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# Integrated Science Assessment for Ozone and Related Photochemical Oxidants




**Office of Research and Development**
National Center for Environmental Assessment-RTP Division

U.S. Environmental Protection Agency
Research Triangle Park, NC

United States Environmental Protection Agency

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# TABLE OF CONTENTS


**OZONE PROJECT TEAM** xxiv

**AUTHORS, CONTRIBUTORS, AND REVIEWERS** xxvii

**CLEAN AIR SCIENTIFIC ADVISORY COMMITTEE OZONE NAAQS REVIEW PANEL** xxxiii

**ACRONYMS AND ABBREVIATIONS** xxxv

**PREAMBLE** li

Process of ISA Development li

Figure I Illustration of the key steps in the process of the review of National Ambient Air Quality Standards. lii

Figure II Illustration of processes for literature search and study selection used for development of ISAs. liii

Figure III Characterization of the general process of ISA development. lvii

EPA Framework for Causal Determination lviii

Evaluating Evidence for Inferring Causation lviii

Consideration of Evidence from Scientific Disciplines lix

Application of Framework for Causal Determination lxiv

Table I Aspects to aid in judging causality. lxv

Determination of Causality lxvi

Table II Weight of evidence for causal determination. lxviii

Quantitative Relationships: Effects on Human Populations lxix

Quantitative Relationships: Effects on Ecosystems or Public Welfare lxx

Concepts in Evaluating Adversity of Health Effects lxx

Concepts in Evaluating Adversity of Ecological Effects lxxi

References lxxiii

**LEGISLATIVE AND HISTORICAL BACKGROUND** lxxv

Legislative Requirements for the NAAQS Review lxxv

History of the NAAQS for Ozone lxxvi

Table III Summary of primary and secondary NAAQS promulgated for $O_3$ during the period 1971-2008. lxxvii

References lxxxi

**1 EXECUTIVE SUMMARY** 1-1

Introduction and Purpose 1-1

Scope and Methods 1-1

Ambient Ozone Concentrations 1-2

Human Exposure to Ozone 1-3

Dosimetry and Modes of Action 1-4

Integration of Ozone Health Effects 1-4

Table 1-1 Summary of $O_3$ causal determinations by exposure duration and health outcome. 1-5

Respiratory Effects 1-6

Mortality Effects 1-7

Cardiovascular Effects 1-7

Populations Potentially at Increased Risk 1-8

Integration of Effects on Vegetation and Ecosystems 1-8

Table 1-2 Summary of $O_3$ causal determination for welfare effects. 1-9

Visible Foliar Injury 1-10
Growth, Productivity, Carbon Storage and Agriculture 1-10
Water Cycling 1-11
Below Ground Processes 1-11
Community Composition 1-11
Air Quality Indices and Exposure-Response 1-12
The Role of Tropospheric Ozone in Climate Change and UV-B Shielding Effects 1-12
Radiative Forcing and Climate Change 1-13
UV-B Shielding Effects 1-13
Table 1-3 Summary of $O_3$ causal determination for climate change and UV-B shielding effects. 1-14
Conclusion 1-14

**2 INTEGRATIVE SUMMARY 2-1**

2.1 ISA Development and Scope 2-1
2.2 Atmospheric Chemistry and Ambient Concentrations 2-4
2.2.1 Physical and Chemical Processes 2-4
2.2.2 Background $O_3$ Concentrations 2-5
Figure 2-1 Mean daily average maximum 8-h avg $O_3$ concentrations in surface air, for spring and summer 2006. 2-7
2.2.3 Monitoring 2-8
2.2.4 Ambient Concentrations 2-8
2.3 Human Exposure 2-10
2.4 Dosimetry and Mode of Action 2-12
2.5 Integration of Ozone Health Effects 2-14
2.5.1 Conclusions from Previous $O_3$ AQCDs 2-15
2.5.2 Summary of Causal Determinations 2-16
Table 2-1 Summary of evidence from epidemiologic, controlled human exposure, and animal toxicological studies on the health effects associated with short- and long-term exposure to $O_3$. 2-20
2.5.3 Integrated Synthesis of Evidence for Health Effects 2-24
2.5.4 Policy Relevant Considerations 2-29
2.6 Integration of Effects on Vegetation and Ecosystems 2-35
2.6.1 Visible Foliar Injury 2-35
Figure 2-2 An illustrative diagram of the major endpoints that $O_3$ may affect in plants and ecosystems. 2-36
Table 2-2 Summary of $O_3$ causal determinations for vegetation and ecosystem effects. 2-37
2.6.2 Growth, Productivity, Carbon Storage and Agriculture 2-38
2.6.3 Water Cycling 2-40
2.6.4 Below-ground Processes 2-41
2.6.5 Community Composition 2-42
2.6.6 Policy Relevant Considerations 2-43
2.7 The Role of Tropospheric $O_3$ in Climate Change and UV-B Shielding Effects 2-45
2.7.1 Tropospheric Ozone as a Greenhouse Gas 2-45
Figure 2-3 Schematic illustrating the effects of tropospheric $O_3$ on climate; including the relationship between precursor emissions, tropospheric $O_3$ abundance, radiative forcing, climate response, and climate impacts. 2-46
2.7.2 Tropospheric Ozone and UV-B Shielding Effects 2-47
2.8 Summary of Causal Determinations for Health Effects and Welfare Effects 2-48
Table 2-3 Summary of $O_3$ causal determinations by exposure duration and health outcome. 2-49
Table 2-4 Summary of $O_3$ causal determination for welfare effects. 2-50
Table 2-5 Summary of $O_3$ causal determination for climate and UV-B shielding effects. 2-51
References 2-52

**3 ATMOSPHERIC CHEMISTRY AND AMBIENT CONCENTRATIONS 3-1**

3.1 Introduction 3-1

3.2 Physical and Chemical Processes 3-1

Figure 3-1 Schematic overview of photochemical processes influencing stratospheric and tropospheric $O_3$. 3-3

3.2.1 Sources of Precursors Involved in $O_3$ Formation 3-5

Figure 3-2 Estimated anthropogenic emissions of $O_3$ precursors for 2005. 3-6

3.2.2 Gas Phase Reactions Leading to $O_3$ Formation 3-10

3.2.3 Multiphase Processes 3-14

3.2.4 Temperature and Chemical Precursor Relationships 3-17

Figure 3-3 Measured concentrations of $O_3$ and $NO_Z$. 3-21

3.3 Atmospheric Modeling 3-22

Figure 3-4 Sample Community Multi-scale Air Quality (CMAQ) modeling domains. 3-23

Figure 3-5 Main components of a comprehensive atmospheric chemistry modeling system, such as the U.S. EPA's Community Multi-scale Air Quality (CMAQ) modeling system. 3-24

3.3.1 Global Scale CTMs 3-27

Figure 3-6 Comparison of global chemical-transport model (CTM) predictions of daily maximum 8-h avg $O_3$ concentrations and multi-model mean with monthly averaged CASTNET observations in the Intermountain West and Southeast Regions of the U.S. 3-29

3.4 Background $O_3$ Concentrations 3-30

Figure 3-7 Schematic overview of contributions to North American (NA) background concentrations of $O_3$. 3-32

3.4.1 Contributions from Natural Sources 3-32

3.4.2 Contributions from Anthropogenic Emissions 3-36

Figure 3-8 Time series of MDA8 $O_3$ concentrations (ppm) measured at Trinidad Head, CA, from April 18, 2002 through December 31, 2009. 3-38

3.4.3 Estimating Background Concentrations 3-40

Figure 3-9 Mean MDA8 $O_3$ concentrations in surface air for spring and summer 2006 calculated by GEOS-Chem for the base case (Base), U.S. background (USB), and NA background (NAB). 3-42

Figure 3-10 Spring and summer mean Canadian and Mexican (CM) contributions to MDA8 $O_3$ determined as the difference between the U.S. background and NA background. 3-44

Figure 3-11 MDA8 $O_3$ concentrations for spring (March-May) and summer (June-August) 2006 simulated by GEOS-Chem vs. measured by the ensemble of CASTNET sites in the Intermountain West, Northeast, Great Lakes, and Southeast. 3-45

Figure 3-12 Frequency distributions of MDA8 $O_3$ concentrations in March- August 2006 for the ensemble of low-altitude (<1,500 meters) and high-altitude CASTNET sites (>1,500 meters) in the U.S. 3-48

Figure 3-13 Mean MDA8 $O_3$ concentrations in surface air during spring and summer 2006 (top) calculated by GEOS-Chem/CAMx for the base case (Base, top) and NA background (NAB, bottom). 3-50

Figure 3-14 Monthly average MDA8 $O_3$ concentrations observed (Obs) and predicted for the base case and NA background (NAB) by GEOS-Chem (GC) and GEOS-Chem/CAMx (CX) at CASTNET sites above 1,500 meters elevation (upper panel) and CASTNET sites below 1,500 meters elevation (lower panel). 3-51

Figure 3-15 Annual 4th-highest MDA8 $O_3$ predicted by GEOS-Chem (0.5° × 0.667°) for the base case (Base) with corresponding U.S. background (USB) and NA background (NAB) MDA8 $O_3$ for the same days in 2006. 3-56

Figure 3-16 Annual 4th-highest MDA8 $O_3$ predicted by CAMx for the base case (Base) and corresponding NA background (NAB) MDA8 $O_3$ for the same days in 2006. 3-57

Table 3-1 Comparison of Zhang et al. (2011) and Emery et al. (2012) results for MDA8 $O_3$ concentrations (ppbv) with measurements at selected CASTNET sites. 3-60

Table 3-2 Comparison of annual 4th-highest MDA8 $O_3$ concentrations measured at CASTNET sites in 2006 with MDA8 $O_3$ concentrations simulated by the GEOS-Chem and CAMx base case models. 3-61

3.5 Monitoring 3-63
3.5.1 Routine Monitoring Techniques 3-63
3.5.2 Precision and Bias 3-66
Table 3-3 Summary of $O_3$ monitors meeting 40 CFR Part 58, Appendix A Precision and Bias Goals. 3-66
Figure 3-17 Box plots of precision data by year (2005-2009) for all $O_3$ monitors reporting single-point QC check data to AQS. 3-67
Figure 3-18 Box plots of percent-difference data by year (2005-2009) for all $O_3$ monitors reporting single-point QC check data to AQS. 3-67
Figure 3-19 Box plots of RPD data by year for the co-located $O_3$ monitors at two sites in Missouri from 2006-2009. 3-68
Figure 3-20 Box plots of RPD data by year for all of the United States. Ozone sites reporting single-point QC check data to AQS from 2005-2009. 3-69
3.5.3 Performance Specifications 3-69
Table 3-4 Performance specifications for $O_3$ based in 40 CFR Part 53. 3-70
3.5.4 Monitor Calibration 3-70
3.5.5 Other Monitoring Techniques 3-71
3.5.6 Ambient $O_3$ Network Design 3-75
Figure 3-21 U.S. $O_3$ sites reporting data to AQS in 2010. 3-77
Figure 3-22 U.S. Rural NCore, CASTNET and NPS POMS $O_3$ sites in 2010. 3-79
3.6 Ambient Concentrations 3-80
3.6.1 Measurement Units, Metrics, and Averaging Times 3-80
Figure 3-23 Distribution in nation-wide year-round site-level correlations between daily $O_3$ metrics including 24-h avg, 1-h daily max and 8-h daily max using AQS data, 2007-2009. 3-82
3.6.2 Spatial Variability 3-82
Figure 3-24 Required $O_3$ monitoring time periods (ozone season) identified by monitoring site. 3-83
Table 3-5 Summary of $O_3$ data sets originating from AQS. 3-84
Figure 3-25 Location of the 457 $O_3$ monitors meeting the year-round data set completeness criterion for all 3 years between 2007 and 2009. 3-85
Figure 3-26 Location of the 1,064 $O_3$ monitors meeting the warm-season data set completeness criteria for all 3 years between 2007 and 2009. 3-85
Table 3-6 Nationwide distributions of $O_3$ concentrations (ppb) from the year-round data set. 3-87
Table 3-7 Nationwide distributions of $O_3$ concentrations (ppb) from the warm-season data set. 3-88
Table 3-8 Seasonally stratified distributions of 8-h daily max $O_3$ concentrations (ppb) from the year-round data set (2007-2009). 3-90
Figure 3-27 Highest monitor (by county) 3-year avg (2007-2009) of the 8-h daily max $O_3$ concentration based on the year-round data set (the top map) with seasonal stratification (the four bottom maps). 3-91
Figure 3-28 Highest monitor (by county) 3-year avg (2007-2009) of the 8-h daily max $O_3$ concentration based on the warm-season data set (the top map) with annual stratification (the three bottom maps). 3-92
Table 3-9 Focus cities used in this and previous assessments. 3-95
Table 3-10 City-specific distributions of 8-h daily max $O_3$ concentrations (ppb) from the warm-season data set (2007-2009). 3-96
Figure 3-29 Map of the Atlanta, Georgia, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-97
Figure 3-30 Map of the Boston, Massachusetts, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-97
Figure 3-31 Map of the Los Angeles, California, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-98
Figure 3-32 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Atlanta CSA. 3-100
Figure 3-33 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Boston CSA. 3-100

