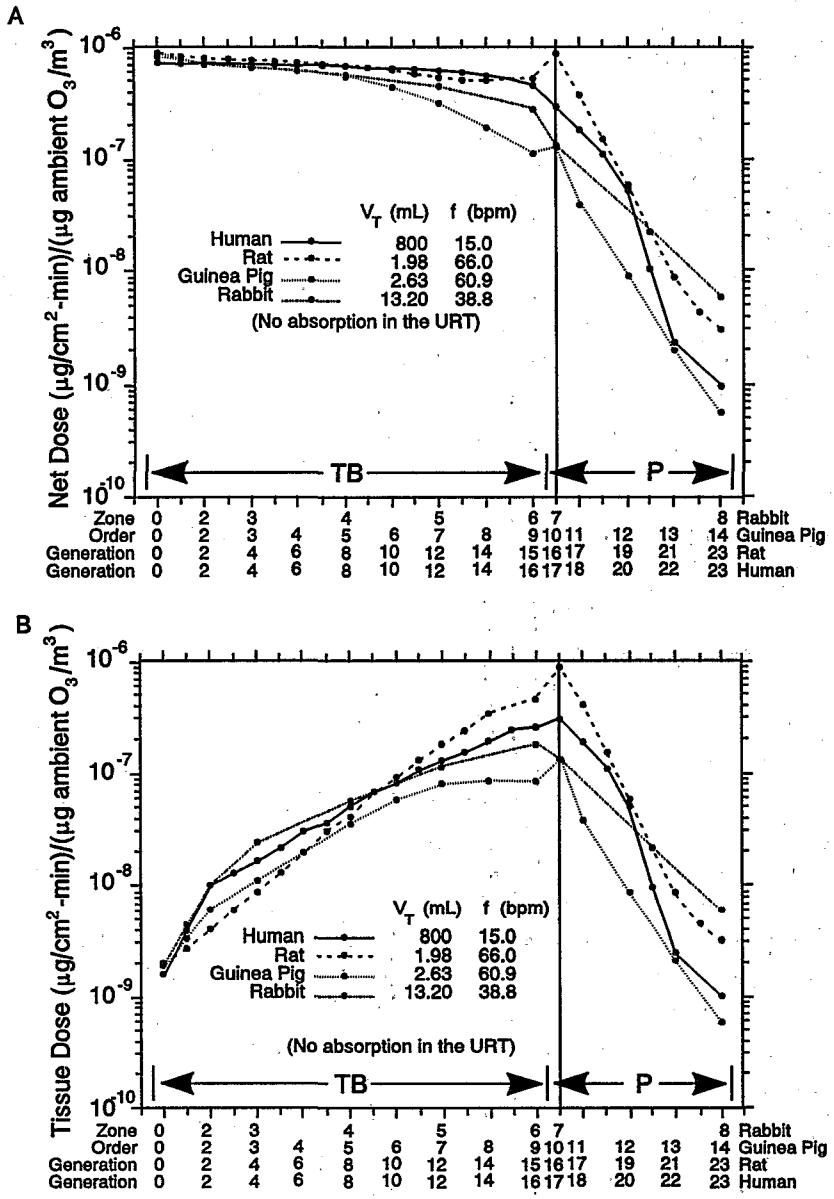


Figure 8-5. Net dose (A) and tissue dose (B) versus sequential segments along anatomical model airway paths for human, rat, guinea pig, and rabbit. In general, each segment represents a group of airways or ducts, with common features as defined by the designers of the anatomical model (human and rat: generation; guinea pig: order; rabbit: zone). For a given species, the plotted dots represent a predicted dose that corresponds to a given segment. The dots have been joined by lines for ease of interpreting the plots; these lines do not represent predicted values except where they intercept the dots. TB = tracheobronchial region; P = pulmonary region.

Source: Overton and Miller (1988).

(3) *Effect of respiratory tract inhomogeneity.* Models have predicted that the further the proximal alveolar region is from the trachea, the less the O_3 tissue dose (mass of O_3 absorbed per unit surface area) to the proximal alveolar region. (For modeling purposes, the proximal alveolar region has been defined as the first pulmonary generation or the first pulmonary region model segment along a path; this region is a part of the CAR.) Overton et al. (1989) predicted a threefold greater proximal alveolar region dose for the shortest path relative to the longest path in rats. Ultman and Anjilvel (1990) simulated O_3 distribution in a small segment (<1%) of the distal airways of an asymmetric anatomic model of the human lung. They found that the O_3 tissue dose (mass per square centimeter) in the proximal alveolar region along the shortest path was approximately 50% greater than that along the longest path. Mercer et al. (1991) found that path distance and ventilatory unit size affect dose: predicted doses in the proximal segments (essentially, the proximal alveolar region) of the larger ventilatory units (with the smallest relative dead space) are significantly larger than the average proximal segment doses. Further, for the small sample of ventilatory units modeled (43), Mercer et al. (1991) predicted a range of proximal segment doses of greater than a factor of 6. Because the proximal alveolar regions of Ultman and Anjilvel (1990) and of Mercer et al. (1991) belonged to a "local cluster", and there are many clusters with varying distances from the trachea, a variability greater than 50% and a factor greater than 6, respectively, are expected in proximal alveolar region doses. Mercer and Crapo (1993) illustrated the effect of ventilatory unit volume alone on the distribution of dose, predicting that a 2.3 times larger unit receives 1.9 times the dose (mass per surface area) of the smaller unit at the entrance of the unit.

The variability of predicted proximal alveolar region doses and, by inference, CAR doses suggests that the magnitude of toxicological effects for different CARs are different. This prediction is consistent with the observations of Schwartz et al. (1976) and Boorman et al. (1980) of damage variation among different CARs of the same rat. It is reasonable to assume that variable damage at equivalent but different morphological locations also occurs in humans.

Specific Topics

Effect of Assumptions About Anatomical Dimensions. For rats and guinea pigs, Overton et al. (1987) used two morphometrically based anatomical models (rat anatomical models: Kliment, 1973, and Yeh et al., 1979; guinea pig anatomical models: Kliment, 1973, and Schreider and Hutchens, 1980) to investigate the influence of anatomical model formulation on predicted uptake. Results with all four anatomical models in combination with different ventilatory parameters showed a qualitative similarity in the shapes of the dose profiles, but the two anatomical models for the same species resulted in considerable differences in predicted percent RT and pulmonary region uptakes.

Respiratory Tract Uptake in Human Adults and Children. Overton and Graham (1989) used several sources of data on age-dependent LRT dimensions and structure to construct theoretical LRT anatomical models for humans from birth to adulthood. The O_3 dosimetry model of Miller et al. (1985) was used to estimate the regional and local uptake

of O_3. For the percent uptake (84 to 88%) during quiet breathing, the LRT distribution of absorbed O_3 and the centriacinar O_3 tissue dose are not very sensitive to age. Regional percent uptakes are more dependent on age during heavy exercise or work than during quiet breathing, and total uptakes range from 87 to 93%. Generally, the total quantity of O_3 absorbed per minute increases with age. For all conditions simulated, the largest O_3 tissue dose is predicted to occur in the CAR. Miller and Overton (1989) present similar results. Because uptake by the URT was not simulated and because this region can be assumed to have an important effect on LRT uptake, a comparison of predictions of LRT uptakes in children and adults should be viewed with caution. On the other hand, URT uptake probably has little effect on the shape of the dose curves.

Dosimetry Modeling Results Compared to Dosimetry Data

Based on the experimental conditions discussed in Gerrity et al. (1988) and using the model and parameters of Miller et al. (1985), Miller et al. (1988) simulated the uptake of O_3 distal to the oropharynx of human subjects. For the target f's of 12 and 24 bpm and V_Ts ranging from 0.4 to >1.6 L, the simulation results were in good agreement with the breath-by-breath experimental data. The average experimental LRT uptake efficiency was ≈ 0.91 as compared to the 0.89 prediction given by Miller et al. (1985) for the region distal to the oropharynx. It should be remembered, however, that values for uptake efficiency from the Gerrity et al. (1988) study were derived from the raw data using a steady-state method, whereas the models of Miller et al. (1985) and Miller et al. (1988) utilize cyclic flow, thus making the predictions more appropriate for comparison with uptake data from non-steady state methods. From an analysis in Gerrity et al. (1994), it appears that total RT uptake computed by either steady-state or non-steady state methods differ by only about 10% in relative terms.

There have been major improvements to the original model, as described by Miller et al. (1985) and Overton et al. (1987), including the addition of the URT and establishing a regional mass transfer coefficient based on experimental data. Table 8-6 summarizes the assumptions that underpin these improvements, as well as other relevant assumptions used for simulating the uptake of several human dosimetry experiments. A discussion of the assumptions is given in Section 8.5.2.

After taking into consideration the assumptions of Table 8-6, Table 8-7 compares experimental total RT uptake efficiency data and model predictions for humans. Use of the rat assumptions in conjunction with the model will be discussed later (Section 8.4.3). The model predictions show good agreement with the total RT uptake efficiency data of Gerrity et al. (1988), Gerrity et al. (1994), and Hu et al. (1992b). In all cases, the predictions are within 10% of the measured values. The agreement with the data of Hu et al. (1992b) is even better, as expected.

The model prediction for the data of Wiester et al. (1996) is less accurate. Comparison of the Wiester et al. (1996) data with the Hu et al. (1994) data (Figure 8-1) shows, however, that the results are in good agreement with each other. Thus, it would appear that the V_T dependence of the model does not necessarily reflect the real world. However, the general agreement between the model predictions and data are quite good.

Although the models are capable of making reasonable predictions of total RT uptake efficiency, their accuracy for specific regions remains uncertain. The O_3 bolus data (Hu et al., 1992b, 1994; Ultman et al., 1993) and the airway uptake efficiency data

Table 8-6. Assumption for Application of Dosimetry Model to Breathing Frequency Responses to Ozone[a]

Species	Mode of Breathing	Respiratory Tract Morphology	Mass Transfer Coefficients
Human	Oral	- LRT structure from Weibel (1963). - Volume of oral cavity through larynx and surface to volume ratio (S/V) from Hu et al. (1992a,b). - Dead space volume (V_d) and FRC from Hart et al. (1963); TB region volume at FRC equals V_d minus oropharyngeal volume. - Proximal alveolar region defined as first respiratory bronchiole. - Pulmonary region expands; TB does not expand.	- Mass transfer coefficients for each oropharyngeal segment and each TB generation defined as Ka/(S/V) where S/V for the TB region is from Weibel (1963) dimensions reduced to FRC; Ka from Hu et al. (1992b). - Pulmonary mass transfer coefficient is 0.10 cm/s (Miller et al., 1985).
Rat	Nasal	- NP dimensions from Schreider and Raabe (1981). - TB region from Yeh et al. (1979). - Volumes and surface areas of LRT isotropically scaled to FRC. - Pulmonary region from Mercer et al. (1991). - TB and pulmonary regions expand uniformly during breathing. - Proximal alveolar region is first generation of pulmonary region.	- NP and TB mass transfer coefficients estimated using data of Hatch et al. (1989). - Mass transfer coefficient of pulmonary region = 0.137 cm/s; inferred from Pinkerton et al. (1992).

[a]See Appendix A for abbreviations and acronyms.

Table 8-7. Comparison of Total Respiratory Tract Uptake Data with Model Predictions[a]

V_T (mL)	f (bpm)	Measured F_t	Predicted F_t	Data Source
832	12	0.97	0.96	Gerrity et al. (1988)
832	24	0.96	0.93	Gerrity et al. (1988)
500	15	0.86	0.89	Hu et al. (1992b)
1,000	7.5	0.93	0.94	Hu et al. (1992b)
1,650	25	0.88	0.95	Gerrity et al. (1994)
1,239	35	0.87	0.93	Gerrity et al. (1994)
631	16	0.76	0.94	Wiester et al. (1996)

[a]See Appendix A for abbreviations and acronyms.

(Gerrity et al., 1995) raise questions about the model predictions of uptake in the conducting airways. These latter data sets suggest that the models of Miller et al. (1985, 1988) may underestimate the O_3 uptake coefficients in the conducting airways. Ultman et al. (1993) show in their analysis of the bolus data that the reactivity of O_3 with the lung liquid lining decreases with increasing depth into the lung. This could imply that more O_3 is taken up in central airways than previously had been thought. However, the predictions presented in Table 8-7, which are based on the assumptions of Table 8-6, represent a revision of the Miller et al. (1985) model in that the mass transfer coefficients are derived from the actual human data of Hu et al. (1992b).

Because the utility of dosimetry models is their usefuleness in facilitating interspecies extrapolation, it is important to compare predictions with animal data as well as with human data. Using the Overton et al. (1987) model formulation and parameters, Overton et al. (1989) developed a formula that can be used to calculate LRT uptakes in rats, given their V_Ts and f's. For comparison purposes, the data of Wiester et al. (1987, 1988) and Hatch et al. (1989) can be used. The average uptake efficiency for the rat from these data is 0.45. Based on the V_Ts and f's of these animals, an average LRT uptake of 0.61 is computed using Overton et al. (1989). If Overton et al. (1989) had included the effects of URT uptake, their model would have predicted more than 0.61.

It is important to note that these results are based on the older model assumptions, not those presented in Table 8-6. If the newer assumptions had been used, the agreement between predictions and actual results would have been much better (i.e., total uptake would have been in the 50 to 60% range, depending on ventilation).

8.3 Species Sensitivity: Lung Function and Inflammatory Endpoints Exemplifying an Approach

8.3.1 Introduction

Quantitative extrapolation of animal-based O_3 toxicity data to the human circumstance requires a paradigm that includes both an estimate of target tissue dose (dosimetry) and an algorithm that relates the responsiveness of the test species to that of the human (species sensitivity). This paradigm can be depicted as an extrapolation parallelogram (Figure 8-6), which conceptualizes a relationship between chronic animal study data and long-term human health effects based on an understanding of acute effects in both species (Graham and Hatch, 1984). Although recent studies have begun to elucidate the underlying mechanisms determining response, the bulk of the present O_3 toxicity database in animals and humans remains largely descriptive. Hence, only a simplified application of this paradigm is feasible at this time. The following section will attempt to harmonize selective literature on acute human and animal responses to O_3 exposure (already reviewed in detail in Chapters 6 and 7) with what is known about the dosimetry of O_3, in an effort to discern relative species sensitivity. To construct an argument that is plausible for this test application, focus is on endpoints for which there are sufficient data in both humans and test animal species and for which exposure scenarios are similar. The endpoints compared include measures of pulmonary function and markers of lung inflammation, most notably BAL protein and cells. When possible, other influencing parameters, such as ventilation augmentation and antioxidants within the lung also will be discussed. The body of in vitro cell studies has not been included because of the difficulties in interpretation associated with

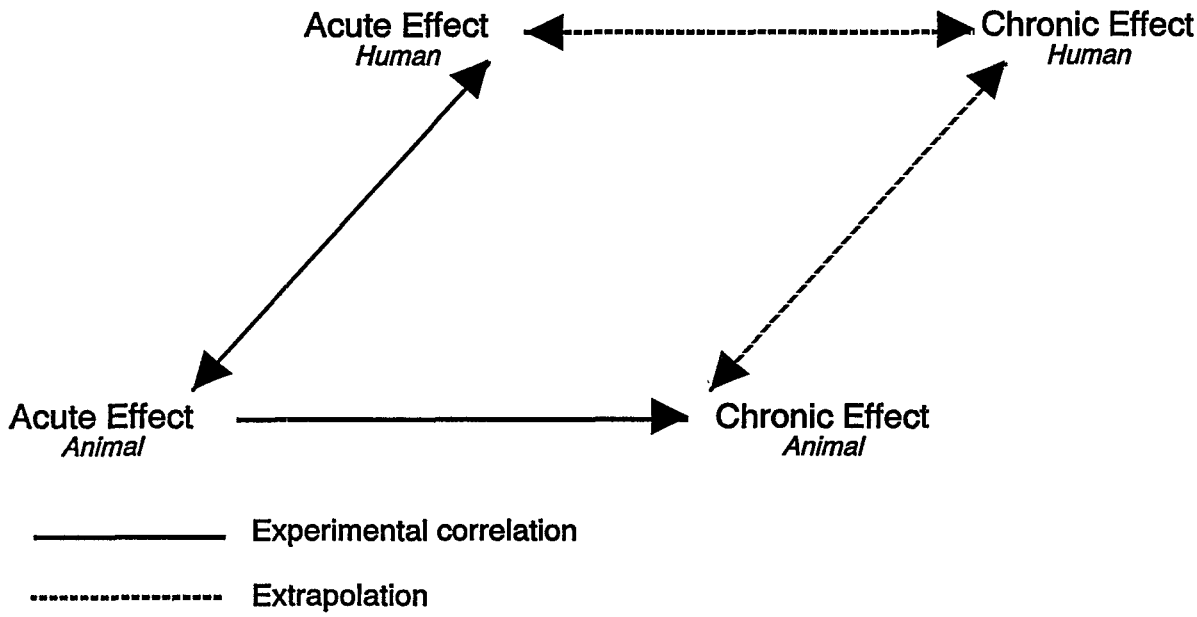


Figure 8-6. Parallelogram paradigm for utilizing animal data for human health predictions. Acute homologous endpoints serve as the basis for extrapolating chronic effects in humans from animal data.

dosimetry and culturing systems. The reader is referred to Chapter 7, Section 7.2.5, and the recent review by Koren et al. (1994). The goal here is to develop a hypothetical model for the assessment of species-specific sensitivity with acute O_3 exposure that can serve as a framework to better predict human responses, especially with regard to chronic effects. The complex issue of whether controlled human clinical studies accurately reflect population-based responses also will not be considered in this discussion.

8.3.1.1 Dosimetry

This topic has been discussed in detail in Section 8.2. Recent studies by Hatch et al. (1994), utilizing the nonradioactive isotope of oxygen, ^{18}O, to label O_3, have shown that exposure of exercising humans (60 L/min) and resting rats to 0.4 ppm O_3 for 2 h resulted in 4 or 5 times the ^{18}O dose (as adduct) to the BAL constituents of humans as compared to those of F344 male rats. This four- to fivefold difference appeared to be due to the exercise-stimulated hyperventilation of the humans when compared to the rat and compared favorably with indices of effect (i.e., BAL cells and protein at 24 h). Only when the rats were exposed to 2 ppm O_3 for 2 h did the $^{18}O_3$ labeling of BAL constituents approximate that of the human. Thus, on the basis of this study of cellular and protein influx due to O_3 injury, the rat and human appear to have similar sensitivity to O_3 when exercise is considered. Additional related studies with $^{18}O_3$ indicate that deposition in the RT is a cumulative function of ventilation over the initial period of exposure, which would lend support to these findings (Santrock et al., 1989). Attempts to compare animal data obtained

without exercise to human study data with exercise thus would underestimate the dose to the lung and presumably the resultant risk of effect. Studies of O_3 effects (but without assessment of dose) in exercising rodents have confirmed this conclusion (Mautz et al., 1985).

Exercise is only one factor that can alter dose and effect. Studies in laboratory animals that incorporate other factors, such as time of day or diurnal rhythms (Van Bree et al., 1992), animal strain (Pino et al., 1991; Costa et al., 1993), or nutrition (Chapter 6, Section 6.2.5), also show substantial modification of response to O_3, and thus emphasize the need for careful consideration of exogenous factors when attempting to compare or extrapolate study findings. It is likely that a similar range of factor-dependent variability exists within human test subjects.

8.3.2 Homology of Response

The concept of species sensitivity actually consists of two integrated components. The first, homology of response, indicates whether the outcome seen in the animal test species represents the same biological response in the human. In many cases, a measurement of the same endpoint in both species can be presumed to reflect the same toxic phenomenon or mechanism (i.e., the pulmonary irritant-induced tachypnea) (see below). On the other hand, there may be endpoints that, although homologous, are not expressed similarly; for example, the burning discomfort of sensory irritation in the human and the pause on tidal expiration seen in rodents. The second component of species sensitivity relates the dose-response curve to given homologous responses. Alterations in permeability of the air-blood barrier of the lung appear to reflect true species differences in sensitivity to pulmonary irritants such as O_3 (Hatch et al., 1986). Ideally, these elements of species sensitivity should flow directly into extrapolation formulae developed to integrate animal and human research data.

8.3.2.1 Lung Function Endpoints as Homologous Indicators

Lung function studies of small mammals have provided basic physiological information important to the understanding of both normal and diseased lungs (Snider and Sherter, 1977; Harkema et al., 1982; Raub et al., 1982; Mauderly, 1984; Costa, 1985). Animal lung function tests, adapted from those used clinically, have proven useful in describing the nature and severity of lung injury and in distinguishing toxicant-induced effects in the central or peripheral airways from those effects in the parenchyma.
In practice, the interpretation of functional changes detected in animals derives from knowledge and experience in human pulmonary medicine. Supporting this view, in theory, is the allometric database for normal mammals, in which the lung function variables associated with ventilation and aerobic metabolism scale systematically to body mass over nearly seven orders of magnitude (Stahl, 1967; Leith, 1976). The lung function studies of O_3 toxicity in animals and humans considered in the present discussion are described in detail in Chapters 6 and 7, Sections 6.2.5 and 7.2, respectively, of this document and in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986).

8.3.2.2 Inflammatory and Antioxidant Endpoints as Homologous Indicators

Inflammation of pulmonary airways and airspaces is best described as a cascade of events that network infiltrating leukocytes, plasma proteins, and cell-derived mediators,

which function presumably to defend or repair (but may further damage) the injured lung (see Chapters 6 and 7, Sections 6.2.2 and 7.2.4, respectively; Koren et al., 1994). Key markers of the basic inflammatory process include plasma-derived proteins, such as albumin, globulins, and plasmin, and a primary inflammatory cell, the polymorphonuclear leukocyte (PMN). Of these two markers, plasma-derived proteins in the acute phase generally are thought to represent a "leak" from the vasculature to the airspace lumen. Hence, under controlled temporal conditions, plasma protein residing in the airspace and accessible by BAL can be used as a proportional marker of effect that, in turn, should be related to dose. The presence of PMNs in the airspaces is a bit more complex because of the signals involved in recruiting these cells into the lung lumen after injury and the cascade of events apparently involved in their poiesis from the vasculature to the lung lumen. For the purposes of species comparison, plasma-derived protein (nonspecific) and the proportion of PMNs among total cells as sampled by BAL will be emphasized as primary indices of damage and inflammation within the lung.

Antioxidant substances in lung tissue (Slade et al., 1985) and BAL fluid and cells (Slade et al., 1993) have been identified and quantified for humans and several laboratory animal species. The species profiles of these antioxidants in the lung tissue and their respective BAL cells and fluid can differ appreciably (Table 8-8), but collectively they appear to play a significant role in defense of the lung against both endogenous and exogenous oxidant challenge. In particular, ascorbate and vitamin E appear to have major functions in protecting the lung from O_3 challenge (Chapter 6, Section 6.2.1; Slade et al., 1989; Crissman et al., 1993; Koren et al., 1989b; Elsayed et al., 1988), and, when their levels are manipulated in vivo, either can influence the degree of toxic outcome. Hence, the measurement of basal and O_3 response levels of these antioxidants in BAL cells and fluids is useful in assessing the qualitative and quantitative responses among humans and laboratory test species.

8.3.3 Studies of Lung Function

8.3.3.1 Confounding Influences in Lung Function Studies

Ideally, a system for measuring pulmonary function in small animals would be approximately the same as that used in humans for cooperative, unrestrained subjects. However, in animal studies, this is usually not possible. Fortunately, certain measures (e.g., static lung volumes, diffusion capacity) appear to be minimally influenced by sensory reflex or muscular activity in spite of unnatural stresses or blunting of responses caused by anesthetic or physical immobilization. On the other hand, some measurements, typically those involved in the assessment of ventilatory mechanics, can be profoundly influenced by these and other factors, such as ambient and toxicant-altered body core temperature, thus confounding cross-species comparisons. Because a major emphasis of this section is the comparison of lung function data of animals and humans, it is important that the reader realize potentially confounding influences borne by studies of lung function in rodents when compared to analogous measurements in humans. These are discussed briefly below.

Anesthesia

Anesthesia alters pulmonary function measurements in both humans (Rehder et al., 1975) and laboratory animals (Skornick and Brain, 1990; Lamm et al., 1982; Rich et al.,

Table 8-8. Pulmonary Antioxidant Substances in Various Laboratory Animal Species and Humans[a]

Antioxidant	Mouse	Hamster	Rat	Guinea Pig	Rabbit	Human
Ascorbate						
Tissue[b]	41±4	26±2	34±2	39±1	27±3	22±7
BAL cells[c]	—	—	50.3±5.4	17.9±1.4	—	3.5±0.1
BAL fluid[c]	—	—	199.4±9.1	28.8±2.2	—	21.4±2.8
Glutathione						
Tissue[b]	62±3	61±2	50±2	83±3	83±3	7±1
BAL cells[c]	—	—	14.8±2.7	14.6±2.4	—	2.9±0.5
BAL fluid[c]	—	—	12.1±5.0	11.2±1.9	—	20.4±3.8
Tocopherol						
Tissue[b]	1.0±0.1	1.0±0.1	2.1±0.1	2.0±0.2	1.4±0.2	0.8±0.1
BAL cells[d]	—	—	577.7±83.1	454.5±58.2	—	95.1±23.4
BAL fluid[d]	—	—	0.6±0.2	1.4±0.5	—	47.2±3.8
Uric Acid						
Tissue[b]	—	—	0.35±0.05	4.14±0.24	—	—
BAL cells[c]	—	—	<0.01	0.8±0.1	—	0.07±0.03
BAL fluid[c]	—	—	4.3±0.6	2.7±0.4	—	15.9±2.5

[a]See Appendix A for abbreviations and acronyms; data (mean ± SE) extracted and summarized from Slade et al. (1985, 1993).
[b]Data expressed as mg/100 g wet tissue.
[c]Data expresses as nmol/mg protein.
[d]Data expressed as nmol/mg lipid phosphorus.

1979). In general, ventilation is reduced and changes in ventilatory patterns occur (Pavlin and Hornbein, 1986; Bellville et al., 1960; Hunter et al., 1968; Siafakas et al., 1983). In humans, anesthesia can decrease compliance and FRC, and it also can increase airway resistance (R_{aw}) (Rehder et al., 1974, 1975). In small laboratory mammals, an analogous decrease in FRC occurs, although apparently via a different physiological mechanism (Lamm et al., 1982). Additional anesthesia-related effects include a blockade of irritant reflexes (Weissberg et al., 1976) and alteration of ventilatory patterns in response to CO_2 (Martin-Body and Sinclair, 1985). Hence, although not invalidating experimental results, choice of anesthetic agent may affect the measured response and may confound cross-species comparison.

Restraint

Collection of small animal pulmonary function data without the use of anesthesia usually requires some type of physical immobilization. Restraint may range from minimally restrictive, allowing turning and some locomotion, to extremely confining, as occurs when

animals are inserted into nose-only exposure tubes. Although restraint reduces movement artifacts and permits attachment of delicate probes or sensors, immobilization can also produce undesirable physiological disturbances such as changes in body core temperature (T_{co}) (Nagasaka et al., 1979), hypermetabolism (Nagasaka et al., 1980), increased expiratory CO_2 (Jaeger and Gearhart, 1982), changes in ventilation and ventilatory pattern (Lai et al., 1978; Mauderly, 1986), and gastric response (Toraason et al., 1980). Such stress-related responses are poorly understood, and their influence on toxicologic responses may well pass unnoticed unless specifically examined.

Temperature

Although not widely appreciated, toxicant-induced changes in thermoregulatory function can modify the results of toxicological studies (Gordon et al., 1988; Gordon, 1991). Recent studies indicate that exposure to 0.37, 0.50, and 1.00 ppm O_3 also can decrease T_{co}, heart rate, and blood pressure over 2 or more hours in unrestrained, unanesthetized rodents maintained at normal room temperature (Uchiyama et al., 1986; Watkinson et al., 1995). On the other hand, when rats were restrained in a head-out body plethysmograph and exposed to the same concentration of O_3 (1 ppm) as in the Uchiyama et al. (1986) and Watkinson et al. (1995) studies, no change in blood pressure was observed (Tepper et al., 1990). The discordance between these findings may be the result of restraint stress, which has been shown to increase T_{co} (Nagasaka et al., 1979) and, in this circumstance, could have blunted the decrease in T_{co} associated with O_3 exposure.

Although O_3-induced changes in heart rate and T_{co} may be unique to rodents, this phenomenon has not been well studied in humans. It is possible that because of their larger thermal mass and different thermoregulatory mechanisms, humans do not exhibit similar changes in these parameters on exposure. For example, rectal temperature increased by the same amount in both air and 0.4 ppm O_3 groups of humans during a 2-h exposure at 35 °C (Bedi et al., 1982). The effects on T_{co} may have been confounded because the subjects performed moderate exercise during alternate 15-min periods during exposure. On the other hand, women exercising intermittently in moderate (24 °C) and hot (35 °C) ambient conditions showed no change in T_{co} related to O_3 exposure, but did show less of an increase in heart rate (2.7%) than did air-exposed (8.1%) subjects at 35 °C (Gibbons and Adams, 1984). It should be noted, however, that other studies have shown potentiation of human lung function responses associated with increased ambient temperature and O_3 exposure (Folinsbee et al., 1977; Gibbons and Adams, 1984). The full importance of temperature in relating rodent and human responsiveness to O_3 remains to be understood.

Exercise and Ventilation

Exercise has long been employed in human studies to enhance the effects of air pollutants, especially O_3 (Folinsbee and Raven, 1984). Exercise appears to exacerbate functional effects by increasing the inhaled dose (Hatch et al., 1994) and possibly by shifting the deposition of the pollutant to more sensitive pulmonary sites (Gerrity and Wiester, 1987). Although exercise can be used in laboratory animals to enhance deposition of O_3, no direct methods for measuring ventilation or breathing mechanics are available for small animals during exercise. Alternatively in an attempt to mimic the increase in ventilation produced by exercise in humans, studies employing restrained animals have used CO_2 as a ventilatory stimulant. Carbon dioxide (8 to 10%) maximally increases $\dot{V}_E$ three to five times in rodent species; CO_2 in excess of 10% will result in a reduction in ventilation (Wong and Alarie,

1982; Tepper et al., 1988). This increase in $\dot{V}_E$ is equivalent to light (2 to 3 × resting $\dot{V}_E$) or moderate exercise (4 to 6 × $\dot{V}_E$) in humans (U.S. Environmental Protection Agency, 1986). In many O_3 studies in humans, both heavy (7 to 8 × $\dot{V}_E$) or very heavy (>9 × $\dot{V}_E$) exercise have been used. Similar increases in ventilation cannot be attained in small animals using the CO_2 challenge technique, thus posing a limitation in attempting to make direct comparisons between animal and human studies.

The application of this CO_2-challenge methodology in O_3-exposed rats (0.25 to 1.0 ppm for 2.25 h with 15 min alternating hyperventilation) clearly demonstrates enhanced pulmonary irritation, as reflected in breathing pattern changes during exposure (Tepper et al., 1988, 1990). The breathing pattern alterations typical of O_3 exposure appeared to be larger than would be predicted based solely on increased dose, suggesting that CO_2 challenge during O_3 exposure may have enhanced deposition at critical lung sites (Tepper et al., 1989). This augmented response was reflected clearly in the large increases in protein observed in the BAL fluid (Costa et al., 1988b). In postmortem studies, rats exercised during exposure have been found to have exacerbated lung pathology, thus appearing to confirm this hypothesis (Mautz et al., 1985) and suggesting that exercise may, in fact, enhance toxicity disproportionate to the apparent dose of toxicant.

8.3.3.2 Acute Exposure Data

Two corners of the parallelogram paradigm can be constructed readily from data gathered in empirical studies of acute O_3 exposure in humans and laboratory animals. These studies have the bulk of the data with the highest frequency of common endpoints that can be compared. Hence, the following discussion will focus on several categories of homologous lung function and BAL study data that have been obtained from humans and animals exposed similarly to O_3. The human studies were drawn from the large existing database on lung function and represent typical responses. The corresponding BAL data are more limited and are used to the extent possible. In contrast, the animal studies selected for comparison are highly selective and represent a rather small database involving similar exposure scenarios and homologous endpoints. This approach, of necessity, excludes the large majority of animal studies, not because they do not contain important toxicologic data on O_3, but rather, they are disparate in their exposure parameters or biologic endpoints that readily can be tied to those available in humans.

Tidal Breathing

In humans, O_3 produces pulmonary irritation, a response associated with cough and substernal soreness (Chapter 7). Although these symptoms are difficult to assess in animals, exposure to sufficient concentrations of O_3 produces reflex alterations in tidal breathing that can be measured objectively. Most notably, the response is an increase in f that is usually accompanied by a decrease in V_T (tachypnea), whereas $\dot{V}_E$ may not be altered. Although the magnitude of the tachypneic response is variable, depending on the species and exposure conditions, this endpoint is quite sensitive and consistent across many species (e.g., guinea pigs, cats, dogs, rats, monkeys, humans).

To examine the cross-species response to O_3, data were evaluated from human and animal studies that reporting immediate postexposure alterations in f. Most human studies employed an exercise regimen during O_3 exposure to increase dose. On the other hand, few animal studies have used exercise, relying rather on high exposure concentrations or CO_2-induced hyperventilation. Three representative human studies were selected because

they used a large range of concentrations and ventilation rates. Selected data from these three human studies are compared to the available animal data in Figure 8-7 and discussed further below.

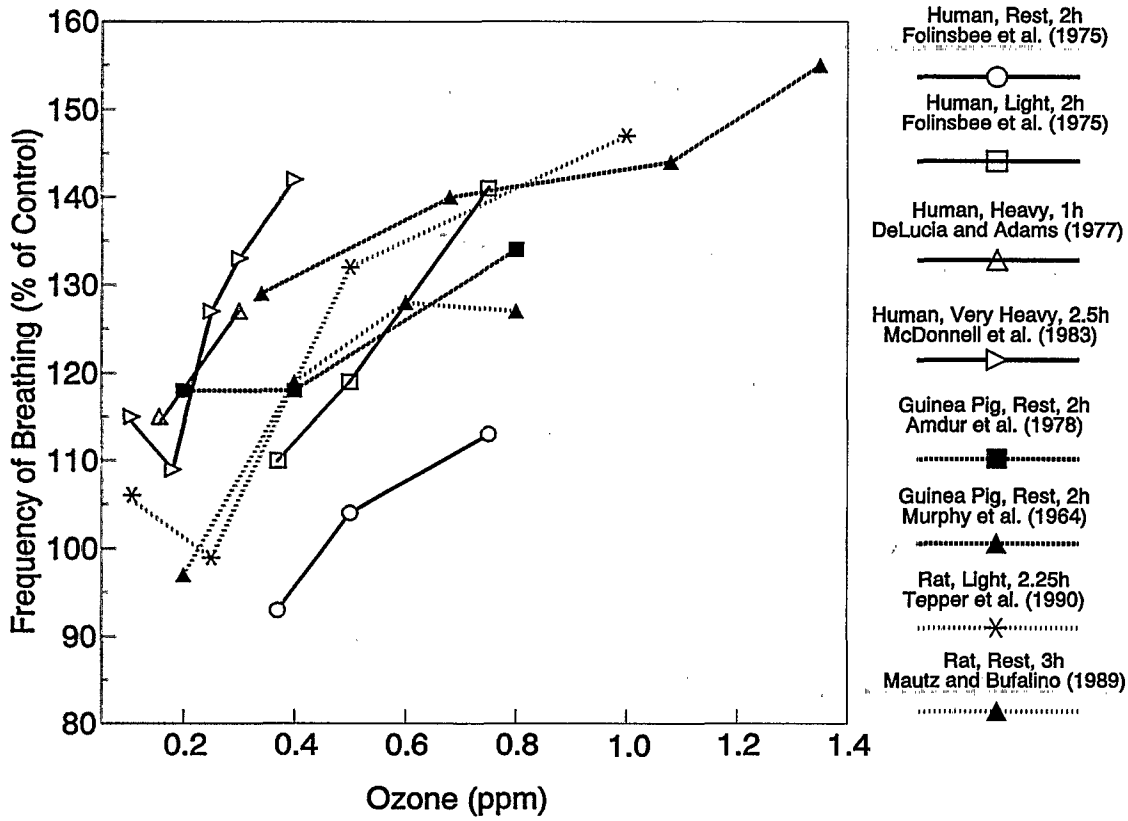


Figure 8-7. Comparison of changes in frequency of breathing after ozone exposure in humans and animals. Data are expressed as percent of the control response. Right-hand legend indicates species, exercise level, exposure duration, and the reference. Human data are plotted with solid lines and open symbols, whereas animal data are plotted with broken lines (differentiated by species) and closed symbols.

Folinsbee et al. (1975) examined the upper limits of the O_3 concentration-response curve in human subjects exposed either at rest or while performing light to moderate intermittent exercise (IE; 29 L/min; $\approx 5 \times$ resting $\dot{V}_E$). Measurements of f were obtained during exercise following a 2-h exposure to 0.37, 0.50, or 0.75 ppm O_3. Concentration-dependent increases in f were observed in both the resting and exercising group, although the magnitude of the change in the exercising group was greater. The role of exercise in altering the O_3-induced changes in f was examined further by DeLucia and Adams (1977), who exposed humans to 0.15 or 0.30 ppm O_3 for 1 h while the subjects exercised at one of four

ventilation levels (1, 3, 4, or 6 × resting $\dot{V}_E$). The magnitude of the f response increased with concentration and exercise level, but was significant only in the highest exercise group at 0.15 and 0.30 ppm. Lastly, the lower limits of the concentration-response were explored by McDonnell et al. (1983), where subjects performing very heavy exercise (65 L/min; ≈10 × resting $\dot{V}_E$) were exposed to 0.12, 0.18, 0.24, 0.30, or 0.40 ppm O_3 for 2.5 h. Significant changes in f were observed at all exposure levels.

Although several animal studies have evaluated tidal breathing changes during and after O_3 exposure, only four studies have examined multiple concentrations such that comparisons to human data can be made. Unanesthetized, restrained guinea pigs were exposed for 2 h to 0.34, 0.68, 1.08, or 1.34 ppm O_3 via nose cones, while tidal breathing was measured using a constant-volume plethysmograph (Murphy et al., 1964). A similar experimental preparation was used by Amdur et al. (1978) to evaluate the respiratory response of guinea pigs to 0.2, 0.4, and 0.8 ppm O_3. In both of these experiments, a monotonic increase in f was observed; however, the animals studied by Murphy et al. (1964) were uniformly more sensitive to O_3 than those of Amdur et al. (1978). Mautz and Bufalino (1989) measured breathing patterns in awake, restrained rats exposed for 3-h to 0.2, 0.4, 0.6, and 0.8 ppm O_3. Concentration-related increases in f were observed up to 0.6 ppm, but the responses to 0.6 and 0.8 ppm were the same. In another study, awake rats were exposed to 0.12, 0.25, 0.50, and 1.00 ppm O_3 for 2.25 h in head-out pressure plethysmographs where CO_2-stimulated breathing was incorporated to augment ventilation (Tepper et al., 1990). With the added CO_2, rats and guinea pigs appeared to be similarly responsive to O_3. In general, as depicted in Figure 8-7, restrained guinea pigs and rats appeared to be as responsive as the lightly exercising humans, and clearly more responsive than the humans exposed at rest. Only with strenuous exercise does the response of humans appear to exceed that of rodents.

In addition to similar concentration-related effects in humans and animals, the time-related effects of O_3 exposure appear to be similar. To demonstrate this homology, Mauderly (1984) compared the time course of response to O_3 in humans and guinea pigs exposed under somewhat similar conditions. Humans were exposed to 0.75 ppm O_3 for 2 h while engaging in nonstrenuous IE at 15-min intervals (Folinsbee et al., 1975). In another study, respiratory parameters were measured at 30-min intervals during exposure and for 4 h postexposure (Bates and Hazucha, 1973). Similarly, unanesthetized, restrained guinea pigs were exposed to 0.68 ppm O_3 for 2 h as part of a concentration-response study (described above), with respiratory function assessed at 15-min intervals during exposure and for 3.5 h postexposure (Murphy et al., 1964). In both guinea pigs and humans, f increased and V_T decreased; both parameters then returned toward control values during the postexposure period. The percent change from control in f and V_T was nearly the same throughout the exposure and postexposure periods, indicating that a similar concentration of O_3 (≈0.7 ppm) produced similar temporal alterations in ventilation. Again, the guinea pigs would appear to be slightly more responsive than humans because the guinea pigs were exposed to a lower concentration (0.68 ppm) at rest, whereas the humans were exposed to 0.75 ppm with light IE.

Mechanics

Breathing mechanics have been examined in several animal and human-O_3 exposure studies, but there is little similarity between the databases for the concentrations or the specific techniques used. Bates et al. (1972) examined breathing mechanics in resting and in

lightly exercising (2 × resting $\dot{V}_E$) humans exposed for 2 h to 0.75 ppm O_3. Although no concentration-response data were obtained, increased total pulmonary resistance (R_L) and decreased dynamic compliance (Cdyn) were found for both resting (+22 and −12%) and exercising (+67 and −51%) subjects exposed to O_3. In a similar study, Hazucha et al. (1989) exposed men for 2 h to 0.5 ppm O_3 using moderate IE (40 L/min), and found a significant 12.5% increase in R_{aw}, although no concomitant change in Cdyn was detected. McDonnell et al. (1983), using a broad range of O_3 concentrations (0.12, 0.18, 0.24, 0.30, and 0.40 ppm; 2 h) and very heavy exercise (65 L/min), reported concentration-dependent increases in R_{aw}. In humans at rest or performing light exercise, however, a 2-h exposure at near-ambient O_3 concentrations would be expected to induce only modest increases in R_{aw} and no changes in Cdyn (Hazucha, 1987).

Although relatively high O_3 concentrations (≥1.0 ppm) produced effects on R_L and Cdyn in animals (Murphy et al., 1964), only three studies in animals have evaluated these parameters at lower, more relevant O_3 concentrations. Watanabe et al. (1973) studied anesthetized, paralyzed, and mechanically ventilated cats exposed via a steel tracheal tube to either 0.25, 0.50, or 1.00 ppm O_3 for between 2 and 6.5 h. Measurements of breathing mechanics were recorded every 30 min. With increasing O_3 concentration and exposure duration, R_L increased and, to a lesser extent, Cdyn decreased. Bronchoconstriction at 0.25 ppm O_3 was reversed following atropine (a parasympathetic receptor blocker), but only partially reversed at the two higher concentrations, suggesting the involvement of more than bronchoconstriction in the increase in R_L at these levels. Unfortunately, relating the concentrations used in this study to other animal or human studies is difficult because exposure through a tracheal tube would eliminate scrubbing of O_3 by the nose and oropharynx and likely would exaggerate the pulmonary O_3 dose (Gerrity et al., 1988).

Other studies have attempted to examine breathing mechanics in unanesthetized animals with natural nasal breathing and avoidance of potential anesthesia-related blunting of reflex responses. Murphy et al. (1964) exposed unanesthetized guinea pigs to several concentrations of O_3 for 2 h and measured ventilation, as previously discussed, and R_L. At concentrations less than 1 ppm, O_3 had no effect, but R_L increased 48 and 147% at 1.08 and 1.34 ppm, respectively. Using a similar test system, Amdur et al. (1978) observed no significant alteration of R_L in unanesthetized guinea pigs during a 2-h exposure to 0.2, 0.4, or 0.8 ppm O_3. However, Cdyn decreased significantly at 0.4 and 0.8 ppm O_3. In analogous studies in unanesthetized rats, Tepper et al. (1990) observed no significant changes in R_L or Cdyn after a 2.25 h exposure to 0.12, 0.25, 0.50, or 1.00 ppm O_3, in spite of intermittent 15-min periods of exercise-like hyperventilation induced by CO_2.

Although increased resistance is demonstrable in guinea pigs, cats, dogs, and humans, a comparison of percent change in resistance from control measurements after an acute (≈2 h) O_3 exposure (Figure 8-8) suggests that humans are more likely to bronchoconstrict due to O_3 exposure than rodents. Neither of the guinea pig studies (Murphy et al., 1964; Amdur et al., 1978) nor the rat study (Tepper et al., 1990) showed a significant increase in R_L at less than 1 ppm. However, closer examination of the human data reveals that the McDonnell et al. (1983) study employed very heavy exercise, and most of the studies included in the Hazucha (1987) model used moderate to heavy exercise. Thus, the inhaled dose likely would be greater than in spontaneously breathing animals. A more comparable study in humans that employed only light exercise reported that 0.25, 0.37, and 0.50 ppm for 2 h resulted in minimal, nonsignificant 118, 124, and 104% increases in R_{aw} (Hackney

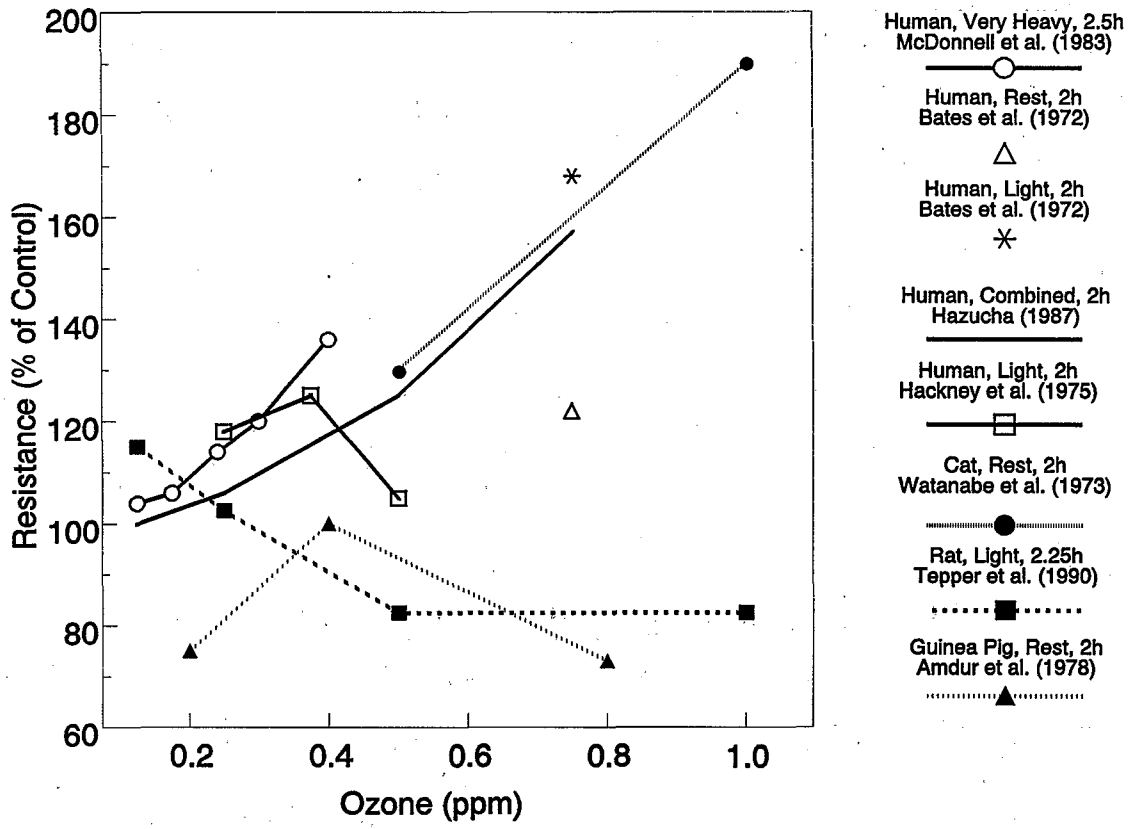


Figure 8-8. Comparison of changes in resistance after ozone exposure in humans and animals. Data are expressed as percent of the control response. Right-hand legend indicates species, exercise level, exposure duration, and the reference. Human data are plotted with solid lines and open symbols. The line labeled "Hazucha (1987)" is a model of predicted response. Animal data are plotted with dashed lines (differentiated by species) and closed symbols.

et al., 1975). Likewise, the Bates et al. (1972) data obtained in subjects at rest and with light exercise (0.75 ppm O_3) also argue against an unusually high O_3 responsiveness in humans relative to test animals for this endpoint when exercise-related dose is considered.

In general, similar findings have been observed using the measurement of Cdyn; however, the response decrements were more variable and of smaller magnitude. Given the distal deposition of O_3, as indicated by morphological studies (Chapter 6, Section 6.2.4), it is surprising that so little attention has been given to this parameter. Available data suggest that these changes in Cdyn are of little biological significance for ambient exposures.

Airway Responsiveness

The ability of O_3 to increase airway responsiveness to nonspecific bronchoconstricting stimuli in humans and other mammalian species has been known for at least a decade (Chapters 6 and 7, Sections 6.2.5 and 7.2.4, respectively). However, airway responsiveness is perhaps the least understood response to O_3, particularly in the context of species comparisons. Humans clearly exhibit increases in airway responsiveness at environmental O_3 exposure levels (Gong et al., 1986; McDonnell et al., 1987; Folinsbee et al., 1988; Horstman et al., 1990), whereas analogous responses in animals at O_3 concentrations below 1 ppm are controversial. Most studies of airway responsiveness in laboratory animals focus on the development of asthma-like models to elucidate generic mechanisms of airway responsiveness and utilize concentrations as high as 3 ppm for brief periods of time to injure the airways. Hence, anything more than a qualitative comparison between animal species and humans is tenuous and, thus, will not be discussed further in this section. Details of the methodologies of the laboratory animal and human bronchoreactivity studies can be obtained in the reviews of pulmonary function found in Chapters 6 and 7, Sections 6.2.5 and 7.2.3, respectively.

Elasticity and Diffusion

The integrity of the pulmonary air-blood barrier is essential for efficient exchange of oxygen and CO_2. This fragile epithelial interface with matrixed interstitial connective tissues and capillaries possesses inherent elastic properties and presents a finite resistance to oxygen diffusion to the blood. Although the elastic and diffusionary properties of the blood-air barrier are not linked implicitly to one another functionally, both properties can be quantified readily and compared between humans and laboratory animals (Costa, 1985). When combined, assessment of these functional properties is often sufficient to evaluate pathologic or toxic events in the distal reaches of the lung. For this reason and because of the fact that O_3 deposits in the deep lung, the effects of O_3 on these parameters will be discussed together.

Inhaled O_3 is known to penetrate to the depths of the lung and preferentially deposit in the smallest airways and its proximal acini (Section 8.2). Somewhat surprisingly, relatively few studies in humans have sought to characterize potential functional impairments at the air-blood interface. The reasons for this are likely twofold. First, in the early studies of the health effects of O_3 on humans, static compliance and diffusing capacity for carbon monoxide (DL_{CO}) were affected at only very high concentrations, well above what would be considered environmentally relevant. Second, from a practical perspective, these measurements proved to be considerably more tedious to perform than the forced expiratory measurement, which sensitively detects O_3-induced alterations (discussed below). Nevertheless, there are sufficient data on humans exposed acutely to O_3 to allow a reasonable comparison of these endpoints with their more abundant animal homologues.

The earliest studies leave little doubt that O_3 is edemagenic at high concentrations in virtually all mammalian species. In the past, occupational exposures of 2 to 3 ppm O_3 were not uncommon, and a 9-ppm peak exposure has been reported (Kleinfeld et al., 1957; Challen et al., 1958). The resultant worker symptoms and signs, including chest radiograms, were consistent with the manifestations of edema reported in experimental animals (i.e., increased lung weight and stainable edema in the airspaces) (Stokinger, 1965). Lung function, however, typically was not measured in these work-related exposures. In a later study of arc welders exposed to 0.2 to 0.3 ppm O_3, little, if any, convincing evidence

of functional impairment, in terms of altered lung volumes, maximal expiratory flow rates, and DL_{CO} was obtained (Young et al., 1963). To further explore the possible effects of O_3, Young and coworkers (1964) subjected 11 human volunteers at rest to controlled atmospheres of 0.6 to 0.8 ppm O_3 via mouthpiece for 2 h. Small reductions in vital capacity (VC), forced expiratory volume in 0.75 s, dynamic and static lung compliance, and intrapulmonary gas distribution were observed, but only the 25% fall in DL_{CO} proved to be statistically significant. Similar, but considerably more variable effects on lung function were reported by Hallett (1965) in 10 subjects exposed to 1 to 4 ppm O_3 for 30 min. Nonetheless, of 10 exposed subjects, seven showed at least a 20% drop in DL_{CO}. Like Young and coworkers, Hallett (1965) interpreted these changes to indicate lung edema, in agreement with the hypothesis that the deep lung irritant O_3 was having its effect at the alveolar level. Interestingly, additional work from the same laboratory of the Young study (Bates et al., 1972) found that resting subjects receiving nasal exposure to 0.75 ppm O_3 for 2 h resulted in a nonsignificant 3% reduction in DL_{CO}. However, in a limited test group, the co-imposition of light exercise, which doubled ventilation, enhanced this response ($\approx$12%). It appears that the nasal (Bates et al., 1972) versus mouthpiece (Young et al., 1964) routes of exposure were instrumental in the differential response, because it is likely that the mouthpiece diminished what scrubbing occurs when exposure is via the unencumbered mouth in human test subjects (as reported by Gerrity et al., 1988). Since these early studies, there have been no additional controlled human acute studies that have examined alterations in DL_{CO} at O_3 concentrations below 0.6 ppm.

Analogous animal studies of acute O_3 exposure indicate that the general pattern of functional impairment is similar to that reported in human studies. Anesthetized and ventilated cats showed a general decline in VC, static lung compliance, or DL_{CO} with exposures up to 6.5 h of 0.26 to 1.00 ppm O_3 (Watanabe et al., 1973). The responses of the 20 animals were variable, and these declines, which did not achieve overall statistical significance, were thought to be largely secondary to the substantial (36 to 200%) increases in R_{aw}. In a more complex study design, rats were exposed for 2 or 7 h to 0.5 or 0.8 ppm O_3 with intermittent 8% CO_2 to hyperventilate ($\approx$2 to 3 $\times$ resting $\dot{V}_E$) the animals as an exercise analogue to human exposures (Costa et al., 1988a). The DL_{CO} values were reduced by about 10% at both 0.5 ppm time-points and by about 12% with a 2-h exposure to 0.8 ppm. Exposure to 0.8 ppm for 7 h, however, greatly exacerbated the alveolar effect with a resultant 40% reduction in DL_{CO}. Static compliance, unaffected by the other exposure conditions, was affected only at this latter exposure duration. These O_3-induced effects, particularly the reductions in DL_{CO}, appeared to correlate with the degree of lung edema in affected animals, as had been surmised for the acutely exposed humans. With the multitude of more recent studies of O_3 at ambient levels, alterations in static lung compliance or DL_{CO} rarely are reported in either humans or animals.

Forced Expiration

Reductions in FVC and FEV_1 have become the hallmarks of acute lung dysfunction in humans after O_3 exposure (Chapter 7). These measures are sensitive to O_3 levels as low as 0.12 ppm for as little as 2 h when heavy IE is included during the exposure (McDonnell et al., 1983) and show cumulative dysfunction resulting from 6.6 h of lower levels of this oxidant (0.08 and 0.10 ppm) when nearly continuous, moderate exercise is employed (Horstman et al., 1990). Reductions in FEV_1 and FVC induced by O_3 are believed to be partly the result of pain-mediated interruption of maximal inspiration (Hazucha

et al., 1989). Exactly what level of tissue injury or inflammation correlates with these functional deficits is unclear and is an active area of research (Chapter 7, Section 7.2.4).

Most studies of O_3 in experimental animals make little effort to mimic human study designs, thereby impeding the extrapolation of experimental animal study results to humans. Recently, however, rat studies involving periods of intermittent CO_2-induced hyperventilation to enhance dosimetry have attempted to capitalize on the qualitative similarity of the rat and human maximum expiratory flow volume (MEFV) curves as a potentially sensitive endpoint of toxicity (Costa et al., 1988a; Tepper et al., 1989). In the rat, FVC does indeed decrease with O_3 exposure, although the magnitude of response is apparently less than that observed in humans. As in the human, the reduced FVC in the animal appears not to be the result of a change in residual volume. Total lung capacity may be reduced slightly, but lung compliance does not change. However, it is premature to assume a common mechanism for the FVC reductions in the rat and human. Unlike the human, pain on inspiration in the animal model is likely not an issue because the animal is anesthetized during the procedure and is brought to TLC by a defined airway pressure ($\approx$30 cm H_2O). Because general anesthesia is known to diminish sensory afferent stimuli, an analogous O_3-induced fall in rat FVC should expectedly have been blunted, if not totally eliminated. To what extent anesthesia mitigates the rat response or that there are inherent species differences in dosimetry or sensitivity is not clear from these studies. Nevertheless, comparison of model-predicted FVC changes in humans (Hazucha, 1987) with analogous rat data (Costa et al., 1988a) would suggest that this response in the anesthetized rat is about half that of the human (Figure 8-9).

Studies of Inflammation and Antioxidant Content of Bronchoalveolar Lavage Fluid

Both humans and animals exhibit a PMN inflammatory response with associated changes in lung permeability after acute exposure to O_3. Recent studies indicate that humans exposed to O_3 concentrations as low as 0.08 ppm for 6.6 h with moderate exercise (40 L/min) exhibit a fourfold increase in the percentage PMNs when BAL is obtained 18 h postexposure (Devlin et al., 1991). To date, animal studies at comparable exposure levels are rare, (Hotchkiss et al., 1989), and exercise enhancement of exposure dose has yet to be incorporated. As noted above, the issue of dosimetry is critical if extrapolation at such levels is to be attempted. Nevertheless, in the one acute rat study at 0.12 ppm O_3 for 6 h, an increase in nasal-lavage-derived PMNs was noted 18 h postexposure, with no similar change in PMN number in the BAL (Hotchkiss et al., 1989). In contrast, in the same study when higher concentrations of O_3 (0.8 and 1.5 ppm) were used, BAL PMNs were elevated, but no changes were observed in the nose washings. Such a "competitive" nasal-pulmonary response has yet to be studied directly in humans. Nevertheless, the data support the general hypothesis that there is comparability between the inflammatory responsiveness of rats and humans. More direct comparison of laboratory animal inflammatory responses with those of humans can be drawn from studies at higher concentrations when the nasal/lung competitive response in the rat is skewed to the lung, and, like in the lung-function comparison, analogous exposure conditions can be more directly compared. These studies are tabulated in Table 8-9 and discussed in more detail below.

Four representative human studies of lung inflammatory responses after acute O_3 exposure can be compared with existing acute animal data from studies of analogous design. Seltzer et al. (1986) exposed moderately exercising (83 to 100 W) subjects to 0.4 or 0.6 ppm O_3 for 2 h, with BAL obtained 3 h postexposure. The BAL fluid from the

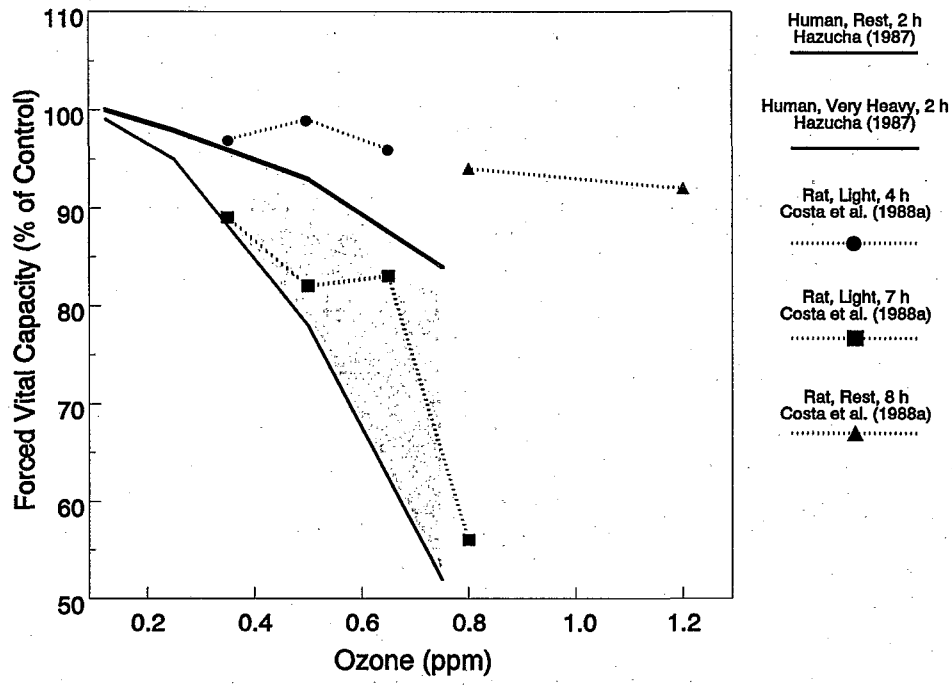


Figure 8-9. Comparison of changes in forced vital capacity after ozone exposure in humans and animals. Data are expressed as percent of the control response. Right-hand legend indicates species, exercise level, exposure duration, and the reference. Human data are plotted with solid lines from the equation of Hazucha (1987), with the shaded area representing the predicted range of decrements expected between light exercise (top line) and very heavy exercise (bottom line). Rat data are plotted with dashed lines and closed symbols.

combined 0.4- and 0.6-ppm O_3 groups contained an average 7.8-fold more PMNs than did BAL fluid after filtered air. Protein levels in the BAL fluid were not assayed in this study. In separate studies, humans were exposed to 0.4 ppm O_3 for 2 h with heavy exercise (60 L/min), with BAL samples collected at 1 and 18 h postexposure (Koren et al., 1989b, 1991). These two studies showed that the inflammatory response is quickly initiated postexposure. The PMN and protein content were elevated at both times (1 h: PMN 18.2×; protein 1.2×), with the 18-h timepoint (PMN 8×; protein 2.2×) being higher for protein, but somewhat less for PMNs. Schelegle et al. (1991) followed the time course of the inflammatory response (1, 6, and 24 h postexposure) after a 1-h exposure of 0.3 ppm O_3 with exercise (60 L/min). The PMN content of the combined airway and alveolar BAL fluid was elevated at 6 h (3×) and 24 h (2.5×) only, with the 6-h point representing the peak. Protein content of the BAL fluid did not change significantly at any point.

The many studies of O_3-induced inflammation in laboratory animals are reviewed in Chapter 6, Section 6.2.2. For the purpose of species comparison, only selected studies

Table 8-9. Polymorphonuclear Leukocyte and Protein Permeability Response to Ozone by Species[a]

Exposure Parameters	Exposure Conditions	Species (Strain)	Postexposure BAL Time	PMN[b] Increase	Protein[b] Increase	Reference
0.4-0.6 2 h	Exercise (15 min/alt; 83-100 W)	Human	3 h	7.8	Not done	Seltzer et al. (1986)
0.4 2 h	Exercise (15 min/alt; 60 L/min)	Human	18 h	8.0	2.2	Koren et al. (1989a,b)
0.4 2 h	Exercise (15 min/alt; 60 L/min)	Human	1 h	18.2	1.2	Koren et al. (1991)
0.3 1 h	Exercise (15 min/alt; 60 L/min)	Human	1, 6, 24 h	3.0 at 6 h 2.5 at 24 h	No change	Schelegle et al. (1991)
0.96 8 h	Daytime; rest	Rhesus monkey	1, 12, 24, 72, 168 h	27 at 1 h 19 at 12 h 24 at 24 h 6 at 72 h 3 at 168 h	3 at 1 h 3 at 12 h 8 at 24 h 3 at 72 h 1 at 168 h	Hyde et al. (1992)
0.2 0.5 1.0 2.0 4 h	Daytime; rest	(a) Mouse (Swiss Albino) (b) Guinea pig (Hartley) (c) Rat (S-D) (d) Hamster (Golden Syrian) (e) Rabbit (NZW) M	16 h	Not done	(a) 1.8 at 1.0 ppm 3.2 at 2.0 ppm (b) 1.4 at 0.2 ppm 2.0 at 0.5 ppm 4.1 at 1.0 ppm 4.5 at 2.0 ppm (c) 2.1 at 1.0 ppm 3.6 at 2.0 ppm (d) 1.5 at 1.0 ppm 2.6 at 2.0 ppm (e) 2.7 at 2.0 ppm	Hatch et al. (1986)
0.5 1.0 4 h	Daytime; rest; Vitamin C (AH_2+/−)	Guinea pig (Hartley) M	16 h	Not done	For 0.5 ppm; 1.1 for AH_2^+, 2.1 for AH_2^-. For 1.0 ppm; 2.4 for AH_2^+, 2.7 for AH_2^-	Slade et al. (1989)
0.5 0.8 2 or 7 h	Daytime; (15 min/alt for 2 h; 45 min/alt for 7 h); 3-5× $\dot{V}_E$	Rat (F344) M	1 h	Not done	For 0.5 ppm; 1.2 at 6 h, 2.1 at 7 h. For 0.8 ppm; 1.5 at 2 h, 3.3 at 7 h	Costa et al. (1988a)

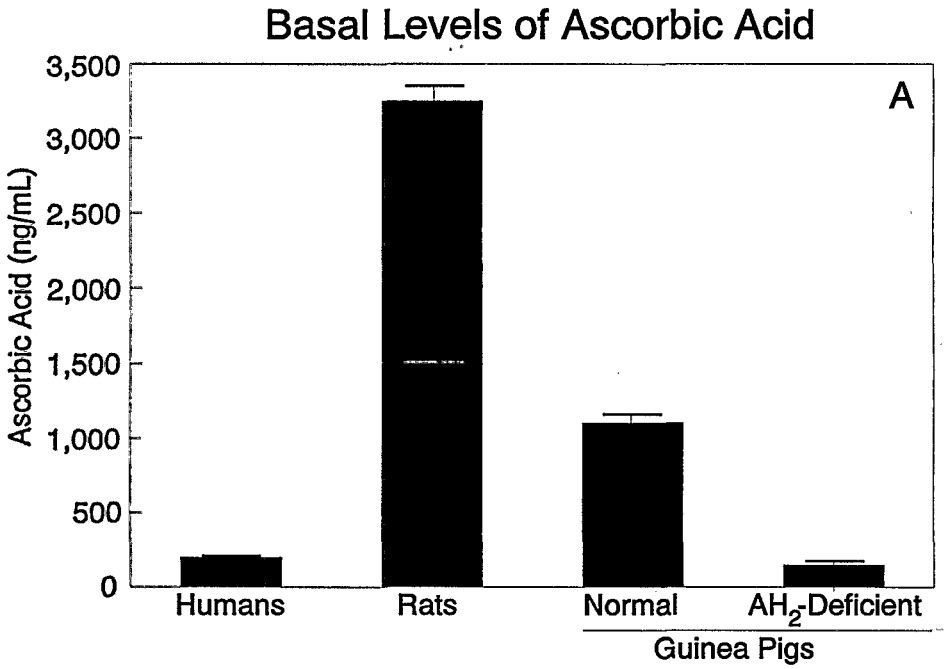
[a]See Appendix A for abbreviations and acronyms.
[b]Ozone response/air response.

(Table 8-9) will be considered. The spectrum of exposure conditions used in the various animal studies makes difficult the direct comparison among laboratory test species. However, one study by Hatch and coworkers (1986) specifically addressed this question by exposing mice, guinea pigs, rats, rabbits, and hamsters under identical conditions (0.2, 0.5, 1.0, and 2.0 ppm O_3 for 4 h), followed at 18 h postexposure with BAL and assay for protein. Guinea pigs were most responsive, responding at 0.2 ppm, whereas mice, hamsters, and rats began responding at 1.0 ppm, and rabbits responded only to 2.0 ppm. Only one study involving BAL assessment of PMNs and protein in exposed monkeys has been published (Hyde et al., 1992). At 0.96 ppm O_3 (8 h), the monkeys had a significant inflammatory response, but it is difficult to assess monkey responsiveness relative to the human for this endpoint. Assuming a linear concentration times duration relationship, the monkey data appear similar to the guinea pig response. However, none of these species showed BAL protein increases approximating those reported in human studies.

In recent studies (0.4 ppm O_3 for 2 h, with BAL 16 to 18 h postexposure) (Slade et al., 1989; Crissman et al., 1993), guinea pigs made vitamin C-deficient exhibited enhanced responsiveness to O_3, this result is comparable to that of the exercised humans of Koren et al. (1989b) (Figure 8-10). Similarly, when rats were exposed to 0.5 ppm O_3 for 2 h with intermittent CO_2-induced hyperventilation (Tepper et al., 1993) to mimic mild/moderate exercise (three- to fivefold $\dot{V}_E$), the BAL protein, as well as PMN responses at 18 h postexposure compared favorably with those data of Koren and coworkers (1989a,b) (Figure 8-11).

Within a given laboratory animal species, responses among strains also can differ appreciably, as demonstrated in rats by Pino et al. (1991) and Costa et al. (1993). These studies indicated that Wistar rats exhibit greater inflammatory responses (protein and PMN) to O_3 than S-D and F344 rats after an 8-h exposure to 0.5, 1.0, and 1.5 ppm with BAL sampled at 2 or 24 h later. Similarly, mouse strains (C3H/HeJ and B6C57/6; Kleeberger et al., 1990) and S-D rat substrains (Costa et al., 1985) have been shown to possess specific genetic susceptibility to high levels of O_3 (2 ppm). In the case of the mice, the responsive strain is seven times (at the 6-h postexposure peak) as susceptible as the databases in animals and, particularly, in humans with regard to these antioxidants are quite limited. Supplementation and deprivation studies with vitamins C and E have shown that these antioxidants have some role in protecting against the effects of O_3 in animals (Elsayed et al., 1988; Slade et al., 1989; Crissman et al., 1993) and in humans (Chatham et al., 1987). Of the animal models, ascorbate-deprived guinea pigs appear to have BAL ascorbate levels most like humans, with a protein permeability response (without exercise in the animal) very similar to the human exposed to the same concentration (0.4 ppm O_3 for 2 h) with exercise. However, Crissman et al. (1993) also resistant strain for the PMN response to 2 ppm O_3; the protein response is twice (at 24 h postexposure) that of the resistant strain. In the S-D substrain, protein extravasation into the alveolar lumen immediately postexposure is 40% higher in the responsive strain than the resistant (no other time points were examined).