Figure 3-34 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Los Angeles CSA. 3-101
Figure 3-35 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Atlanta CSA. 3-103
Figure 3-36 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Boston CSA. 3-104
Figure 3-37 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Los Angeles CSA. 3-105
Figure 3-38 Pair-wise monitor coefficient of divergence (COD) expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Atlanta CSA. 3-106
Figure 3-39 Pair-wise monitor coefficient of divergence (COD) expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Boston CSA. 3-107
Figure 3-40 Pair-wise monitor coefficient of divergence (COD) expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Los Angeles CSA. 3-108
Figure 3-41 Terrain map showing the location of two nearby AQS $O_3$ monitoring sites (red dots) along the western edge of the Los Angeles CSA. 3-110
Figure 3-42 Terrain map showing the location of four AQS $O_3$ monitoring sites (red dots) located in or near the city limits in the center of the Boston CSA. 3-111
Table 3-11 Rural focus areas. 3-114
Figure 3-43 Rural focus area site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the rural focus areas. 3-115
Figure 3-44 Terrain map showing the location of five AQS $O_3$ monitoring sites (green/black stars) in Great Smoky Mountain National Park, NC-TN (SMNP). 3-116
Figure 3-45 Pair-wise monitor correlations (left) and coefficients of divergence (CODs) (right) expressed as a histogram (top), contour matrix (middle) and scatter plot vs. distance between monitors (bottom) for 8-h daily max $O_3$ in Great Smoky Mountain National Park, NC-TN (SMNP). 3-117
Figure 3-46 Terrain map showing the location of the AQS $O_3$ monitoring site in Rocky Mountain National Park, Colorado (black/green star) and the Denver, Colorado, CSA (red dots) along with $O_3$ monitoring sites used in the Brodin et al. (2010) study (blue circles). 3-118
Figure 3-47 Terrain map showing the location of two AQS $O_3$ monitoring sites (black/green stars) in Sequoia National Park, CA. 3-119
3.6.3 Temporal Variability 3-120
Figure 3-48 National 8-h daily max $O_3$ trend and distribution across 870 U.S. $O_3$ monitors, 1998-2010 (annual 4th-highest 8-h daily max $O_3$ concentrations in ppm). 3-121
Figure 3-49 National 1-h daily max $O_3$ trend and distribution across 875 U.S. $O_3$ monitors, 1998-2010 (annual 2nd-highest 1-h daily max $O_3$ concentrations in ppm). 3-122
Figure 3-50 Trend in mean 8-h daily max $O_3$ by region, 1998-2010 (mean of the annual 4th-highest 8-h daily max $O_3$ concentrations in ppm). 3-123
Figure 3-51 Trend in mean 1-h daily max $O_3$ by region, 1998-2010 (mean of the annual 2nd-highest 1-h daily max $O_3$ concentrations in ppm). 3-124
Figure 3-52 Individual monitor 8-h daily max $O_3$ design values displayed: (A) for the 2008-2010 period, and (B) as the change since the 2001-2003 period. 3-125
Figure 3-53 Individual monitor 1-h daily max $O_3$ design values displayed: (A) for the 2008-2010 period, and (B) as the change since the 2001-2003 period. 3-126
Figure 3-54 Diel patterns in 1-h avg $O_3$ for Atlanta, Boston and Los Angeles between 2007 and 2009. 3-130
Figure 3-55 Diel patterns in 1-h avg $O_3$ for six rural focus areas between 2007 and 2009. 3-133
3.6.4 Associations with Copollutants 3-134
Figure 3-56 Distribution of Pearson correlation coefficients for comparison of 8-h daily max $O_3$ from the year-round data set with co-located 24-h avg CO, $SO_2$, $NO_2$, $PM_{10}$ and $PM_{2.5}$ from AQS, 2007-2009. 3-135

Figure 3-57 Distribution of Pearson correlation coefficients for comparison of 8-h daily max $O_3$ from the warm-season (May-Sept) data set with co-located 24-h avg CO, $SO_2$, $NO_2$, $PM_{10}$ and $PM_{2.5}$ from AQS, 2007-2009. 3-136

3.7 Chapter Summary 3-137

3.7.1 Physical and Chemical Processes 3-137

3.7.2 Atmospheric Modeling 3-138

3.7.3 Background Concentrations 3-139

3.7.4 Monitoring 3-141

3.7.5 Ambient Concentrations 3-142

3.8 Supplemental Information on $O_3$ Model Predictions 3-144

Figure 3-58 Comparison of time series of measurements of MDA8 $O_3$ concentrations at four CASTNET sites in the Northeast with GEOS-Chem predictions for the base case and for the North American background case during March-August, 2006. 3-145

Figure 3-59 Comparison of time series of measurements of MDA8 $O_3$ concentrations at four CASTNET sites in the Southeast with GEOS-Chem predictions for the base case and for the North American background case during March-August, 2006. 3-146

Figure 3-60 Comparison of time series of measurements of MDA8 $O_3$ concentrations at four CASTNET sites in the Upper Midwest with GEOS-Chem predictions for the base case and for the North American background case during March-August, 2006. 3-146

Figure 3-61 Comparison of time series of measurements of MDA8 $O_3$ concentrations at four CASTNET sites in the Intermountain West with GEOS-Chem predictions for the base case and the North American background case during March-August, 2006. 3-147

Figure 3-62 Comparison of time series of measurements of MDA8 $O_3$ concentrations at four CASTNET sites in the Intermountain West with GEOS-Chem predictions for the base case and the North American background case during March-August, 2006. 3-147

Figure 3-63 Comparison of time series of measurements of MDA8 $O_3$ concentrations at four CASTNET sites in the West with GEOS-Chem predictions for the base case and the North American background case during March-August, 2006. 3-148

Figure 3-64 Comparison of time series of measurements of MDA8 $O_3$ concentrations at three CASTNET sites and the Trinidad Head site in California with GEOS-Chem predictions for the base case and the North American background case during March-August, 2006. 3-148

Figure 3-65 Comparison of MDA8 $O_3$ predicted using GEOS-Chem at 0.5° × 0.667° (and 2° × 2.5° resolution; left figure only) with measurements at Mount Bachelor, Oregon (left); and at Trinidad Head, California (right) from March to August 2006. 3-149

Figure 3-66 Comparison of monthly mean (± 1 standard deviation) $O_3$ calculated GEOS-Chem (in red) with ozonesondes (in black) at Trinidad Head, CA (top) and Boulder, Colorado (bottom) during April and August 2006. 3-149

Figure 3-67 A deep stratospheric $O_3$ intrusion over California on May 28 to May 29, 2010. 3-150

Figure 3-68 A deep stratospheric $O_3$ intrusion over California on June 7 to June 12, 2010. 3-151

Figure 3-69 Box plots showing maximum, interquartile range and minimum $O_3$ concentrations measured at CASTNET sites (black) in the Northeast and predictions from GEOS-Chem at ~50 × 50 km resolution (green) and CAMx at 12 × 12 km resolution (blue) for May-August 2006. 3-152

Figure 3-70 Box plots showing maximum, interquartile range and minimum $O_3$ concentrations measured at CASTNET sites (black) in the Southeast and predictions from GEOS-Chem at ~50 × 50 km resolution (green) and CAMx at 12 × 12 km resolution (blue) for May-August 2006. 3-153

Figure 3-71 Box plots showing maximum, interquartile range and minimum $O_3$ concentrations measured at CASTNET sites (black) in the Central U.S. and predictions from GEOS-Chem at ~50 × 50 km resolution (green) and CAMx at 12 × 12 km resolution (blue) for May-August 2006. 3-154

Figure 3-72 Box plots showing maximum, interquartile range and minimum $O_3$ concentrations measured at CASTNET sites (black) in the Northern Rockies and predictions from GEOS-Chem at ~50 × 50 km resolution (green) and CAMx at 12 × 12 km resolution (blue) for May-August 2006. 3-155
Figure 3-73 Box plots showing maximum, interquartile range and minimum $O_3$ concentrations measured at CASTNET sites (black) in the Southern Rockies and predictions from GEOS-Chem at ~50 × 50 km resolution (green) and CAMx at 12 × 12 km resolution (blue) for May-August 2006. 3-156
Figure 3-74 Box plots showing maximum, interquartile range and minimum $O_3$ concentrations measured at CASTNET sites (black) in the West and predictions from GEOS-Chem at ~50 × 50 km resolution (green) and CAMx at 12 × 12 km resolution (blue) for May-August 2006. 3-157
Figure 3-75 MDA8 $O_3$ in surface air at Gothic, Colorado for March through August 2006. 3-158
3.9 Supplemental Figures of Observed Ambient $O_3$ Concentrations 3-158
3.9.1 Ozone Monitor Maps for the Urban Focus Cities 3-158
Figure 3-76 Map of the Atlanta, Georgia, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-159
Figure 3-77 Map of the Baltimore, Maryland, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-159
Figure 3-78 Map of the Birmingham, Alabama, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-160
Figure 3-79 Map of the Boston, Massachusetts, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-160
Figure 3-80 Map of the Chicago, Illinois, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-161
Figure 3-81 Map of the Dallas, Texas, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-161
Figure 3-82 Map of the Denver, Colorado, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-162
Figure 3-83 Map of the Detroit, Michigan, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-162
Figure 3-84 Map of the Houston, Texas, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-163
Figure 3-85 Map of the Los Angeles, California, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-163
Figure 3-86 Map of the Minneapolis, Minnesota, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-164
Figure 3-87 Map of the New York City, New York, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-164
Figure 3-88 Map of the Philadelphia, Pennsylvania, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-165
Figure 3-89 Map of the Phoenix, Arizona, CBSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-165
Figure 3-90 Map of the Pittsburgh, Pennsylvania, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-166
Figure 3-91 Map of the Salt Lake City, Utah, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-166
Figure 3-92 Map of the San Antonio, Texas, CBSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-167
Figure 3-93 Map of the San Francisco, California, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-167
Figure 3-94 Map of the Seattle, Washington, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-168
Figure 3-95 Map of the St. Louis, Missouri, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways. 3-168
3.9.2 Ozone Concentration Box Plots for the Urban Focus Cities 3-169
Figure 3-96 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Atlanta, Georgia, CSA. 3-169
Figure 3-97 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Baltimore, Maryland, CSA. 3-170

Figure 3-98 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Birmingham, Alabama, CSA. 3-170
Figure 3-99 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Boston, Massachusetts, CSA. 3-171
Figure 3-100 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Chicago, Illinois, CSA. 3-171
Figure 3-101 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Dallas, Texas, CSA. 3-172
Figure 3-102 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Denver, Colorado, CSA. 3-172
Figure 3-103 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Detroit, Michigan, CSA. 3-173
Figure 3-104 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Houston, Texas, CSA. 3-173
Figure 3-105 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Los Angeles, California, CSA. 3-174
Figure 3-106 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Minneapolis, Minnesota, CSA. 3-175
Figure 3-107 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the New York City, New York, CSA. 3-175
Figure 3-108 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Philadelphia, Pennsylvania, CSA. 3-176
Figure 3-109 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Phoenix, Arizona, CBSA. 3-176
Figure 3-110 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Pittsburgh, Pennsylvania, CSA. 3-177
Figure 3-111 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Salt Lake City, Utah, CSA. 3-177
Figure 3-112 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the San Antonio, Texas, CBSA. 3-178
Figure 3-113 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the San Francisco, California, CSA. 3-178
Figure 3-114 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Seattle, Washington, CSA. 3-179
Figure 3-115 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the St. Louis, Missouri, CSA. 3-179
3.9.3 Ozone Concentration Relationships for the Urban Focus Cities 3-180
Figure 3-116 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Atlanta, Georgia, CSA. 3-180
Figure 3-117 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Baltimore, Maryland, CSA. 3-181
Figure 3-118 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Birmingham, Alabama, CSA. 3-182

Figure 3-119 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Boston, Massachusetts, CSA. 3-183
Figure 3-120 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Chicago, Illinois, CSA. 3-184
Figure 3-121 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Dallas, Texas, CSA. 3-185
Figure 3-122 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Denver, Colorado, CSA. 3-186
Figure 3-123 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Detroit, Michigan, CSA. 3-187
Figure 3-124 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Houston, Texas, CSA. 3-188
Figure 3-125 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Los Angeles, California, CSA. 3-189
Figure 3-126 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Minneapolis, Minnesota, CSA. 3-190
Figure 3-127 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the New York City, New York, CSA. 3-191
Figure 3-128 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Philadelphia, Pennsylvania, CSA. 3-192
Figure 3-129 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Phoenix, Arizona, CBSA. 3-193
Figure 3-130 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Pittsburgh, Pennsylvania, CSA. 3-194
Figure 3-131 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Salt Lake City, Utah, CSA. 3-195
Figure 3-132 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the San Antonio, Texas, CBSA. 3-196
Figure 3-133 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the San Francisco, California, CSA. 3-197
Figure 3-134 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Seattle, Washington, CSA. 3-198
Figure 3-135 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the St. Louis, Missouri, CSA. 3-199
Figure 3-136 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Atlanta, Georgia, CSA. 3-200
Figure 3-137 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Baltimore, Maryland, CSA. 3-201
Figure 3-138 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Birmingham, Alabama, CSA. 3-202
Figure 3-139 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Boston, Massachusetts, CSA. 3-203

Figure 3-140 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Chicago, Illinois, CSA. 3-204
Figure 3-141 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Dallas, Texas, CSA. 3-205
Figure 3-142 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Denver, Colorado, CSA. 3-206
Figure 3-143 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Detroit, Michigan, CSA. 3-207
Figure 3-144 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Houston, Texas, CSA. 3-208
Figure 3-145 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Los Angeles, California, CSA. 3-209
Figure 3-146 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Minneapolis, Minnesota, CSA. 3-210
Figure 3-147 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the New York City, New York, CSA. 3-211
Figure 3-148 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Philadelphia, Pennsylvania, CSA. 3-212
Figure 3-149 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Phoenix. Arizona, CBSA. 3-213
Figure 3-150 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Pittsburgh, Pennsylvania, CSA. 3-214
Figure 3-151 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Salt Lake City, Utah, CSA. 3-215
Figure 3-152 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the San Antonio, Texas, CBSA. 3-216
Figure 3-153 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the San Francisco, California, CSA. 3-217
Figure 3-154 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Seattle, Washington, CSA. 3-218
Figure 3-155 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the St. Louis, Missouri, CSA. 3-219
3.9.4 Hourly Variations in $O_3$ for the Urban Focus Cities 3-220
Figure 3-156 Diel patterns in 1-h avg $O_3$ for select CSAs between 2007 and 2009 using the year-round data set for the cold month/warm month comparison (left half) and the warm-season data set for the weekday/weekend comparison (right half). 3-221
Figure 3-157 Diel patterns in 1-h avg $O_3$ for select CSAs between 2007 and 2009 using the year-round data set for the cold month/warm month comparison (left half) and the warm-season data set for the weekday/weekend comparison (right half). 3-222

Figure 3-158 Diel patterns in 1-h avg $O_3$ for select CSAs between 2007 and 2009 using the year-round data set for the cold month/warm month comparison (left half) and the warm-season data set for the weekday/weekend comparison (right half). 3-223
Figure 3-159 Diel patterns in 1-h avg $O_3$ for select CSAs/CBSAs between 2007 and 2009 using the year-round data set for the cold month/warm month comparison (left half) and the warm-season data set for the weekday/weekend comparison (right half). 3-224
Figure 3-160 Diel patterns in 1-h avg $O_3$ for select CSAs/CBSAs between 2007 and 2009 using the year-round data set for the cold month/warm month comparison (left half) and the warm-season data set for the weekday/weekend comparison (right half). 3-225
References 3-226

**4 EXPOSURE TO AMBIENT OZONE 4-1**

4.1 Introduction 4-1
4.2 General Exposure Concepts 4-1
4.3 Exposure Measurement 4-4
4.3.1 Personal Monitoring Techniques 4-4
4.3.2 Indoor-Outdoor Concentration Relationships 4-5
Table 4-1 Relationships between indoor and outdoor $O_3$ concentration. 4-6
4.3.3 Personal-Ambient Concentration Relationships 4-12
Figure 4-1 Variation in hourly personal and ambient concentrations of $O_3$ and $PM_{2.5}$ in various microenvironments during daytime hours. 4-13
Table 4-2 Correlations between personal and ambient $O_3$ concentration. 4-16
Table 4-3 Ratios of personal to ambient $O_3$ concentration. 4-21
4.3.4 Co-exposure to Other Pollutants and Environmental Stressors 4-26
Figure 4-2 Correlations between 1-week concentrations of $O_3$ and copollutants measured near roadways. 4-29
4.4 Exposure-Related Metrics 4-30
4.4.1 Activity Patterns 4-30
Table 4-4 Mean fraction of time spent in outdoor locations by various age groups in the NHAPS study. 4-31
Table 4-5 Mean ventilation rates (L/min) at different activity levels for different age groups. 4-32
Figure 4-3 Distribution of time that NHAPS respondents spent in ten microenvironments based on smoothed 1-min diary data. 4-33
4.4.2 Ozone-Averting Behavior 4-34
4.4.3 Population Proximity to Fixed-Site Ozone Monitors 4-36
Figure 4-4 Map of the Atlanta CSA including $O_3$ monitor locations and major roadways with respect to census block group population density estimates for 2009. 4-37
Figure 4-5 Map of the Boston CSA including $O_3$ monitor locations and major roadways with respect to census block group population density estimates for 2009. 4-38
Figure 4-6 Map of the Los Angeles CSA including $O_3$ monitor locations and major roadways with respect to census block group population density estimates for 2009. 4-39
Table 4-6 Fraction of the 2009 population living within a specified distance of an $O_3$ monitor in selected U.S. cities. 4-42
4.5 Exposure Modeling 4-43
Table 4-7 Characteristics of exposure modeling approaches. 4-44
4.5.1 Concentration Surface Modeling 4-44
4.5.2 Residential Air Exchange Rate Modeling 4-47
4.5.3 Microenvironment-Based Models 4-48
4.6 Implications for Epidemiologic Studies 4-50
4.6.1 Non-Ambient Ozone Exposure 4-51
4.6.2 Spatial and Temporal Variability 4-51
4.6.3 Exposure Duration 4-55
4.6.4 Relationship between Personal Exposure and Ambient Concentration 4-57

4.6.5 Exposure to Copollutants and Ozone Reaction Products 4-58
4.6.6 Averting Behavior 4-58
Figure 4-7 Adjusted asthma hospital admissions by age on lagged $O_3$ by alert status, ages 5-19 years old. 4-60
Figure 4-8 Adjusted asthma hospital admissions by age on lagged $O_3$ by alert status, ages 20-64 years old. 4-60
4.6.7 Exposure Estimation Methods in Epidemiologic Studies 4-61
4.7 Summary and Conclusions 4-62
References 4-65

**5 DOSIMETRY, MODE OF ACTION, AND SPECIES HOMOLOGY 5-1**

5.1 Introduction 5-1
Figure 5-1 Schematic of the $O_3$ exposure and response pathway. 5-2
5.2 Human and Animal Ozone Dosimetry 5-2
5.2.1 Introduction 5-2
Figure 5-2 Representation of respiratory tract regions in humans. 5-3
Figure 5-3 Structure of lower airways with progression from the large airways to the alveolar region. 5-5
5.2.2 Ozone Uptake 5-6
Table 5-1 Human respiratory tract uptake efficiency data. 5-7
Figure 5-4 Total $O_3$ uptake efficiency as a function of breathing frequency at a constant minute ventilation of 30 L/min. 5-13
Table 5-2 General adult human inhalation rates by activity levels. 5-16
Figure 5-5 Modeled effect of exercise on tissue dose of the LRT. 5-18
5.2.3 Ozone Reactions and Reaction Products 5-19
Figure 5-6 Schematic overview of $O_3$ interaction with PUFA in ELF and lung cells. 5-20
Figure 5-7 Details of the $O_3$ interaction with the airway ELF to form secondary oxidation products. 5-28
5.3 Possible Pathways/Modes of Action 5-29
5.3.1 Introduction 5-29
5.3.2 Activation of Neural Reflexes 5-29
5.3.3 Initiation of inflammation 5-33
5.3.4 Alteration of Epithelial Barrier Function 5-38
5.3.5 Sensitization of Bronchial Smooth Muscle 5-40
5.3.6 Modification of Innate/Adaptive Immune System Responses 5-43
5.3.7 Airways Remodeling 5-46
5.3.8 Systemic Inflammation and Oxidative/Nitrosative Stress 5-48
5.3.9 Impaired Alveolar-Arterial Oxygen Transfer 5-50
5.3.10 Summary 5-50
Figure 5-8 The modes of action/possible pathways underlying the health effects resulting from inhalation exposure to $O_3$. 5-51
5.4 Interindividual Variability in Response 5-54
5.4.1 Dosimetric Considerations 5-54
5.4.2 Mechanistic Considerations 5-55
Figure 5-9 Some factors, illustrated in yellow, that likely contribute to the interindividual variability in responses resulting from inhalation of $O_3$. 5-70
5.5 Species Homology and Interspecies Sensitivity 5-71
5.5.1 Interspecies Dosimetry 5-71
Figure 5-10 Humans and animals are similar in the regional pattern of $O_3$ tissue dose distribution. 5-74
Figure 5-11 Oxygen-18 incorporation into different fractions of BALF from humans and rats exposed to 0.4 and 2.0 ppm $^{18}O_3$. 5-75
5.5.2 Interspecies Homology of Response 5-76
5.5.3 Summary 5-79
5.6 Chapter Summary 5-79
References 5-81