Humans have an order of magnitude less ascorbate in BAL fluid as compared to the rat, but they have nearly twice the glutathione, four times the uric acid, and 80 times the vitamin E, as normalized to lipid P-surfactant (Slade et al., 1993) (Table 8-8). However, on a BAL-derived cell/protein basis, the ratios clearly favor the rat for all of these antioxidants, with the exception of uric acid, which is generally not high in rats because of species differences in protein and prime nucleotide catabolism (urea being the major nitrogenous



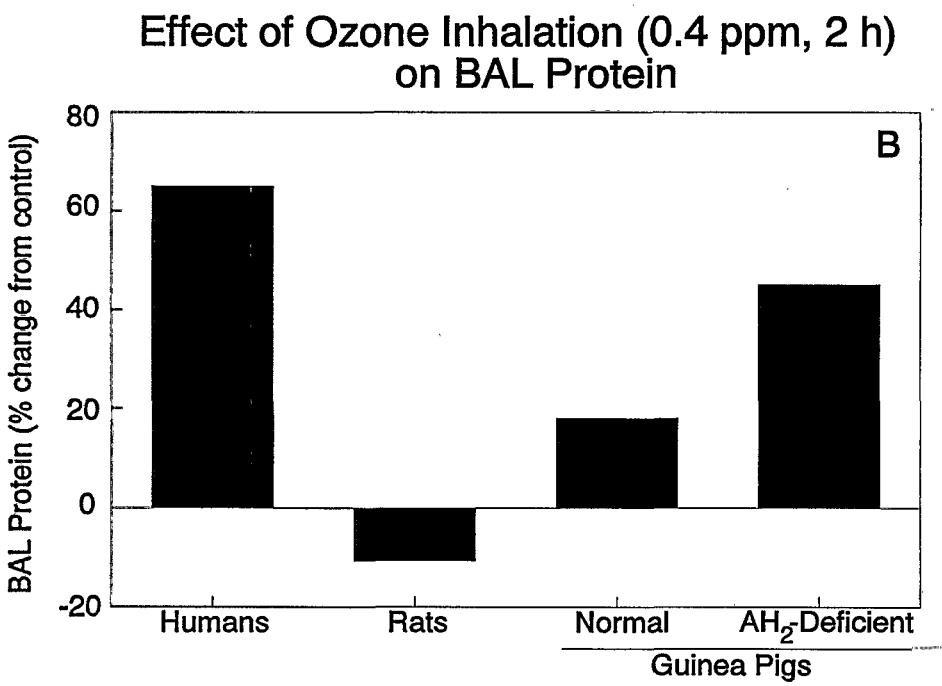


Figure 8-10. ***Composite of data from Slade et al. (1989), Koren et al. (1989b), and Crissman et al. (1993) comparing basal bronchoalveolar lavage (BAL) ascorbate levels (A) to ozone-induced changes in BAL protein (B). Ozone-exposures (0.4 ppm; 2 h; 16 to 18 h BAL) of humans (exposed with exercise), rats (exposed resting), and guinea pigs (exposed resting) with (ascorbic acid [AH_2]-deficient) and without (normal) AH_2 deficiency.***

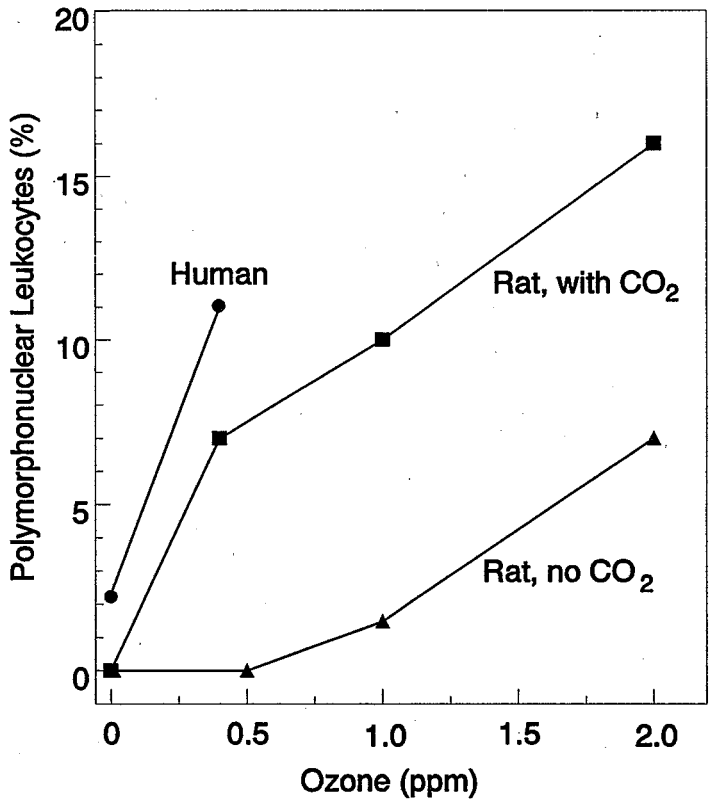


Figure 8-11. Composite of data comparing polymorphonuclear leukocytes obtained by bronchoalveolar lavage 16 to 18 h after ozone (O_3) exposure (0.4 ppm; 2 h) of humans (with exercise) (Koren et al., 1989b) to those of rats exposed to O_3 (0.5 to 2.0 ppm) at rest or hyperventilated with carbon dioxide (CO_2) (Tepper et al., 1993).

by-product for rats). The guinea pig most closely resembles the human for ascorbate. Because these antioxidants are thought to function in the defense against oxidant challenge, it would appear critical to appreciate their presence and function when attempting to interpret data for extrapolation. Unfortunately, the reported that reduced ascorbate levels in BAL fluid (18 h postexposure) increase in the human, whereas those levels decrease in the rat. Whether this relates to the distinctly different basal levels of this vitamin and is associated with the disparate protein responsiveness (ignoring exercise) is unclear because deficiency in animals (guinea pigs) appears more critical to the responsiveness at low (ambient-like) concentrations than at higher concentrations (1.0 ppm). Although it would appear that vitamin C is involved in the interplay between O_3 and the exposed subject (human or animal), there is not full coherence of the data. For example, Hatch et al. (1986) showed that the rabbit was the least responsive to O_3 in terms of BAL protein, but this species has among the lowest tissue levels of vitamin C (Slade et al., 1985). However, rabbits apparently have a low propensity to form lipid peroxides (Arakawa et al., 1986), an expected

product of lung lipid-O_3 interaction (Pryor, 1992). Thus, to interpret interspecies sensitivity only in terms of basal antioxidant levels, however tempting this may be, would be overly simplistic and premature at this time.

8.3.3.3 Prolonged Exposure Studies

The previous sections of this text have utilized lung function responses to acute O_3 exposure in an attempt to elucidate the relative sensitivity among laboratory test species and humans and, thereby, to complete two corners of the parallelogram paradigm (Figure 8-6). Because acute responses represent only part of the extrapolation paradigm, temporal-based exposure responsiveness also will be considered, despite the relative paucity of comparative data between laboratory animals and humans for prolonged exposures. Again, in an effort to best extract species-specific response differences, the criterium for selection of studies was similarity in exposure scenario. The discussion will focus on relative adaptability of acute functional changes and associated BAL-derived findings after repeated exposures and the coherence of the findings from prolonged human and animal exposure studies and epidemiological results.

Lung Function Studies

Reversal ("attenuation") of pulmonary function decrements using a scenario of repeated exposure to O_3 has been reported for both humans and laboratory animals. At least nine studies between 1977 and 1984 have documented that, for repeated exposures between 0.2 and 0.5 ppm O_3 (2 h/day, up to 5 days), spirometric changes were most severe on the first or second day of exposure, waned over the next 3 days of exposure, and, by the fifth day, had returned to control preexposure levels (Chapter 7, Section 7.2.1.4). In the only animal study using a similar exposure protocol and analogous experimental design, Tepper et al. (1989) showed that rats initially displayed a tachypneic response to O_3 that attenuated after 5 consecutive days of exposure, a pattern quite similar to that of humans. Exposures were for 2.25 h and included challenge with CO_2 during alternate 15-min periods to augment ventilation (2 to 3 $\times$ resting $\dot{V}_E$, which is equivalent to light exercise in humans). As in the human studies, the functional changes were largest on Day 1 or 2, depending on the parameter and the O_3 concentration (0.35, 0.50 and 1.00 ppm). Attenuation of the changes in shape constant of the flow-volume curve of the rats also was observed over this period. Thus, under analogous conditions of exposure, both the humans and the rats exhibited similar initial functional responses to O_3 with full and kinetically similar reversal of effects.

More difficult is the direct comparison between human and animal lung function responses to prolonged (several-week) O_3 exposure, largely because of the limited availability of controlled human study data. In the only study of its kind, Bennett (1962) exposed 12 human subjects at rest to 0.2 or 0.5 ppm O_3 for 3 h/day, 6 days/week for 12 consecutive weeks. Although no effects were discernable early in the exposure, there appeared to be small, but significant O_3-induced reductions in FEV_1 (and small, nonsignificant reductions in FVC), particularly in the last weeks of the study. This reduction in FEV_1 more likely would reflect obstructive changes within the lung at these points late in time rather than the pain-mediated reductions that are seen with acute O_3 exposure, which attenuate after a few days of exposure. The lack of concentration-related decrements in FEV_1 and FVC is somewhat unsettling, but, regardless, after 9 postexposure weeks in clean air, all measured effects had reversed.

Unfortunately, there are no directly parallel animal studies to compare to this limited database. But, if the two-fold sensitivity difference between the rat and human FVC response (see Figure 8-12) is assumed, a number of animal studies may be considered comparable for the purposes of this discussion. On the one hand, rats exposed to 0.2 or 0.8 ppm O_3 for 6 h/day, 5 days/week for 12 weeks were reported to exhibit some degree of small airway obstruction based on the MEFV curves, but little, if any, reduction in FVC or DL_{CO} was observed (Costa et al., 1983). Others have reported analogous marginal increases in rat TLC or its component volumes (Bartlett et al., 1974; Costa et al., 1983; Raub et al., 1983) or in regional R_{aw} (Yokoyama et al., 1984) after intermittent or continuous exposures to $\geq$0.25 ppm O_3 for 4 to 12 weeks, which would not be unexpected with distal airway or lung damage. Actual pathology in the distal lung tends to be focal and difficult to correlate precisely with the marginal functional impairment.

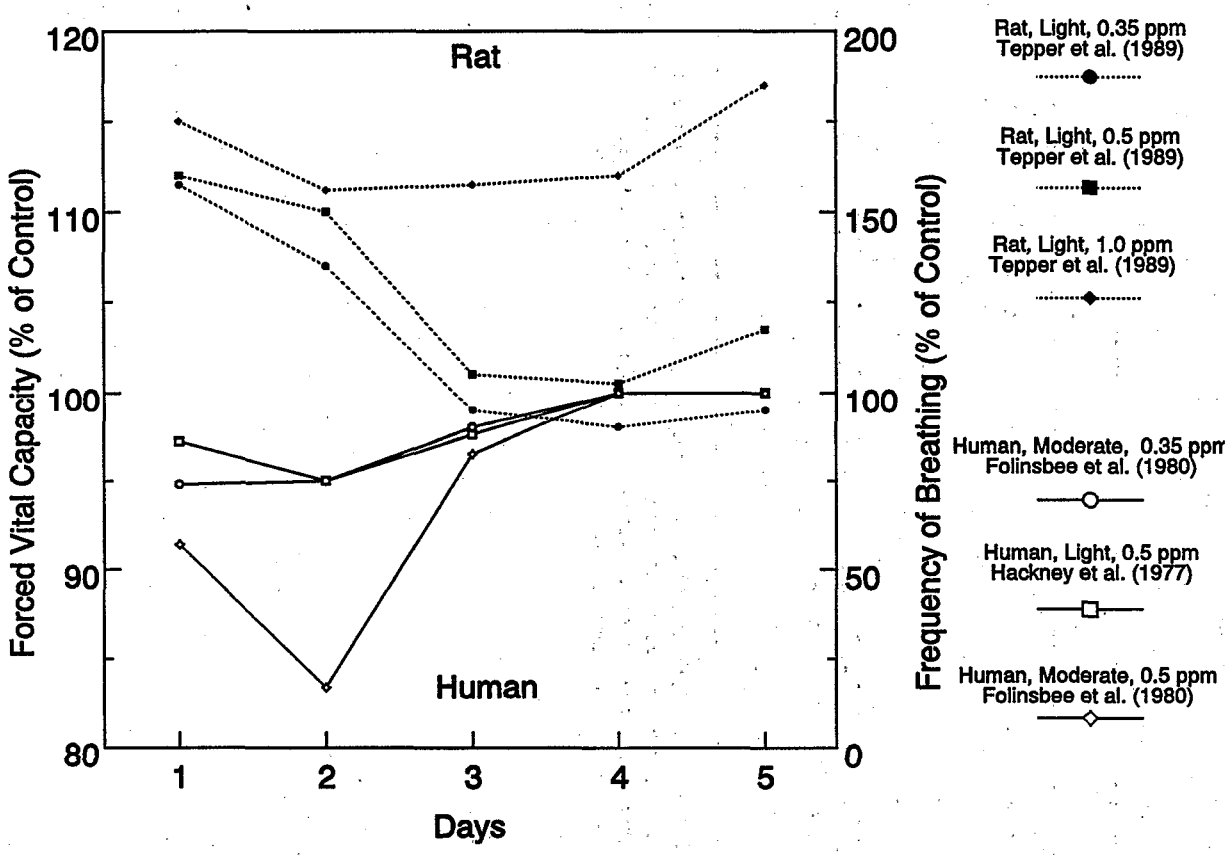


Figure 8-12. Comparison of changes in forced vital capacity in humans (left ordinate) and frequency of breathing in rats (right ordinate) with up to 5 consecutive days of ozone (O_3) exposure. Data are expressed as percent of the control response. Right hand legend indicates species, exercise level, O_3 concentration, and the reference. Human data are plotted with solid lines and open symbols, whereas rat data are plotted with broken lines and closed symbols.

The limited functional data available in monkeys generally agree with the pattern of distal lung pathophysiology reported in rats. When exposed to 0.5 ppm O_3 for 90 days (8 h/day), monkeys exhibited a slight increase in lung distensibility (Eustis et al., 1981). Likewise, monkeys exposed to 0.25 ppm (8 h/day for 18 mo) exhibited increased chest wall (but not lung) compliance and lung volumes, which was most marked in the monkeys exposed to O_3 only in alternate months during that 18-mo period (Tyler et al., 1988). Recall, however, that little or no change in lung elasticity has been associated with controlled O_3 exposures in humans, whether the exposures are acute or repeated. On the other hand, higher concentrations (0.64 ppm for 1 year) resulted in the alteration of distal airway mechanics in exposed monkeys, as gleaned from local resistances measured using oscillatory methods; again, this is in general agreement with the presence of distal lung injury or disease. Morphometric analyses of the end-airways and distal lung regions of O_3 exposed monkeys consistently show altered cell profiles and interstitial restructuring, even when functional changes are marginal, which, like in the rat, likely reflects the large functional reserve of the integrated lung. Thus, although these collective data from subchronic animal studies suggest a reasonably homologous distal lung response to O_3, many of these linkages in functional outcomes remain uncertain in terms of what to anticipate in the human response.

Clearly, the question of potential lung impairment resulting from a near-lifetime exposure to O_3 ranks among the most pressing concerns about this toxicant. The animal data, although demonstrating that chronic O_3 exposure can induce changes in the structure and function of the lung, have yet to provide evidence of potential disease or disability in humans exposed to O_3 over prolonged periods of their lives. The existing epidemiologic studies (Chapter 7, Section 7.4.2), too, merely provide suggestive evidence that persistent or progressive deterioration in lung function may be associated with long-term oxidant pollutant exposure (Detels et al., 1981, 1987). Detels and coworkers (1991) reported decrements in FEV_1 and nitrogen washout across all age groups in areas where oxidant pollution is high. Similarly, analysis of the pulmonary function data from the National Health and Nutrition Examination Survey (NHANES) II showed loss of lung function when annual averages ofambient O_3 exceeded 0.04 ppm (Schwartz, 1989). This pattern of impairment is consistent qualitatively with the chronic animal studies (Costa et al., 1994).

Studies of Inflammation and Antioxidant Content of the Bronchoalveolar Lavage Fluid

The virtual absence of human BAL study data after repeated or prolonged exposures to O_3 hinders the comparison of nonacute human and animal inflammatory responses. However, the recent study of Devlin et al. (1995) suggests that the PMN and protein responses to repeated daily exposures to 0.4 ppm O_3 (2 h with IE for 5 consecutive days) attenuate, much as do the functional responses. Hence, for most of the BAL parameters (with the exception of lactate dehydrogenase activity [a marker of cell injury]), there is indeed an apparent reversal of acute inflammation when exposures are continued over the 5-day exposure period. Rat studies largely appear to show similar attenuation to O_3, but this response seems to be influenced by exposure patterns or conditions (Bassett et al., 1988; Tepper et al., 1989; Van Bree et al., 1989). The study with the most similar design to the human protocol (Tepper et al., 1989) showed some reduction in BAL protein with repeated exposure involving intermittent hyperventilation with CO_2, but over the 5-day period, the protein levels remained significantly elevated; cells were not evaluated in this study. Interestingly, vitamin C and glutathione levels in the BAL fluid increased over the 5-day

course of exposure, a response consistent with an unregulated antioxidant role in the adaptative mechanism. Van Bree and coworkers (1992), on the other hand, reported that 5 consecutive days (12 h/day) of 0.4 ppm O_3 resulted in complete reversal of both BAL albumin and PMN measures to the control values. It should be noted, however, that other biomarkers and mediators within the BAL were not fully recovered, which might suggest a slower reversal time frame or continued O_3-induced pathogenesis, a conclusion of the Tepper et al. (1989) study.

In guinea pigs made deficient in vitamin C and exposed to O_3 (0.2, 0.4, or 0.8 ppm) continuously for 7 days, attenuation of the functional and inflammatory endpoints appeared nearly complete in spite of the deficiency (Kodavanti et al., 1995). Other antioxidants, not altered basally, were unregulated more by the O_3 challenge; the small residual reservoirs of ascorbate, which persisted in the nearly 98% deficiency state of the animals, were apparently mobilized to the site of injury, allowing repair to proceed. Likewise, chronically exposed rats have elevated BAL ascorbate indicative of the oxidant burden and the ongoing repair (Grose et al., 1988). Prolonged exposures up to 18 mo appear to sustain a low-grade interseptal inflammation and evidence of lung matrix remodeling in both rats and monkeys, suggesting that humans would behave similarly. However, such data are not presently available from humans.

8.4 Quantitative Extrapolation of Acute Ozone Effects

8.4.1 Introduction

Advances in dosimetry since the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986) fall into five major areas: (1) greater sophistication of model applications (e.g., Overton et al., 1989; Mercer et al., 1991), (2) the appearance of experimental uptake data that can be compared to model predictions (Wiester et al., 1987, 1988, 1996; Hatch et al., 1989; Gerrity et al., 1988, 1994, 1995), (3) experiments specifically designed to estimate model parameters (Hu et al., 1992b), (4) a better understanding of the role of O_3 in the liquid linings and tissues of the RT (Pryor, 1992), and (5) a better understanding of anatomical aspects (Mercer et al., 1991). The role of these advances in interspecies dosimetric extrapolation follows.

With the information available for rats, reasonably reliable predictions of the flux of O_3 to the air-liquid lining interface of toxicologically important regions, such as the CAR, is possible. There are two main investigations that make this feasible: (1) Hatch et al. (1989), who estimated the percent uptake and the fraction of the retained O_3 that is in the URT, trachea, and lung of rats, and (2) Pinkerton et al. (1992) (with elaboration by Miller et al., 1993) who illustrated the basic correctness of modeling assumptions for ventilatory units. (The judgment of basic correctness is based on the assumption that the dose causing the response is proportional to the flux of O_3 to the air-liquid lining interface.) Using this information, regional mass transfer coefficients could be estimated, which would allow the prediction of local respiratory tract O_3 doses in rats exposed under general conditions.

The results of the investigation of Hu et al. (1992b) can be used to estimate URT and TB model parameters, but may not be sensitive enough to determine pulmonary region parameters. If uptake is not confined to the URT and TBs, then their mass transfer coefficients alone would not be sufficient to account for total RT uptake (e.g., as measured by Wiester et al. [1996] or Gerrity et al. [1988]), and the difference in predicted (without

pulmonary region uptake) and experimental uptakes could be used to estimate the pulmonary region mass transfer coefficient. Unfortunately, no dosimetry data for the human pulmonary region are available.

Despite limitations, the O_3 dosimetry data that have been obtained over the past several years, coupled with the advances in modeling, suggest that there has been continual convergence between the model predictions and experimental observations. Given the many areas of consistency between models and experiments, it is valuable to begin to employ these models to provide the dosimetric basis for animal-to-human extrapolation. One of the greatest sources of uncertainty in such an application of dosimetry models is the lack of full understanding of the appropriate target site for O_3 toxicity (e.g., upper or lower airways or pulmonary region) that initiates a particular response, especially the functional changes in the lung. However, reasonable assumptions can be made to narrow the target site.

The first application of dosimetry models given in Section 8.4.2 is an examination of delivered dose versus response within a given species. This is followed in Section 8.4.3 by some interspecies comparisons of delivered dose versus response.

8.4.2 Intraspecies Delivered Dose Response

Assuming that changes in FEV_1 in humans exposed to O_3 mainly are the result of O_3 pulmonary tissue dose, Miller et al. (1988) constructed a dose-response curve. They plotted decrements in FEV_1 versus predicted cumulative pulmonary region O_3 tissue dose scaled to body mass (Figure 8-13). The concentration-response data are from McDonnell et al. (1983), in which 135 healthy subjects were exposed to 0, 0.12, 0.18, 0.24, 0.30, and 0.40 ppm O_3. Exposure was for 2.5 h with heavy IE. Miller et al. (1988) used the average weight and height of the subjects to estimate the FRC that was used in the model to simulate O_3 dose. The exercise breathing parameter data were used along with an estimate of resting breathing parameters. Figure 8-13 is similar in shape to the concentration-response curve of McDonnell et al. (1983). Differences between these two curves, however, are accounted for by the translation between exposure concentration and O_3 dose.

As another example, Pinkerton et al. (1992) examined the relation between actual tissue response of rats chronically exposed to O_3 and a prediction of O_3 dose as a function of distance from the bronchiole-alveolar duct junction (BADJ) to ventilatory units. Using these data, Miller and Conolly (1995) plotted the predicted O_3 dose and the observed change in wall thickness due to the exposure versus distance from the BADJ (Figure 8-14). Even though considerable variability in the thickness change can be inferred from the data, the two curves, scaled to their values at the junction, show a remarkable similarity and suggest a basic correctness in regards to the ventilatory unit model parameters.

8.4.3 Interspecies Delivered Dose Response

The illustrations presented in this section are based on dosimetric estimates for humans and rats using existing or modified theoretical models (Miller et al., 1985; Miller et al., 1988; Overton et al., 1987). One functional and one inflammatory endpoint will be provided drawing from the f and BAL protein data described in Section 8.3. Because the diversity of exposure scenarios across species is so great, the window of exposure parameters has been narrowed, minimizing exposure-based differences in relating species responsiveness.

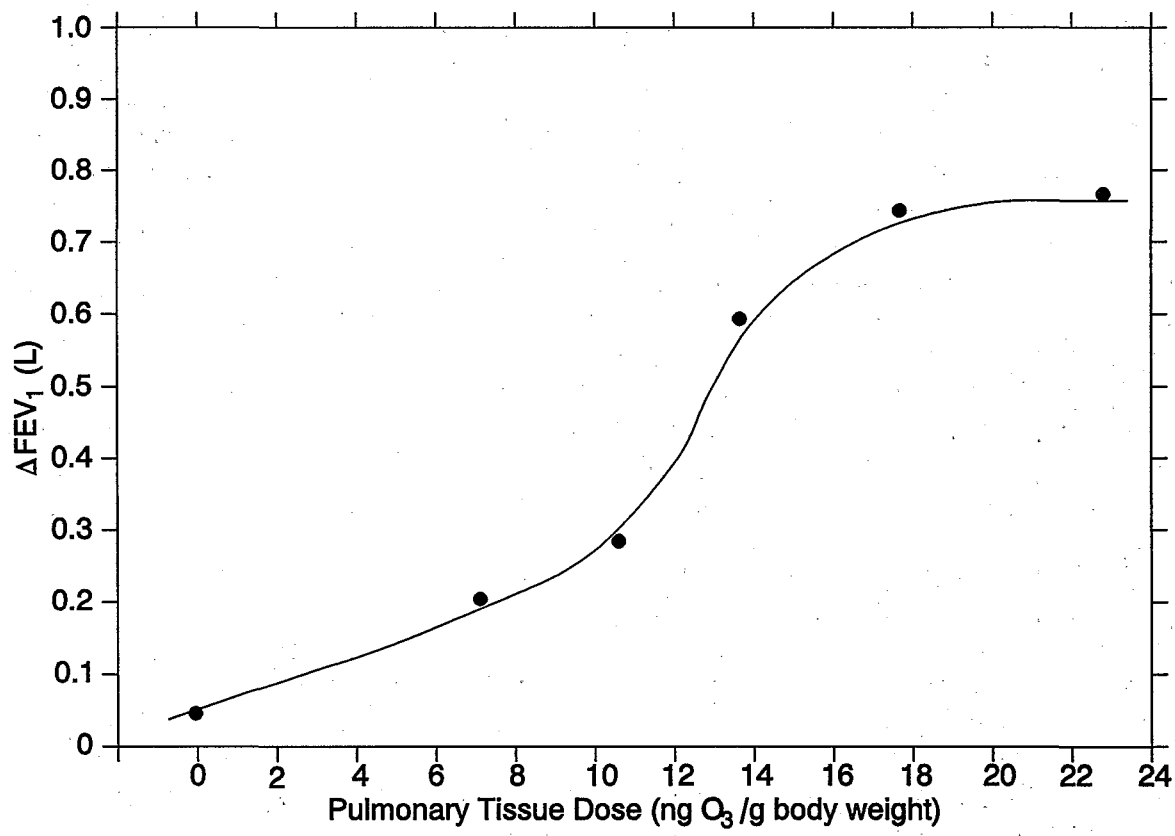


Figure 8-13. Changes in forced expiratory volume in 1 s (ΔFEV_1) versus pulmonary tissue dose. Plotted are decrements in FEV_1 (liters) for human subjects against predicted pulmonary tissue dose normalized to body weight. In order, from left to right, the dose values correspond to 0, 0.12, 0.18, 0.24, 0.30, and 0.40 ppm ozone (O_3) exposure concentrations. The continuous curve was an "eye fit".

Source: Miller et al. (1988)

Because the theoretical models developed by Miller et al. (1985, 1988) and Overton et al. (1987) estimate the dose distribution to the RT on a per breath basis, a minimum quantitative description of tidal breathing (V_T and f) is needed to utilize the theoretical models of O_3 deposition. The need for detailed breathing parameters, therefore, severely restricts the application of the model to studies providing such data. Unfortunately, breath-by-breath parameters over the course of an exposure normally are not measured or reported in most publications. Two studies each in humans and rats, providing adequate detail over the course of the exposure, allowed the model computations to be performed for this illustration (Figure 8-15). The human studies examined were DeLucia and Adams (1977), who exposed the subjects to 0.15 and 0.30 ppm O_3 for 1 h with continuous exercise (65% oxygen uptake to the body) periods, and Beckett et al. (1985), who exposed the

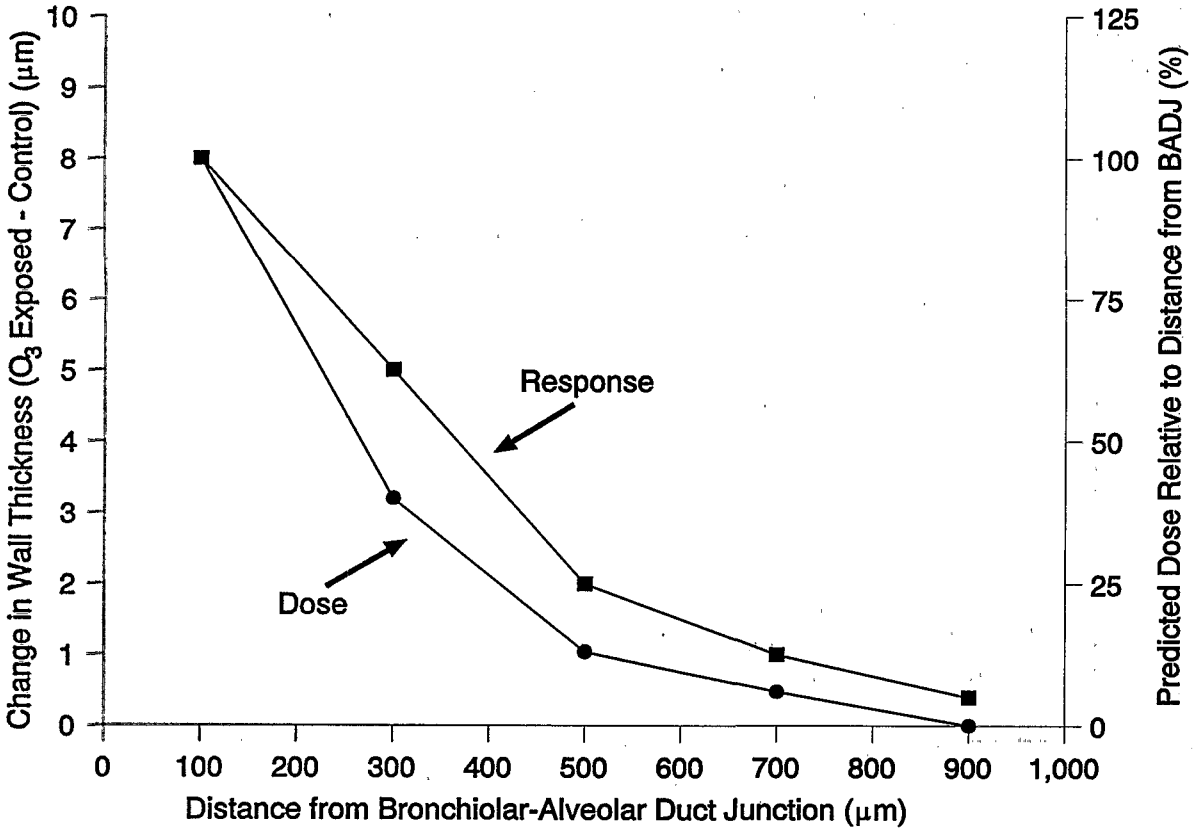


Figure 8-14. Relationship between change in alveolar wall thickness and predicted ozone (O_3) dose as a function of distance from the bronchiole-alveolar duct junction (BADJ). Rats were exposed to 0.98 ppm O_3 for 8 h/day for 90 days.

Source: Miller and Conolly (1995).

subjects to 0.4 ppm O_3 with alternating 15 min exercise (70 L/min). Three rat studies were evaluated: (1) Tepper et al. (1989), who exposed rats to 0.35, 0.50, and 1.00 ppm O_3 for 2.25 h with alternating 15-min periods of CO_2-induced hyperventilation (2 to 3 × resting $\dot{V}_E$); (2) Tepper et al. (1990), who exposed rats to 0.12, 0.25, 0.50, and 1.00 ppm O_3 for 2.25 h with alternating 15-min periods of CO_2, such that subsequent periods of CO_2 exposure had higher CO_2 concentrations than previous periods; and (3) Mautz and Bufalino (1989), who exposed rats to 0.8 ppm O_3 for 3 h (at rest). The response parameter was the ratio of O_3-altered f (f_{O_3}) to control f (f_{cnt}). Dose rate was the average dose to the proximal alveolar region (PAR) computed from time 0 to the time of the maximum f_{O_3}/f_{cnt}. The PAR was chosen as the target based on the perception that O_3 acts on deep lung stretch receptors

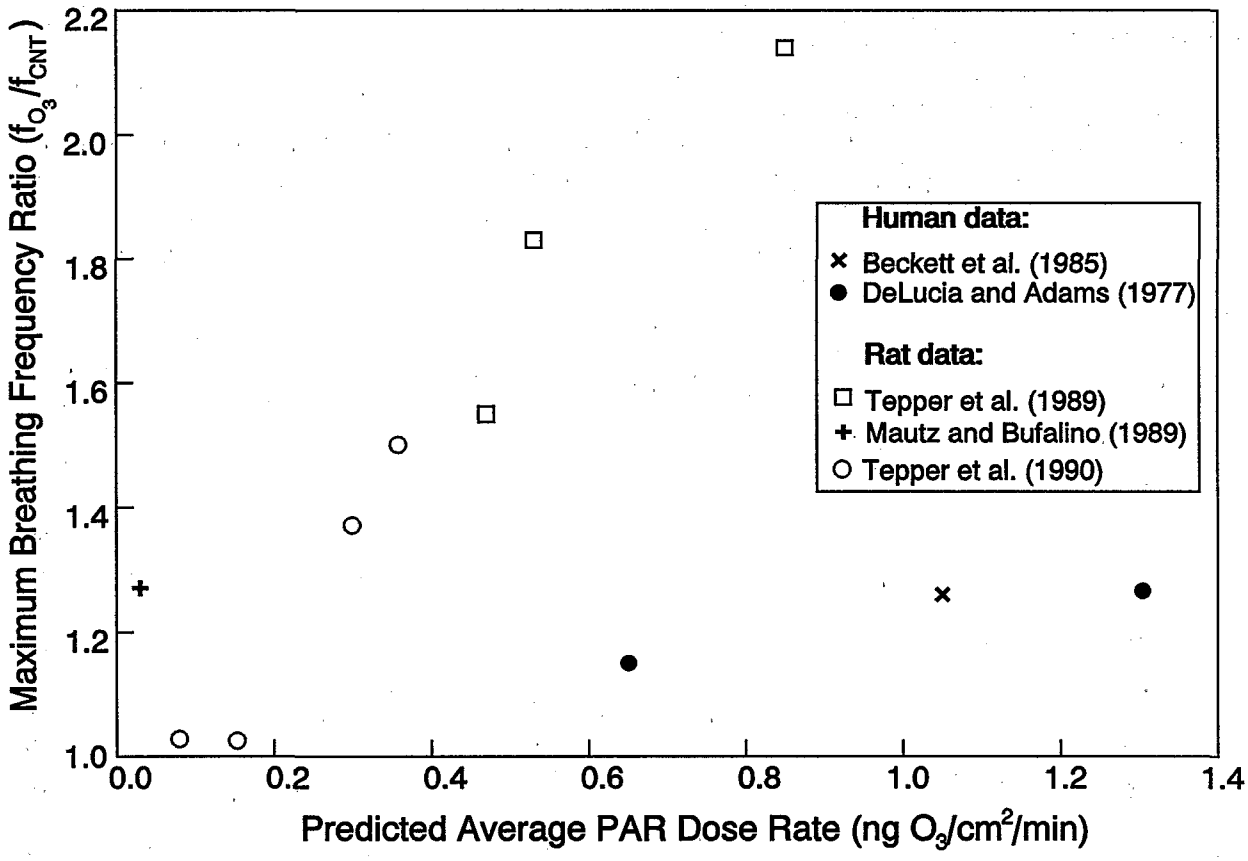


Figure 8-15. The maximum ratio of ozone (O_3)-altered breathing frequency (f_{O_3}) to control breathing frequency (f_{cnt}) at various O_3 concentrations versus predicted average dose rate to the proximal alveolar region (PAR; first generation distal to the terminal bronchioles). The average is for the time from the initiation of exposure to the time of occurrence of the maximum ratio. Beckett et al. (1985) and Mautz and Bufalino (1989) provided sufficient ventilatory data for only one O_3 concentration.

directly or indirectly (via tissue fluid pressure [edema]) as the stimulus for tachypnea. A number of assumptions were necessary to implement the deposition model for comparison of the human and the rat in this context; these are noted in Table 8-6. Although admittedly not validated, Figure 8-15 suggests that the response of the rats not only exceeded that of humans, but was initiated at a lower dose rate to the targeted lung region. This conclusion is in general agreement with that emanating from Figure 8-7, but the dose-to-target approach appears to accentuate the apparent difference in sensitivity for this endpoint in favor of the rat.

Using an analysis similar to that applied above for f, Miller et al. (1988) related pulmonary tissue dose (normalized to body weight) to BAL protein from rats, guinea pigs, and rabbits (Hatch et al., 1986) for O_3 concentrations of 0 to 2.0 ppm for 4 h. These values

have been supplemented in Figure 8-16 to include the results of the human study of Koren et al. (1989b). As can be seen in the illustration, there appears to be a log-normal relationship between BAL protein and dose to the pulmonary region, the purported site of plasma leakage to the airspace lumen. This relationship would support the contention that there is a mechanistic consistency in response across species that may exhibit a quantitative sensitivity factor for use in further quantitative interspecies extrapolation. This sensitivity factor is evident from the clustering of data from different animal species.

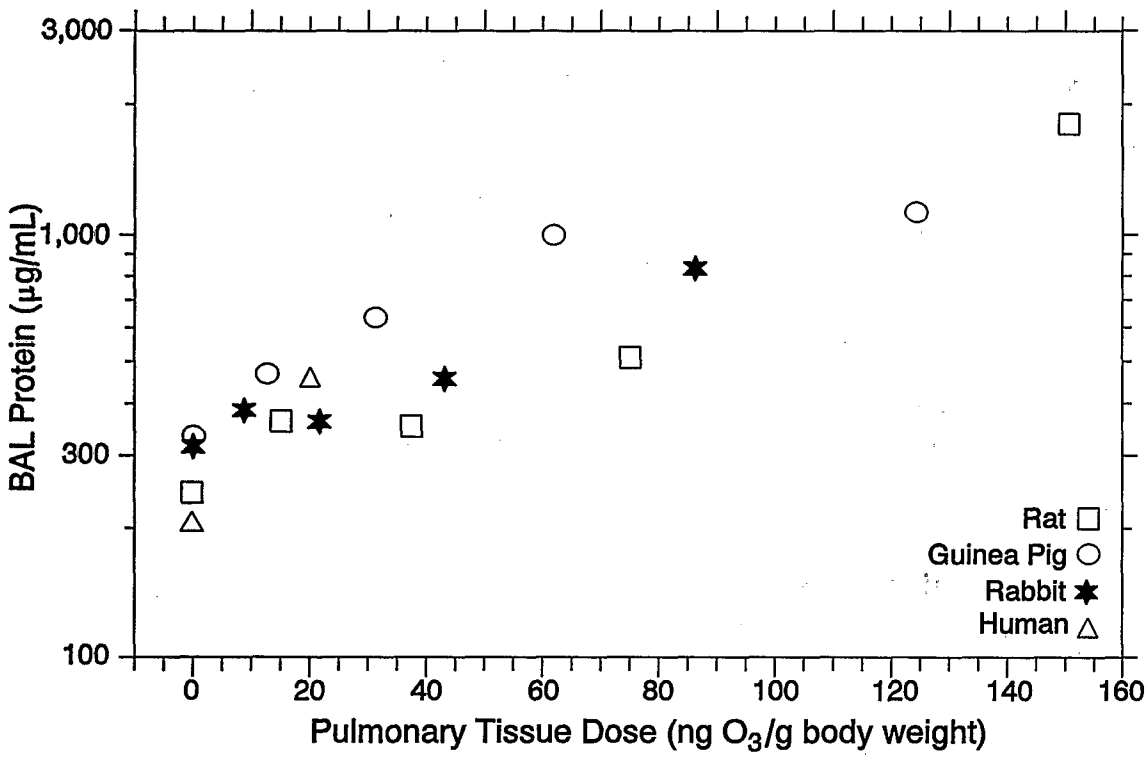


Figure 8-16. Protein in the bronchoalveolar lavage (BAL) for several laboratory animal species and humans, as related to the estimated pulmonary dose (normalized per gram of body weight).

Source: Modified from Miller et al. (1988).

8.5 Quantitative Extrapolation of Chronic Ozone Effects

8.5.1 Introduction

In previous sections, the homology between laboratory animal responses to acute O_3 exposure and effects observed in human subjects was demonstrated. Most of these responses were related to lung function and, to a more limited extent, recovery of

extravasated protein in BAL fluid, because these effects are measurable in both human and nonhuman species. Of laboratory species commonly used to address questions of acute toxicity, rats have the strongest and most compatible database for such comparisons. Studies in nonhuman primates typically are limited to morphology endpoints and to longer exposure periods, thereby limiting the practical utility of these studies in this same context.
A conclusion drawn from the discussion of acute effects is that there is reasonable semi-quantitative agreement and homology between species with regard to their functional and permeability responses to short-term O_3 exposure. Where data from long-term exposures exist, cross-species relationships among similar endpoints (usually functional) are considerably weaker, although there is the suggestion that long-term exposure to O_3 can alter the distal lung (that zone where theoretical models would predict greatest O_3 deposition).

This section continues the rationale founded in the extrapolation paradigm illustrated in Figure 8-6 in an attempt to quantitatively address the question of potential chronic alteration to the lungs of O_3-exposed people. Direct data from epidemiological studies remain suggestive at best (see Chapter 7). On the other hand, the extensive database of morphometric effects on the distal lung of exposed animals (rodents and nonhuman primates) reveals the sensitivity of such endpoints and clearly relates to the site of the lung associated with incipient chronic lung diseases associated with known toxic inhalants (e.g., tobacco smoke). Unfortunately, there is a lack of reliable morphometric data on the human lung that are associated with O_3 or other air pollutants. Thus, the goal here is to draw from the extensive and reliable animal database, which demonstrates chronic effects of long-term exposures to O_3, and project from it the potential for chronic effects in humans utilizing the linkage provided by newer, refined O_3 dosimetry estimates.

In the long-term studies selected for detailed analysis, great importance was placed on the relevance of the exposure concentrations, the site specificity of the morphometric analysis, and the consistency of analysis within species. Two rat studies were selected that represent near-lifetime exposures to O_3 over a range of concentrations and scenarios (Chang et al., 1992; Chang et al., 1995). The former study was conducted in conjunction with the U.S. Environmental Protection Agency (EPA) and involved a weekday urban pattern of exposure represented by a 9-h spike (5 days/week) slowly rising to 0.25 ppm from a near-continuous baseline of 0.06 ppm O_3 for 78 weeks. The latter study was conducted by the same morphometrists but on rats exposed weekdays (6 h/day) to 0, 0.12, 0.50, or 1.00 ppm O_3 for 87 weeks. Both studies utilized F344 rats. Likewise, the primate studies were conducted by the same investigators, but involved two strains of monkeys. In one study, the bonnet monkey was exposed for 90 days (8 h/day) to 0, 0.15, or 0.30 ppm O_3 (Harkema et al., 1993), whereas the second study consisted of daily (8 h/day) exposures to the smaller fascicularis monkey of 0 or 0.25 ppm O_3 for 18 consecutive mo or 9 mo with alternating months of clean air (Tyler et al., 1988). The assumptions needed to model the specific species dosimetry of each study are provided in the following section. Growth was compensated where appropriate (for rats), and allometric anatomic adjustments or assumptions were made to estimate unavailable anatomic data (as in the case of the monkey) for the dosimetry models. Ventilation was assumed to be unaffected by O_3 after the first 2 days of exposure because adaptive events are known to occur in that time frame. This simplified the model formulations for the animal studies, although varied activity and exposure profiles were considered throughout for the human dose estimates. Allometric equivalent life-span estimates also were made in an effort to relate the duration of the exposure period relative to life-span and cumulative dose.

In each experiment, a subregion of the CAR (where major lesions have been observed) was chosen by the investigators for study. For the two rat studies, the site was the PAR, which was defined by Chang et al. (1992) as "the alveolar tissue surrounding the alveolar ducts beginning at the bronchiolar-alveolar duct junction and ending at the second alveolar duct bifurcation." For modeling purposes, the first generation distal to the terminal bronchioles corresponded to the PAR. The monkey studies focused on an analogous airway region, the respiratory bronchioles. Thus, in three of the four experiments, the first generation distal to terminal bronchioles was the explicit site of the observed effects. For simplicity, similar assumptions were made for the Tyler et al. (1988) monkey study that came from the same laboratory as Harkema. For discussion purposes, the term PAR has been used for the monkey sites, even though the airway morphology of this site differs between the rat and monkey. The simulated dose of the PAR was chosen for comparison to the reported effect.

Because the PAR generally is thought to be the site of incipient lung disease, it is of particular interest with regard to the potential role of O_3-induced chronic lung disease. Effects in the PAR can be evaluated specifically using morphometric techniques with an electron microscope, and, likewise, dosimetric models can estimate surface-area-normalized focal tissue doses within the same region. The dose-response relationships constructed in this extrapolation focus on the theoretical dose to the PAR of rats and monkeys, with their corresponding impact on the PAR interstitial structure (as total and acellular thickness). Analogous estimated cumulative doses to the PAR of a 9-year-old child and of an outdoor worker exposed to a New York City summer O_3 season then are interpolated from the effects in the experimental animals at nominally similar doses.

In this attempt to extrapolate chronic O_3 effects from the experimental animals to humans, the two foremost assumptions are that (1) there exists coherence of analogous dose-response data across species with regard to acute exposure effects (as represented by one side of the parallelogram model [Figure 8-6]), and (2) there exists some relationship of effect to the total cumulative dose to the target tissue. The essential hypothesis is that the rate of change of interstitial thickness is related to the rate of O_3 uptake. Clearly, the latter assumption ignores potential adaptive or reparative processes, but rather assumes that continuing exposure of any scenario imparts irreversible or slowly reversible changes within the constitutive structure of the target area. There is little to substantiate this assumption other than the commonly believed irreversibility of fibrogenesis and degenerative lung disease.

8.5.2 Factors Considered in Estimating Dose

For each of the animals for which doses were simulated, Table 8-10 lists information or parameters needed for the dosimetry simulations and the source of the information. Also, details are provided as to how this information was modified, scaled, or used to correspond to the experimental or hypothetical animals for which dose was estimated. An expanded discussion follows.

8.5.2.1 Human

The dose simulations were for a hypothetical New York City adult outdoor worker and a hypothetical 9-year-old New York City child, who is active out of doors. Their

Table 8-10. The Basis of Information for Model Parameters[a]

Information Needed	F344 Rat (EPA Chronic Study)	F344 Rat (NTP/HEI Chronic Study)	Bonnet Monkey (*Macaca radiata*)	Monkey (*Macaca fascicularis*)	Human Child	Human Adult
Characteristics of simulated animal	Chang et al. (1992); Tepper et al. (1991): 0.21-0.47 kg, 10-88 weeks old, males.	Chang et al. (1995); Last et al. (1994): 0.13-0.53 kg, 7-93 weeks old, males.	Harkema et al. (1993): 2.3-9.7 kg (used 6-kg), 22-35 weeks old, males and females.	Tyler et al. (1988): 1-2 kg (used 1.6-kg), 30-107 weeks old, males.	Present simulation: 31 kg, 9 years, 132-cm height, male or female.	Present simulation: 73 kg, 181-cm height, adult male.
Exposure regime	Chang et al. (1992); 0.06 ppm continuous background, 22 h/day, 7 days/week; 0.25 ppm ramped peak over 9 h, 5 days/week. 3-, 13-, and 78-week exposures.	Chang et al. (1995); 0, 0.12, 0.50, and 1.00 ppm 6 h/day, 5 days/week for 87 weeks.	Harkema et al. (1993); 8 h/day for 90 days; 0, 0.15, and 0.30 ppm.	Tyler et al. (1988); 8 h/day for 18 mo; 0 ppm, 0.25 ppm (daily), and 0.25 ppm (daily, alternating months).	Johnson (1994); continuous exposure pattern for a New York outdoor child; April-October, 1991. Avg. exposure 22 ppb; range, 0-238 ppb.	Johnson (1994); continuous exposure pattern for a New York outdoor worker. April-October, 1991. Avg. exposure, 23 ppb; range, 0-227 ppb.
LRT structure	PUL: Mercer et al., 1991 (0.283-kg S-D rat). TB: Yeh et al., 1979 (0.33-kg Long-Evans rat).		Weibel (1963): adult human LRT.			
Dead space volume at FRC	Dead space volume = URT + TB volumes.		Dead space volume = URT + TB volumes.		Hart et al. (1963): height: 92-198 cm, age: 4-42 years, weight: 16-115 kg.	
FRC	Mercer et al., 1987 (0.291-kg S-D rat). FRC $\propto$ weight$^{0.55}$ (Takezawa et al., 1980).		Kosch et al., 1979 (radiata monkeys, FRC = 52.8 mL/kg).	Tyler et al., 1988 (2-kg fascicularis). FRC $\propto$ weight$^{0.86}$ (relation for combined rodent species, Takezawa et al. (1980).		
URT volume (V) and surface area (S)	Patra et al., 1987 (F344 rat, 0.012-0.366 kg, 1-63 weeks old). Graphical interpolation and extrapolation with respect to age.		Schreider and Raabe, 1981 (7-kg rhesus monkey). V $\propto$ weight; S $\propto$ $V^{2/3}$.		V: The ratio of URT and dead space volumes are the same as in the adult human. S $\propto$ $V^{2/3}$	Hu et al. (1992a).
TB volume (V) and surface area (S)	V: Mercer et al., 1994a (0.293-kg S-D rat). S: Yeh et al., 1979 (0.33-kg Long-Evans rat). S $\propto$ $V^{2/3}$.		V: Pulmonary and TB volumes in same ratio as human. S $\propto$ $V^{2/3}$.		V: volume = dead space volume minus URT volume. S $\propto$ $V^{2/3}$.	
PUL region volume (V) and surface area (S)	V: FRC minus TB volume. S $\propto$ $V^{2/3}$.		Pulmonary and TB volumes in same ratio as human. S: Mercer et al. (1994b), interspecies interpolation.	V: Pulmonary and TB volumes in same ratio as human. S: Tyler et al., 1988 (2-kg fascicularis). S $\propto$ $V^{2/3}$.	V: volume = FRC volume minus dead space volume. S $\propto$ $V^{2/3}$.	

Table 8-10 (cont'd). The Basis of Information for Model Parameters[a]

Information Needed	F344 Rat (EPA Chronic Study)	F344 Rat (NTP/HEI Chronic Study)	Bonnet Monkey (*Macaca radiata*)	Monkey (*Macaca fascicularis*)	Human Child	Human Adult
Tidal volume and breathing frequency	Costa (1994): measurements made at 1, 3, 13, 52, and 78 weeks of exposure (interpolation for other time points).	None reported. Assumed similar to that of the EPA chronic study.	None reported. A range of parameters used. See Table 8-11 and Section 8.5.2.2.		Johnson (1994): activity pattern. Hofmann et al. (1989): tidal volumes and breathing frequences.	Johnson (1994): activity pattern. ICRP (1975): tidal volumes and breathing frequences.
Mass transfer coefficients	Parameter estimation using the rat data of Hatch et al. (1989) and an assumed pulmonary region coefficient. See Table 8-6 and Section 8.5.2.3.		For corresponding generations or model segments, the same as for the adult human. See Table 8-6 and Section 8.5.2.1.			Hu et al. (1992b); Miller et al. (1985); Weibel (1963). See Table 8-6.

[a]See Appendix A for abbreviations and acronyms. PUL, V, and S indicate pulmonary, volume, and surface area, respectively. In some cells of this table, the information is ordered as follows: characteristics of the species and the reference to the basis of the information, followed by an indication as to how the reference information was used or modified for the present simulations. The proportion symbol $\propto$ indicates that one parameter is proportional to another (e.g., $S \propto V^{2/3}$ implies that $S = S_0 (V/V_0)^{2/3}$, where the subscripted parameters are known; V is the new or desired volume and S is the estimated surface area of the new volume.

residences were not air-conditioned, and they lived, worked, or played in the same area. These two groups of people would have tended to experience higher outdoor O_3 concentrations than other New York City people because the chosen area had higher outdoor O_3 concentrations than other areas, and O_3 levels are higher in homes without air-conditioning (Johnson, 1994). These scenarios were selected because they involve the same at-risk population groups used for the O_3 risk analysis in the staff paper (U.S. Environmental Protection Agency, 1995).

The activity patterns and O_3 exposure estimates were generated by the probabilistic National Ambient Air Quality Standards Exposure Model for O_3 (pNEM/O_3) (Johnson, 1994) for April through October 1991. The modeling approach for outdoor workers can be described as follows. Outdoor workers residing in New York City were divided into nonoverlapping sets of cohorts, such that each cohort could be identified by a residential location, a work location, and a residential air conditioning system. The pNEM/O_3 model generated an "exposure event sequence" (EES) for each cohort based on data obtained from activity diary studies involving outdoor workers. Each exposure event in the EES assigned the cohort to a geographic location, a microenvironment, and an exertion level. Algorithms within the pNEM/O_3 model provided estimates of O_3 exposure and equivalent ventilation rate (EVR) for each event that lasted from 1 to 60 min. The EVR is ventilation rate (liters per minute) divided by body surface area (square meters). Exposures were calculated on a minute-by-minute basis; however, the concentration and EVR values of all minutes within a given exposure event were held constant during the event. The pNEM/O_3 model used for outdoor children was generally consistent with the model for outdoor workers but relied on human activity data from children. Another difference was that the cohorts of children were identified by residential location and air conditioning system only; workplace location was not specified.

Figures 8-17A and 8-17B give an idea of the exposure concentrations for the outdoor worker and child, respectively, with plots of the average hourly and monthly O_3 concentrations. The exposure concentrations of the adult and child differ because their daily activities and locations are different. Note that in a few cases, the hourly average exceeds the 0.12 ppm (120 ppb) standard.

Anatomical Aspects

The adult New York City worker was assumed to have characteristics similar to the subjects of an investigation by McDonnell et al. (1983). The body surface area of the worker (needed to convert EVR to minute volume) was assumed to be 2 m^2 (Koren et al.,1989b $\approx$1.90 and $\approx$1.98 m^2; Johnson, 1994: 1.90 to 1.95 m^2). The New York City child had a body surface area of 1.07 m^2, which was consistent with both 9-year-old males and females (Johnson, 1994). The assumed values for the child's height (132 cm) and weight (31 kg) were based on Phalen et al. (1985) and Johnson (1994).

For the simulations, no distinction was made between mouth and nose breathing with respect to URT uptake. The significance of this assumption is not clear. If both modes have approximately the same uptake efficiency, then the assumption of no distinction between mouth and nose breathing is appropriate for predicting PAR doses. Whether this is a valid assumption has not been settled (see Section 8.2.3.4). The same URT anatomical model was used for both ages, but was isotropically scaled as necessary. Surface areas and lengths were assumed to be proportional to the volume to the two-thirds and one-third powers for adults and children, respectively (e.g., if x is proportional to volume V to the power p, then

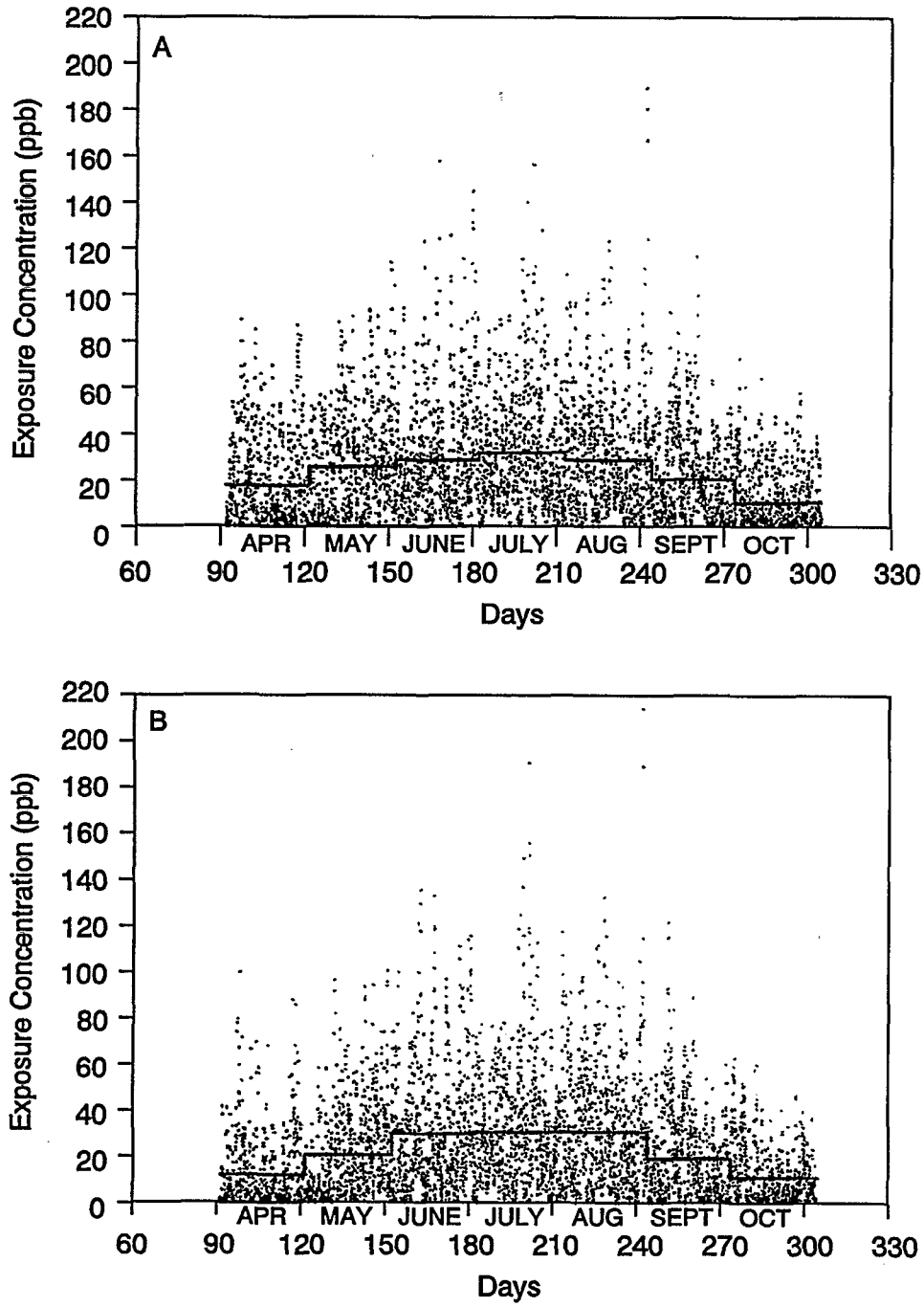


Figure 8-17. ***The variation in exposure concentration for the New York City adult and child. Plotted are the hourly and monthly O_3 averages for the New York City outdoor worker (A) and the 9-year-old child (B). The hourly averages are represented by the "dots" and the monthly average by the continuous solid line.***

$x_n = x_0(V_n/V_0)^p$, where the subscripts n and o indicate the new and original values, respectively). Dimensions used for the adult were from Hu et al. (1992b), who based their values on the acoustic reflection measurements of Fredberg et al. (1980) that corresponded to the oropharyngeal (mouth through larynx) region. The LRT model developed by Weibel (1963) was used for LRT structure and dimensions, but was scaled isotropically to FRC and dead-space volumes, as appropriate.

Mass Transfer Coefficients

This parameter is necessary to determine the local dose, expressed in terms of the flux of O_3 to respiratory tract surfaces. Specifically, this dose is related to the product of the mass transfer coefficient and gas-phase concentration of O_3 integrated over the time of interest. Good estimates of mass transfer coefficients are needed to predict a reliable dose at specific respiratory tract sites.

A brief description of how the human URT and TB region mass transfer coefficients were derived from Hu et al. (1992b) and Weibel (1963) is presented in Table 8-6. These coefficients were "validated" by simulating the bolus-response experiment of Hu et al. (1992b). Simulation results of the bolus uptakes overpredicted by $\approx 5\%$; results for the other two measured variables deviated from the experimental values by $\approx 30\%$.

As a result, simulations with inhaled and exhaled flow rates of ≈ 250 mL/s are expected to overpredict total uptake by no more than 5%. The significance of the poor results in simulating the other two variables is not clear. Possibly, predictions of the distribution of absorbed O_3 within the RT are questionable; also, predicted total uptakes for flow rates different from the experimental value of 250 mL/s may be suspect. For very different reasons, this latter speculation is probably valid. Measurements by Hu et al. (1994) at rates higher than 250 mL/s indicate that local mass transfer coefficients increase with increasing flow rates. In addition, naive subjects were used for the bolus experiments. Chronic exposure to O_3, which is more appropriate to the present simulation scenarios, could alter mass transfer coefficients due to chemical reactions changing the properties of biochemical constituents. See Section 8.2.4.2 and Table 8-7 for a comparison of dosimetry modeling results with dosimetry data, using coefficients based on Hu et al. (1992b).

The experimental data of Hu et al. (1992b) were not sensitive enough to allow an estimate, with confidence, for the pulmonary region or for the PAR mass transfer coefficient. For this reason, these coefficients were assumed to be the same as used by Miller et al. (1985). The value of this parameter is approximately the same as used for the rat. Although the value of the pulmonary region mass transfer coefficient of rats also is unknown, a comparison by Pinkerton et al. (1992) of morphometric effects and dosimetry model predictions suggests that the rat value is not unreasonable.

Ventilation

Activity patterns for the adult and the child (Johnson, 1994) were used to estimate $\dot{V}_E$s. The set of estimates had over 300,000 minute-by-minute cases, each case consisting of the day of the year, hour, minute, and equivalent ventilatory rate ($\dot{V}_E$ divided by body surface area). The generated patterns were for 214 sequential days, from the beginning of April through October 1991.

For the adult, V_Ts were estimated by interpolation from a plot of V_T versus $\dot{V}_E$ that was drawn using information from Table 120 in International Commission on Radiological Protection (1975). Tidal volumes and f's for the child were estimated using a

relationship between V_T and $\dot{V}_E$ developed by Hofmann et al. (1989). For both humans, f's were defined as $\dot{V}_E$ divided by the estimated V_T.

Estimating Proximal Alveolar Region Dose

The PAR doses were estimated by simulation for each of the minute-by-minute equivalent $\dot{V}_E$s and personal concentration estimates; these doses were averaged.

8.5.2.2 Monkeys

Anatomical Aspects

Because there were no data on the uptake of O_3 by the URT of monkeys, no distinction was made between oral and nasal breathing. The URT dimensions were based on Schreider and Raabe (1981) for the nasopharyngeal region of a 7-kg rhesus monkey. Volumes were proportional to body weight and dimensions were scaled isotropically. For the nasopharyngeal region, the dimensions used may have been too large because the casting procedure may have resulted in a larger volume (Yeh et al., 1989) than in a live animal, and air flow streamlining (Morgan et al., 1991) may have resulted in a smaller effective volume. The structure of the LRT was based on Weibel (1963) for humans (the same as used for the human simulations), which was isotropically scaled to monkey FRC values, assuming that TB and pulmonary region volumes were in the same proportion as the human FRC values. The use of the human LRT structure for the monkey simulations is considered to be of less importance to reasonable predictions than the correct specification of volumes and surface areas of the different regions.

Mass Transfer Coefficients

The human mass transfer coefficient values were assumed because there were no reported values nor uptake data that would have allowed estimates of these coefficients. For corresponding model segments or model generations, the monkey coefficients were the same as those used for humans.

Ventilation

No ventilation parameters were reported for the monkey experiments. For this reason, four states of ventilation were used to calculate the doses (see Table 8-11). The first three in the table were extrapolated from human values of V_Ts and f's. These human parameters were consistent with the algorithm used for the human simulations and correspond approximately to sedentary, low, and light activity as categorized by Hofmann et al. (1989) for an adult human. The last set of parameters correspond to measurements made by Moss (1995) on five awake female adult cynomolgus monkeys (*Macaca fascicularis*).

Estimating Proximal Alveolar Region Doses

For the two monkey experiments and corresponding ventilatory parameters, scaled PAR doses were estimated and multiplied by the average exposure concentrations to obtain estimated average PAR doses.

8.5.2.3 Rats

Anatomical Aspects

Rats are nasal breathers; therefore, the URT model corresponds to and is based on the F344 rat nasopharyngeal region. The dimensions may be too large, however, because

Table 8-11. Estimating Monkey Ventilatory Parameters[a]

Human Activity Level[b]	Extrapolation from a 73-kg Human with the Following Parameters[c]			To a 1.6-kg Monkey			To a 6-kg Monkey		
	$\dot{V}_E$ (L/min)	V_T (mL)	f (breaths/min)	$\dot{V}_E$ (L/min)	V_T (mL)	f (breaths/min)	$\dot{V}_E$ (L/min)	V_T (mL)	f (breaths/min)
Sedentary	6	500	12.0	0.34	11.0	31.2	0.92	41.1	22.4
Low	12	900	13.3	0.68	19.7	34.7	1.84	74.0	24.9
Light	20	1,300	15.4	1.14	28.5	40.0	3.07	107.0	28.7
	Extrapolation from a 4.4-kg *Macaca fascicularis* with the following parameters[d]								
	1.78	52.9	33.6	0.83	19.3	43.2	2.25	72.3	31.1

[a]See Appendix A for abbreviations and acronyms. For extrapolation: $(V_T)_1 = (V_T)_2 \times (\text{weight}_1/\text{weight}_2)$; $f_1 = f_2 \times (\text{weight}_1/\text{weight}_2)^{-0.25}$; $\dot{V}_E = V_T \times f$. Subscript 1 corresponds to the 1.6- or 6-kg monkey, and subscript 2 corresponds to either the human or the 4.4-kg monkey.

[b]These characterizations for the human parameters are consistent with those of the International Commission on Radiological Protection (1975) and Hofmann et al. (1989).

[c]For a given human $\dot{V}_E$, the human V_T and f are based on the International Commission on Radiological Protection (1975). For the selected $\dot{V}_E$, V_T and f are the same as used for the adult human.

[d]Moss (1995); parameters are the average for five monkeys.

the casting procedure can result in a larger volume than in a live animal (Yeh et al., 1989), and air flow streamlining (Morgan et al., 1991) may result in a smaller effective volume. The structure of the LRT is a composite of the TB model of the Long-Evans rat (Yeh et al., 1979) and a pulmonary region based on the Mercer et al. (1991) ventilatory unit model of the S-D rat. Given the dosimetry model used, the volumes and surface areas of the different regions are more important than the structural differences of the three strains used to construct the rat RT.

Mass Transfer Coefficients

Mass transfer coefficients for the nasopharyngeal region and TB of the rat were estimated using the uptake data of Hatch et al. (1989) and an assumed pulmonary region mass transfer coefficient. Hatch et al. (1989) reported the average uptake of O_3 for eight F344 rats and the average fraction of $^{18}O_3$ in the nasopharyngeal region, the trachea, and the lungs. (Hatch et al. [1989 and 1994] discuss issues related to using $^{18}O_3$ dose as a measure of O_3 dose.) Based on a discussion in Miller et al. (1993) concerning the investigation of Pinkerton et al. (1992), the pulmonary region mass transfer coefficient was defined as that used by Mercer et al. (1991). This, combined with information from Hatch et al. (1989), allowed an estimate of mass transfer coefficients for the nasopharyngeal region and the TB. A common estimate of mass transfer coefficients also was made using the data for the individual rats. For these coefficients, whose values were essentially the same as the first set, the individual rat simulations deviated from the experimental data by an average of $\approx 23\%$.

Ventilation

For the EPA chronic study, V_T and f were measured at Exposure Weeks 1, 3, 13, 52, and 78. For intermediate weeks, these parameters were estimated by interpolation and assumed constant during each of those weeks. Because ventilatory parameters were not reported for the National Toxicology Program/Health Effects Institite (NTP/HEI) chronic study, parameters similar to those of the EPA chronic study were assumed (see below).

Estimating Proximal Alveolar Region Doses

For the EPA chronic study, the exposure pattern was variable during the week, but repeated each week (Tepper et al., 1991). The PAR doses were simulated for each week and averaged. The ventilatory, physiological, and anatomical characteristics of the NTP/HEI study rats with respect to time were assumed to be similar to the EPA chronic study rats, and the average scaled PAR dose ([g/cm^2-min]/[g/m^3 ambient O_3]) was defined as the same that was predicted for the EPA study rats. Given this and the average exposure concentration, the average PAR doses were estimated.

8.5.3 Results and Discussion

8.5.3.1 Simulation Results

The simulation results are presented in Table 8-12. The first and second columns identify the laboratory experiments and the hypothetical human exposures that were simulated. Column 4 applies only to the monkeys and is discussed above (see Section 8.5.2.2) in the discussion of monkey ventilation. The "Average PAR Dose" (column 5) is the predicted average flux of O_3 to the surfaces of the alveoli or respiratory

Table 8-12. Summary of Simulation Results[a]

Simulation of	Source for Simulation	Average Weight (kg)	Minute Volume[b] (L/min)	Average PAR Dose ($\mu g/cm^2$-min)	Cumulative PAR Dose ($\mu g/cm^2$)	Duration of Experiment (weeks)	Equivalent Human Child Time[c] (weeks)	Equivalent Human Adult Time[c] (weeks)
EPA rat (3 weeks)	Chang et al. (1992) Tepper et al.(1991)	0.24		4.11e-5	1.24	3	8.9	11
EPA rat (13 weeks)		0.29		3.85e-5	5.1	13	39	48
EPA rat (78 weeks)		0.4		3.43e-5	27	78	232	286
NTP/HEI rat (0.12 ppm)[d]	Chang et al. (1995)	0.4		0.828e-5	7.3	87	259	319
NTP/HEI rat (0.5 ppm)[d]		0.4		3.45e-5	30.3	87	259	319
NTP/HEI rat (1 ppm)[d]		0.4		6.89e-5	60.5	87	259	319
Bonnet monkey (0.15 ppm)	Harkema et al. (1993)	6		1/2 below[e]	1/2 below[e]	12.9	19.5	24
Bonnet monkey (0.3 ppm)		6	0.92	0.61e-5	0.79	12.9	19.5	24
			1.84	3.99e-5	5.17	12.9	19.5	24
			3.07	9.92e-5	12	12.9	19.5	24
			2.25	5.65e-5	7.3	12.9	19.5	24
Fascicularis monkey (daily; 0.25 ppm)	Tyler et al. (1988)	1.6		2 × below[f]	2 × below[f]	78	164	203
Fascicularis monkey ("seasonal"; 0.25 ppm)		1.6	0.34	0.197e-5	1.55	78	164	203
			0.68	1.26e-5	9.91	78	164	203
			1.14	3.25e-5	25.6	78	164	203
			0.83	1.81e-5	14.3	78	164	203
9-year-old NYC[g] child	U.S. Environmental Protection Agency (1995)	31		3.2e-5	9.9	30	30	NA
Adult NYC[g] outdoor supervisor		73		2.78e-5	8.6	30	NA	30

[a]See Appendix A for abbreviations and acronyms.
[b]Only relevant for the monkeys. See Table 8-11.
[c]Given the nonhuman exposure time, the number of weeks the human would have to be exposed for equal human and nonhuman physiological times: $weeks_{HUMAN} = weeks_{NONHUMAN} \times (weight_{HUMAN}/weight_{NONHUMAN})^{0.25}$.
[d]The relative PAR dose [$(g/cm^2\text{-min})/(g/m^3\ O_3)$] was assumed to be the same as the average for the EPA rat.
[e]For each minute volume, the exposure concentration for these bonnet monkeys was one-half those listed below.
[f]For each minute volume, the fascicularis monkeys exposed "daily" were exposed to O_3 twice as long as the "seasonal" fascicularis monkeys.
[g]NYC = New York City.

bronchioles in the first model generation distal to the terminal bronchioles. For each experiment, the average is over the total time or duration of the experiment (given in column 7, "Duration"); the average includes times of no exposure. Column 6, "Cumulative PAR Dose," is the total quantity of O_3 predicted to be absorbed by the tissue and liquids of the PAR during the experiment; these dose values are the same as the duration (in minutes) multiplied by the average PAR dose (column 5).

The entries in columns 8 and 9 are the total times a human would have to be exposed to reach the equivalent physiologic times of a specific laboratory animal species. Basically, the concept of physiological time is that the time courses of equivalent processes (e.g., a breath, a heartbeat) across species are approximately the same with respect to the time span of the process divided by the body mass raised to the one-fourth power (Travis et al., 1990). If this concept is applicable to the time course of O_3-induced biological effects, then the entries in the two columns are the human exposure durations necessary to obtain the same cumulative PAR dose biological effect as that of the corresponding laboratory animal duration. Note that for only two or three of the laboratory animal experiments are the human durations greater than the laboratory animals' equivalent or real (duration) times.

8.5.3.2 Interpretation of Chronic Site-Specific Dose-Effect Estimates

Because the PAR is considered the primary site of O_3 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 selected "effect" relates to the thickness of the interstitium at the PAR, which is indicative of fundamental structural remodeling. The indices of thickness provided in the noted studies include both cellular and acellular constituents, a distinction that was not always clear; however, interstitial arithmetic thickness, areal volume (cubic micrograms per square microgram), or volume density was available and could be represented in terms of percent change from control.

Dose-response curves for O_3-induced thickening of the PAR are represented in Figures 8-18A (rats) and 8-18B (monkeys). The cumulative PAR dose for the specific exposure scenario (from Table 8-12; Cumulative PAR Dose) for each study is provided on the abscissa as is its corresponding percent change in total and acellular interstitial (PAR) thickness on the ordinate (detailed in Table 8-13). The rat studies are plotted separately from the monkey studies because of an approximate three- to fivefold difference in their responses, with the monkey being more responsive than the rat. Although this difference could represent innate sensitivity differences between the species, it should be noted that estimates of daytime rat exposures (a period of quiescence for the rat), in contrast to the daytime-active monkeys, may have been substantially lower in terms of dose than that predicated on the basis of rat ventilatory measurements that were derived when they were in an aroused, awakened state. Recent studies comparing rat and human $^{18}O_3$ dosimetry indicate that exercise can account for up to a fivefold difference in acute responsiveness between species (Hatch et al., 1994). These explanations remain speculative, however, in the absence of direct data. Ventilation values for the monkeys were selected a priori as light activity to be similar to the human model being used, which had ventilation values only slightly higher than the empirically derived values (see Table 8-11) from comparable awake, resting monkeys (when adjusted for size).