**6 INTEGRATED HEALTH EFFECTS OF SHORT-TERM OZONE EXPOSURE 6-1**

6.1 Introduction 6-1

6.2 Respiratory Effects 6-1

6.2.1 Lung Function 6-3

Table 6-1 Activity levels used in controlled exposures of healthy young adults to $O_3$. 6-7

Figure 6-1 Cross-study comparison of mean $O_3$-induced $FEV_1$ decrements following 6.6 hours of exposure to $O_3$. 6-8

Figure 6-2 Frequency distributions of $FEV_1$ decrements observed by Schelegle et al. (2009) in young healthy adults (16 F, 15 M) following 6.6-hour exposures to $O_3$ or filtered air. 6-17

Figure 6-3 Proportion of individuals predicted to have greater than 10%, 15%, and 20% $O_3$-induced $FEV_1$ decrements a following 6.6-hour exposure to $O_3$ with moderate exercise. 6-19

Table 6-2 Mean and upper percentile $O_3$ concentrations in epidemiologic studies of lung function in populations with increased outdoor exposures. 6-30

Figure 6-4 Changes in $FEV_1$ (mL) or PEF (mL/sec) in association with ambient $O_3$ concentrations among children attending summer camp. 6-33

Table 6-3 Changes in $FEV_1$ or PEF in association with ambient $O_3$ concentrations among children attending summer camp for studies presented in Figure 6-4. 6-34

Figure 6-5 Percent change in $FEV_1$ in association with ambient $O_3$ concentrations among adults exercising outdoors. 6-36

Table 6-4 Percent change in $FEV_1$ in association with ambient $O_3$ concentrations among adults exercising outdoors for studies presented in Figure 6-5, and among children exercising outdoors. 6-37

Figure 6-6 Percent change in lung function in association with ambient $O_3$ concentrations among outdoor workers. 6-40

Table 6-5 Percent change in $FEV_1$ or $FEV_1$/FVC in association with ambient $O_3$ concentrations among outdoor workers for studies presented in Figure 6-6. 6-41

Table 6-6 Associations between ambient $O_3$ concentration and $FEV_1$ decrements in different ranges of ambient $O_3$ concentrations. 6-42

Table 6-7 Mean and upper percentile concentrations of $O_3$ in epidemiologic studies of lung function in children with asthma. 6-43

Figure 6-7 Percent change in $FEV_1$ in association with ambient $O_3$ concentrations among children with asthma. 6-45

Table 6-8 Percent change in $FEV_1$ in association with ambient $O_3$ concentrations among children with asthma for studies presented in Figure 6-7 plus others. 6-46

Figure 6-8 Percent change in PEF or $FEF_{25-75\%}$ in association with ambient $O_3$ concentrations among children with asthma. 6-48

Table 6-9 Percent change in PEF or $FEF_{25-75\%}$ in association with ambient $O_3$ concentrations among children with asthma for studies presented in Figure 6-8 plus others. 6-49

Table 6-10 Mean and upper percentile concentrations of $O_3$ in epidemiologic studies of lung function in adults with respiratory disease. 6-56

Table 6-11 Mean and upper percentile concentrations of $O_3$ in epidemiologic studies of lung function in populations not restricted to individuals with asthma. 6-59

Figure 6-9 Percent change in $FEV_1$ or FVC in association with ambient $O_3$ concentrations in studies of children in the general population. 6-60

Table 6-12 Percent change in $FEV_1$ or FVC in association with ambient $O_3$ concentrations in studies of children in the general population presented in Figure 6-9 plus others. 6-61

Table 6-13 Associations between ambient $O_3$ concentration and lung function in studies of adults. 6-63

Figure 6-10 Comparison of $O_3$-associated changes in lung function in single- and copollutant models. 6-67

Table 6-14 Comparison of $O_3$-associated changes in lung function in single- and copollutant models for studies presented in Figure 6-10 plus others. 6-68

6.2.2 Airway Hyperresponsiveness 6-72

6.2.3 Pulmonary Inflammation, Injury and Oxidative Stress 6-76

Table 6-15 Mean and upper percentile $O_3$ concentrations in epidemiologic studies of biological markers of pulmonary inflammation and oxidative stress. 6-84

Figure 6-11 Percent change in exhaled nitric oxide (eNO) in association with ambient $O_3$ concentrations in populations with and without asthma. 6-85
Table 6-16 Percent change in exhaled nitric oxide (eNO) in association with ambient $O_3$ concentrations in populations with and without asthma for studies presented in Figure 6-11. 6-86
Table 6-17 Associations between short-term ambient $O_3$ exposure and biological markers of pulmonary inflammation and oxidative stress. 6-87
Table 6-18 Morphometric observations in non-human primates after acute $O_3$ exposure. 6-98
6.2.4 Respiratory Symptoms and Medication Use 6-100
Table 6-19 Mean and upper percentile $O_3$ concentrations in epidemiologic studies of respiratory symptoms, medication use, and activity levels in children with asthma. 6-103
Figure 6-12 Associations between ambient $O_3$ concentrations and respiratory symptoms in children with asthma. 6-105
Table 6-20 Associations between ambient $O_3$ concentrations and respiratory symptoms in children with asthma for studies presented in Figure 6-12. 6-106
Figure 6-13 Associations between ambient $O_3$ concentrations and asthma medication use. 6-110
Table 6-21 Associations between ambient $O_3$ concentrations and asthma medication use for studies presented in Figure 6-13. 6-111
Table 6-22 Mean and upper percentile $O_3$ concentrations in epidemiologic studies of respiratory symptoms and medication use in adults with respiratory disease. 6-113
Table 6-23 Mean and upper percentile $O_3$ concentrations in epidemiologic studies of respiratory symptoms in populations not restricted to individuals with asthma. 6-115
Figure 6-14 Associations between ambient $O_3$ concentrations and respiratory symptoms in children in the general population. 6-116
Table 6-24 Associations between ambient $O_3$ concentrations and respiratory symptoms in children in the general population for studies represented in Figure 6-14. 6-117
Table 6-25 Associations between ambient $O_3$ concentrations and respiratory symptoms in single- and copollutant models. 6-120
6.2.5 Lung Host Defenses 6-122
6.2.6 Allergic and Asthma-Related Responses 6-128
6.2.7 Hospital Admissions, Emergency Department Visits, and Physicians Visits 6-130
Table 6-26 Mean and upper percentile concentrations of respiratory-related hospital admission and emergency department (ED) visit studies evaluated. 6-132
Figure 6-15 Percent increase in respiratory hospital admissions from natural spline models with 8 df/yr for a 40 ppb increase in 1-h max $O_3$ concentrations for each location of the APHENA study. 6-137
Table 6-27 Corresponding effect estimates for Figure 6-15. 6-138
Figure 6-16 Estimated relative risks (RRs) of asthma hospital admissions for 8-h max $O_3$ concentrations at lag 0-1 allowing for possible nonlinear relationships using natural splines. 6-144
Figure 6-17 Risk ratio for respiratory ED visits and different $O_3$ exposure metrics in Atlanta, GA, from 1993-2004. 6-146
Figure 6-18 Loess C-R estimates and twice-standard error estimates from generalized additive models for associations between 8-h max 3-day average $O_3$ concentrations and ED visits for pediatric asthma. 6-149
Figure 6-19 Percent increase in respiratory-related hospital admission and ED visits in studies that presented all-year and/or seasonal results. 6-153
Table 6-28 Corresponding Effect Estimates for Figure 6-19. 6-154
Figure 6-20 Percent increase in respiratory-related hospital admissions and ED visits for studies that presented single and copollutant model results. 6-156
Table 6-29 Corresponding effect estimates for Figure 6-20. 6-157
6.2.8 Respiratory Mortality 6-158
6.2.9 Summary and Causal Determination 6-159
6.3 Cardiovascular Effects 6-165
6.3.1 Controlled Human Exposure 6-165
6.3.2 Epidemiology 6-168

Table 6-30 Characterization of $O_3$ concentrations (in ppb) from studies of arrhythmias. 6-169
Table 6-31 Characterization of $O_3$ concentrations (in ppb) from studies of heart rate variability. 6-172
Figure 6-21 Odds ratio (95% confidence interval) for ischemic stroke by quintiles of $O_3$ exposure. 6-177
Table 6-32 Characterization of $O_3$ concentrations (in ppb) from studies of biomarkers. 6-178
Table 6-33 Characterization of $O_3$ concentrations (in ppb) from studies of blood pressure. 6-184
Table 6-34 Characterization of $O_3$ concentrations (in ppb) from studies of hospital admissions and ED visits. 6-186
Figure 6-22 Effect estimate (95% CI) per increment ppb increase in $O_3$ for over all cardiovascular ED visits or hospital admissions. 6-191
Table 6-35 Effect estimate (95% CI) per increment ppb increase in $O_3$ for overall cardiovascular ED visits or hospital admissions in studies presented in Figure 6-22. 6-192
Figure 6-23 Effect estimate (95% CI) per increment ppb increase in $O_3$ for congestive heart failure ED visits or hospital admissions. 6-194
Table 6-36 Effect estimate (95% CI) per increment ppb increase in $O_3$ for congestive heart failure ED visits or hospital admissions for studies in Figure 6-23. 6-195
Figure 6-24 Effect estimate (95% CI) per increment ppb increase in $O_3$ for ischemic heart disease, coronary heart disease, myocardial infarction, and angina pectoris ED visits or hospital admissions. 6-196
Table 6-37 Effect estimate (95% CI) per increment ppb increase in $O_3$ for ischemic heart disease, coronary heart disease, myocardial infarction, and angina pectoris ED visits or hospital admissions for studies presented in Figure 6-24. 6-197
Figure 6-25 Effect estimate (95% CI) per increment ppb increase in $O_3$ for stroke ED visits or hospital admissions. 6-198
Table 6-38 Effect estimate (95% CI) per increment ppb increase in $O_3$ for stroke ED visits or hospital admissions for studies presented in Figure 6-25. 6-199
Figure 6-26 Effect estimate (95% CI) per increment ppb increase in $O_3$ for arrhythmia and dysrhythmia ED visits or hospital admissions. 6-200
Table 6-39 Effect estimate (95% CI) per increment ppb increase in $O_3$ for arrhythmia and dysrhythmia ED visits or hospital admissions for studies presented in Figure 6-26. 6-201
6.3.3 Toxicology 6-203
Table 6-40 Characterization of study details for Section 6.3.3. 6-209
6.3.4 Summary and Causal Determination 6-210
6.4 Central Nervous System Effects 6-211
Table 6-41 Central nervous system and behavioral effects of short-term $O_3$ exposure in rats. 6-216
6.4.1 Neuroendocrine Effects 6-217
6.4.2 Summary and Causal Determination 6-218
6.5 Effects on Other Organ Systems 6-219
6.5.1 Effects on the Liver and Xenobiotic Metabolism 6-219
6.5.2 Effects on Cutaneous and Ocular Tissues 6-220
6.6 Mortality 6-220
6.6.1 Summary of Findings from 2006 $O_3$ AQCD 6-220
6.6.2 Associations of Mortality and Short-Term $O_3$ Exposure 6-221
Figure 6-27 Summary of mortality risk estimates for short-term $O_3$ exposure and all-cause (nonaccidental) mortality from all-year and summer season analyses. 6-221
Table 6-42 Corresponding effect estimates for Figure 6-27. 6-222
Table 6-43 Range of mean and upper percentile $O_3$ concentrations in previous and recent multicity studies. 6-223
Table 6-44 Correlations between PM and $O_3$ by season and region. 6-226
Figure 6-28 Scatter plots of $O_3$ mortality risk estimates with versus without adjustment for $PM_{10}$ in NMMAPS cities. 6-227