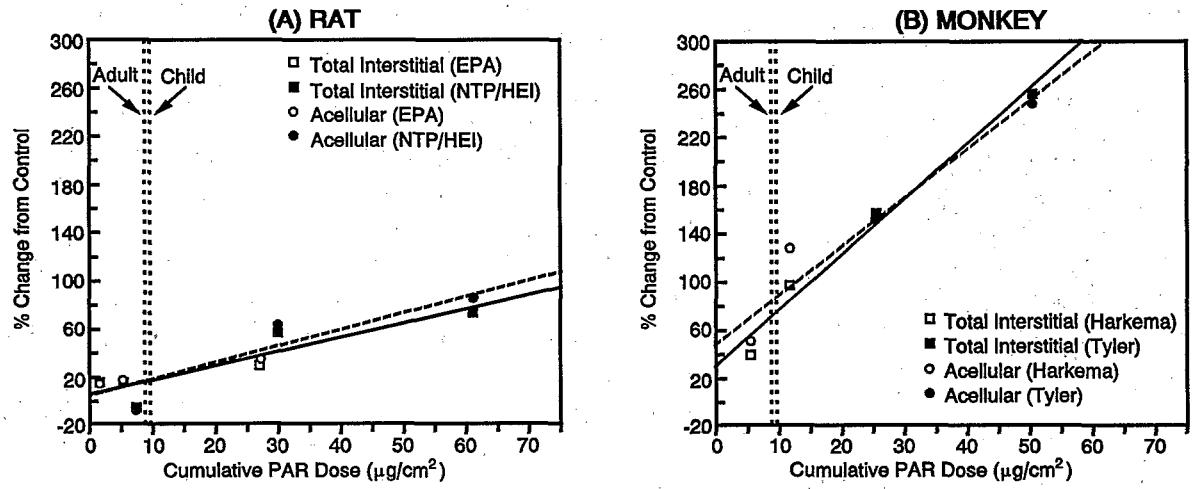


Figure 8-18. ***(A) Change from control of total interstitial and acellular thickness for the rats exposed to ozone (O_3) in the U.S. Environmental Protection Agency (Chang et al., 1992) and National Toxicology Program/Health Effects Institite (Chang et al., 1995) studies. Solid line represents the linear regression for the total interstitial thickness, and the dashed line represents the linear regression for the acellular thickness across the various cumulative dose estimates for both studies. The respective correlation coefficients (r^2) are 0.84 and 0.80. (B) Change from control of total interstitial and acellular thickness for the monkeys exposed to O_3 in the Tyler et al. (1988) and Harkema et al. (1993) studies. Solid line represents the linear regression for the total interstitial thickness, and the dashed line represents the linear regression for the acellular thickness across the various cumulative dose estimates for both studies. The respective correlation coefficients (r^2) are 0.97 and 0.93. Vertical dashed line with arrows for child and adult denote proximal alveolar region (PAR) dose level for interpolation of PAR thickness effect from the monkey upper estimate of response.***

What is remarkable in both Figures 8-18A and 8-18B is the apparent linear dose-response relationships within species. This may be due, in part, to the fact that there was consistency in the methods of the respective investigatory team studying each species. It is interesting to note that the estimated total cumulative PAR dose for the EPA chronic study (27 $\mu g\ O_3/cm^2$) is quite similar to that estimated for the NTP/HEI study group of 0.5 ppm O_3 (30.3 $\mu g\ O_3/cm^2$). An examination of the respective PAR thickness response from each study indicates an apparent similarity between the studies, with the NTP/HEI results being a bit larger in value. At 0.5 ppm O_3, the NTP/HEI investigators also reported the observation of bronchiolarization into the PAR, whereas this process was absent or not significant at 0.12 ppm O_3 or in the EPA chronic study. It is not clear whether this indicates qualitatively, rather than quantitatively, different responses initiated at 0.12 and 0.50 ppm O_3. However, it is important to note that the correlation coefficients (r^2) for PAR dose to response across

Table 8-13. Summaries of Study Data Used in Extrapolation of Chronic Ozone Effects[a]

Study Species	Total Interstitial Volume (mean value — $\mu m^3/\mu m^2$)	Cellular Volume ($\mu m^3/\mu m^2$)	Acellular Volume ($\mu m^3/\mu m^2$)
F344 Male Rat; Chang et al., 1992 (EPA)—78 weeks (urban profile)			
• Profile: 0.06-ppm continuous background, 22 h/day, 7 days/week; 0.25-ppm ramped peak over 9 h (avg. 0.19 ppm for peak; 0.09 ppm daily), 5 days/week			
• 3-week exposure (from Figure 4)	(+16% estimate from figure)	(+12%)	(+15%)
• 13-week exposure (from Table 4)	0.398[b] 0.462 (+16%)[c]	0.153[b] 0.174 (+14%)	0.245[b] 0.288 (+18%)
• 78-week exposure (from Table 4)	0.473[b] 0.619 (+31%)	0.149[b] 0.179 (+20%)	0.325[b] 0.440 (+35%)
• 16-week recovery after 78 weeks air • 16-week recovery after 78 weeks O_3	0.531[b] 0.580 (+9%)	0.171[b] 0.168 (0%)	0.360[b] 0.410 (+14%)
F344 Male Rat; Chang et al., 1995 (NTP/HEI)—87 weeks (6h/day, 5 days/week)	From Appendix D1—HEI Report		
• 0 ppm	0.520	0.161	0.360
• 0.12 ppm	0.497 (−5%)	0.162 (0%)	0.335 (−7%)
• 0.50 ppm	0.826 (+58%)	0.239 (+48%)	0.587 (+63%)
• 1.00 ppm	0.902 (+73%)	0.233 (+45%)	0.669 (+85%)
Fascicularis Monkey; Tyler et al., 1988 —18 mo (8 h/day)	Volume fraction of lung occupied by respiratory bronchiolar wall ($\times 10^{-3}$)—from Table II		
• 0 ppm	0.716	0.110	0.606
• 0.25 ppm (daily)	2.550 (+256%)	0.437 (+297%)	2.113 (+248%)
• 0.25 ppm (daily; alternating months)	1.830 (+156%)	0.297 (+170%)	1.533 (+152%)
Bonnet Monkey; Harkema et al., 1993—90 days (8 h/day)	Arithmetic mean thickness (μm)—from Figure 3 and text		
• 0 ppm	6.5	3.2	3.3
• 0.15 ppm	9.2 (+41%)	4.2 (+30%)	5.0 (+51%)
• 0.30 ppm	13.1 (+102%)	5.6 (+74%)	7.5 (+128%)

[a]See Appendix A for abbreviations and acronyms.
[b]Control value.
[c]Parenthetical values indicate percent difference from respective control.

both studies as illustrated in Figure 8-18A are 0.80 and 0.84 for the total and acelluar interstitial changes, respectively. In the case of the monkey data represented in Figure 8-17B, the analogous correlation coefficients (r^2) were 0.97 and 0.93 for the total and acellular interstitial effects, respectively. These highly significant correlations suggest that interstitial injury is indeed a cumulative process throughout the exposure history. Moreover,in the case of monkeys with seasonal (intermittent monthly) exposures, there was no loss of effect (i.e., reversal) during the air periods. The response was strictly a function of cumulative dose.

The availability of exposure/activity data for a 9-year-old child and adult outdoor worker over an "exposure-season" of 214 days in New York City provides the opportunity to estimate an analogous PAR dose in these human individuals for comparison to the animal dose-response relationships. Because the rat exposure scenario extends over the majority of a lifetime, an attempt was made to determine the equivalent human exposure time using standard, accepted algorithms for lifetime transformations (see Table 8-12). However, the extrapolation of human biologic exposure time from that of the rat did not yield reasonable results. The primate-based extrapolation of exposure-time fared slightly better but also was less than adequate for the purposes of this exercise. Estimates of accumulated dose, however, were in general agreement (see Table 8-12) and served as the basis for the cross-species extrapolation of effects described below.

Unfortunately, there are no hard data to substantiate whether the rat or monkey better represents the human in the context of the endpoint being addressed herein. The monkey could be favored on the basis of responsiveness at low levels of O_3, corresponding to those in human spirometric tests following exposures involving exercise; however, the dose-adjusted BAL protein data are comparable for humans and rodents. The apparent differences in sensitivity between the rat and monkey may, on the other hand, reflect more rapid repair in the rat than in the primate. This concept would be consistent with the algorithms of Travis et al. (1990), which are based on intrinsic metabolic rates and thus may reasonably apply to repair processes after injury or damage from oxidant-lung surface interactions. Thus, the responses for the rat and monkey may best be considered as bracketing the human response. The linearity of the dose-response relationships in both the rat and monkey models lends credibility to interpolation (Figures 8-18A and 8-18B) of the estimated dose to the human PAR to its corresponding response. For example, in the case of the child, the predicted seasonal response could range from about a 20 to 75% increase in PAR thickness; the adult human response would be slightly less with a 15 to 70% increase. Although the actual changes in the human may not be as large as the monkey, the graphical data from both species suggest that recovery may not be complete (in fact, there was no reversal) when exposure is interrupted during alternating months. Likewise, in rats exposed long-term, reversal was incomplete ($\approx$66%; see Table 8-13) after a 4-mo postexposure period in clean air. In the case of the rat, 4 mo represents a larger proportion of its life span than a similar period in either the monkey or human, thereby suggesting that even 6 to 8 mo of background "off season" levels of ozone would not be sufficient for complete recovery in primates.

This attempt to extrapolate results of animal studies for the chronic effects of O_3 to the human obviously must be considered preliminary because of lingering questions regarding relative dosimetry across species and the uncertainties associated with episodic (typical human scenario) versus continual, repeated exposures (typical of animal studies). Yet, this extrapolation provides a foundation from which additional questions can be derived and addressed to reduce these uncertainties. The coherent evidence in hand suggests that

there is a real possibility that chronic exposure to O_3 can lead to interstitial thickening at the PAR, that region of the lung involved in chronic diseases such as chronic bronchiolitis associated with cigarette smoke or occupational fibrogenesis. The apparent lack of reversal of effects during periods of clean air raises concern that seasonal exposures have a cumulative impact over many years. The role of adaptive processes in this response is unknown but may be critically dependent on the temporal frequency or profile of exposure. Furthermore, the interspecies diversity in apparent sensitivity to the chronic effects of O_3 is notable, but the issue of dosimetry may be explanatory, in part. It would appear that the rat probably represents no less than the lower limit of response and the monkey the upper limit, if not a direct 1:1 correlate as could be speculated on the basis of relative equivalent lifetime estimates and their phylogenetic relationship.

8.6 Summary and Conclusions

8.6.1 Ozone Dosimetry

There have been significant advances in O_3 dosimetry since publication of the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986) that better enable quantitative extrapolation with marked reductions in uncertainty. Prior to 1986, there were limited data on O_3 uptake in laboratory animals (Yokoyama and Frank, 1972; Miller et al., 1979), essentially no reliable data in humans (Clamann and Bancroft, 1959; Hallett, 1965), only one realistic model of O_3 dose (Miller et al., 1978, 1985), and no data on O_3 reaction kinetics in lung lining fluids. At the present time, data gaps in all of these areas have begun to fill in. Experiments and models describing the uptake efficiency and delivered dose of O_3 in the RT of animals and humans are beginning to present a clearer picture than previously has existed.

The total RT uptake efficiency of rats at rest is approximately 0.50 (Wiester et al., 1987, 1988; Hatch et al., 1989). Data from excised rat lungs support these in vivo findings, and further indicate that O_3 uptake efficiency is chemical reaction dependent (Postlethwait et al., 1994). Of the O_3 taken up by the total RT of the rat, 0.50 is removed in the head, 0.07 in the larynx/trachea, and 0.43 in the lungs (Hatch et al., 1989). The regional uptake efficiency data from the rat have been useful in estimating O_3 mass transfer coefficients for the rat.

Ozone dosimetry models require input of regional mass transfer coefficients. Limited studies have been conducted to quantitate the mass transfer coefficients of lung tissue directly using excised animal tissue. In pig and sheep tracheae, mass transfer coefficients were determined for unidirectional flow conditions and were found to be independent of flow, suggesting a lack of dependence of O_3 uptake on gas-diffusion processes (Ben-Jebria et al., 1991). These findings contrast with Aharonson et al. (1974), who found that the mass transfer coefficient in dog NP increased as a function of increasing flow.

In humans at rest, the total RT uptake efficiency is between 0.80 and 0.95 (Gerrity et al., 1988; Hu et al., 1992b; Wiester et al., 1996). At V_Ts around 500 mL, total RT uptake efficiency falls from about 0.9 to 0.75 as flow increases from 250 to 1,000 mL/s. As V_T increases, uptake efficiency increases and flow dependence lessens, suggesting that, at high V_T, uptake may be gas diffusion limited. At a V_T around 1,500 mL, total RT uptake falls from 0.96 at a flow of 250 mL/s to 0.92 at a flow of 1,000 mL/s. The studies of Gerrity et al. (1988) and Wiester et al. (1996) indicate that the mode of breathing

(oral versus nasal versus oronasal) has little effect on URT or on total RT uptake efficiency. This observation is supported by experiments comparing pulmonary function response as a function of mode of breathing (Adams et al., 1989; Hynes et al., 1988). Kabel et al. (1994), however, found that URT uptake efficiency was lower with mouthpiece breathing as compared with nasal breathing. One possible explanation of the discrepancy among the studies is that a mouthpiece may decrease URT uptake efficiency in comparison with unencumbered breathing. The enhanced physiologic response to O_3 with mouthpiece breathing, shown by Adams et al. (1989), supports this concept.

To obtain data on regional O_3 uptake efficiency in humans, Gerrity et al. (1995) measured O_3 concentrations at various anatomical sites (from the mouth to bronchus intermedius) in spontaneously breathing humans. They found that the unidirectional uptake efficiency of the human trachea was similar to that of the sheep and pig trachea (Ben-Jebria et al., 1991), suggesting a similar mass transfer coefficient behavior in the human trachea. Gerrity et al. (1995) also found that the uptake efficiencies between the mouth and various anatomical sites in the total RT agreed well with the O_3 bolus data of Hu et al. (1992). Both the Hu et al. (1992b) and Gerrity et al. (1995) data indicate that the mass transfer coefficients of the large conducting airways are larger than had been thought previously.

When all of the animal and human in vivo O_3 uptake efficiency data are compared, there is a good degree of consistency across data sets. This agreement raises the level of confidence with which these data sets can be used to support dosimetric model formulations.

In the area of mathematical model formulation, there have been several models developed since 1986. They can be grouped according to how transport and chemical reactions are modeled: instantaneous reactions or quasi-steady, first-order reactions. The models (Overton et al., 1987; Miller et al., 1988; Overton et al., 1989; Hanna et al., 1989; Grotberg et al., 1990) predict that net O_3 dose to lung lining fluid plus tissue gradually decreases distally from the trachea toward the end of the TB, and then rapidly decreases in the pulmonary region. When the theoretical dose of O_3 to lung tissue is computed, it is low in the trachea, increases to a maximum in the terminal bronchioles of the first generation of the pulmonary region, and then decreases rapidly distally into the pulmonary region. The models also provide insight into the role that increased ventilation plays in enhancing O_3-induced responses. The increased V_T and flow, associated with exercise in humans or CO_2-stimulated ventilation increases in rats, shifts O_3 dose further into the periphery of the lung, causing a disproportionate increase in distal lung dose. This prediction is supported by the data of Postlethwait et al. (1994) in excised rat lungs and of Hu et al. (1992b) and Gerrity et al. (1995) in human lungs.

Ozone dosimetry models also have enabled examination of regional dosimetry among parallel and serial anatomical structures. When asymmetric lung morphology is used in dosimetric models, the variation of O_3 dose among anatomically equivalent ventilatory units as a function of path length from the trachea has been predicted to vary as much as sixfold (Overton et al., 1989; Mercer et al., 1991; Mercer and Crapo, 1993); units with the shorter paths are expected to have the greater damage. This could have significant implications for regional or localized damage to lung tissue. Whereas the average lung dose might be at a level that would be considered insignificant, local regions of the lung may receive significantly higher than average doses and therefore be at greater risk for chronic effects.

Theoretical models also have been applied to make predictions about delivered doses from exposure scenarios that are not necessarily achievable experimentally. Overton and Graham (1989) and Miller and Overton (1989) have scaled the human lung dimensions to account for age variations. They predicted that LRT uptake efficiency is not sensitive to age at resting ventilation, but is age dependent when exercise conditions are invoked. The total quantity of O_3 absorbed per minute is predicted to increase with age during heavy work or exercise.

8.6.2 Species Homology and Sensitivity

Examining functional parameters measured analogously in humans and various animal species discloses remarkable similarity in acute O_3-induced effects. The tachypneic response to this oxidant is clearly concentration-dependent in both humans and animals and shows parallel exacerbation when hyperventilation (e.g., exercise or CO_2) is superimposed. Indeed, rodents appear to be slightly more responsive than humans in this regard. What is not known is whether this is evidence of pulmonary irritant sensitivity, perhaps as a prelude to toxicity, or whether tachypnea is a defensive posture taken by the respiratory system to minimize distal lung O_3 deposition. Airway or lung resistance in humans is not affected appreciably by acute exposure to O_3, except under conditions of heavy exercise; animals appear to need high-level exposures or special preparations that bypass nasal scrubbing. Dynamic lung compliance, on the other hand, tends to decrease across species. However, the evidence in both animals and humans is not as strong as one might expect, given the distal lung deposition of this poorly soluble oxidant.

Ozone-induced spirometric changes, the hallmark of response in humans, also occur in exposed rats, although the relative responsiveness of these alterations in the rodent appears to be about half that of the human. It is unclear, however, the degree to which anesthesia (rat) and the comparability of hyperventilation induced by CO_2 (rat) or exercise (human) may influence this difference in responsiveness. Collectively, the acute functional response of laboratory animals to O_3 appears quite homologous to that of the human. Likewise, the studies of BAL constituents indicate that the influx of inflammatory cells and protein from the serum is influenced by species, but perhaps to a less extent than by ventilation and antioxidant status, because adjustment for these factors can modulate responses to approximate animal responses to those of humans. Unfortunately, these influential factors are rarely measured and, even less often, controlled.

When humans are exposed repeatedly for several consecutive days, lung function decrements subside, and normal spirometric parameters are regained. This phenomenon of functional attenuation also has been demonstrated in rats, not only in terms of spirometry, but also in terms of the classic tachypneic ventilatory response. Full or partial attenuation of the BAL parameters also appears to occur in both rats and humans, but exposure scenario appears to play a role; other cellular changes in animals do not attenuate. Existing epidemiologic studies provide only suggestive evidence that persistent or progressive deterioration in lung function is associated with long-term oxidant-pollutant exposure. These long-term effects are thought to be expressed in the form of maximum airflow or spirometric abnormalities, but the foundation for this conclusion remains weak and hypothetical. Animal study data, although suggesting that O_3 has effects on lung function at near-ambient levels, present a variable picture of response that may or may not relate to technical conditions of exposure or some other, yet undiscovered variable of response. Thus, a cogent interpretation

of the animal findings as definitive evidence of chronic deterioration of lung function would be difficult at this time. However, the subtle functional defects apparent after 12 to 18 mo of exposure and the detailed morphometric assessments of the O_3-induced lesions do appear consistent with the modicum of studies focusing on long-term effects in human populations. Based on the apparent homology of these responses between humans and laboratory animals, animal studies provide a means to more directly assess such chronic health concerns.

8.6.3 Quantitative Extrapolation

The agreement between theoretical models of O_3 uptake and experimental determinations of O_3 uptake efficiency now provide a basis on which responses may be examined as a function of delivered O_3 dose instead of O_3 exposure concentration. By examining responses as a function of delivered dose, the goal of quantitative extrapolation between species can be approached.

The use of delivered dose to investigate responses has been examined in two contexts: (1) intraspecies comparisons and (2) interspecies comparisons. With respect to intraspecies comparisons, Miller et al. (1988) assumed that the relevant dose mediating the human pulmonary function response was the pulmonary tissue dose. They then utilized the breathing patterns, exposure concentrations, and pulmonary function responses from the human studies of McDonnell et al. (1983) to predict the dose-response. They found that there was general agreement between the shapes of the concentration-response curves and the dose-response curves and that differences could be accounted for by the translation between exposure concentration and O_3 dose. In another example dealing with intraspecies comparisons, Miller and Conolly (1995) compared the distribution of predicted O_3 tissue dose to a ventilatory unit in a rat as a function of distance from the BADJ, with the distribution of alveolar wall thickening as a function of the same distance measure. Miller and Conolly (1995) found remarkable consistency between the predicted dose distribution and the response distribution (i.e., as predicted delivered dose declined, response declined).

In an attempt to make an interspecies comparison of dose and response, existing or modified models of Miller et al. (1985), Miller et al. (1988), and Overton et al. (1987) were used to predict doses among species for two different types of responses. In the first case, the tachypneic response to O_3 as a function of dose was analyzed. The maximum ratio of O_3-altered f to control f was plotted as a function of the average centriacinar dose over the period from the beginning of exposure to the point of maximum f ratio. Rat and human data were used for this comparison. It was found that, at comparable O_3 doses, the responses of rats greatly exceeded that of humans and were initiated at lower doses. By examining the dose response instead of the concentration response, the difference in tachypneic response between rats and humans is magnified. In another example, an analysis similar to Miller et al. (1988) was performed to examine recovered BAL protein as a function of O_3 dose to the pulmonary region. The species considered were the rat, guinea pig, rabbit, and human. In all cases, the BAL protein response followed a log-linear relationship, suggesting a consistency of response across species. Yet the data from different species tended to cluster together, suggesting species-specific sensitivity factors.

An attempt was made to address quantitatively the question of potential chronic alteration of the lungs of people exposed to O_3 by integrating dosimetry model predictions and biological effects observed in laboratory animals. In the long-term exposure studies selected for analysis, importance was placed on the relevance of exposure concentrations, the

site of specificity of the morphometric analysis, and the consistency of analysis within species. Two rat (F344) studies were selected that represented near-lifetime exposures to O_3 over a range of concentrations and scenarios (Chang et al., 1992, 1995). Two monkey studies were also considered: (1) bonnet monkeys exposed for 90 days (Harkema et al., 1993) and (2) fascicularis monkeys exposed daily for 18 mo or daily every other month for 18 mo (Tyler et al., 1988). The biological effect chosen for extrapolation was the increased thickness of the acellular and total interstitial volumes in the PAR region of the lung; measurements were made in all four of these investigations. The quantity of O_3 predicted to be absorbed per square centimeter of PAR surface area was chosen as the dose.

Generally, the information needed to carry out the dosimetry predictions was not provided by the studies. This required assumptions such as the scaling of ventilation parameters, volumes, and surface areas from one species or strain to another. The assumption that had the greatest impact on the modeling results dealt with the pulmonary region mass transfer coefficient. The value used for this parameter has very little experimental justification and was chosen to be approximately the same for all species (i.e., the values calculated by Miller et al. [1985] for humans and by Overton et al. [1987] for rats). If the value of this coefficient is in fact approximately the same for all the species, the extrapolation of effects is not expected to be affected by the value itself. For the human simulations, a 9-year-old child and an adult outdoor supervisor living in New York City were considered. The activity and exposure patterns for these hypothetical people were generated by an exposure model (Johnson, 1994) for April through October 1991. The laboratory animal dose-response curves showed an apparent linear relationship within species with relatively high correlation coefficients, from 0.80 to 0.98 depending on species and effect. Assuming the relationships depicted in Figure 8-18, 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 laboratory species used for the prediction (the higher range corresponds to the monkey). For the monkeys, there seemed to be little reversal with postexposure to air, which was consistent with the cumulative dose hypothesis. Although the reader should note the number of assumptions that underlie these predictions, this exercise, nevertheless, suggests that long-term O_3 exposure could impart a chronic effect in humans.

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9

Integrative Summary of Ozone Health Effects

9.1 Introduction

Characterization of the health risks associated with pollutant exposure requires an integrative interpretation of the continuum from air quality to exposure to dose to effects, with full consideration of the actual exposure and susceptibility of different subgroups in the population. The currently published information on population exposure to ozone (O_3) (see Chapter 4) is superseded by recent analyses of the U.S. Environmental Protection Agency (EPA) in a staff paper prepared by the Office of Air Quality Planning and Standards (U.S. Environmental Protection Agency, 1996). Thus, the staff paper contains the risk assessment for O_3.

This chapter characterizes the hazard and dose-response components of risk assessment by integrating the animal toxicological, human clinical and epidemiological, and extrapolation studies of O_3 that were discussed in Chapters 6, 7, and 8, respectively, of this criteria document. Because each of these approaches has different strengths and weaknesses, they were evaluated separately in the respective base chapters; however, a combined evaluation can better describe the full array of effects that are known to occur with exposure to O_3.

The chapter begins with an overview of the relationship between exposure and dose, as this lays a foundation for inter- and intraspecies extrapolation. The chapter then is organized according to biological outcomes, beginning with the effects of short- and long-term exposures of O_3 alone or in ambient air and ending with experimental exposures to binary mixtures with O_3. The section on short-term exposures (i.e., <8 h) presents descriptively symptoms and effects on lung function, exacerbation of existing disease, and cellular-biochemical responses. Quantitative exposure-response relationships for the effects of O_3 on pulmonary function (e.g., changes in lung volume) are summarized separately because the large number of studies allows more complex evaluation and modeling. For the other classes of effects, the more limited exposure-response information is integrated with the description of the effects. The section on long-term exposures encompasses repeated exposures (i.e., 1 to 5 days), prolonged exposures (i.e., months), and genotoxicity and carcinogenicity. Because the available database on binary exposure studies has little predictive value of effects in populations exposed to complex pollutant mixtures, the emphasis is placed on the principles of interaction. The conclusions section is organized according to key questions about the health consequences of O_3 exposure and the population groups that are most likely to be affected.

Because this chapter integrates the results of a large number of studies from the current and earlier O_3 criteria documents (U.S. Environmental Protection Agency, 1978, 1986), it is not practical to provide experimental details or cite specific references. Rather, emphasis is given to main findings that are supported by published, confirmatory studies, unless noted otherwise. Comprehensive details and references are provided in the base chapters (Chapters 6 through 8). A discussion of selective, key references can be found in the summary and conclusion sections of those chapters.

9.2 Exposure-Dose Relationships

Qualitative and quantitative health assessments require, among other things, the ability to relate exposure to dose and dose to effect. In the case of O_3 health assessment, this ability is necessary for two major reasons: (1) to develop unified predictive models of human population responses based on exposure, and (2) to enable extrapolation from animals to humans for chronic effects. Physically and biologically based models of dose simplify the methods of predicting population responses and, in turn, significantly reduce the uncertainty of these predictions. For animal-to-human extrapolations, splitting the problem of exposure and response into an exposure-dose problem and a dose-response problem separates the issue of interspecies sensitivity from purely dosimetric considerations. Responses in animals may be homologous with responses in humans but follow different dose-response curves. By measuring or computing delivered O_3 dose to relevant tissues in animals and humans, transfer functions can, in principle, be developed relating dose-response curves among different species, assuming tissues from different species react in identical fashion. This section discusses the understanding of exposure-dose relationships and how they improve the ability to interpret and predict O_3 responses.

Historically, the first step beyond describing responses solely in terms of exposure concentration was the use of the product of concentration $\times$ time $\times$ minute ventilation ($C \times T \times \dot{V}_E$), yielding what often has been referred to as an "effective dose". Response modeling has examined the interaction of individual pairs of variables. However, no single model has been able to simply unify any response in terms of the product of $C \times T \times \dot{V}_E$. This is due to the fact that $C \times T \times \dot{V}_E$ is a metric of exposure dose rather than delivered dose and, furthermore, does not account for the mediation of responses in localized regions of the lung that would be responding to local O_3 doses. Advances in O_3 dosimetry modeling and experimental determinations of regional O_3 dose in animals and humans have enabled extensions beyond simple $C \times T \times \dot{V}_E$ modeling to interpret responses.

Ozone dosimetry models provide predictions of the dose distribution of O_3 in the respiratory tract from the trachea to the alveolar spaces of the lung. These models utilize the best available anatomical, physiological, and biochemical data available for animals and humans. These data are incorporated into mathematical formulations of convection, diffusion, and chemical reaction processes in the lung. The models predict that, under resting ventilatory conditions, the O_3 dose per airway generation to all respiratory tract constituents (tissue plus fluid) slowly decreases from the trachea to the terminal and respiratory bronchioles and then declines in the alveolated generations. When dose of O_3 to tissue alone is considered (accounting for reaction and diffusion kinetics in the liquid lining layer), there is a three order of magnitude increase in tissue dose from the trachea to the proximal alveolar regions (PARs), after which the tissue and total dose are virtually equal

and fall rapidly in the alveolated generations. Currently, relationships between delivered regional dose and response are derived assuming that O_3 is the active agent directly responsible for effects; however, there is uncertainty as to the correctness of this assumption. Reactive intermediates, such as peroxides and aldehydes formed when O_3 interacts with constituents of lung lining fluid, may be the agents mediating responses. Thus, the dose of the reactive intermediates may be relevant. Even in the presence of uncertainty over the relevant dose agent, the histopathological findings from chronic O_3 exposures in animals match the predicted distribution of O_3 dose (i.e., the sites of the highest predicted O_3 doses correspond with those regions of the lung with the greatest tissue alterations).

Experimental studies in humans have revealed some important features needed for health assessment. Among these is the observation that the dose of O_3 delivered to the lower respiratory tract is independent of the mode of breathing (i.e., oral versus nasal versus oronasal). This observation simplifies health assessment by eliminating the need for precise information on modes of breathing when considering population responses. Experimental studies in humans also have shown that increasing $\dot{V}_E$ with exercise (increasing both breathing frequency and tidal volume) causes only a small decrease in O_3 uptake efficiency by the total respiratory tract. Based on models of O_3 dose, it appears that the increased $\dot{V}_E$ in exercise, although having little effect on uptake efficiency by the total respiratory tract, causes the distribution of delivered O_3 dose to shift deeper into the respiratory tract. The shift in O_3 dose as a function of $\dot{V}_E$ could help explain the complex relationships seen between response and C, T, and $\dot{V}_E$. An important observation from the human experimental dosimetry studies is the general agreement between O_3 dosimetry models and the measured data.

Experiments in laboratory animals (particularly rats) have been valuable in providing, in conjunction with human experimental data and mathematical dosimetry models, the basis for dosimetric extrapolation. Whereas the human total respiratory tract has an O_3 uptake efficiency between 70 and 100%, the respiratory tract of the rat takes up only about 50% of the inhaled O_3. Unlike the case with humans, the dosimetry models overestimate the uptake efficiency of the rat respiratory tract by approximately 25 to 50% (i.e., the predicted uptake efficiency is between 65 and 75%), but the models are still highly valuable for extrapolation purposes. An important finding has been that the models correctly relate the regional dose of O_3 to the increase in alveolar wall thickness, both of which decline with distance from the junction of the conducting airways and the alveolar regions of the lung.

Experimental O_3 dosimetry and predictive O_3 dosimetry models are informative about the feasibility of extrapolating animal responses to humans. Some acute responses to O_3 can be compared across species on a strict dose-response basis. For example, both animals and humans respond to O_3 in a dose-dependent manner by increasing breathing frequency and decreasing tidal volume (tachypnea). A qualitative comparison between rat and human tachypneic responses at a variety of O_3 concentrations and exercise levels indicates that when exercising, rats and humans have a similar response, but rats are somewhat more responsive at rest. However, when dose to the proximal alveolar region of the lung (normalized to body weight) is considered as the dose metric for tachypneic responses, rats appear to be much more responsive than humans. Another example is influx of protein into the alveolar spaces following O_3 exposure as measured in bronchoalveolar lavage (BAL) fluid. When BAL protein is plotted as a function of pulmonary tissue dose, the rat, guinea pig, rabbit, and human all respond with a similar dose-response pattern,

suggesting a common mechanism of response. However, each curve is offset from the other, reflecting overall sensitivity differences among the species, with the human and guinea pig being more responsive than the rat and rabbit.

Available data on chronic responses to O_3 are considerably more difficult to compare across species. Specific assumptions are required to model exposure-dose relationships. For example, allometric anatomic adjustments provide estimates of unavailable dosimetric data for nonhuman primates, and allometric equivalent life-span estimates better relate the duration of exposure to life-span and cumulative dose. Studies of long-term exposure in monkeys and rats show a near-linear dose-response pattern when alveolar interstitial thickness was related to cumulative dose estimates for the PAR of the lung. Analogous estimates of PAR dose in humans predict similar increases in interstitial thickness at the PAR, with the monkey being more responsive, and the rat less responsive.

9.3 Effects of Short-Term Ozone Exposures

9.3.1 Physiological Responses to Ozone Exposure

Typical acute physiological responses to O_3 exposure observed in both human clinical and field studies include a reduction in forced vital capacity (FVC), decreased expiratory flow rates, and increased respiratory symptoms. The most common symptoms include cough, airway irritation, and chest discomfort associated with deep inhalation. These responses are often accompanied by increased airway resistance and tachypnea. The voluntary spirometry and symptom responses cannot be elicited from animals, but their tachypneic response is well documented. Ozone exposure also increases airway responsiveness to nonallergenic airway stimuli (e.g., histamine) in humans and animals. There is a large range of physiological responses among humans, with at least a 10-fold difference between the most and least responsive individuals.

9.3.1.1 Respiratory Symptom Responses

An association between O_3 exposure and the presence of symptoms has been shown in human clinical, field, and epidemiological studies. Prevalent symptoms include cough, irritation of the airways (described as a scratchy throat or discomfort under the sternum), and discomfort when taking a deep breath (described as chest tightness or pain in the chest). Eye irritation, sometimes reported as a symptom in field or epidemiological studies with exposure to oxidant mixtures including peroxyacyl nitrates, is not associated with exposure to O_3 alone. The most prevalent respiratory symptoms have a much higher incidence in young adults than in older adults and generally are not reported in children or adolescents. Asthmatics have symptoms similar to nonasthmatics but also report a higher incidence of wheezing. The receptors responsible for cough may be unmyelinated C-fibers or rapidly adapting receptors located in the larynx and the largest conducting airways. Thus, there appears to be a potential mechanistic linkage between coughing and changes in spirometry. Field and epidemiological studies also indicate an association between hourly or daily ambient O_3 levels and the presence of respiratory symptoms, particularly cough. Such associations may be most evident in asthmatic children. Although symptoms cannot be elicited from animals, indirect measures of symptom responses in animals include behavioral responses (e.g., decreased wheel-running activity, decreased activity associated with obtaining food) indicative of aversion to O_3 exposure.

Symptom responses to O_3 exposure follow a monotonic exposure-response relationship that has a similar form to that for spirometry responses. Increasing exposure levels elicit increasingly more severe symptoms that persist for longer periods. Symptom and spirometry responses follow a similar time course during an acute exposure and the subsequent recovery, as well as over the course of several days in a repeated exposure study. Furthermore, medication interventions that block or reduce spirometry responses have a similar effect on symptom responses. Levels at which symptoms occur under various exposure conditions are discussed in Section 9.3.4.2. As with spirometry responses, symptom responses vary considerably among subjects, although the individual correlations between spirometry and symptom responses are relatively low. Ozone induces interference with exercise performance, either by reducing maximal sustainable levels of activity or by reducing the duration of activity that can be tolerated at a particular work level; this is likely related to symptoms. In several heavy or severe exercise studies of athletes exposed to O_3, the discomfort associated with the respiratory symptoms caused by O_3 concentrations in excess of 0.18 ppm was of sufficient severity that the athletes reported that they would have been unable to perform maximally if the conditions of the exposure were present during athletic competition. In workers or active people exposed to ambient O_3, respiratory symptoms may cause reduced productivity or may curb the desire to pursue certain leisure activities.

9.3.1.2 Lung Function Responses

Epidemiological, field, and chamber studies have demonstrated that acute exposure to O_3 decreases FVC and forced expiratory volume in 1 s (FEV_1). In humans, O_3 exposure reduces FVC primarily by decreasing inspiratory capacity. This is believed to be the result of neurogenic inhibition of maximal inspiration, possibly caused by stimulation of C-fiber afferents, either directly or from O_3-induced products of inflammation. C-fibers are also thought to be the receptors responsible for the cough reflex in humans. After exposure to O_3, coughing frequently is elicited during the deep inspiration prior to the forced expiratory maneuver used in dynamic spirometry tests such as FVC, FEV_1, and forced expiratory flow at 25 to 75% of FVC. The observation that nonsteroidal anti-inflammatory drugs (e.g., indomethacin, ibuprofen) reduce or block spirometric responses to O_3 exposure and reduce levels of prostaglandin E_2 (PGE_2) within the lung suggests that mediators released by damaged epithelial cells and alveolar macrophages may play a role in the inhibition of maximal inspiration. Although it seems clear that the reduction in total lung capacity is not attributable to reduced static compliance (i.e., a stiffer lung) or inspiratory muscle weakness, other mechanisms may be involved. Increased interstitial fluid in patients with heart disease causes a decrease in vital capacity and frequency-dependent decreases in lung compliance. The O_3-induced tachypneic response, seen in many animal species and in exercising humans, may be related to the decrease in vital capacity. In humans, the pulmonary reflexes that inhibit maximal inspiration may also limit tidal volume during exercise, which leads to a compensatory tachypnea. The tachypneic response in humans may not be entirely involuntary because it has been reported that O_3-exposed subjects may consciously modify their breathing pattern to relieve discomfort.

The time course of the spirometry responses to O_3 exposure depends on the exposure conditions. At low levels of exposure (e.g., light exercise and O_3 concentration <0.18 ppm), responses are induced slowly and progressively and they may or may not reach a plateau of response, depending on the duration of the exposure. At higher levels of

exposure (e.g., very heavy exercise and O_3 concentration >0.25 ppm), responses occur rapidly (within 15 min), and the largest portion of the response tends to occur early in exposure, indicative of a plateau of response that typically is not achieved because of termination of the exposure within 1 to 2 h. The quantitative exposure-response relationships are discussed more extensively in Section 9.3.4.

In both chamber and field studies, the responses of healthy children to acute O_3 exposure are similar to those seen in adults. Responses of children and adolescents exposed to ambient O_3 (and other copollutants) at summer camps in at least six different locations in the northeastern United States, southeastern Canada, and Southern California indicate changes in spirometry similar to those found in individuals exposed to O_3 under controlled experimental conditions. There is a substantial range of response among individuals in camp studies and between various locations; however, the average FEV_1 was lower when ambient O_3 was higher. Although direct comparisons cannot be made because of incompatible differences in experimental design and analytical approach, this range of response is comparable to the range of response seen in chamber studies at low O_3 concentrations. In the "camp studies", a key measurement is the slope of the relationship between FEV_1 and the measured O_3 concentration during the previous hour, without consideration of the background O_3 levels (even though exposures occurred over multiple hours). The average slope from six studies was −0.50 mL/ppb within an O_3 concentration range of 0.01 to 0.16 ppm (see Chapter 7). For an exposure to 0.12 ppm O_3, this corresponds to a decrease in FEV_1 of 60 mL from a base level of approximately 2,000 to 2,500 mL, or a 2.4 to 3.0% decrease in FEV_1. This is comparable to the findings of McDonnell et al. (1985) for 8- to 11-year-old boys who experienced a 3.4% decrease in FEV_1 after being exposed to 0.12 ppm O_3 for 2 h. Recent studies in adults performing outdoor exercise also have shown an association between decreased spirometric responses and increased ambient O_3 levels.

A consistent finding across many animal species is that O_3 causes rapid shallow breathing (O_3-induced tachypnea), which, in humans, may be related to a sensation of discomfort associated with taking large tidal breaths. Of particular interest for comparing interspecies responses is that the responses of rats and guinea pigs fall within the same range as seen for humans from rest to heavy exercise.

Common pulmonary function tests do not measure changes in the small airways of the centriacinar region of the lung (that segment between the last conducting airway and the gas exchange region), which is highly susceptible to damage by O_3 and is the site of epithelial cell necrosis and remodeling of respiratory bronchioles. Numerous pulmonary function tests reputed to measure responses in small airways (e.g., closing volume, aerosol bolus) have been used in O_3 studies. Responses have been demonstrated, but it is not clear that these tests correlate with the morphological lesion observed in the small airways of experimental animals (see Section 9.4), which is predicted to occur in humans but has not been confirmed reliably through comparable morphologic data from humans residing in O_3-polluted areas. Even if small airways disease is demonstrated in humans, there is as yet no compelling evidence that it will progress to chronic lung disease.

An increase in airway resistance is an indication of the response of large airways to O_3 exposure and is mechanistically different from lung volume responses. Also, higher O_3 concentrations are required to change airway resistance compared to FEV_1. Changes in specific airway resistance (SR_{aw}) of healthy subjects following O_3 exposure are small relative to those seen in asthmatics with an inhalation exposure to a bronchoconstricting drug

(methacholine), a specific antigen, or sulfur dioxide (SO_2). In rats exposed to O_3, changes in resistance also tend to be small. The observation that changes in airway resistance are modest clearly indicates that reductions in maximum expiratory flow are not caused primarily by narrowing of large airways. The increase in airway resistance appears to be vagally mediated because it is sensitive to inhibition by atropine.

9.3.1.3 Changes in Airway Responsiveness

Ozone exposure also causes increased responsiveness of the pulmonary airways to subsequent challenge with bronchoconstrictor drugs such as histamine or methacholine. This phenomenon is seen even after recovery from spirometric changes, but it typically is no longer present after 24 h. Although changes in airway responsiveness tend to resolve somewhat more slowly and appear to be less likely to be attenuated with repeated exposure, the evidence for a persistent increase in responsiveness from animal studies is inconsistent. Changes in airway responsiveness in rats and guinea pigs tend to occur at higher O_3 concentrations and, as in humans, tend to be most pronounced shortly after the exposure and less so 24 h postexposure. Changes in airway responsiveness appear to occur independently of changes in pulmonary function. This response may not be due to the presence of polymorphonuclear leukocytes (PMNs) in the airway or to the release of arachidonic acid metabolites, but could possibly be due to epithelial damage and the consequent increased access of these chemicals to smooth muscle in the airways or to the receptors in the airways responsible for reflex bronchoconstriction. The clinical relevance of this observation is that, after O_3 exposure, human airways may be more susceptible to a variety of stimuli, including antigens, chemicals, and particles. One animal study has demonstrated decreased antigen-induced bronchoconstriction after O_3 exposure, and a human study in allergic asthmatics is suggestive of an increase in such a response. An increased response to inhalation of a specific antigen to which a human is sensitized is a plausible outcome of O_3 exposure. However, ongoing studies of this phenomenon will need to be evaluated in order to determine the exposure-response relationship for alterations in responses to inhaled antigens, especially with regard to sensitive asthmatics. Enhanced response to antigens in asthmatics could lead to increased morbidity (i.e., medical treatment, emergency room visits, hospital admissions) or to more persistent alterations in airway responsiveness.

9.3.2 Exacerbation of Respiratory Disease

People with preexisting pulmonary disease may be at increased risk from O_3 exposure. Because of their existing functional limitations, any further decrease in function would lead to a greater overall functional decline. Furthermore, some individuals with pulmonary disease may have an inherently greater sensitivity to O_3. Asthmatics, by definition, have inherently greater bronchial responsiveness, but, depending on the severity of their disease and its clinical status, their FEV_1 can be within the normal range ($100 \pm 20\%$ predicted) or may be less than 50% predicted. Patients with chronic obstructive pulmonary disease (COPD) can have FEVs ranging from 30 to 80% of predicted, again depending on disease severity. Because of their depressed functional state, small absolute changes in lung function have a larger relative impact. For example, a 500-mL FEV_1 decrease in a healthy young man with an FEV_1 of 4,000 mL causes only a 12% decline. In a 55-year-old COPD patient with an FEV_1 that is 50% of predicted, or about 1,670 mL, a 500-mL decline in FEV_1 would result in a 30% decline in FEV_1. Asthmatics

with depressed baseline function would have similarly magnified relative responses and, because of increased bronchial responsiveness, may also experience larger changes in airway resistance. Evaluating the intersection of risk factors and exposures is more complex. For example, an individual with more severe lung disease is unable to exercise heavily and thus would be less likely to encounter an effective exposure.

About 10 million people in the United States (4% of the population) are estimated to have asthma (National Institutes of Health, 1991). The prevalence is higher among African Americans, older (8- to 11-year-old) children, and urban residents (Schwartz et al., 1990). Death due to asthma is an infrequent event; on an annual basis, about one death occurs per 10,000 asthmatic individuals. Mortality rates are higher among males and are at least 100% higher among nonwhites. In two large urban centers (New York and Chicago), mortality rates from asthma among nonwhites may exceed the city average by up to fivefold (Sly, 1988; Evans et al., 1987; National Institutes of Health, 1991; Weiss and Wagener, 1990; Carr et al., 1992). Although some innercity areas may have lower O_3 concentrations than some suburban areas, the concentrations are much higher than those in most rural areas. The impact of ambient O_3 on asthma morbidity and mortality in this apparently susceptible population is not well understood. The few epidemiological studies are subject to confounding factors and have rarely focused on innercity nonwhite asthmatics. Furthermore, controlled human exposure studies of asthmatics typically include mild to moderate asthmatics and have not dealt specifically with nonwhite asthmatics.

A number of epidemiological studies have shown a consistent relationship between ambient oxidant exposure and acute respiratory morbidity in the population. Small decreases in forced expiratory volumes and increased respiratory symptoms, including exacerbation of asthma, occur with increasing ambient O_3, especially in children. Modifying factors, such as ambient temperature, aeroallergens, and other copollutants (e.g., particles) also can contribute to this relationship. Ozone air pollution can account for a portion of summertime hospital admissions and emergency department visits for respiratory causes; studies conducted in various locations in the eastern United States and Canada consistently have shown a relationship with increased incidence of visits and admissions, even after controlling for modifying factors, as well as when considering only concentrations <0.12 ppm O_3. It has been estimated from these studies that O_3 may account for roughly one to three excess summertime respiratory hospital admissions per hundred parts per billion O_3, per million persons.

The association between elevated ambient O_3 concentrations during the summer months and increased hospital visits and admissions has a plausible biologic basis in the physiologic, symptomatic, and field study evidence discussed earlier. Specifically, increased airway resistance, airway permeability, and incidence of asthma attacks and airway inflammation suggest that ambient O_3 exposure could be a cause of the increased hospital admissions, particularly for asthmatics.

The associations found in the epidemiological studies are supported by chamber studies. Asthmatics and nonasthmatics have qualitatively similar responses to chamber O_3 exposures. Although symptom and volume-related responses (i.e., decreased FVC) tend to be similar, airway resistance increases relatively more, from an already higher baseline, in asthmatics exposed to O_3. Ozone-induced alterations in responsiveness to bronchoconstrictor drugs show similar changes in asthmatics and nonasthmatics. There is no evidence at this time that O_3 induces a persistent increase in airway responsiveness or that O_3-exposed asthmatics are more likely to have a late-phase response to specific antigen challenge.

Symptom responses also have been reported in asthmatics exposed to O_3. In contrast to nonasthmatics, wheezing, a typical finding in asthma, is a prevalent symptom in addition to the cough, chest tightness, and shortness of breath that are reported by subjects without asthma.

9.3.3 Morphological and Biochemical Abnormalities

9.3.3.1 Inflammation and Cell Damage

Ozone-induced cell injury may lead to effects including inflammation, altered permeability of the epithelial barrier, impaired host defense and particle clearance, irreversible structural alterations in the lung, exacerbation of preexisting disease (e.g., asthma), and increased sensitivity to biocontaminants (e.g., allergens). Of these, O_3-induced inflammation of the respiratory tract has been best documented and occurs in all species that have been studied. The mechanisms leading to the observed inflammatory responses induced by O_3 are just beginning to be understood. Both animal morphological studies and in vitro studies indicate that airway ciliated epithelial cells and Type 1 cells are the most O_3-sensitive cells and are initial targets of O_3. These cells are damaged by O_3 and produce a number of proinflammatory mediators (e.g., interleukins [IL-6, IL-8], PGE_2) capable of initiating a cascade of events leading to PMN influx into the lung, activation of alveolar macrophages, inflammation, and increased permeability across the epithelial barrier.

Ozone-Induced Inflammation

Ozone causes inflammatory changes throughout the respiratory tract, including the nose. Humans and laboratory animals exposed to O_3 develop inflammation and increased permeability in the nasal passages. A recent study reported a positive correlation between nasal inflammation in children and measured ambient O_3 concentrations. Studies with rats suggest a potential competing mechanism between the nose and lung, with inflammation occurring preferentially in the nose at low O_3 concentrations and shifting to the lung at higher concentrations. It is unclear if this represents a specialization restricted to rats or is a more general phenomenon.

In general, inflammation can be considered as the host response to injury and the induction of inflammation as evidence that injury has occurred. Inflammation induced by exposure of humans to O_3 can have several potential outcomes: (1) inflammation induced by a single exposure (or several exposures over the course of a summer) can resolve entirely; (2) continued acute inflammation can evolve into a chronic inflammatory state; (3) continued inflammation can alter the structure and function of other pulmonary tissue, leading to diseases such as fibrosis; (4) inflammation can alter the body's host defense response to inhaled microorganisms, particularly in potentially vulnerable populations such as the very young and old; and (5) inflammation can alter the lung's response to other agents such as allergens or toxins. Except for outcome (1), the possible chronic responses have not been identified with inflammation induced by exposure of humans to O_3. It is also possible that the profile of response can be altered in persons with preexisting pulmonary disease (e.g., asthma, COPD) or smokers.

The recent use of BAL as a research tool in humans has afforded the opportunity to sample cells and fluid from the lung and lower airways of humans exposed to O_3 and to ascertain the extent and course of inflammation and its constitutive elements. Several studies have shown that humans exposed acutely (1 to 3 h) to 0.2 to 0.6 ppm O_3 had O_3-induced

inflammation, cell damage, and altered permeability of epithelial cells lining the respiratory tract (allowing components from plasma to enter the lung). For individuals acutely exposed to 0.4 to 0.6 ppm O_3, PMNs (the hallmark cells of inflammation) make up 8 to 10% of the recovered BAL cells. This represents a five- to eightfold increase in PMNs compared with similar individuals exposed to clean air, who typically have 1 to 2% PMNs in their BAL fluid. The lowest concentration of O_3 tested, 0.08 ppm for 6.6 h with moderate exercise, also induced small but statistically significant increases in a number of inflammatory mediators, including PMNs.

The percent of PMNs in BAL fluid taken from individuals exposed to 0.4 ppm O_3 for 2 h equals or exceeds those found in individuals exposed to other environmental toxicants, such as asbestos or silica, or in individuals with idiopathic pulmonary fibrosis (IPF) or connective tissue disorders (CTD) (Cherniak et al., 1990). For example, individuals with a history of occupational exposure to asbestos (>10 years) have 3.3 ± 1.3% BAL PMNs, and individuals with a history of occupational exposure to silica (>2 years) have 1.4 ± 0.4% PMNs. Untreated patients newly diagnosed with IPF have 6.7 ± 2.5% PMNs, whereas those with CTD have 16 ± 11.6% PMNs. Baseline levels of PMNs in patients with asthma do not differ significantly from healthy individuals, although PMN levels can increase following allergen bronchoprovocation (Smith and Deshazo, 1993). In contrast, PMNs can make up as much as 80% of BAL cells in patients with acute bacterial infections (Stanley, 1991).

Short-term (<8 h) exposure of animals to O_3 also results in cell damage, inflammation, and altered permeability, although, in general, higher O_3 concentrations are required to elicit a response equivalent to that of humans. Because humans were exposed to O_3 while exercising and most animal studies were done at rest, differences in ventilation likely play a significant role in the different response of humans and rodents to the same O_3 concentration. Studies in which animals were exposed at night (during their active period) or in which ventilation was increased with CO_2 tend to support this idea.

Studies utilizing BAL techniques sample only free or loosely adherent cells in the lung; thus, it is possible that cellular changes have occurred in the interstitium that are not reflected in BAL studies, or that BAL changes exist in the absence of interstitial changes. However, morphometric analyses of inflammatory cells present in lung and airway tissue sections of animals exposed to O_3 are in general agreement with BAL studies. Short-term O_3 exposure (<8 h) causes similar types of alterations in lung morphology in all laboratory animal species studied. The most affected cells are the ciliated epithelial cells of the airways and Type 1 cells in the alveolar region. The centriacinar region (the junction of the conducting airways and gas exchange region) is a primary target, possibly because it receives the greatest dose of O_3 delivered to the lower respiratory tract. Sloughing of ciliated epithelial and Type 1 cells occurs within 2 to 4 h of exposure of rats to 0.5 ppm O_3.

Time Course of Ozone-Induced Inflammatory Response

Findings from human and animal studies agree that the O_3-induced inflammatory response occurs rapidly and persists for at least 24 h. Increased levels of PMNs and protein are observed in the BAL fluid within 1 h following a 2-h exposure of humans to O_3 and continue for at least 20 h. The kinetics of response during this time have not been well studied in humans, although a single study shows that PMN levels are higher at 6 h postexposure than at 1 or 20 h in different individuals. Several animal studies suggest that BAL PMN and protein levels peak 12 to 16 h after an acute O_3 exposure and begin to

decline by 24 h, although some studies report detectable BAL PMNs even 36 h after exposure. It is also clear that in humans the pattern of response differs for different inflammatory mediators. Mediators of acute inflammation, such as IL-6 and PGE_2, are more elevated immediately after exposure; whereas mediators that potentially could play a role in resolving inflammation, such as fibronectin and plasminogen activator, are preferentially elevated 18 h after exposure. The rapidity with which cellular and biochemical mediators are induced by O_3 makes it conceivable that some of them may play a role in O_3-induced changes in lung function—indeed there is some evidence that BAL PGE_2 levels are correlated with decrements in FEV_1, and anti-inflammatory medications that block PGE_2 production also reduce or block the spirometric responses to O_3. Although earlier studies suggested that O_3-induced PMN influx might contribute to the observed increase in airway hyperreactivity, animal studies show that when PMNs are prevented from entering the lung, O_3-induced hyperreactivity or increases in many inflammatory mediators still occur. In addition, studies in which anti-inflammatory drugs are used to block O_3-induced lung function decrements still show increases in PMNs and most other inflammatory mediators (although PGE_2 is not increased).

Individuals and Populations Susceptible to Ozone

To date, there have been no studies that have examined the cellular/biochemical response to O_3 of potentially susceptible subpopulations, such as asthmatics, nor are there any data in humans addressing whether age, gender, or racial differences can modify the inflammatory response to O_3. Increased susceptibility of asthmatics or chronic bronchitics could be hypothesized on the basis that they have an underlying inflammatory disease that may be exacerbated with an otherwise small magnitude of change. Inflammation is not induced to the same extent in all individuals. In moderately exercising humans exposed to 0.08 ppm O_3 for 6.6 h, the mean changes in inflammatory indices were low, but some individuals had increases comparable to those reported in heavily exercising subjects exposed to 0.4 ppm O_3 for 2 h, suggesting that some segments of the population may be more responsive to low levels of O_3. It has not yet been studied whether intersubject differences in inflammatory response to O_3 are reproducible over time for the same subject, as has been shown for intersubject differences in lung function. There seems to be no strong correlation between the various mediators of inflammation, cell damage, and permeability (i.e., those individuals with the greatest PMN response are not necessarily those with the greatest BAL protein, PGE_2, or IL-6 response). Furthermore, the magnitude of lung function decrements and respiratory symptoms has not yet been shown to be correlated with mediators of inflammation, with the possible exception of PGE_2.

Animal studies also show large interspecies and interstrain differences in response to O_3 and suggest that genetic factors may play a role in susceptibility to O_3. Different rat strains respond to O_3 differently; for example, Wistar rats have the greatest PMN influx, whereas Fischer rats demonstrate the most epithelial cell damage. In addition, limited data suggest that dietary antioxidant levels may affect the response of rodents to O_3 and that very young rats produce more PGE_2 in response to O_3 than do older rats. Taken as a whole, the human and animal studies suggest that the inflammatory response to O_3 is complex and that determinants of susceptibility may occur at several different genetic loci.

9.3.3.2 Host Defense

The mammalian respiratory tract has a number of closely integrated defense mechanisms that, when functioning normally, provide protection from the adverse effects of a wide variety of inhaled particles and microbes. Impaired mucociliary clearance can result in unwanted accumulation of cellular secretions and increased numbers of particles and microorganisms in the lung, leading to increased infections and bronchitis.

Mucociliary Clearance of Inhaled Particles

Animal studies show that clearance of inhaled insoluble particles is slowed after acute exposure to O_3. Ozone-induced damage to cilia and increased mucus secretion likely contribute to a slowing of mucociliary transport rates. Interestingly, retarded mucociliary clearance is not observed in animals exposed repeatedly to O_3. The effects of O_3 on mucociliary clearance in humans have not been well studied, and the results are somewhat conflicting; one study reports an O_3-induced increase in particle clearance in subjects exposed to 0.4 ppm O_3 for 2 h, and another study reports no O_3-induced change in particle clearance with a similar exposure regimen.

Alveolar Macrophage Function

Macrophages represent the first line of defense against inhaled microorganisms and particles that reach the lower airways and alveoli. Studies in both humans and animals have shown that there is an immediate decrease in the number of BAL macrophages following O_3 exposure. Alveolar macrophages also have been shown to be crucial to the clearance of certain gram-positive bacteria from the lung. Several studies in both humans and laboratory animals also have shown that O_3 impairs the phagocytic capacity of alveolar macrophages, and some studies suggest that mice may be more impaired than rats. The production of superoxide anion (an oxygen radical used in bacterial killing) by alveolar macrophages also is depressed in both humans and animals exposed to O_3, and the ability of alveolar macrophages to kill bacteria directly is impaired. Decrements in alveolar macrophage function have been observed in moderately exercising humans exposed to the lowest concentration tested, 0.08 ppm O_3 for 6.6 h.

Interaction with Infectious Agents

Concern about the effect of O_3 on susceptibility to respiratory infection derives primarily from animal studies in which O_3-exposed mice die following a subsequent challenge with aerosolized bacteria. Increased mortality of experimental laboratory animals has been shown to be concentration-dependent, and exposure to as little as 0.08 ppm O_3 for 3 h can increase mortality of mice to a subsequent challenge with streptococcus bacteria. In addition, younger mice are more susceptible to infection than older mice; this has been related to increased PGE_2 production in these animals, which likely decreases alveolar macrophage activity.

It has been suggested that impaired alveolar macrophage function is the mechanism likely responsible for enhanced susceptibility to bacteria. However, mortality is not observed with other rodent species, raising the question of whether this phenomenon is restricted to mice. Although both mice and rats show impaired macrophage killing of inhaled bacteria following O_3 exposure, rats mount a faster PMN response to O_3 to compensate for the deficit in alveolar macrophage function. The slower clearance time in mice allows the streptococcus strain to persist in lung tissue and, subsequently, to elaborate a

number of virulence factors that evade secondary host defense and lead to bacterial multiplication and death of the host. Although increased mortality in laboratory animals is not directly relevant to humans, laboratory animals and humans share many host defense mechanisms being measured by mortality in the mouse model. Thus, the category of effect (i.e., decrement in antibacterial defenses) can be qualitatively extrapolated to humans.

There is no compelling evidence from animal toxicological, human clinical, or epidemiological studies that O_3 increases the incidence of respiratory viral infection in humans. A study of experimental rhinovirus infection in susceptible volunteers failed to show any effect of 5 consecutive days of O_3 exposure (0.3 ppm, 8 h/day) on the clinical picture or on host response. Studies in which O_3-exposed mice were challenged with influenza virus have conflicting results: some studies show increased mortality, some show decreased mortality, and still others show no change at all. However, even when increased mortality was demonstrated, there was no difference in viral titers in the lung, suggesting virus-specific immune functions were not altered. One animal study found that, although subchronic O_3 exposure did not affect the acute course of a viral infection, it did enhance postinfluenzal alveolitis.

Taken as a whole, the data clearly indicate that an acute O_3 exposure impairs the host defense capability of both humans and animals, primarily by depressing alveolar macrophage function and perhaps also by decreasing mucociliary clearance of inhaled particles and microorganisms. This suggests that humans exposed to O_3 could be predisposed to bacterial infections in the lower respiratory tract. The seriousness of such infections may depend on how quickly bacteria develop virulence factors and how rapidly PMNs are mobilized to compensate for the deficit in alveolar macrophage function.

Ozone also has been reported to suppress natural killer cell activity in the lung, to suppress proliferative responses to bacterial antigen (Listeria) in both spleen and bronchial lymph nodes, and to induce delayed hypersensitivity responses to Listeria antigen. However, these effects occur at higher exposure levels (0.75 to 1.0 ppm O_3) than those that affect macrophage function.

9.3.4 Quantitative Ozone Exposure-Response Relationships

A quantitative understanding of the relationship between O_3 exposure and subsequent response is useful both for a better understanding of the processes underlying outcomes of interest and for purposes of prediction. Examples of the utility of the latter include identification of exposures unlikely to produce effects, risk and benefits assessment, and prediction of responses based on exposures for which empirical data do not exist. In general, exposure-response relationships have been better characterized for populations than for individuals, and, although the form of the relationships may differ, those for individuals are likely to be qualitatively similar to those of populations. On the other hand, because of large differences in responsiveness among individuals, exposure-response relationships for the population may not reflect quantitatively the experience of a given individual.

Relationships between short-term exposure and acute response have been described for lung function changes, induction of symptoms, and BAL outcomes in experimental exposure studies and for lung function, symptoms, hospital and emergency room admissions, and mortality in epidemiologic studies. Exposure in the experimental studies can be defined in terms of concentration, dose rate of exposure, total inhaled dose, and dose at the active

site. The limiting factor in modeling exposure-response with experimental data has been that no single study has included a wide enough range of the three exposure variables of interest (i.e., C, $\dot{V}_E$, T) to choose between models or to identify the appropriate method of describing exposure.

Exposure-response models in the epidemiologic studies generally have included only O_3 concentration measured at a central monitoring site in the study area as the exposure variable. With some exceptions, characterization of exposure-response relationships in these studies has been limited by little information on activity level or duration of exposure, a generally narrow range of exposure concentrations, the need for complex models to control for potential confounding by other pollutants and extraneous variables, and outcomes for which only a small fraction of the variance is explained by exposure to pollutants. These factors make selection among various models of response difficult.

A number of exposure-response functions have been proposed to describe the results of experimental studies. No single exposure-response model form, however, has been adequately tested and identified as providing an accurate, precise description of the relationship between exposure and response in both humans and laboratory animals for lung function or BAL endpoints. Rather, for a given study, a particular model may have been selected a priori to describe the exposure-response data or may have been identified as providing the best fit among several competing models. In many cases, models have been found to be deficient, but rarely has the performance of a number of possible models been systematically compared.

From the individual studies, several important observations have been made that are qualitatively true for describing BAL and pulmonary function responses in both humans and laboratory animals and that should be considered in the selection of a model to describe population response as a function of exposure. Response increases monotonically with C, $\dot{V}_E$, and T, with C generally being a stronger predictor of response than $\dot{V}_E$ or T. The relationship between response and one of the exposure variables is dependent on the level of the other two variables. The relationship between response and each of the exposure variables is curvilinear over a wide range of exposure conditions, although it may appear linear over certain narrow ranges of exposure. With increasing duration of response (and possibly with increasing concentration), the FEV_1 response may approach a plateau in humans. Some evidence exists suggesting that the level of the plateau with T is a function of C. This plateau has not been observed in animal studies or for BAL endpoints. Respiratory symptom responses generally follow a pattern similar to that observed for spirometry (e.g., mean responses increase with increasing C, $\dot{V}_E$, and T). As with spirometry responses, large individual differences in symptom responses occur. Little analytical work, however, has been performed that mathematically describes either the mean or individual responses as multivariable functions of C, $\dot{V}_E$, and T.

Exposure-response models of FEV_1 and BAL responses in laboratory animals that have been proposed and that fit to varying degrees include linear and polynomial models of C, $\dot{V}_E$, and T, with and without cross-product terms (e.g., $C \times T$); exponential models utilizing $C \times T$ as the exposure variable at constant $\dot{V}_E$; and cumulative normal probability or logistic models utilizing $C^y \times T$ as the exposure variable at constant $\dot{V}_E$. Models of these types have been found under some circumstances to describe the relationship between exposure and response for a particular data set. Most single data sets, however, do not include a wide enough range of data to test adequately the performance of a particular model across a wide range of exposure conditions or to identify an appropriate exposure metric.

In particular, recent efforts have focused on the relationship between response and C and T at constant $\dot{V}_E$. No definitive work has addressed the modeling of response and $\dot{V}_E$ for a given endpoint or for consideration of $\dot{V}_E$ changing as a function of T. Because animal and human studies often are conducted at different relative levels of $\dot{V}_E$, and because techniques to adjust mathematically for these differences only now are being developed, efforts to compare responses across species or to develop extrapolation models have been hampered. As noted earlier, quantitative models currently do not exist for respiratory symptoms.

Evidence indicates that, for humans and animals, the exposure-response relationship of BAL and pulmonary function outcomes may be modified by previous recent exposure to O_3, and the relationship for FEV_1 changes in humans may be modified by age. Previous exposure to O_3 has not been included in any exposure-response models. For young adults, the modification of the exposure-response relationship by age has been modeled.

Exposure-response models of lung function in epidemiologic studies generally have been limited to linear models of response as functions of various O_3 exposure metrics (e.g., 1-h or 8-h daily maximum, etc.). A number of studies have demonstrated significant negative mean linear relationships between O_3 exposure and lung function. Exposure-response models of respiratory symptoms in epidemiologic studies generally have employed logistic regression techniques with O_3 or total oxidant concentration as the exposure variable. These latter models generally have been chosen a priori reflecting the categorical nature of the outcome variable rather than by comparison of the performance of several candidates.

Ecological studies of the relationship between daily rates of emergency room or hospital admissions or mortality and O_3 exposure have utilized a variety of complex exposure-response models with some metric of daily O_3 concentration as the exposure variable. The complexity of the models results from, among other things, the need to control for potentially confounding long-wave patterns in the health outcomes in relationship to other potential confounders, such as other air pollutants, seasonal and meteorological factors, holidays, and day-of-week effects. In various studies, both linear and nonlinear functions have been used to describe the relationship between adjusted health outcome and concentration.

In summary, O_3 is no exception to the general problems encountered in all studies of environmental epidemiology. No single universal model form has been identified that accurately and precisely describes the relationship between population exposure and response under all circumstances. In general, the ability of a predictive model based on one study to predict responses from an independent study has not been studied adequately. For purposes of prediction or risk estimation, the adequacy of fit of a given model in a given data set and the size and representativeness of the sample should be assessed. Extrapolation beyond the range of observed data introduces additional uncertainty into predictions or risk estimates.

9.3.4.1 Prediction and Summary of Mean Responses

A selection of published reports in which models of population or mean responses have been developed is listed below, along with figures summarizing examples of predicted quantitative exposure-response relationships. Reports numbered 5 and 11 are epidemiological studies, and the remainder are experimental studies. Because no currently available single model is sufficient to accurately describe all major scenarios, the key models are presented without weighting. Following this section is a further section that describes models of individual responses within the population.

1. Hazucha (1987) predicts mean FEV_1 decrements in humans as a function of C (0.0 to 0.75 ppm O_3) for four levels of $\dot{V}_E$ for 2-h exposures (Figure 9-1).

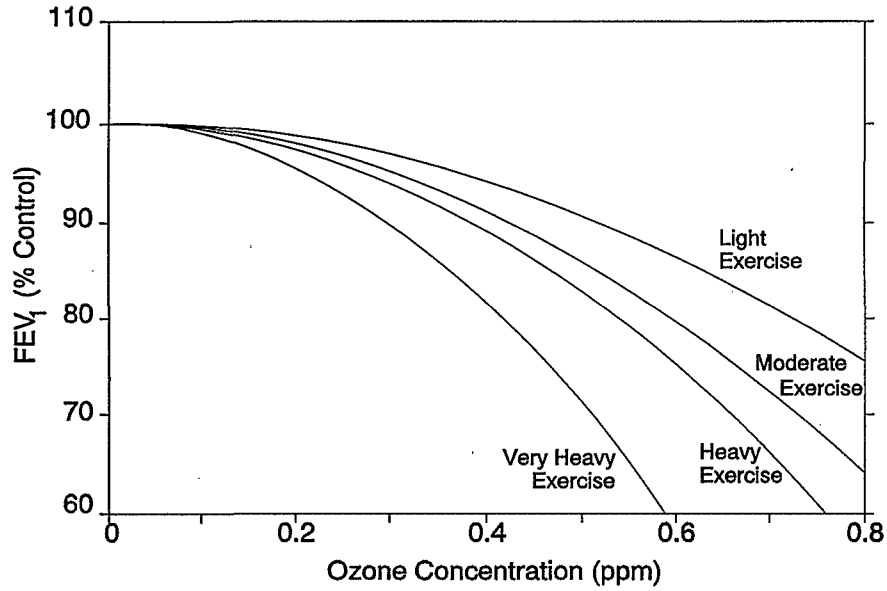

Figure 9-1. Mean predicted changes in forced expiratory volume in 1 s following 2-h exposures to ozone with increasing levels of intermittent exercise.

Source: Hazucha (1987).

2. McDonnell and Smith (1994) predict mean FEV_1 decrements in heavily exercising humans as a function of C (0.0 to 0.4 ppm O_3) and T (1.0 to 6.6 h) (Figure 9-2).
3. Highfill et al. (1992) predict the BAL responses of resting rats and guinea pigs as a function of C (0.0 to 0.8 ppm O_3) and T (2 to 8 h) (Figure 9-3).
4. Tepper et al. (1994) predict the FVC changes as a function of C (0.0 to 0.8 ppm O_3) and T (2 to 7 h) for exposures conducted with rats breathing at three times resting $\dot{V}_E$ (Figure 9-4).
5. Burnett et al. (1994) predict the frequency of hospital admissions (adjusted for covariates) as a linear function of C (previous day 1-h O_3 maximum) for Ontario hospitals (Figure 9-5).

Other reports in which models are developed or that contain data potentially useful for further development or testing of models are listed below.

6. Seal et al. (1993) present data that would allow modeling of FEV_1 decrements in humans as a function of C (0.0 to 0.4 ppm O_3) for 2-h exposures with moderate exercise.
7. Folinsbee et al. (1978) predict lung function changes in humans as a function of C (0.0 to 0.50 ppm O_3) and $\dot{V}_E$ (10 to 65 L/min) for 2-h exposures.

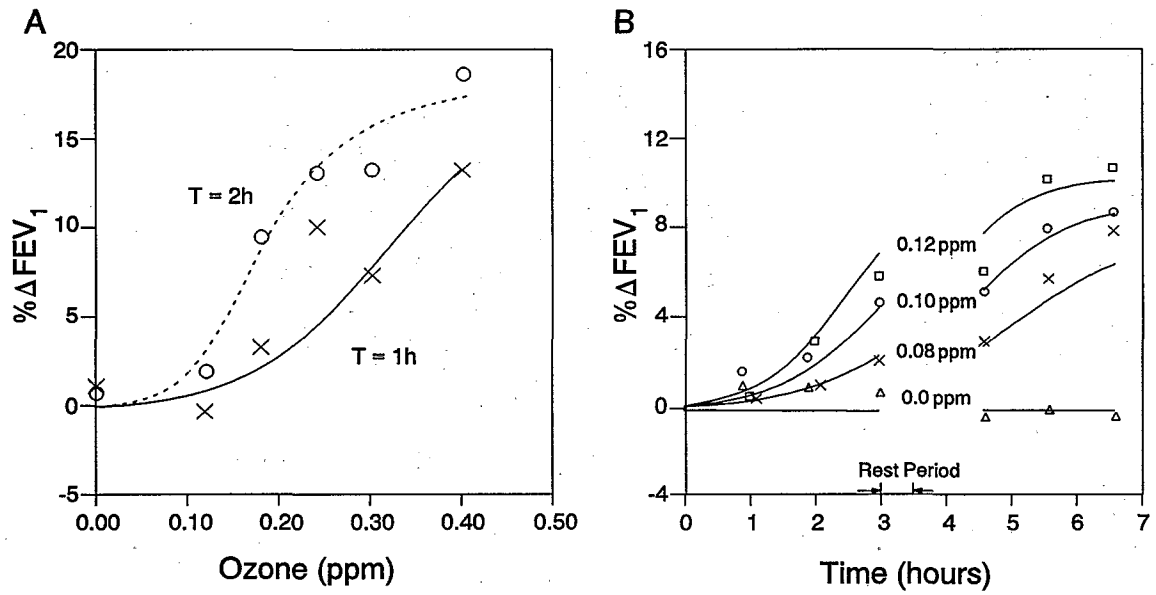

Figure 9-2. Predicted mean decrements in forced expiratory volume in 1 s for 1- and 2-h exposures to ozone with intermittent heavy exercise (A) and 6.6-h exposures with moderate prolonged exercise (B).