Figure 6-29 Community-specific $O_3$-mortality risk estimates for nonaccidental mortality per 10 ppb increase in same-day 24-h average summertime $O_3$ concentrations in single-pollutant models and copollutant models with sulfate. 6-229
Figure 6-30 Percent increase in all-cause (nonaccidental) and cause-specific mortality from natural spline models with 8 df/yr from the APHENA study for single- and copollutant models. 6-231
Table 6-45 Corresponding effect estimates for Figure 6-30. 6-232
Table 6-46 Sensitivity of $O_3$ risk estimates per 10 μg/$m^3$ increase in 24-h average $O_3$ concentrations at lag 0-1 to alternative methods for adjustment of seasonal trend, for all-cause mortality using Berkey MLE and TLNISE Hierarchical Models. 6-234
Table 6-47 Additional percent change in $O_3$-related mortality for individual-level characteristics. 6-237
Figure 6-31 Ozone mortality risk estimates and community-specific characteristics, U.S., 1987-2000. 6-239
Table 6-48 Percent change in all-cause mortality, for all ages, associated with a 40ppb increase in 1-h max $O_3$ concentrations at Lag 0–1 at the 25th and 75th percentile of the center-specific distribution of selected effect modifiers. 6-240
Table 6-49 Percentage increase in daily mortality for a 10 ppb increase in 24-h average $O_3$ concentrations during the previous week by geographic region in the U.S., 1987-2000. 6-242
Figure 6-32 Community-specific Bayesian $O_3$-mortality risk estimates in 98 U.S. communities. 6-242
Figure 6-33 Map of spatially dependent $O_3$-mortality coefficients for 8-h max $O_3$ concentrations using summer data. 6-243
Table 6-50 Estimated effect of a 10 ppb increase in 8-h max $O_3$ concentrations on mortality during the summer months for single-day and distributed lag models. 6-247
Figure 6-34 Estimated combined smooth distributed lag for 48 U.S. cities during the summer months. 6-248
Table 6-51 Estimated percent increase in cause-specific mortality (and 95% CIs) for a 10-μg/$m^3$ increase in 8-h daily max $O_3$ during June-August. 6-249
Figure 6-35 Estimated combined smooth distributed lag in 21 European cities during the summer (June-August) months. 6-251
Table 6-52 Percent excess all-cause mortality per 10 ppb increase in daily 8-h max $O_3$ on the same day, by season, month, and age groups. 6-252
Figure 6-36 Estimated combined C-R curve for nonaccidental mortality and 24-hour average $O_3$ concentrations at lag 0-1 using the nonlinear (spline) model. 6-255
Figure 6-37 Percent increase in cause-specific mortality. 6-259
Table 6-53 Corresponding effect estimates for Figure 6-37. 6-260
6.6.3 Summary and Causal Determination 6-261
6.7 Overall Summary 6-264
Table 6-54 Summary of causal determinations for short-term exposures to $O_3$. 6-264
References 6-265

**7 INTEGRATED HEALTH EFFECTS OF LONG-TERM OZONE EXPOSURE 7-1**

7.1 Introduction 7-1
7.2 Respiratory Effects 7-2
7.2.1 Asthma 7-3
Figure 7-1 Interaction of heme-oxygenase genetic variants and $O_3$ level on the Hazard Ratio (HR) of new-onset asthma in the 12 Children's Health Study communities. 7-7
Figure 7-2 Ozone modifies the effect of TNF GG genotype on bronchitic symptoms among children with asthma in the CHS. 7-11
7.2.2 Asthma Hospital Admissions and ED Visits 7-14
Figure 7-3 Ozone-asthma concentration-response relationship using the mean concentration during the entire follow-up period for first asthma hospital admission. 7-16
7.2.3 Pulmonary Structure and Function 7-17

Table 7-1 Respiratory effects in nonhuman primates and rodents resulting from long-term $O_3$ exposure. 7-25
7.2.4 Pulmonary Inflammation, Injury, and Oxidative Stress 7-27
7.2.5 Allergic Responses 7-29
7.2.6 Host Defense 7-30
7.2.7 Respiratory Mortality 7-31
7.2.8 Summary and Causal Determination 7-31
Table 7-2 Summary of selected key new studies examining annual $O_3$ exposure and respiratory health effects. 7-33
Table 7-3 Studies providing evidence concerning potential confounding by PM for available endpoints. 7-35
7.3 Cardiovascular Effects 7-36
7.3.1 Cardiovascular Disease 7-36
Table 7-4 Characterization of study details for Section 7.3.1.2. 7-39
7.3.2 Cardiovascular Mortality 7-39
7.3.3 Summary and Causal Determination 7-40
7.4 Reproductive and Developmental Effects 7-40
7.4.1 Effects on Sperm 7-42
7.4.2 Effects on Reproduction 7-44
7.4.3 Birth Weight 7-45
Figure 7-4 Birthweight deficit by decile of 24-h avg $O_3$ concentration averaged over the entire pregnancy compared with the decile group with the lowest $O_3$ exposure. 7-46
Table 7-5 Brief summary of epidemiologic studies of birth weight. 7-48
7.4.4 Preterm Birth 7-49
Table 7-6 Brief summary of epidemiologic studies of preterm birth (PTB). 7-53
7.4.5 Fetal Growth 7-54
Table 7-7 Brief summary of epidemiologic studies of fetal growth. 7-57
7.4.6 Postnatal Growth 7-57
7.4.7 Birth Defects 7-58
Table 7-8 Brief summary of epidemiologic studies of birth defects. 7-61
7.4.8 Developmental Respiratory Effects 7-61
7.4.9 Developmental Central Nervous System Effects 7-65
7.4.10 Early Life Mortality 7-67
Table 7-9 Brief summary of infant mortality studies. 7-71
Table 7-10 Summary of key reproductive and developmental toxicological studies. 7-73
7.4.11 Summary and Causal Determination 7-74
7.5 Central Nervous System Effects 7-75
7.5.1 Effects on the Brain and Behavior 7-75
Table 7-11 Central nervous system effects of long-term $O_3$ exposure in rats. 7-79
7.5.2 Summary and Causal Determination 7-79
7.6 Carcinogenic and Genotoxic Potential of Ozone 7-80
7.6.1 Introduction 7-80
7.6.2 Lung Cancer Incidence and Mortality 7-82
7.6.3 DNA Damage 7-82
7.6.4 Summary and Causal Determination 7-85
7.7 Mortality 7-85
Figure 7-5 Adjusted $O_3$-mortality relative risk estimates (95% CI) by time period of analysis per subject-weighted mean $O_3$ concentration in the Cancer Prevention Study II by the American Cancer Society. 7-86
Table 7-12 Relative risk (and 95% CI) of death attributable to a 10-ppb change in the ambient $O_3$ concentration. 7-89
7.7.1 Summary and Causal Determination 7-89
7.8 Overall Summary 7-90
Table 7-13 Summary of causal determinations for long-term exposures to $O_3$. 7-91
References 7-92

**8 POPULATIONS POTENTIALLY AT INCREASED RISK FOR OZONE-RELATED HEALTH EFFECTS 8-1**

Table 8-1 Classification of Evidence for Potential At-Risk Factors. 8-3

8.1 Genetic Factors 8-3

Table 8-2 Summaries of results from epidemiologic and controlled human exposures studies of modification by genetic variants. 8-5

Table 8-3 Summaries of results from animal toxicology studies of modification by genetic variants. 8-7

8.2 Pre-existing Disease/Conditions 8-10

Table 8-4 Prevalence of respiratory diseases, cardiovascular diseases, and diabetes among adults by age and region in the U.S. 8-11

8.2.1 Influenza/Infections 8-11

8.2.2 Asthma 8-12

Table 8-5 Prevalence of asthma by age in the U.S. 8-12

8.2.3 Chronic Obstructive Pulmonary Disease (COPD) 8-15

8.2.4 Cardiovascular Disease (CVD) 8-15

8.2.5 Diabetes 8-17

8.2.6 Hyperthyroidism 8-17

8.3 Sociodemographic Factors 8-18

8.3.1 Lifestage 8-18

8.3.2 Sex 8-24

8.3.3 Socioeconomic Status 8-26

8.3.4 Race/Ethnicity 8-28

8.4 Behavioral and Other Factors 8-30

8.4.1 Diet 8-30

8.4.2 Obesity 8-31

8.4.3 Smoking 8-32

8.4.4 Outdoor Workers 8-33

8.4.5 Air Conditioning Use 8-34

8.5 Summary 8-35

Table 8-6 Summary of evidence for potential increased risk of $O_3$-related health effects. 8-36

References 8-38

**9 ENVIRONMENTAL EFFECTS: OZONE EFFECTS ON VEGETATION AND ECOSYSTEMS 9-1**

9.1 Introduction 9-1

Figure 9-1 An illustrative diagram of the major endpoints that $O_3$ may affect in plants and ecosystems. 9-3

9.2 Experimental Exposure Methodologies 9-3

9.2.1 Introduction 9-3

9.2.2 “Indoor,” Controlled Environment, and Greenhouse Chambers 9-4

9.2.3 Field Chambers 9-4

9.2.4 Plume and FACE-Type Systems 9-6

9.2.5 Ambient Gradients 9-7

9.2.6 Comparative Studies 9-8

9.3 Mechanisms Governing Vegetation Response to Ozone 9-10

9.3.1 Introduction 9-10

9.3.2 Ozone Uptake into the Leaf 9-11

Figure 9-2 Ozone uptake from the atmosphere (A), and The anatomy of a dicot leaf (B). 9-14

Figure 9-3 Possible reactions of $O_3$ within water. 9-15

Figure 9-4 The Crigee mechanism of $O_3$ attack of a double bond. 9-15

9.3.3 Cellular to Systemic Responses 9-16

Figure 9-5 Composite diagram of major themes in the temporal evolution of the genetic response to $O_3$ stress. 9-21

Figure 9-6 The oxidative cell death cycle. 9-24
9.3.4 Detoxification 9-24
9.3.5 Effects on Primary and Secondary Metabolism 9-28
9.3.6 Summary 9-34
9.4 Nature of Effects on Vegetation and Ecosystems 9-36
9.4.1 Introduction 9-36
9.4.2 Visible Foliar Injury and Biomonitoring 9-38
9.4.3 Growth, Productivity and Carbon Storage in Natural Ecosystems 9-42
Table 9-1 Ozone effects on plant reproductive processes. 9-47
Table 9-2 Comparison of models used to simulate the ecological consequences of $O_3$ exposure. 9-50
Table 9-3 Modeled effects of $O_3$ on primary production, C exchange, and C sequestration. 9-56
9.4.4 Crop Yield and Quality in Agricultural Systems 9-57
Table 9-4 Summary of recent studies of $O_3$ effects on crops (exclusive of growth and yield). 9-64
Table 9-5 Modeled effects of $O_3$ on crop yield loss at regional and global scales. 9-67
9.4.5 Water Cycling 9-67
Figure 9-7 The potential effects of $O_3$ exposure on water cycling. 9-68
9.4.6 Below-Ground Processes 9-71
Figure 9-8 Conceptual diagram showing where $O_3$ alters C, water and nutrient flow in a tree-soil system, including transfer between biotic and abiotic components below ground that influence soil physical and chemical properties. 9-72
Table 9-6 The effect of elevated $O_3$ on leaf/litter nutrient concentrations. 9-74
Table 9-7 The temporal variation of ecosystem responses to $O_3$ exposure at Aspen FACE site 9-77
9.4.7 Community Composition 9-80
9.4.8 Factors that Modify Functional and Growth Response 9-84
Table 9-8 Response of plants to the interactive effects of elevated $O_3$ exposure and nitrogen enrichment. 9-89
9.4.9 Insects and Other Wildlife 9-92
9.5 Effects-based Air Quality Exposure Indices and Dose Modeling 9-98
9.5.1 Introduction 9-98
9.5.2 Description of Exposure Indices Available in the Literature 9-99
Figure 9-9 Diagrammatic representation of several exposure indices illustrating how they weight concentration and accumulate exposure. 9-100
9.5.3 Important Components of Exposure Indices 9-104
Figure 9-10 Trends in May to September: 12-hour SUM06, Peak 1-hour $O_3$ concentration and number of daily exceedances of 95 ppb for the Crestline site in 1963 to 1999; in relation to trends in mean daily maximum temperature for Crestline and daily reactive organic gases (ROG) and oxides of nitrogen ($NO_X$) for San Bernardino County. 9-107
Figure 9-11 The number of hourly average concentrations between 50 and 89 ppb for the period 1980-2000 for the Crestline, San Bernardino County, CA, monitoring site. 9-108
Figure 9-12 Diurnal (a) conductance through boundary layer and stomata (gbs), (b) ozone concentration, and leaf-level stomatal $O_3$ flux (Fst0l) in control plots from mid-June through August, in (c) 2004 and (d) 2005 in the Aspen FACE experiment. 9-111
Figure 9-13 Maximum 3-month, 12-h W126 plotted against maximum 6-month, 12-h W126. 9-113
9.5.4 Ozone Uptake/Dose Modeling for Vegetation 9-114
9.5.5 Summary 9-116
9.6 Ozone Exposure-Plant Response Relationships 9-117
9.6.1 Introduction 9-117
9.6.2 Estimates of Crop Yield Loss and Tree Seedling Biomass Loss in the 1996 and 2006 Ozone AQCDs 9-120
Figure 9-14 Quantiles of predicted relative yield loss for 34 NCLAN crop experiments. 9-122
Figure 9-15 Quantiles of predicted relative yield loss for 4 crop species in NCLAN experiments. 9-123

Figure 9-16 Quantiles of predicted relative biomass loss for 49 studies of 11 tree species in NHEERL/WED experiments. 9-124
Figure 9-17 Quantiles of predicted relative biomass loss for 4 tree species in NHEERL/WED experiments. 9-125
Table 9-9 Ozone exposures at which 10 and 20% yield loss is predicted for 50 and 75% of crop species. 9-126
Table 9-10 Ozone exposures at which 10 and 20% yield loss is predicted for 50 and 75% of crop species (Droughted versus Watered conditions). 9-126
Table 9-11 Ozone exposures at which 10 and 20% biomass loss is predicted for 50 and 75% of tree species. 9-127
9.6.3 Validation of 1996 and 2006 Ozone AQCD Models and Methodology Using the 90-day 12-h W126 and Current FACE Data 9-127
Table 9-12 Comparison between change in yield observed in the SoyFACE experiment between elevated and ambient $O_3$, and change predicted at the same values of $O_3$ by the median composite function for NCLAN. 9-130
Table 9-13 Comparison between yield observed in the SoyFACE experiment and yield predicted at the same values of $O_3$ by the median composite function for NCLAN. 9-130
Figure 9-18 Comparison of yield observed in SoyFACE experiment in a given year with yield predicted by the median composite function based on NCLAN. 9-131
Figure 9-19 Comparison of composite functions for the quartiles of 7 curves for 7 genotypes of soybean grown in the SoyFACE experiment, and for the quartiles of 11 curves for 5 genotypes of soybean grown in the NCLAN project. 9-132
Table 9-14 Comparison between change in above-ground biomass elevated and ambient $O_3$ in Aspen FACE experiment in 6 year, and change predicted at the same values of $O_3$ by the median composite function for NHEERL/WED. 9-134
Table 9-15 Comparison between above-ground biomass observed in Aspen FACE experiment in 6 year and biomass predicted by the median composite function based on NHEERL/WED. 9-134
Figure 9-20 Comparison between above-ground biomass observed in Aspen FACE experiment in 6 year and biomass predicted by the median composite function based on NHEERL/WED. 9-135
Figure 9-21 Above-ground biomass for one genotype of cottonwood grown in seven locations for one season in 3 years. 9-137
Table 9-16 Meta-analyses of growth or yield studies published since 2005. 9-138
9.6.4 Summary 9-140
Table 9-17 Summary of studies of effects of $O_3$ exposure on growth and yield of agricultural crops. 9-141
Table 9-18 Summary of studies of effects of $O_3$ exposure on growth of natural vegetation. 9-145
9.7 Summary and Conclusions 9-147
Table 9-19 Summary of $O_3$ causal determinations for vegetation and ecosystem effects. 9-148
References 9-149

**10 THE ROLE OF TROPOSPHERIC OZONE IN CLIMATE CHANGE AND UV-B SHIELDING EFFECTS 10-1**

10.1 Introduction 10-1
10.2 Physics of the Earth's Radiation Budget 10-1
Figure 10-1 Diagram of the factors that determine human exposure to ultraviolet radiation. 10-3
10.3 Effects of Tropospheric $O_3$ on Climate 10-4
10.3.1 Background 10-4
10.3.2 Climate Change Evidence and the Influence of Tropospheric $O_3$ 10-5
Figure 10-2 Schematic illustrating the effects of tropospheric $O_3$ on climate. 10-8
Figure 10-3 Global average radiative forcing (RF) estimates and uncertainty ranges in 2005 for anthropogenic $CO_2$, $CH_4$, $O_3$, and other important agents and mechanisms. 10-9
10.3.3 Factors that Influence the Effect of Tropospheric $O_3$ on Climate 10-10

10.3.4 Competing Effects of $O_3$ Precursors on Climate 10-15
Figure 10-4 Components of radiative forcing for emissions of principal gases, aerosols, aerosol precursors, and other changes. 10-17
10.3.5 Calculating Radiative Forcing and Climate Response to Past Trends in Tropospheric $O_3$ Concentrations 10-18
Figure 10-5 Ensemble average 1900-2000 radiative forcing and surface temperature trends (ºC per century) in response to tropospheric $O_3$ concentration changes. 10-19
10.3.6 Calculating Radiative Forcing and Climate Response to Future Trends in Tropospheric $O_3$ Concentrations 10-19
Table 10-1 Changes in anthropogenic emissions, $CH_4$ and tropospheric $O_3$ concentrations between 2000 and 2030, and the associated tropospheric $O_3$ radiative forcing for three scenarios. 10-22
Figure 10-6 Global mean radiative forcing estimates calculated by a set of models for the 2000-2100 change in tropospheric $O_3$ concentrations. 10-24
10.4 UV-B Shielding Effects and Tropospheric $O_3$ 10-25
10.4.1 Background 10-25
10.4.2 Human Exposure and Susceptibility to Ultraviolet Radiation 10-25
10.4.3 Human Health Effects due to UV-B Radiation 10-26
10.4.4 Ecosystem and Materials Damage Effects Due to UV-B Radiation 10-27
10.4.5 UV-B Shielding Effects Associated with Changes in Tropospheric $O_3$ Concentrations 10-28
10.5 Summary and Causal Determinations 10-30
10.5.1 Summary of the Effects of Tropospheric $O_3$ on Climate 10-30
10.5.2 Summary of UV-B Related Effects on Human Health, Ecosystems, and Materials Relating to Changes in Tropospheric $O_3$ Concentrations 10-31
10.5.3 Summary of $O_3$ Causal Determinations 10-32
Table 10-2 Summary of $O_3$ causal determinations for climate and UV-B shielding effects. 10-32
References 10-33

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# ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| 129 | mouse strain (129S1/SvlmJ) |
| α | alpha, ambient exposure factor |
| α-ATD | alpha 1-antitrypsin deficiency |
| α-SMA | alpha-smooth muscle actin |
| α-tocopherol | alpha-tocopherol |
| α-TOH | alpha tocopherol |
| a | air exchange rate of the microenvironment |
| A2 | climate scenario in IPCC |
| AADT | annual average daily traffic |
| A1B | climate scenario in IPCC |
| ABA | abscisic acid |
| ABI | abscisic acid insensitive |
| ABI2 | abscisic acid insensitive Arabidopsis mutant |
| A1c | glycosylated hemoglobin blood test |
| Ach | acetylcholine |
| ACM | (Harvard University) Atmospheric Chemistry Modeling (Group) |
| ACS | American Cancer Society |
| ACS-CPSII | ACS Cancer Prevention Study II |
| ADC | arginine decarboxylase |
| ADSP | Adirondack State Park, NY |
| AER | air exchange rate |
| $AH_2$ | ascorbic acid; ascorbate |
| AHR | airway(s) hyperresponsiveness, airway(s) hyperreactivity |
| AhR | aryl hydrocarbon receptor |
| AHSMOG | (California Seventh Day) Adventist Heath and Smog (Study) |
| AI | alveolar interstitial |
| AIC(s) | Akaike's information criterion |
| AIRS | Aerometric Information Retrieval System; Atmospheric Infrared Sounder (instrument) |
| A/J | mouse strain |
| Ala-9Val | genotype associated with Manganese superoxide dismutase (MnSOD) gene |
| AM | alveolar macrophage(s) |
| ANF | atrial natriuretic factor |
| AOT20 | seasonal sum of the difference between an hourly concentration at the threshold value of 20 ppb, minus the threshold value of 20 ppb |
| AOT30 | seasonal sum of the difference between an hourly concentration at the threshold value of 30 ppb, minus the threshold value of 30 ppb |
| AOT40 | seasonal sum of the difference between an hourly concentration at the threshold value of 40 ppb, minus the threshold value of 40 ppb |
| AOT60 | seasonal sum of the difference between an hourly concentration at the threshold value of 60 ppb, minus the threshold value of 60 ppb |
| AOTx | family of cumulative, cutoff concentration-based exposure indices |
| AP | activated protein |
| A2p | climate scenario in IPCC (preliminary version of A2) |
| APEX | Air Pollutants Exposure (model) |
| APHEA(2) | Air Pollution on Health: a European Approach (study) |
| APHENA | Air Pollution and Health: A European and North American Approach |
| ApoB | apolipoprotein B |
| ApoE | apolipoprotein E |
| APX | ascorbate peroxidase |
| aq | aqueous form: $(aq)O_3$ |
| AQCD | Air Quality Criteria Document |
| AQI | Air Quality Index |
| AQS | (U.S. EPA) Air Quality System (database) |
| AR | acoustic rhinometry |
| AR4 | Fourth Assessment Report (AR4) from the IPCC |
| AR5 | Fifth Assessment Report (AR5) from the IPCC |
| ARG | arginase variants (ex., ARG1, ARG2, ARG1h4) |
| ARIC | Atherosclerosis Risk in Communities |
| ARIES | (Atlanta) Aerosol Research and Inhalation Epidemiology Study |
| atm | atmosphere |
| ATP | adenosine triphosphate |
| ATPase | adenosine triphosphatase; adenosine triphosphate synthase |
| ATS | American Thoracic Society |
| avg | average |
| AVHRR | advanced very high resolution radiometer |
| AX | Annex |
| β | beta, beta coefficient; regression coefficient; standardized coefficient; shape parameter; scale parameter |
| B | boron |
| B1 | climate scenario in IPCC |