Source: McDonnell and Smith (1994).

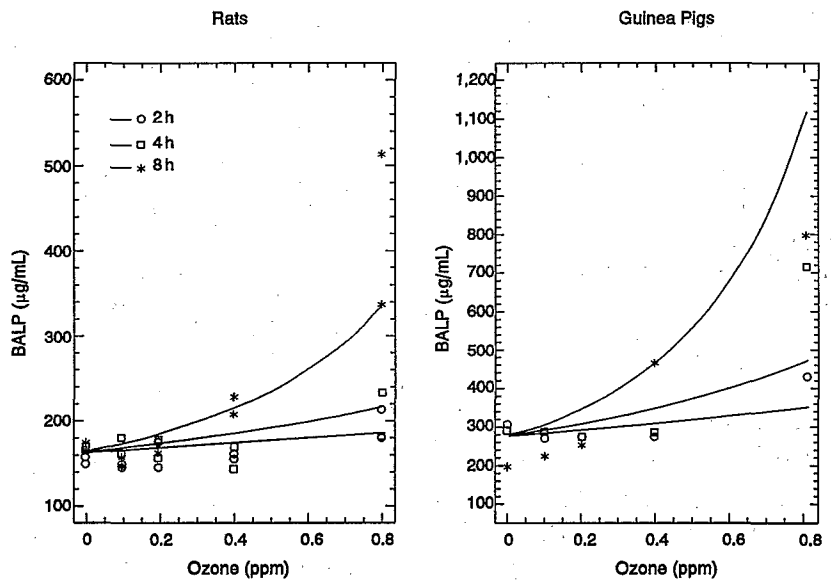

Figure 9-3. Derived means of BAL protein (BALP) denoted by symbols and the exponential model shown by lines as time of exposure varies from 2 to 8 h.

Source: Highfill et al. (1992).

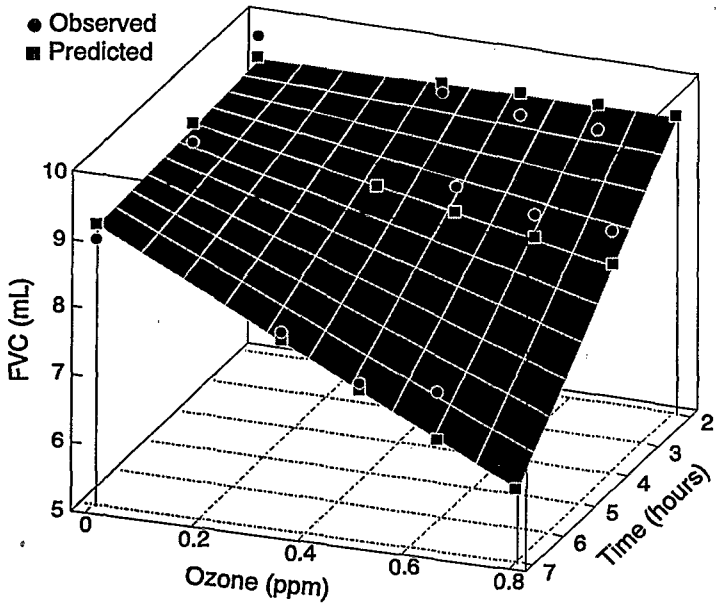

Figure 9-4. Predicted mean forced vital capacity for rats exposed to ozone while undergoing intermittent carbon dioxide-induced hyperpnea.

Source: Tepper et al. (1994).

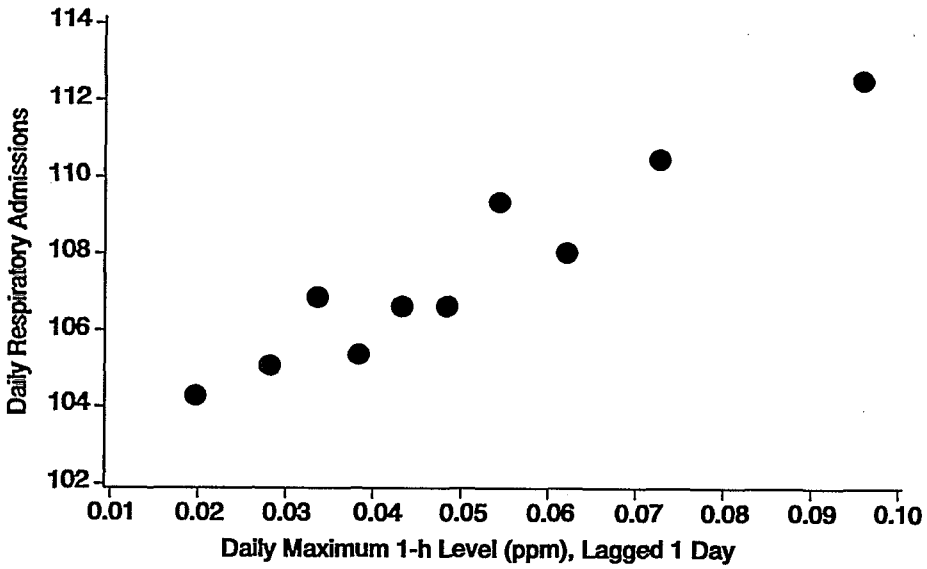

Figure 9-5. Average number of adjusted respiratory admissions among all 168 hospitals by decile of the daily 1-h maximum ozone level (ppm), lagged 1 day.

Source: Burnett et al. (1994)

8. Adams et al. (1981) predict lung function changes in humans as a function of the product of $C \times T \times \dot{V}_E$ for C = 0 to 0.4 ppm O_3, T = 30 to 80 min, and $\dot{V}_E$ = 33 or 66 L/min.
9. Rombout et al. (1989) predict the concentration of protein in BAL fluid of rats as a function of C (0.25 to 4.0 mg/m^3 O_3) and T (0 to 12 h) for daytime and nighttime exposures.
10. Highfill and Costa (1995) compare the fits of quadratic, exponential, and sigmoid-shaped models to published human lung function data and O_3 laboratory animal BAL data.
11. Thurston et al. (1994) predict hospital admissions in Toronto as a function of C (previous day 1-h O_3 maximum) for Toronto hospitals.

9.3.4.2 Prediction and Summary of Individual Responses

It is well known that considerable interindividual differences in the magnitude of response to O_3 exposure exist. The individual lung function and, to a lesser extent, respiratory symptom responses to O_3 have been demonstrated to be reproducible over a period of time, indicating that some individuals are consistently more responsive than others to O_3. The basis for these differences is not known, with the exception that young adults have been observed to be more responsive than older adults (see Figure 9-6).

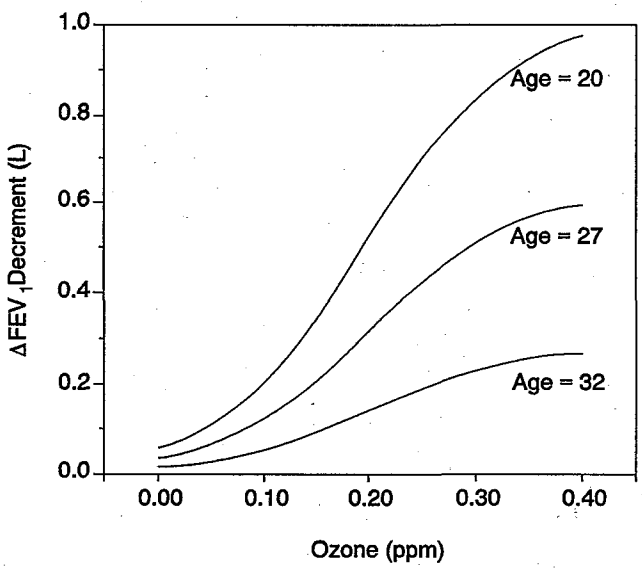

Figure 9-6. Predicted mean decrements in forced expiratory volume in 1 s (ΔFEV_1) following 2-h exposures to ozone while undergoing heavy intermittent exercise for three ages. (Note: To convert ΔFEV_1 to $\%\Delta FEV_1$, multiply by 22.2%.)

Source: McDonnell et al. (1993).

Calculation of group mean responses for a population that includes both more and less responsive individuals is useful for making inferences regarding the probability that a population effect is present or absent for a given exposure. Because the frequency distribution of individual responses to O_3 changes with changing exposure conditions, however, knowledge of the mean and variance of population responses does not provide reliable information on the distribution of individual responses for a given exposure, and, hence, is not particularly useful for estimating risks to members of the population. One method of presenting individual data is illustrated in Figure 9-7 in which histograms are presented for individual responses of subjects participating in four 6.6-h studies of low-level O_3 exposure.

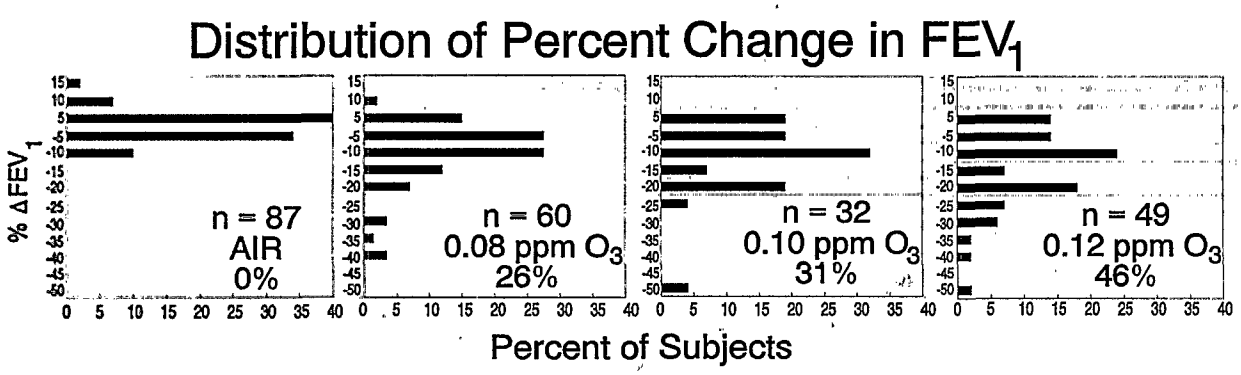

Figure 9-7. ***The distribution of response for 87 subjects exposed to clean air and at least one of 0.08, 0.10, or 0.12 ppm ozone (O_3). The O_3 exposures lasted 6.6 h, during which time the subjects exercised for 50 min of each hour, with a 35-min rest period at the end of the third hour. Decreases in forced expiratory volume in 1 s (FEV_1) are expressed as percent change from baseline. For example, the bar labeled, "−10" indicates the percent of subjects with a decrease in FEV_1 of >5% but ≤10%, and the bar labeled "5" indicates improvement in FEV_1 of >0% but ≤5%. Each panel of the figure indicates the percentage of subjects at each O_3 concentration with a decrease of FEV_1 in excess of 10%.***

Similarly, the histograms of regression slopes of the lung function-O_3 concentration relationship for children participating in a camp study illustrate a large range of variability in response (Figure 9-8).

Another method that allows interpolation between observed data points involves definition of the effect of interest (e.g., a 10% decrement in FEV_1) and modeling of the proportion of individuals who experience such an effect as a function of exposure conditions. Figures 9-9 and 9-10 show the predicted proportion of individuals (humans) experiencing 10% FEV_1 decrements and respiratory symptom responses, respectively, as a function of C (0.0 to 0.4 ppm O_3) for independent studies conducted at either 1 or 2 h of exposure with heavy exercise.

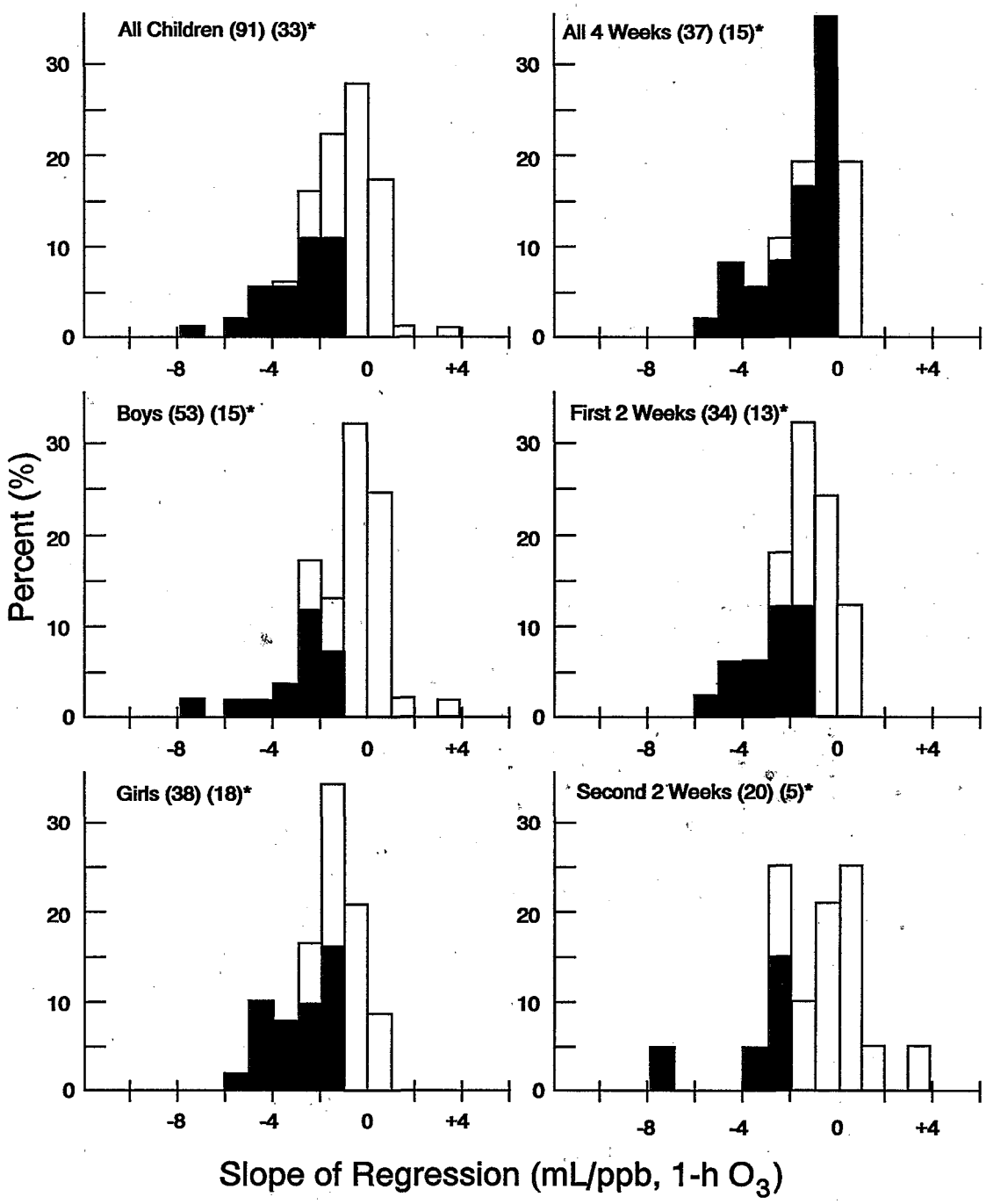

Figure 9-8. ***Histograms of regression slopes for FEV_1 versus 1-h ozone (O_3) concentration in children attending a summer camp in northwestern New Jersey. The numbers of children in each group are indicated in parentheses; an asterisk identifies the number of children with slopes that were significantly different from zero ($p < 0.05$). Shading represents the percent of significant ($p < 0.05$) slopes across the distributions.***

Source: Spektor et al. (1988).

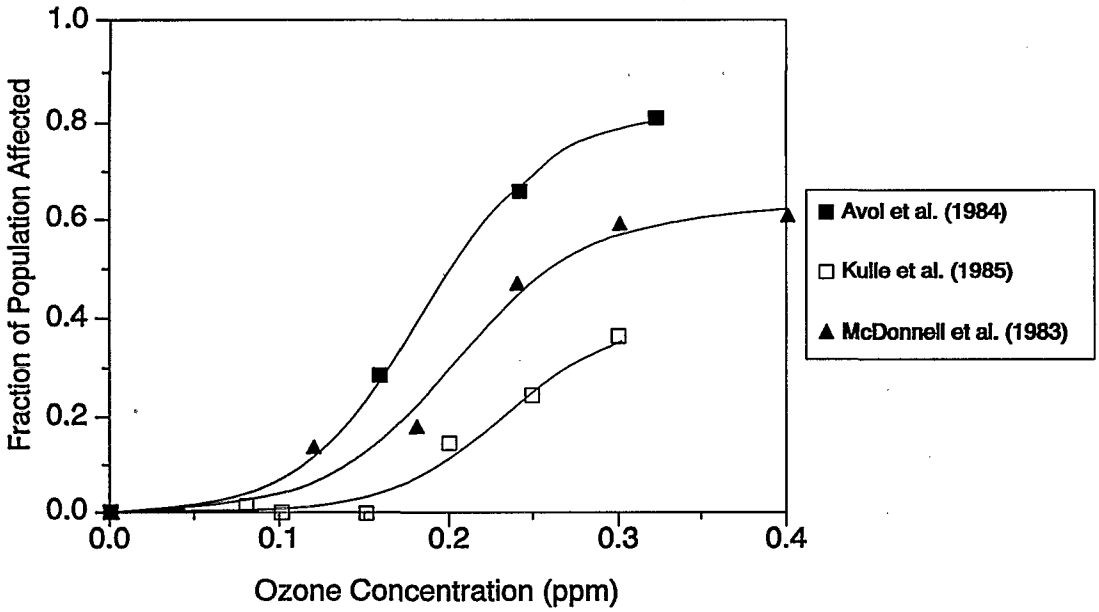

Figure 9-9. Proportion of heavily exercising individuals predicted to experience a 10% decrement in forced expiratory volume in 1 s following a 1- or 2-h exposure to ozone.

Source: U.S. Environmental Protection Agency (1989).

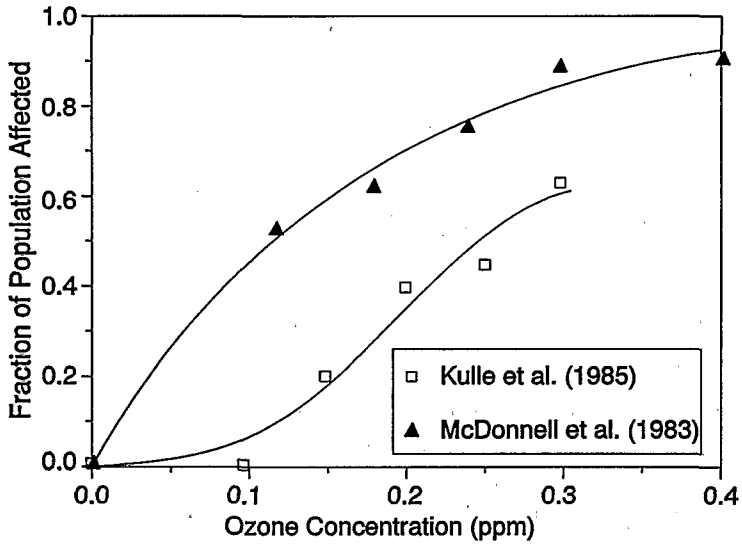

Figure 9-10. Proportion of heavily exercising individuals predicted to experience mild cough following a 2-h ozone exposure.

Source: U.S. Environmental Protection Agency (1989).

Predictions of the proportion of individuals experiencing 5, 10, or 15% FEV_1 decrements as a function of C (0.0 to 0.12 ppm O_3), T (1 to 6.6 h), at a specific age (24 years) for exposures with moderate exercise are shown in Figure 9-11.

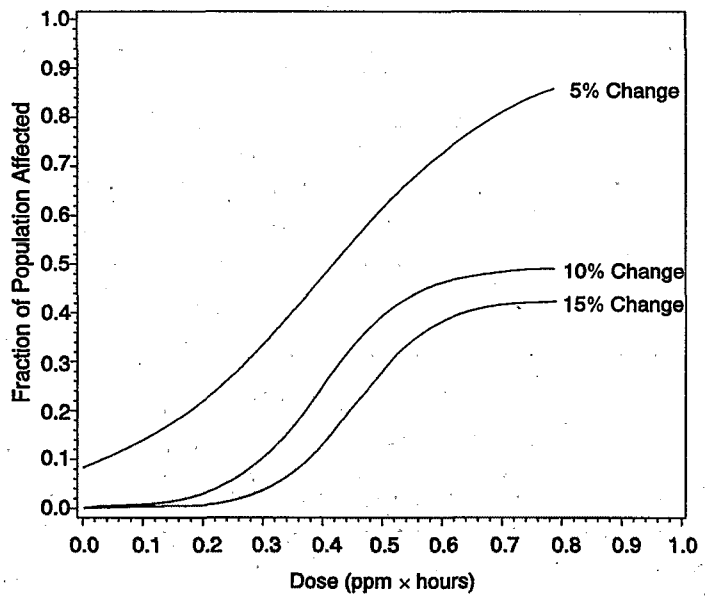

Figure 9-11. Proportion of moderately exercising individuals exposed to ozone for 6.6 h predicted to experience 5, 10, or 15% decrements in forced expiratory volume in 1 s as a function of C × T for age = 24 years.

Source: McDonnell et al. (1995).

As an example of differences between the mean and individual responses, it was stated earlier that exposure for 5.6 h to 0.08 ppm O_3 was the shortest duration for which a 5% mean decrement in FEV_1 was observed. For those same exposure conditions, 41, 17, and 10% of the subjects studied experienced FEV_1 decrements larger than 5, 10, and 15%, respectively.

The clinical significance of individual responses to O_3 exposure depends on the health status of the individual, the magnitude of the changes in pulmonary function, the severity of respiratory symptoms, and the duration of the response. Tables 9-1 and 9-2 categorize individual functional and symptomatic responses to O_3 exposure as normal (or none) and by increasing levels of severity in healthy persons and in persons with impaired respiratory systems, respectively. Pulmonary function responses are represented in these tables by changes in spirometry (e.g., FEV_1), SR_{aw}, and nonspecific bronchial responsiveness. Respiratory symptom responses include cough, pain on deep inspiration, and wheeze. The changes in spirometry that have been focused on most frequently are O_3-induced decrements in FEV_1 because they are easily quantified, have a continuous distribution, and have been used to provide most of the exposure-response relationships described in this section. The combined impact of both functional and symptomatic

Table 9-1. Gradation of Individual Responses to Short-Term Ozone Exposure in Healthy Persons[a]

Functional Response	None	Small	Moderate	Large
FEV_1	Within normal range (±3%)	Decrements of 3% to ≤10%	Decrements of >10% but <20%	Decrements of ≥20%
Nonspecific bronchial responsiveness[b]	Within normal range	Increases of <100%	Increases of ≤300%	Increases of >300%
Duration of response	None	<4 hours	>4 hours but ≤24 hours	>24 hours
Symptomatic Response	Normal	Mild	Moderate	Severe
Cough	Infrequent cough	Cough with deep breath	Frequent spontaneous cough	Persistent uncontrollable cough
Chest pain	None	Discomfort just noticeable on exercise or deep breath	Marked discomfort on exercise or deep breath	Severe discomfort on exercise or deep breath
Duration of response	None	<4 hours	>4 hours but ≤24 hours	>24 hours
Impact of Responses	Normal	Normal	Mild	Moderate
Interference with normal activity	None	None	A few sensitive individuals choose to limit activity	Many sensitive individuals choose to limit activity

[a]See text for discussion; see Appendix A for abbreviations and acronyms.
[b]An increase in nonspecific bronchial responsiveness of 100% is equivalent to a 50% decrease in PD_{20} or PD_{100} (see Chapter 7, Section 7.2.3).

responses to O_3 exposure generally is displayed as an interference with normal activity or a change in medical treatment (see Tables 9-1 and 9-2).

In healthy individuals, the importance attached to individual changes in FEV_1 and nonspecific bronchial responsiveness depends, in part, on the magnitude and persistence of the response, but it is also important to consider the circumstances in which changes in lung function occur with other responses. For example, a 20% decrement in FEV_1 or a 100 to 200% increase in SR_{aw} that is induced as a result of a nonspecific bronchial responsiveness test and one that is almost completely reversible within an hour is associated with little, if any, airway epithelial damage. If, in addition, there are no respiratory symptoms (except chest discomfort), then this response, in itself, would not be considered clinically significant. On the other hand, a smaller decrement in FEV_1 of 15%, accompanied by marked pain on deep inspiration and persistent cough that is reversed in approximately 24 h, may be considered clinically significant in some individuals. In other words, it is important to

Table 9-2. Gradation of Individual Responses to Short-Term Ozone Exposure in Persons with Impaired Respiratory Systems[a]

Functional Response	None	Small	Moderate	Large
FEV_1 change	Decrements of <3%	Decrements of 3 to ≤10%	Decrements of >10% but <20%	Decrements of ≥20%
Nonspecific bronchial responsiveness[b]	Within normal range	Increases of <100%	Increases of ≤300%	Increases of >300%
Airway resistance (SR_{aw})	Within normal range (±20%)	SR_{aw} increased <100%	SR_{aw} increased up to 200% or up to 15 cm H_2O/s	SR_{aw} increased >200% or more than 15 cm H_2O/s
Duration of response	None	<4 hours	>4 hours but ≤24 hours	>24 hours
Symptomatic Response	Normal	Mild	Moderate	Severe
Wheeze	None	With otherwise normal breathing	With shortness of breath	Persistent with shortness of breath
Cough	Infrequent cough	Cough with deep breath	Frequent spontaneous cough	Persistent uncontrollable cough
Chest pain	None	Discomfort just noticeable on exercise or deep breath	Marked discomfort on exercise or deep breath	Severe discomfort on exercise or deep breath
Duration of response	None	<4 hours	>4 hours, but ≤24 hours	>24 hours
Impact of Responses	Normal	Mild	Moderate	Severe
Interference with normal activity	None	Few individuals choose to limit activity	Many individuals choose to limit activity	Most individuals choose to limit activity
Medical treatment	No change	Normal medication as needed	Increased frequency of medication use or additional medication	Physician or emergency room visit

[a]See text for discussion; see Appendix A for abbreviations and acronyms.
[b]An increase in nonspecific bronchial responsiveness of 100% is equivalent to a 50% decrease in PD_{20} or PD_{100} (see Chapter 7, Section 7.2.3).

consider the pattern of responses and not simply to focus on a single marker of the effect of O_3.

The magnitude of individual changes can become more important in persons with impaired respiratory systems (e.g., asthmatics) who already have reduced baseline lung function. Any change in function that causes these individuals to drop below 40 to 50% of predicted would be considered clinically adverse. For example, O_3-induced changes in SR_{aw}, a measure of airway narrowing, are small and of minimal clinical significance in nonasthmatic individuals. Asthmatics, however, often have baseline airway narrowing and experience larger changes in SR_{aw} on exposure to O_3 than do nonasthmatics. Because of these baseline differences, the clinical significance of increases in SR_{aw} depends both on percent change from baseline and on absolute increases in SR_{aw}.

9.4 Effects of Long-Term Ozone Exposures

In both humans and test animals, the response to a single O_3 exposure nominally can be characterized by lung dysfunction, lung cell injury and inflammation, and leakage of plasma proteins into the airspace lumen. However, when such an exposure is repeated for several consecutive days, many of these effects appear to wane, suggesting attenuation or the development of tolerance to the continued intermittent challenge. In spite of this apparent state of attenuation, long-term O_3 exposures have been linked to subtle pulmonary effects, some of which have irreversible components, thereby enhancing concern about chronic effects. The following section will provide an overview attempting to synthesize the current understanding of the phenomenon of attenuation during brief, repeated exposures and the evidence for potential health impairments resulting from protracted exposures to this oxidant.

9.4.1 Repeated Exposures

It is well established that a brief exposure of laboratory rodents to an O_3 concentration, which causes minimal effects, will protect the animals from a subsequent lethal challenge of O_3 a week later. This phenomenon, called tolerance, bears a similarity to the pattern of attenuated nonlethal effects (sometimes referred to as "adaptation") observed in both human volunteers and animals when exposed to episodic levels of O_3 (≤ 0.5 ppm) for 1 to 7 h/day over a succession of 5 or more days. Generally, over a 5-day exposure period, the effects of Day 1 are accentuated on Day 2 and diminish thereafter. Attenuation of the functional effects include spirometric deficits and associated symptoms as well as irritative alterations of breathing; nonspecific airway responsiveness, however, does not revert to normal levels. Measures of tissue effects that attenuate include inflammation and impaired phagocytic capabilities of alveolar macrophages. However, some evidence from animal studies suggests that tissue alterations persist, although the observed changes may be part of a transition to a chronically affected stage of the lung. Thus, in general, cell-associated indicators of injury or damage within the lung appear to diminish in spite of the continued O_3 exposure.

A number of mechanisms have been shown to be involved in the evolution of this "adapted" state. These mechanisms range from the replacement of sensitive cells in the alveolar lining (epithelium) by more resistant cells (with or without a thickened fluid barrier on the lumenal surface) to the enhancement of antioxidant metabolism providing cell resistance and more biochemical defenses at the lung surface. However, controlled human

studies show that after a 1-week period without O_3 exposure, subjects regain their spirometric responsiveness to O_3 challenge, although this abrupt transition between unresponsiveness and responsiveness appears less distinct in field-related studies. For example, studies of Southern Californians suggest that they are significantly less responsive to the spirometric effects of an acute episodic-like controlled challenge with O_3 when studied for a period after the "high" O_3 season than after the relatively "low" O_3 season. Likewise, there is some evidence that O_3-exposed urban populations are also somewhat more resistant to the oxidant than populations that receive minimal exposure. This would appear to be in conflict with hospital admissions data suggesting the aggravation of respiratory diseases, like asthma, within such populations. It remains to be shown whether these latter data reflect the responsiveness of a sensitive subpopulation, perhaps less adapted or having less reserve function.

9.4.2 Prolonged Exposures

Most long-term exposure studies in animals have evaluated structural and functional changes. In the few investigations of the immune system or antibacterial host defenses, prolonged exposures of animals either caused no effects or did not increase the magnitude of effects observed after acute exposures. Thus, the following discussion centers on the larger body of knowledge on other endpoints.

Epidemiologic studies attempting to associate chronic lung effects in humans with long-term O_3 exposure provide only suggestive evidence that such a linkage exists. Most studies have been cross-sectional in design and have been compromised by incomplete control of confounding variables and inadequate exposure information. Other studies have attempted to follow variably exposed groups prospectively. Studies of such design have been conducted in communities of the Southwest Air Basin as well as in Canada where comparisons could be drawn between lung function changes over several years in populations from high- or low-oxidant pollution. The findings suggest small, but consistent decrements in lung function among inhabitants of the more highly polluted communities. However, associations between O_3 and other copollutants and, in some cases, problems with study population loss undermine the confidence in the study conclusions. Likewise, recent associations found between O_3 and the incidence and severity of asthma over a decade of study, although derived from well-designed studies, also tend to be weakened by the colinearity of O_3 with other air pollutants. Nevertheless, in all of the studies assessing lung function, the pattern of dysfunction associated with the long-term exposure has been consistent with the functional and structural abnormalities seen in laboratory animal studies.

The advantage of laboratory animal studies is the ability to examine closely the distribution and intensity of the O_3-induced morphologic changes that have been identified throughout the respiratory tract (see Chapter 6, Section 6.2.4). Indeed, cells of the nose, like the distal lung, clearly are affected by O_3. Perhaps of greater health concern are the lesions that occur in the small airways and in the centriacinar regions of the lung where the alveoli meet the distal airways (Figure 9-12). Altered function of the distal airways, the proximal conduits of air to the gas-exchange regions, can result in reduced communication of fresh air with the alveoli and air-trapping. In fact, chronic O_3 lesions as found in animal studies are reminiscent of the earliest lesions found in respiratory bronchiolitis, some of which may progress to fibrotic lung disease (Kuhn et al., 1989; King, 1993).

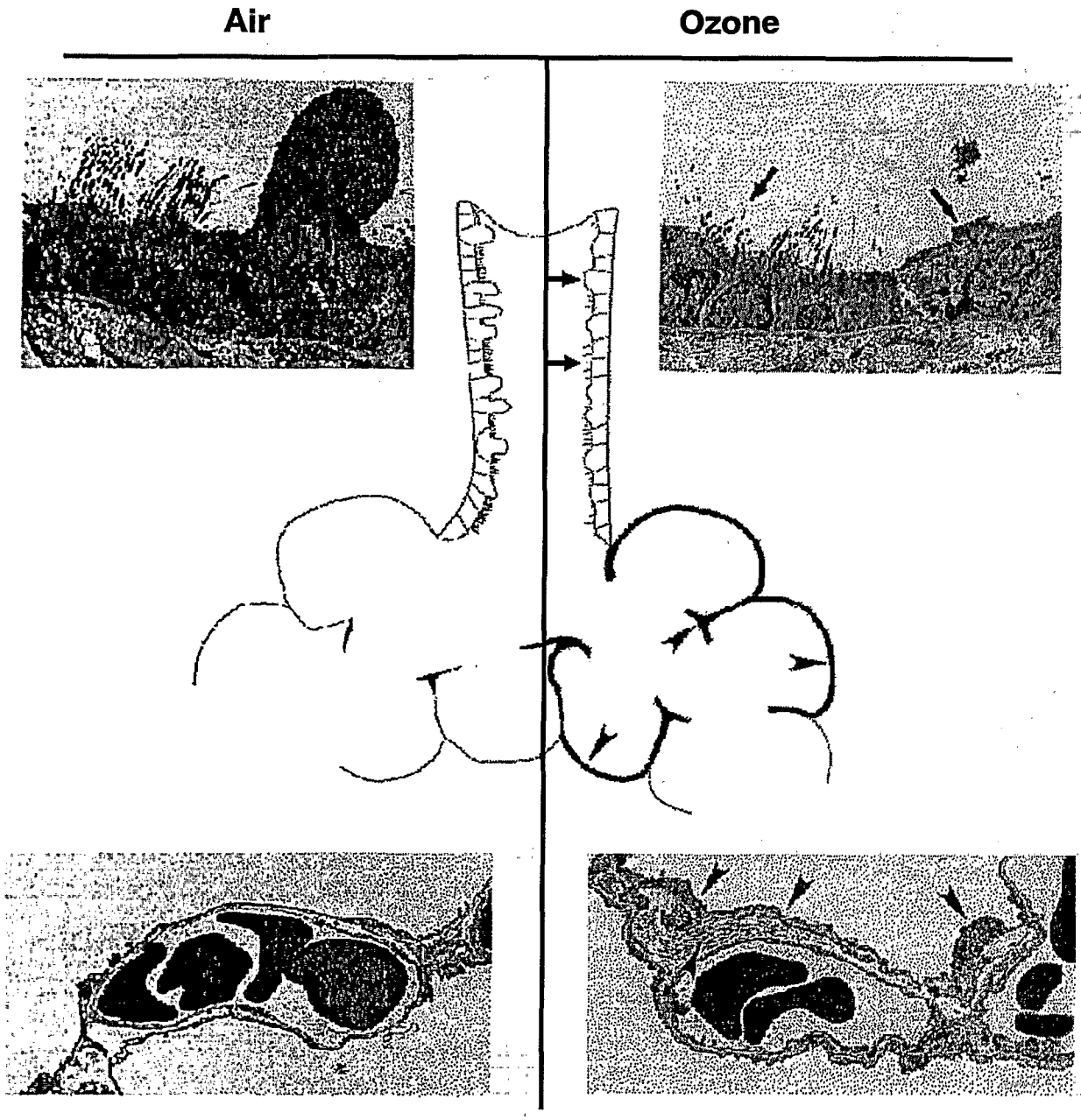

Figure 9-12. A summary of the morphologic lesions found in the terminal bronchioles and centriacinar region (CAR) of the lung following exposure of laboratory rats to filtered air or a simulated ambient pattern of ozone for up to 78 weeks. In the terminal bronchiole, the sizes of the dome of Clara cells became smaller with ozone exposure, and the number of cilia is reduced (arrows). In the CAR, the epithelium becomes thicker, and accumulation of collagen fibers occurs (arrow heads).