| | |
|---|---|
| B6 | mouse strain (C57BL/6J) |
| BAL | bronchoalveolar lavage |
| BALB/c | mouse strain |
| BALF | bronchoalveolar lavage fluid |
| bb | bronchials |
| BB | bronchial airways |
| BC | black carbon |
| B cells | bone-marrow-derived lymphocytes; B lymphocytes |
| B6C3F1 | mouse strain |
| BDNF | brain-derived neurotrophic factor |
| BEAS-2B | human bronchial epithelial cell line |
| BEIS | Biogenic Emissions Inventory System |
| BELD | Biogenic Emissions Landcover Database |
| BIPM | International Bureau of Weights and Measures |
| BM | basement membrane |
| BMI | body mass index |
| BNP | β -type natriuretic peptide |
| BP | blood pressure |
| BPD | biparietal diameter |
| bpm | breaths per minute |
| Br | bromine |
| BRFSS | Behavioral Risk Factor Surveillance System |
| BS | black smoke |
| BSA | bovine serum albumin;<br>body surface area |
| Bsp, BSP | black smoke particles |
| Bt, BT, bt | *Bacillus thuringiensis*; bacterium proteins used in pesticides (or genetically engineered plants produce Bt toxin) |
| BTEX | family of compounds (benzene, toluene, ethylbenzene, and xylene) |
| BW | body weight |
| C | carbon; concentration;<br>(Vitamin C, ascorbate) |
| $^{o}C$ | degrees Celsius |
| $^{13}C$ | carbon-13 isotope |
| C3 | mouse strain (C3H/HEJ) |
| C3 | plants that use only the Calvin cycle for fixing the carbon dioxide from the air |
| C4 | plants that use the Hatch-Slack cycle for fixing the carbon dioxide from the air |
| C16:0 | palmitic acid (saturated fatty acid) |
| C18:1 | unsaturated fatty acid |
| Ca | calcium |
| $C_a$ | ambient concentration |
| [Ca] | calcium concentration |
| $Ca^{2+}$ | calcium ion |
| CA | Canada (ICD-10-CA) |
| CAA | Clean Air Act |
| CALINE4 | California line source dispersion model for predicting air pollutant concentrations near roadways |
| CAM | plants that use crassulacean acid metabolism for fixing the carbon dioxide from the air |
| CAMP | Childhood Asthma Management Program |
| CAMx | Comprehensive Air Quality Model, with extensions |
| CAN | Canada |
| CAP(s) | concentrated ambient particles |
| CAR | centriacinar region |
| CASAC | Clean Air Scientific Advisory Committee |
| CASTNET | Clean Air Status and Trends Network |
| CAT | catalase |
| CB | carbon black; CMAQ mechanisms (ex., CB04, CB05, CB06) |
| C57BL/6 | mouse strain |
| C57BL/6J | mouse strain |
| CBSA | core-based statistical area |
| C/C | carbon of total carbon |
| CCSP | Clara cell secretory protein |
| CD | cluster of differentiation (various receptors on T-cells: CD8+, CD44, etc.);<br>criteria document (see AQCD) |
| CD-1 | mouse strain |
| CDC | Centers for Disease Control and Prevention |
| CF | charcoal-filtered; carbon filtered air |
| CF2 | twice-filtered air (particulate filter and activated charcoal filter) |
| C-fibers | afferent, slow, unmylenated nerves innervating the respiratory system |
| CFR | Code of Federal Regulations |
| CGRP | calcitonin gene-related peptide |
| $CH_3$ | methyl group |
| $CH_4$ | methane |
| $C_2H_2$ | acetylene |
| $C_2H_4$ | ethylene |
| C3H | mouse strain (C3H/HEJ or C3H/OuJ) |
| $C_3H_6$ | propylene |
| CHAD | Consolidated Human Activity Database |
| $CH_3Br$ | methyl bromide |
| $CH_3$-CHO | acetaldehyde |
| $CH_3Cl$ | methyl chloride |
| $CH_3$-CO | acetyl radical(s) |
| CHD | coronary heart disease |
| CHF | congestive heart failure |
| $C_2H_5$–H | ethane |
| C3H/HeJ | mouse strain |
| $CH_3I$ | methyl iodide |

| | |
|---|---|
| CHIP | Effects of Elevated Carbon Dioxide and Ozone on Potato Tuber Quality in the European Multiple Site Experiment |
| $CH_3O_2^{\bullet}$ | methyl peroxy (radical) |
| $CH_3OOH$ | acetic acid; methyl hydroperoxide |
| CHS | Child Health Study |
| CI | confidence interval(s) |
| $C_j$ | airborne $O_3$ concentration at microenvironment j |
| Cl | chlorine |
| $Cl^-$ | chlorine ion |
| $Cl_2$ | chlorine gas |
| CLE | Current Legislation (climate scenario in IPCC) |
| CLM | chemiluminescence method |
| $ClNO_2$ | nitryl chloride |
| cm | centimeter(s) |
| $cm^2$ | square centimeters |
| CM | Clinical Modification (ICD-9-CM) |
| CMAQ | Community Multi-scale Air Quality modeling system |
| CN | constant atmospheric nitrogen deposition (in PnET-CN ecosystem model) |
| CNA | continental North America |
| CNS | central nervous system |
| CO | carbon monoxide; Cardiac output |
| $CO_2$ | carbon dioxide |
| COD | coefficient of divergence; coefficient of determination |
| Col-0 | (Arabidopsis ecotype) Columbia-0 |
| COP | Conference of Parties (to the UNFCCC) |
| COPD | chronic obstructive pulmonary disease |
| COX-2 | cyclooxygenase 2 enzyme |
| C-R | concentration-response |
| CRA | Centro di ricerca per la cerealicoltura (CRA) [The Centre for Cereal Research] – Unit 5: The Research Unit for Cropping Systems in Dry Environments in Bari, Italy (water-stressed conditions) |
| CRP | C-reactive protein |
| CS | corticosteroid |
| CSA | cross-sectional area; combined statistical area |
| csb, Csb | cockayne syndrome (cb) gene/protein group A |
| CSF | colony-stimulating factor |
| CST | central standard time |
| CSTR | continuous stirred tank reactor |
| CSV | comma-separated values (a spreadsheet format) |
| CT | computer tomography |
| CTM(s) | chemical transport model(s) |
| cum avg | cumulative average |
| CUOt | The cumulative stomatal uptake of $O_3$, using a constant $O_3$ uptake rate threshold (t) of nmol/$m^2$/sec |
| CV, C.V. | coefficient of variation |
| cv, c.v. | cultivar |
| CVD | cardiovascular disease |
| CXC | chemokine family of cytokines, with highly conserved motif:cys-xxx-cys (CXC) amino acid group |
| CXCR2 | CXC chemokine receptor 2 (CXCR2) |
| CXR | Chest (x-ray) radiograph(s) |
| CyS | protein cysteines |
| Cys-LT | cysteinyl leukotrienes ($LTC_4$, $LTD_4$, $LTE_4$) |
| cyt | cytosolic-free |
| Δ, δ | delta, difference; change |
| $\Delta FEV_1$ | change in $FEV_1$ |
| $\Delta V_D$ | change in dead space volume of the respiratory tract |
| 2-D | two-dimensional |
| 3-D | three-dimensional |
| DAHPS | 3-deoxy-D-arabino-heptulosonat-7-phosphate synthase |
| DBP | diastolic blood pressure |
| DC(s) | dendritic cell(s) |
| DDM | direct decoupled method |
| DEP(s) | diesel exhaust particle(s) |
| df | degrees of freedom |
| DGGE | denaturing gradient gel electrophoresis |
| DHA | dehydroascorbate |
| DHAR | dehydroascorbate reductase |
| DHBA | 2,3-dihydroxybenzoic acid |
| DLEM | Dynamic Land Ecosystem Model |
| $dm^3$ | cubic decimeter(s) |
| DNA | deoxyribonucleic acid |
| DOAS | differential optical absorption spectroscopy |
| DOC | dissolved organic carbon |
| DR | type of human leukocyte antigens (HLA-DR) |
| *dt* | Portion of time-period spent in microenvironment *j* |
| DTH | delayed-type hypersensitivity |
| DU | Dobson unit(s) |
| DW | dry weight |
| E | embryonic day (ex., E15, E16, etc); [Vitamin] E |
| $E_a$ | exposure to pollutant of ambient origin |
| EBC | exhaled breath condensate (fluid) |
| EC | elemental carbon |
| ECE | endothelin converting enzyme(s) [i.e., ECE-1] |
| ECG | electrocardiogram |

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|---|---|
| ECOPHYS | physiological process modeling to predict the response of aspen forest ecosystems (modeling growth and environmental stress in Populus) |
| ED | emergency department; embryonic day (ex., ED5, ED20) |
| EGEA | (The) Epidemiology (study on) Genetics and Environment of Asthma, (adults and children with asthma) |
| EGEA2 | follow-up study on EGEA (adults with asthma only) |
| EHC-93 | ambient PM reference sample (urban dust [air particles] collected in Ottawa Canada) |
| ELF | extracellular lining fluid |
| EMI | (U.S. EPA) Exposure Model for Individuals |
| $E_{na}$ | exposure to pollutant of nonambient origin |
| ENA-78 | epithelial cell-derived neutrophil-activating peptide 78 |
| eNO | exhaled nitric oxide |
| eNOS | endothelial nitric oxide synthase |
| ENVISAT | (EAS) Earth Observation satellite |
| EOTCP | European Open Top Chamber Programme |
| EP | epithelial cells |
| EPA | U.S. Environmental Protection Agency |
| EPIC | European Prospective Investigation into Cancer and Nutrition |
| ER | emergency room |
| ESA | European Space Agency |
| ET | extrathoracic; endothelin (i.e., ET-1) |
| $ET_1$ | anterior nasal passages within the extrathoracic (ET) region |
| $ET_2$ | oral airway and posterior nasal passages within the extrathoracic (ET) region |
| ETS | environmental tobacco smoke |
| EU | European Union |
| EUS | eastern U.S. |
| Φ | Phi; calculated efficiency |
| ΦPSII-max | maximum photochemical effective quantum yield of PSII |
| f | Fraction of the relevant time period |
| F | female |
| F344 | Fischer 344 (rat strain) |
| F2a | 8-isoprostane (major F2 prostaglandin [8 iso-PGF2a]) |
| FA | filtered air |
| FACE | free-air−$CO_2$ enrichment (system) |
| FACES | Fresno Asthmatic Children's Environment Study |
| $f_B$ | frequency of breathing |
| FC | fibrocartilaginous coat |
| FEF | forced expiratory flow |
| $FEF_{25-75}$ | forced expiratory flow between the times at which 25% and 75% of the vital capacity is reached |
| FEFx | forced expiratory flow after (x)% vital capacity (e.g., after 25, 50, or 75% vital capacity) |
| FEM | Federal equivalent method |
| FeNO | exhaled nitric oxide fraction |
| $FEV_1$ | forced expiratory volume in 1 second |
| FHM | (USDA Forest Service) Forest Health Monitoring Program |
| FIA | (USDA Forest Service) Forest Inventory and Analysis Program |
| $F_{inf}$ | infiltration factor |
| $F_{inf,i}$ | infiltration factor for indoor environment (i) |
| FLAG | Federal land managers' air quality related values workgroup |
| $F_{LRT}$ | fractional uptake efficiency of the lower respiratory tract (LRT) |
| $F_{nose}$ | fractional uptake efficiency via nasal absorption |
| $F_o$ | fraction of time spent in outdoor microenvironments |
| FPM | Forest Pest Management |
| FR | Federal Register |
| FRAP | ferric reducing ability of plasma |
| FRC | functional residual capacity |
| FRM | Federal reference method |
| $F_{RT}$ | fractional uptake efficiency of the respiratory tract (RT) |
| $Fst0_1$ | flux cut off threshold |
| $F_{URT}$ | fractional uptake efficiency of the upper respiratory tract (URT) |
| FVC | forced vital capacity |
| Fv/Fm | a ratio: a measure of the maximum efficiency of Photosystem II |
| FVI | fruits and vegetables index |
| γ | gamma |
| γ-TOH | gamma-tocopherol |
| g, kg, mg, µg, ng, pg | gram(s), kilogram(s), milligram(s), microgram(s), nanogram(s), picogram(s) |
| G | granulocyte; guanosine |
| g | gram(s); gaseous form: (g)$O_3$ |
| GAM | generalized additive model(s) |
| $g_{bs}$ | conductance through boundary layer and stomata |
| GCLC | (glutathione genetic variant) glutamate-cysteine ligase catalytic subunit |
| GCLM | (glutathione genetic variant) glutamate-cysteine ligase modifier subunit |
| G-CSF | granulocyte colony-stimulating factor (receptor) |
| GD | gestational day |
| GEE | generalized estimating equations |