Source: Chang et al. (1992).

The O_3-induced inflammation, cell damage, and altered permeability of epithelial cells lining the respiratory tract allow exudation of fluid, cells, and cellular debris from plasma into lung tissue. The magnitude of intraluminal exudation associated with injury correlates with the initial epithelial necrosis and release of inflammatory mediators. As shown in Figure 9-13, the temporal pattern of effects during and after a chronic exposure is complex. During the early days of exposure, the end-airway lumenal and interstitial inflammation peaks, and, thereafter, appears to subside at a lower plateau of activity sometimes referred to as a "smoldering" lesion. Several cytokines remain elevated beyond the apparent adaptation phase of the response and may be linked conceptually to the development of chronic lesions in the distal lung. To date, however, a clear association of these BAL-derived mediators and cells with long-term toxicity has not been demonstrated. Some evidence of molecular changes within the matrix of the lung may also link to the chronic effects, but these too remain poorly defined. When exposures to O_3 continue for weeks or months, the diminished O_3-induced exudative response in the distal bronchoalveolar areas is supplanted by hyperplastic epithelia in the alveoli and end-airways. Damaged cells in centriacinar alveoli are replaced by metabolically active progenitor cells that are more resistant to oxidant challenge. Junctional areas between conducting and gas-exchange regions, where the O_3 changes are typically most intense, also undergo epithelial hyperplasia, giving the appearance that airway cells are extending into the mouth of the alveolus, hence the term "bronchiolization". The functional result of this concentration-dependent process is the effective elongation of distal bronchioles, which functionally may alter air distribution within the lung during breathing. These hyperplastic cells also are believed to be more resistant to O_3. When exposure to O_3 ceases, most, but not all, of the hyperplasia appears to reverse with time.

In contrast, within the underlying interstitium (tissue between blood and air spaces) of the affected centriacinar region, proliferating fibroblasts appear to evolve excess noncellular fibrous matrices, which may be only partially reversible and may, in fact, progress after removal from O_3 exposure. This would suggest that O_3 can initiate focal interstitial fibrosis of the lung at the regions where O_3 causes epithelial cell damage as a prelude to chronic degenerative lung disease. The crucial question, then, is whether this latter irreversible process, which clearly occurs at relatively high O_3 concentrations, occurs at ambient levels to which humans are typically exposed, in many cases, over a lifetime. Unfortunately, comparable morphologic data from humans residing in O_3-polluted areas are lacking.

Studies of prolonged O_3 exposures in monkeys and rats reveal generally similar morphologic responses, although it appears that the monkey exhibits somewhat more tissue injury than does the rat under roughly similar exposure conditions. Interspecies comparisons of dosimetric data indicate that the monkey, with its similarity to the human in distal airway structure, provides data that may best reflect the potential effects of O_3 in humans exercising out of doors. As such, monkeys exposed to O_3 at 0.15 ppm for 8 h each day for 6 to 90 days exhibit significant distal airway remodeling. Rats show similar but more modest changes at 0.25 ppm O_3 after exposures of longer duration, up to 18 mo and beyond (near-lifetime). The chronic distal lung and airway alterations appear consistent with incipient peribronchiolar fibrogenesis within the interstitium. Attempts to correlate functional deficits have been variable, perhaps due in part to the degree and distribution of the lesions and the general insensitivity of most measures of the distal lung function. The interstitial changes may progress, however. Moreover, one recent primate study revealed evidence that

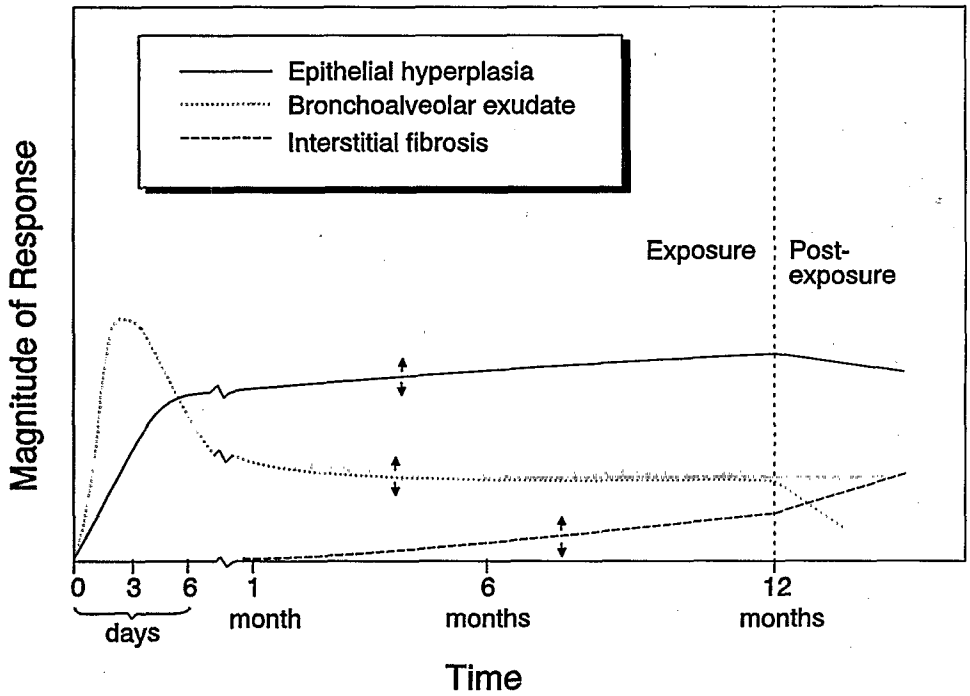

Figure 9-13. Schematic comparison of the duration-response profiles for epithelial hyperplasia, bronchoalveolar exudation, and interstitial fibrosis in the centriacinar region of lung exposed to a constant low concentration of ozone.

Source: Dungworth (1989).

intermittent challenge with a pattern of O_3 exposure more reflective of seasonal episodes, with extended periods of clean air in between extended periods of O_3, actually leads to greater injury. The reasons for this are unclear, but may relate to the known loss of tolerance that occurs in both humans and animal test species with removal of the oxidant burden.

In conclusion, the collective toxicologic data on chronic exposure to O_3 garnered in animal exposure and human population studies have some ambiguities. What is clear is that the distribution of the O_3 lesions is roughly similar across species, is, in part, concentration dependent (and perhaps time or exposure-pattern dependent), and, under certain conditions, has irreversible structural attributes. What is unclear is whether ambient exposure scenarios encountered by humans result in similar lesions and whether there are resultant functional or impaired health outcomes, particularly because the human exposure scenario may involve much longer exposures than can be studied in the laboratory. The epidemiologic lung function data generally parallel those of the animal studies, but they lack the confidence of O_3 exposure history and are frequently confounded by personal or copollutant variables.

9.4.3 Genotoxicity and Carcinogenicity of Ozone

Numerous in vitro exposure studies suggest that O_3 has either weak or no potential to cause mutagenic, cytogenetic, or cellular transformation effects. Most of these experiments utilized high concentrations of O_3 (>5.0 ppm). Because of the exposure systems used, there are unknowns about the formation of artifacts and the dose of O_3. Therefore, these studies are not very useful in health assessment. Cytogenetic effects have been observed in some, but not all, laboratory animal and human studies of short-term O_3 exposure. However, well-designed human clinical cytogenetic studies were negative.

Until recently, in vivo exposure studies of carcinogenicity, with and without co-exposure to known carcinogens, were either negative or ambiguous. A well-designed cancer bioassay study has recently been completed by the National Toxicology Program (NTP) using male and female Fischer 344/N rats and $B6C3F_1$ mice. Animals were exposed for 2 years to 0.12, 0.5, and 1.0 ppm O_3 (6 h/day, 5 days/week). A similar lifetime exposure was conducted, but 0.12 ppm was not used. The NTP evaluated the weight-of-evidence for this study; they found "no evidence" of carcinogenicity in rats but reported "equivocal evidence" of carcinogenicity in O_3-exposed male mice and "some evidence" of carcinogenic activity in O_3-exposed female mice. The increases in adenomas and carcinomas were observed only in the lungs. There was no concentration response. In the male mice, the incidence of neoplasms in the 2-year study was not elevated significantly by O_3 and was within the range of historical controls. The lifetime exposure resulted in an increased incidence of carcinomas that was not statistically significant. When the female mouse data from the two exposure regimens (at 1.0 ppm) were combined, there was a statistically significant increase (almost double) in neoplasms. In a companion study, male rats were treated with a tobacco carcinogen and exposed for 2 years to 0.5 ppm O_3. Ozone did not affect the response and, therefore, had no tumor promoting activity.

In summary, only chronic exposure to a high concentration of O_3 (1.0 ppm) has been shown to evoke a limited degree of carcinogenic activity in the females of one strain of mice. Rats were not affected. Furthermore, there was no concentration response, and there is inadequate information from other research to provide mechanistic support for the finding in mice. Thus, the potential for animal carcinogenicity is uncertain.

9.5 Effects of Combined Pollutant Exposures

In ambient air, people are exposed to mixtures of pollutants, making it important to understand interactions. Epidemiological studies, which inherently evaluate O_3 as part of complex mixtures, are discussed in other subsections dealing with classes of effects. In the laboratory it becomes possible to sort out the role of O_3 in simple mixtures. Complex mixtures are typically not investigated in the laboratory because, even if only six pollutants were involved, the experimental design required to unequivocally sort out which pollutant or pollutant interactions were responsible for the responses or portions of the responses could require as many as 719 additional separate experiments, even if the concentrations of the six pollutants remained the same.

The summary will focus only on binary mixtures because these are by far the predominant type of experiments. Responses to a binary pollutant mixture may represent the sum of the independent responses to the two chemicals (i.e., an additive response). If there is some interaction between either the two responses or the two pollutants, the resultant

response could be larger than additive (synergism) or smaller than additive (antagonism). Interaction between pollutants could result in the production of a more or less toxic byproduct. Alternatively, the response to one pollutant could magnify the response to the other pollutant or could interfere with or block the action of the other pollutant. Binary mixture studies fall into two categories, simultaneous and sequential exposures. In the simultaneous exposures, both the responses and the pollutants can interact. In the sequential exposures, it is primarily the responses that would interact.

In general, controlled human studies of O_3 mixed with other pollutants show no more than an additive response with symptoms or spirometry as an endpoint. This applies to O_3 in combination with nitrogen dioxide (NO_2), SO_2, sulfuric acid (H_2SO_4), nitric acid (HNO_3), or carbon monoxide (CO). Indeed, at the levels of copollutants used in human exposure studies, the responses can be attributed primarily to O_3. In one study, exposure to O_3 increased airway responsiveness to SO_2 in asthmatics. Similarly, other pollutants that may increase airway responsiveness could augment the effect of O_3 on airway responsiveness.

The relatively large number of animal studies of O_3 in mixture with NO_2 and H_2SO_4 shows that additivity, synergism, and antagonism can result, depending on the exposure regimen and the endpoint studied. The numerous observations of synergism are of concern, but the interpretation of most of these studies relative to the real world is confounded by unrealistic exposure designs. For example, ambient concentrations of O_3 often were combined with levels of copollutants substantially higher than ambient, creating the possibility that mechanisms of toxicity unlikely in the real world contributed to the experimental outcome. Nevertheless, the data support a hypothesis that coexposure to pollutants, each at innocuous or low-effect levels, may result in effects of significance.

9.6 Conclusions

This section summarizes the primary conclusions derived from an integration of the known effects of O_3 provided by animal toxicological, human clinical, and epidemiological studies.

1. What are the effects of short-term (< 8-h) exposures to ozone?

Recent epidemiology studies addressing the effects of short-term ambient exposure to O_3 in the population have yielded significant associations with a wide range of health outcomes, including lung function decrements, aggravation of preexisting respiratory disease, increases in daily hospital admissions and emergency department visits for respiratory causes, and increased mortality. Results from lung function epidemiology studies are generally consistent with the experimental studies in laboratory animals and humans.

Short-term O_3 exposure of laboratory animals and humans causes changes in pulmonary function, including tachypnea (rapid, shallow breathing), decreased lung volumes and flows, and increased airway responsiveness to nonspecific stimuli. Increased airway resistance occurs in both humans and laboratory animals, but typically at higher exposure levels than other functional endpoints. In addition, adult human subjects experience O_3 induced symptoms of airway irritation such as cough or pain on deep inspiration. The changes in pulmonary function and respiratory symptoms occur as a function of exposure concentration, duration, and level of exercise. Adult human subjects with mild asthma have

qualitatively similar responses in lung volume and airway responsiveness to bronchoconstrictor drugs as nonasthmatics. Respiratory symptoms are also similar, but wheezing is a prevalent symptom in O_3-exposed asthmatics in addition to the other demonstrated symptoms of airway irritation. Airway resistance, however, increases relatively more in asthmatics from an already higher baseline. Recovery from the effects of O_3 on pulmonary function and symptoms is usually complete within 24 h of the end of exposure, although other responses may persist somewhat longer.

- An association between daily mortality and O_3 concentration for areas with high O_3 levels (e.g., Los Angeles) has been suggested, although the magnitude of such an effect is unclear.
- Increased O_3 levels are associated with increased hospital admissions and emergency department visits for respiratory causes. Analyses from data in the Northeastern United States suggest that O_3 air pollution is associated with a substantial portion (on the order of 10 to 20%) of all summertime respiratory hospital visits and admissions.
- Pulmonary function in children at summer camps in southern Ontario, Canada, in the northeastern United States, and in Southern California is associated with O_3 concentration. Meta-analysis indicates that a 0.50-mL decrease in FEV_1 is associated with a 1 ppb increase in O_3 concentration. For preadolescent children exposed to 120 ppb (0.12 ppm) ambient O_3, this amounts to an average decrement of 2.4 to 3.0% in FEV_1. Similar responses are reported for children and adolescents exposed to O_3 in ambient air or O_3 in purified air for 1 to 2 h while exercising.
- Pulmonary function decrements are generally observed in healthy subjects (8 to 45 years of age) after 1 to 3 h of exposure as a function of the level of exercise performed and the O_3 concentration inhaled during the exposure. Group mean data from numerous controlled human exposure and field studies indicate that, in general, statistically significant pulmonary function decrements beyond the range of normal measurement variability (e.g., 3 to 5% for FEV_1) occur
  (1) at >0.50 ppm O_3 when at rest,
  (2) at >0.37 ppm O_3 with light exercise (slow walking),
  (3) at >0.30 ppm O_3 with moderate exercise (brisk walking),
  (4) at >0.18 ppm O_3 with heavy exercise (easy jogging), and
  (5) at >0.16 ppm O_3 with very heavy exercise (running).
  Smaller group mean changes (e.g., $<5\%$) in FEV_1 have been observed at lower O_3 concentrations than those listed above. For example, FEV_1 decrements have been shown to occur with very heavy exercise in healthy adults at 0.15 to 0.16 ppm O_3, and such effects may occur in healthy young adults at levels as low as 0.12 ppm. Also, pulmonary function decrements have been observed in children and adolescents at concentrations of 0.12 and 0.14 ppm O_3 with heavy exercise. Some individuals within a study may experience FEV_1 decrements in excess of 15% under these exposure conditions, even when the group mean decrement is less than 5%.
- For exposures of healthy subjects performing moderate exercise during longer duration exposures (6 to 8 h), 5% group mean decrements in FEV_1 were observed at
  (1) 0.08 ppm O_3 after 5.6 h,

(2) 0.10 ppm O_3 after 4.6 h, and
(3) 0.12 ppm O_3 after 3 h.
For these same subjects, 10% group mean FEV_1 decrements were observed at 0.12 ppm O_3 after 5.6 and 6.6 h. As in the shorter duration studies, some individuals experience changes larger than those represented by the group mean changes.

- An increase in the incidence of cough has been reported at O_3 concentrations as low as 0.12 ppm in healthy adults during 1 to 3 h of exposure with very heavy exercise. Other respiratory symptoms, such as pain on deep inspiration, shortness of breath, and lower respiratory scores (a combination of several symptoms), have been observed at 0.16 to 0.18 ppm O_3 with heavy and very heavy exercise. Respiratory symptoms also have been observed following exposure to 0.08, 0.10, and 0.12 ppm O_3 for 6.6 h with moderate levels of exercise.
- Increases in nonspecific airway responsiveness in healthy adults have been observed after 1 to 3 h of exposure to 0.40 ppm, but not 0.20 ppm, O_3 at rest and have been observed at concentrations as low as 0.18 ppm, but not to 0.12 ppm, O_3 during exposure with very heavy exercise. Increases in nonspecific airway responsiveness during 6.6-h exposures with moderate levels of exercise have been observed at 0.08, 0.10, and 0.12 ppm O_3.

Short-term O_3 exposure of laboratory animals and humans disrupts the barrier function of the lung epithelium, permitting materials in the airspaces to enter lung tissue, allowing cells and serum proteins to enter the airspaces (inflammation), and setting off a cascade of responses.

- Increased levels of PMNs and protein in lung lavage fluid have been observed following exposure of healthy adults to 0.20, 0.30, and 0.40 ppm with very heavy exercise and have not been studied at lower concentrations for 1- to 3-h exposures. Increases in lung lavage protein and PMNs also have been observed at 0.08 and 0.10 ppm O_3 during 6.6-h exposures with moderate exercise; lower concentrations have not been tested.

Short-term O_3 exposure of laboratory animals and humans impairs alveolar macrophage clearance of viable and nonviable particles from the lungs and decreases the effectiveness of host defenses against bacterial lung infections in animals and perhaps humans. The ability of alveolar macrophages to engulf microorganisms is decreased in humans exposed to 0.08 and 0.10 ppm O_3 for 6.6 h with moderate exercise.

2. *What are the effects of repeated, short-term exposures to ozone?*

During repeated short-term exposures, some of the O_3-induced responses are partially or completely attenuated. Over a 5-day exposure, pulmonary function changes are typically greatest on the second day, but return to control levels by the fifth day of exposure. Most of the inflammatory markers (e.g., PMN influx) also attenuate by the fifth day of exposure, but markers of cell damage (e.g., lactate dehydrogenase enzyme activity) do not attenuate and continue to increase. Attenuation of lung function decrements is reversed following 7 to 10 days without O_3. Some inflammatory markers are also reversed during this time period, but others still show attenuation even after 20 days without O_3. The mechanisms and impacts involved in attenuation are not known, although animal studies show that the underlying cell damage continues throughout the attenuation process.

In addition, attenuation may alter the normal distribution of O_3 within the lung, allowing more O_3 to reach sensitive regions, possibly affecting normal lung defenses (e.g., PMN influx in response to inhaled microorganisms).

3. *What are the effects of long-term exposures to ozone?*

Available data indicate that exposure to O_3 for months and years causes structural changes in several regions of the respiratory tract, but effects may be of the greatest importance in the centriacinar regions (where the alveoli and conducting airways meet); this region typically is affected in most chronic airway diseases of the human lung. This information on O_3 effects in the distal lung is extrapolated from animal toxicological studies because, to date, comparable data are not available from humans. The apparent lack of reversal of effects during periods of clean air exposure raises concern that seasonal exposures may have a cumulative impact over many years. The role of adaptive processes in this response is unknown but may be critically dependent on the temporal frequency or profile of exposure. Furthermore, the interspecies diversity in apparent sensitivity to the chronic effects of O_3 is notable, with the rat representing the lower limit of response, and the monkey the upper limit. Epidemiological studies attempting to associate chronic health effects in humans with long-term O_3 exposure provide only suggestive evidence that such a linkage exists.

Long-term exposure in the females of one strain of mice to high O_3 levels (1 ppm) caused a small, but statistically significant increase in lung tumors. There was no concentration-response relationship, and rats were not affected. Genotoxicity data are either negative or weak. Given the nature of the database, potential carcinogenicity in animals is uncertain. Ozone did not show tumor-promoting activity in a chronic rat study (at 0.5 ppm O_3).

4. *What are the effects of binary pollutant mixtures containing ozone?*

Combined data from laboratory animal and controlled human exposure studies of O_3 support the hypothesis that coexposure to pollutants, each at low-effect levels, may result in effects of significance. The data from human studies of O_3 in combination with NO_2, SO_2, H_2SO_4, HNO_3, or CO show no more than an additive response on lung spirometry or respiratory symptoms. The larger number of laboratory animal studies with O_3 in mixture with NO_2 and H_2SO_4 show that effects can be additive, synergistic, or even antagonistic, depending on the exposure regimen and the endpoint studied. This issue of exposure to copollutants remains poorly understood, especially with regard to potential chronic effects.

5. *What population groups are at risk as a result of exposure to ozone?*

Identification of population groups that may show increased sensitivity to O_3 is based on their (1) biological responses to O_3, (2) preexisting lung disease (e.g., asthma), (3) activity patterns, (4) personal exposure history, and (5) personal factors (e.g., age, nutritional status).

The predominant information on the health effects of O_3 noted above comes from clinical and field studies on healthy, nonsmoking, exercising subjects, 8 to 45 years of age. These studies demonstrate that, among this group, there is a large variation in sensitivity and responsiveness to O_3, with at least a 10-fold difference between the most and least responsive individuals. Individual sensitivity to O_3 also may vary throughout the year, related to seasonal variations in ambient O_3 exposure. The specific factors that contribute to this large

intersubject variability, however, remain undefined. Although differences may be due to the dosimetry of O_3 in the respiratory tract, available data show little difference on O_3 deposition in the lungs for inhalation through the nose or mouth.

Daily life studies reporting an exacerbation of asthma and decrease in peak expiratory flow rates, particularly in asthmatic children, appear to support the controlled studies; however, those studies may be confounded by temperature, particle or aeroallergen exposure, and asthma severity of the subjects or their medication use. In addition, field studies of summertime daily hospital admissions for respiratory causes show a consistent relationship between asthma and ambient levels of O_3 in various locations in the Northeastern United States, even after controlling for independent contributing factors. Controlled studies on mild asthmatics suggest that they have similar lung volume responses but greater airway resistance changes to O_3 than nonasthmatics. Furthermore, limited data from studies of moderate asthmatics suggest that this group may have greater lung volume responses than nonasthmatics.

Other population groups with preexisting limitations in pulmonary function and exercise capacity (e.g., chronic obstructive pulmonary disease, chronic bronchitis, ischemic heart disease) would be of primary concern in evaluating the health effects of O_3. Unfortunately, not enough is known about the responses of these individuals to make definitive conclusions regarding their relative responsiveness to O_3. Indeed, functional effects in these individuals with reduced lung function may have greater clinical significance than comparable changes in healthy individuals.

Currently available data on personal factors or personal exposure history known or suspected of influencing responses to O_3 follow.

- Human studies have identified a decrease in pulmonary function responsiveness to O_3 with increasing age, although symptom rates remain similar. Toxicological studies are not easily interpreted but suggest that young animals are not more responsive than adults.
- Available toxicological and human data have not conclusively demonstrated that males and females respond differently to O_3. If gender differences exist for lung function responsiveness to O_3, they are not based on differences in baseline pulmonary function.
- Data are not adequate to determine whether any ethnic or racial group has a different distribution of responsiveness to O_3. In particular, the responses of nonwhite asthmatics have not been investigated.
- Information derived from O_3 exposure of smokers is limited. The general trend is that smokers are less responsive than nonsmokers. This reduced responsiveness may wane after smoking cessation.
- Although nutritional status (e.g., vitamin E deficiency) makes laboratory rats more susceptible to O_3-induced effects, it is not clear if vitamin E supplementation has an effect in human populations. Such supplementation has no or minimal effects in animals. The role of such antioxidant vitamins in O_3 responsiveness, especially their deficiency, has not been well studied.

Based on information presented in this document, the population groups that have demonstrated increased responsiveness to ambient concentrations of O_3 consist of exercising, healthy and asthmatic individuals, including children, adolescents, and adults.

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Appendix A

Abbreviations and Acronyms

a	Local surface/volume ratio
AD	Alveolar duct
AH_2	Ascorbic acid
AHSMOG	Adventist Health Smog
AIRS	Aerometric Information Retrieval System (U.S. Environmental Protection Agency)
AM	Alveolar macrophage
AMI	Asthma medication index
ANCOVA	Analysis of covariance
AOD	Airway obstructive disease
AR	Autoregressive
ARB	Air Resources Board (California)
ATP	Adenosine triphosphate
BADJ	Bronchiole-alveolar duct junction
BAL	Bronchoalveolar lavage
BF	Black female
BHPN	*N-bis* (2-hydroxypropyl) nitrosamine
bkg	Background
BM	Black male
bpm	Breaths per minute
BrdU	Bromodeoxyuridine
BS	Black smoke
BSA	Body surface area
BW	Body weight
C	Concentration
$C3_a$	Complement protein fragment
CA	Clean air
CAR	Centriacinar region
CC10	Clara cell 10KD protein
Cdyn	Dynamic compliance

CE	Continuous exercise
CI	Confidence interval
CIU	Cumulative methacholine inhalation unit
CL	Confidence limit
CO	Carbon monoxide
CO_2	Carbon dioxide
CoH	Coefficient of haze
ConA	Concanavalin A
COPD	Chronic obstructive pulmonary disease
CORD	Chronic obstructive respiratory disease
C_{st}	Static compliance
$C \times T$	Concentration times duration of exposure
CTD	Connective tissue disorders
C_w	Chest wall compliance
DAAS	Distal airways and alveolar surface fraction
DEN	Dimethylnitrosamine
DHLNL	Dehydrodihydroxylysinonorleucine
DL_{CO}	Diffusing capacity for carbon monoxide
DNA	Deoxyribonucleic acid
DR	Disulfide reductase
DTPA	Diethylenetriaminepentaacetate
DW	Durbin-Watson statistic
Δ	Mean change in a variable
EEG	Electroencephalogram
EES	Exposure event sequence
EPA	U.S. Environmental Protection Agency
EPR	Electron paramagnetic resonance (spectroscopy)
ER	Emergency room
ERV	Expiratory reserve volume
ESC	Epithelial secretory cell
EVR	Equivalent ventilation rate
f	Frequency of breathing
F	Female
F344	Fischer 344
FA	Filtered air
Fc	Antigen-antibody receptor

f_{cnt}	Control breathing frequency
FEF	Forced expiratory flow
$FEF_{25-75\%}$	Forced expiratory flow between 25 and 75% of vital capacity
Fe_2O_3	Ferric oxide
$Fe_2(SO_4)_3$	Iron sulfate
$FEV_{0.5}$	Forced expiratory volume in 0.5 s
$FEV_{0.75}$	Forced expiratory volume in 0.75 s
FEV_1	Forced expiratory volume in 1 s
FEV_3	Forced expiratory volume in 3 s
F_{lrt}	Uptake efficiency of the lower respiratory tract
FP	Fine particle
f_{O_3}	Ozone-altered breathing frequency
FPL55712	Leukotriene D_4 antagonist
FRC	Functional residual capacity
F_t	Total respiratory tract ozone uptake efficiency
F_{urt}	Uptake efficiency of the upper respiratory tract
FVC	Forced vital capacity
GDT	Glutathione-disulfide transhydrogenase
G6PD	Glucose-6-phosphate dehydrogenase
GR	Glutathione reductase
GSH	Glutathione
GSHPx	Glutathione peroxidase
GST	Glutathione-*S*-transferase
H^+	Hydrogen ion
HC	Hydrocarbon
HCHO	Formaldehyde
HEI	Health Effects Institute
HLNL	Hydroxylysinonorleucine
HMV	Half-hour mean value
HNO_3	Nitric acid
H_2O	Water
H_2O_2	Hydrogen peroxide
HR	Heart rate
HR_{max}	Maximum heart rate
H_2SO_4	Sulfuric acid
IC	Inspiratory capacity

ICAM	Intracellular adhesion molecule
ICD	Nicotinamide adenine diphsophate-specific isocitrate dehydrogenase
IE	Intermittent exercise
Ig	Immunoglobulin (IgA, IgE, IgG, IgM^+)
IL	Interleukin (IL-1, IL-6, IL-8)
Inf	Inflammation
ip	Intraperitoneal
IP	Inhalable particles
IPF	Idiopathic pulmonary fibrosis
IU	International Units
iv	Intravenous
K	Mass transfer coefficient
KI	Potassium iodide
LDH	Lactate dehydrogenase
LM	Light microscopy
LOEL	Lowest-observed-effect level
LRT	Lower respiratory tract
LT	Leukotriene (LTB_4, LTC_4, LTD_4, LTE_4)
Λ	Ozone uptake efficiency
M	Male
M	Oral (breathing)
MAP	Mean arterial blood pressure
MC	Methacholine challenge
MEFV	Maximum expiratory flow volume
MLN	Mediastinal lymph node
MMAD	Mass median aerodynamic diameter
MMEF	Maximum mid-expiratory flow
MNNG	*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine
$MnSO_4$	Manganese sulfate
mRNA	Messenger ribonucleic acid
MVV	Maximum voluntary ventilation
n	Number
N	Nasal (breathing)
ΔN_2	Fractional concentration of nitrogen in expired alveolar gas (nitrogen washout)
NA	Not available

N/A	Not applicable
NAAQS	National Ambient Air Quality Standards
NaCl	Sodium chloride
NADH	Reduced nicotinamide adenine dinucleotide
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NADPH-CR	Cytochrome c reductase pertaining to nicotinamide adenine dinucleotide phosphate activity
NCI	National Cancer Institute
NEP	Neutral endopeptidase
NH_4^+	Ammonium ion
NHANES	National Health and Nutrition Examination Survey
$(NH_4)_2SO_4$	Ammonium sulfate
NIH	National Institutes of Health
NK	Natural killer
NL	Nasal lavage
NNK	4-(*N*-methyl-*N*-nitrosomino)-1-(3-pyridyl)-1-butanone
NO	Nitric oxide
NO_2	Nitrogen dioxide
NO_3	Nitrate
NO_x	Nitrogen oxides
NP	Nasopharynx
NPSH	Nonprotein sulfydryl
NS	Nonsmoker
N.S.	Not statistically significant
NTP	National Toxicology Program
NZW	New Zealand White
O_3	Ozone
^{18}O	A stable isotope of oxygen
$^{18}O_2$	^{18}O-labeled oxygen
$^{18}O_3$	^{18}O-labeled ozone
OR	Odds ratio
OX (10)	Number of hours above oxidant threshold of 10 pphm
O_X (KI)	Photochemical oxidants measured by the potassium iodide method
OZ (10)	Number of hours above 10-pphm ozone
OZ (12)	Number of hours above 12-pphm ozone
p	Probability

PA	Proximal airways fraction
PAH	Polycyclic aromatic hydrocarbons
PAN	Peroxyacetyl nitrate
PAR	Proximal alveolar region
PC_{15}	Provocative concentration that produces a 15% decrease in forced expiratory volume in 1 s
PC_{20}	Provocative concentration that produces a 20% decrease in forced expiratory volume in 1 s
$PC_{100SRaw}$	Provocative concentration required to increase specific airway resistance by 100%
PD_{20}	Provocative dose that produces a 20% decrease in forced expiratory volume in 1 s
PD_{100}	Provocative dose that produces a 100% decrease in forced expiratory volume in 1 s
PDI	Pain on deep inspiration
PE	Postexposure
PEFR	Peak expiratory flow rate
PG	Prostaglandin (PGD_2, PGE, PGE_1, PGE_2 $PGF_{1\alpha}$, $PGF_{2\alpha}$)
6PGD	6-Phosphogluconate dehydrogenase
pH	Hydrogen ion concentration
PHA	Phytohemagglutinin
PM	Particulate matter
$PM_{2.5}$	Particulate matter of mass median aerodynamic diameter ≤ 2.5 μm
PM_{10}	Particulate matter of mass median aerodynamic diameter ≤ 10 μm
PM_{15}	Particulate matter of mass median aerodynamic diameter ≤ 15 μm
PMN	Polymorphonuclear leukocyte (also called neutrophil)
$pNEM/O_3$	Probabilistic National Ambient Air Quality Standards Exposure Model for Ozone
PNU	Protein nitrogen unit
p.r.n.	As needed *(pro re nata)*
PUFA	Polyunsaturated fatty acid
r	Linear regression correlation coefficient
r^2	Correlation coefficient
R	Intraclass correlation coefficient
R^2	Multiple correlation coefficient
R_{aw}	Airway resistance

RB	Respiratory bronchiole
RBC	Red blood cell
RH	Relative humidity
R_L	Total pulmonary resistance
RNA	Ribonucleic acid
RR	Relative risk
RSP	Respirable suspended particulate
RT	Respiratory tract
R_T	Total respiratory resistance
RV	Residual volume
S	Smoker
SAROAD	Storage and Retrieval of Aerometric Data (U.S. Environmental Protection Agency) centralized database; superseded by Aerometric Information Retrieval System [AIRS])
SB	Shortness of breath
sc	Subcutaneously
SCE	Sister chromatid exchange
SD	Standard deviation
S-D	Sprague-Dawley
SE	Standard error
SEM	Scanning electron microscopy
SEM	Standard error of the mean
SG_{aw}	Specific airway conductance
SH	Sulphydryl
SHAPE	Simulation of Human Activity and Pollutant Exposure
SHE	Syrian hamster embryo
SMG	Small-mucous-granule
SO_2	Sulfur dioxide
SO_4	Sulfate
$SO_4^=$	Sulfate ion
SOD	Superoxide dismutase
SR_{aw}	Specific airway resistance
SRBC	Sheep red blood cell
σ_g	Geometric standard deviation
σ^2	Difference in volume variance between expired and inspired gas bolus
T	Time (duration of exposure)

T °C	Temperature (degrees)
T_{co}	Core temperature
TB	Terminal bronchioles
TBARS	Thiobarbituric acid reactive substance
^{99m}Tc-DTPA	Radiolabeled diethylene triamine pentaacetic acid
^{99m}Tc-Fe_2O_3	Radiolabeled ferric oxide
Tdb	Dry bulb temperature
TEM	Transmission electron microscopy
THC	Total hydrocarbon content
THI	Temperature-humidity index
TLC	Total lung capacity
TNF	Tumor necrosis factor
TSP	Total suspended particulate
TSP (200)	Number of hours above a total suspended particulate concentration of 200 $\mu g/m^3$
TX	Thromboxane (A_2, B_2)
UCLA	University of California at Los Angeles
UFA	Unsaturated fatty acids
URT	Upper respiratory tract
UV	Ultraviolet
V	Volume
V_B	Breakthrough volume
VC	Vital capacity
V_p	Penetration volume
V_s	Volume per surface area
V_T	Tidal volume
V_{Tmax}	Maximum tidal volume
V_v	Volume fraction
$V_{25\%VC}$	Lung volume at 25% of the vital capacity
$V_{50\%VC}$	Lung volume at 50% of the vital capacity
$\dot{V}_A$	Alveolar ventilation
$\dot{V}_E$	Minute ventilation; expired volume per minute
$\dot{V}_{Emax}$	Maximum minute ventilation
$\dot{V}_I$	Average inspiratory flow
$\dot{V}_{max25\%}$	Maximum expiratory flow at 25% of the vital capacity
$\dot{V}_{max50\%}$	Maximum expiratory flow at 50% of the vital capacity

$\dot{V}_{max75\%}$	Maximum expiratory flow at 75% of the vital capacity
$\dot{V}_{max50\%TLC}$	Maximum expiratory flow at 50% of the total lung capacity
$\dot{V}O_2$	Oxygen uptake by the body
$\dot{V}O_{2max}$	Maximal oxygen uptake (maximal aerobic capacity)
W	Watt
WBGT	Wet bulb globe temperature
WF	White female
WM	White male
ZnO	Zinc oxide

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