| | |
|---|---|
| GEOS | (NASA) Goddard Earth Observing System model |
| GEOS5 | GEOS version 5 |
| GEOS-Chem | GEOS-Chemistry (tropospheric model) |
| GFAP | glial fibrillary acidic protein |
| GH | growth hormone |
| GHG | greenhouse gas |
| GLM(s) | generalized linear model(s) |
| GMAO | (NASA) Global Modeling and Assimilation Office |
| GM-CSF | granulocyte macrophage colony-stimulating factor |
| GOME | (ESA) Global Ozone Monitoring Experiment (spectrometer) |
| GOMOS | Global Ozone Monitoring by Occultation of Stars (ESA ENVISAT spectrometer measuring long-term trends in $O_3$) |
| G6P | glucose-6-phosphate |
| G6PD | glucose-6-phosphate dehydrogenase |
| GPP | gross primary production |
| G-proteins | GTPases |
| GPT | gas phase titration |
| GR | glutathione reductase |
| GSH | glutathione; reduced glutathione |
| $GSO_3^-/GSO_3^{2-}$ | guanine sulfonates |
| GSR | glutathione reductase |
| GSS | glutathione synthetase |
| GSSG | glutathione disulfide |
| GST | glutathione S-transferase |
| GSTM1 | glutathione S-transferase polymorphism M1 genotypes (GSTM1-null, -GSTM1-sufficient) |
| GSTP1 | glutathione S-transferase polymorphism P1 genotypes |
| GTP | guanosine triphosphate |
| GTPases | G-proteins/enzymes |
| GWP | global warming potential |
| GxE | gene-environmental interaction |
| h | hour(s) |
| h/day | hour(s) per day |
| H; $H^+$; H• | atomic hydrogen, hydrogen ion; hydrogen radical |
| $^3H$ | radiolabeled hydrogen; tritium |
| $H_2$ | molecular hydrogen |
| ha | hectare |
| HA | hyaluronic acid, hospital admission |
| HA(s) | hospital admission(s) |
| Hb | hemoglobin |
| HbA1c | glycosylated hemoglobin (blood test) |
| HC(s) | hydrocarbon(s) |
| HCFC(s) | hydrochlorofluorocarbon(s) |
| HCHO | formaldehyde |
| $H_2CO$ | formaldehyde |
| HCO• | formyl (radical) |
| HDM | house dust mite |
| 2HDM | 2nd-highest daily maximum |
| HDMA | house dust mite allergen |
| $^3He$ | non-radioactive isotope of helium |
| HeJ | $O_3$-resistant C3H mouse strain (C3H/HeJ) |
| HEPA | high efficiency particle air (filter) |
| HERO | Health and Environmental Research Online, NCEA Database System |
| 12-HETE | 12-Hydroxyeicosatetraenoic acid |
| HF | (HRV signal) high-frequency power |
| HFCs | hydrofluorocarbons |
| Hg | mercury |
| HHP-C9 | 1-hydroxy-1-hydroperoxynonane |
| HIST | histamine |
| HLA | human leukocyte antigen |
| HLA-DR | human leukocyte antigen receptor genes |
| HMOX | Heme oxygenase |
| HMOX-1 | heme-oxygenase-1 (polymorphism) |
| HNE | 4-hydroxynonenal |
| $HNO_2$ | nitrous acid |
| $HNO_3$ | nitric acid |
| $HNO_4$ | pernitric acid |
| HO | hydroxyl; heme oxygenase |
| HO• | hydroxyl radical |
| HO-1 | heme oxygenase 1 |
| $HO_2$• | hydroperoxyl; hydroperoxy radical; protonated superoxide |
| $HO_3$• | protonated ozone radical |
| $H_2O$ | water |
| $H_2O_2$ | hydrogen peroxide |
| $H_3O^+$ | hydronium ion |
| $HOCH_2OOH$ | hydroxymethylhydroperoxide |
| HONO | nitrous acid |
| $HO_2NO_2$ | peroxynitric acid |
| HOONO | pernitrous acid |
| HOX | hydrogen radical(s) |
| hPa | hectopascal |
| HPLC | high-pressure liquid chromatography |
| HPOT | 13-hydroperoxide linolenic acid |
| HR | heart rate, hazard ratio |
| $HR_{max}$ | maximum heart rate |
| HRP | horseradish peroxidase |
| HRV | heart rate variability |
| HSC | Houston Ship Channel (Texas) |
| hs-CRP | high-sensitivity C-reactive protein |
| $H_2SO_4$ | sulfuric acid |
| HSP | high speed pellet (after centrifuge spin) |
| HSP70 | heat shock protein 70 |

| | |
|---|---|
| HSS | high speed supernatant (after centrifuge spin) |
| 5-HT | 5-hydroxytryptamine |
| hv | Energy per photon of electromagnetic energy at frequency v |
| HVAC | heating, ventilation, and air conditioning |
| Hz | hertz |
| I | iodine |
| IARC | International Agency for Research on Cancer |
| IAS | interalveolar septum |
| IBM | individual-based model or modeling |
| IC | inspiratory capacity; intracloud (lightning flash) |
| ICAM-1 | intercellular adhesion molecule 1 |
| ICARTT | International Consortium for Atmospheric Research on Transport and Transformation |
| ICAS | Inner City Asthma Study |
| ICC | intraclass correlation coefficient |
| ICD | implantable cardioverter defibrillator(s); International Classification of Diseases |
| ICD-9 | International Classification of Disease 9th revision |
| ICD-10 | International Classification of Disease 10th revision |
| ICEM | Indoor Chemistry and Exposure Model |
| ICNIRP | International Commission on Non-ionizing Radiation Protection |
| ICP Forests | International Cooperative Programme on Assessment of Air Pollution Effects on Forests |
| ICU | Intensive Care Unit |
| ICVE | ischemic cerebrovascular events |
| IDW | inverse-distance-weighted |
| IFN | interferon (e.g., IFN-()) |
| IFN-γ | interferon-gamma |
| Ig | immunoglobulin (e.g., IgE) |
| IgA | immunoglobulin A |
| IgE | immunoglobulin E |
| IGF-1 | insulin-like growth factor 1 |
| IgG | immunoglobulin G |
| IgM | immunoglobulin M |
| IHD | ischemic heart disease |
| IL | interleukin (e.g., IL-2, IL-4, IL-6, IL -8, etc.) |
| IL-1β | interleukin-1β |
| Ile | isoleucine |
| i.m. | intramuscular (route) |
| IMPACT | Interactive Modeling Project for Atmospheric Chemistry and Transport |
| IMPROVE | Interagency Monitoring of Protected Visual Environment |
| IN | intranasal |
| INF | interferon |
| inh | inhalation |
| iNKT | invariant (type I) natural killer T-cell |
| iNOS | inducible nitric oxide synthase |
| INRA | National agronomical research institute (INRA) in Thiverval-Grignon. France (adequately-watered conditions) |
| INTRASTAND | a stand-level model designed for hourly, daily and annual integration of forest carbon and water cycle fluxes |
| I/O | indoor-outdoor ratio |
| IOM | Institute of Medicine |
| i.p. | intraperitoneal (route) |
| IPCC | Intergovernmental Panel on Climate Change |
| IPCC-A2 | Intergovernmental Panel on Climate Change 2nd Assessment Report |
| IPCC-AR4 | Intergovernmental Panel on Climate Change 4th Assessment Report |
| IPCC-AR5 | Intergovernmental Panel on Climate Change 5th Assessment Report |
| IPCC-TAR | Intergovernmental Panel on Climate Change Third Assessment Report |
| IPMMI | International Photolysis Frequency Measurement and Modeling Inter-comparison |
| IQR | interquartile range |
| IR | infrared |
| I/R | ischemia-reperfusion |
| IRIS | Integrated Risk Information System |
| IRP | Integrated Review Plan for the Ozone National Ambient Air Quality Standards |
| ISA | Integrated Science Assessment |
| ISCCP | International Satellite Cloud Climatology Project |
| ISO | International Standards Organization |
| 8-iso-PGF | 8-isoprostane |
| IT | intratracheal |
| IU | International Units |
| IUGR | intrauterine growth restriction |
| i.v. | intravenous (route) |
| IVF | in vitro fertilization |
| j | Microenvironment |
| JA | jasmonic acid |
| Jmax | maximum rate of electron transport (for regeneration of RuBP) |
| JNK | jun N-terminal kinase |
| JPL | Jet Propulsion Laboratory |
| κ | kappa |

| | |
|---|---|
| κB | kappa B |
| k | dissociation rate; root:shoot allometric coefficient; rate of $O_3$ loss in the microenvironment |
| K | potassium |
| $K^+$ | potassium ion |
| $K_a$ | intrinsic mass transfer coefficient/parameter |
| KC | keratinocyte-derived chemokine |
| kg | kilogram |
| $K_g$ | mass transfer coefficient for gas phase |
| kHz | kilohertz |
| kJ | kilojoules |
| Kl | mass transfer coefficient for liquid phase |
| km | kilometer |
| KM | particle optical reflectance |
| KML | keyhole markup language |
| KMZ | zipped KML computer language |
| KO | knockout |
| Kr | reaction rate constant |
| KROFEX | Krauzberg Ozone Fumigation Experiment |
| L, dL, mL, µL | Liter, deciLiter, milliLiter, microLiter |
| L0 | Lag (e.x., Lag 0, Lag 1, etc.) |
| LAI | leaf area index |
| LBL | Lawrence Berkeley Laboratory |
| LBLX | Lawrence Berkeley Laboratory model including airflow from natural ventilation |
| Lb(s) | pound(s) |
| LBW | low birth weight |
| $LC_{50}$ | median lethal concentration |
| LCL | lower 95th% confidence limit |
| LDH | lactate dehydrogenase |
| LDL | low-density lipoprotein; lower detectable level |
| LF | (HRV signal) low-frequency power |
| LFHFR | low frequency/high frequency (ratio) |
| LFT | lower free troposphere |
| LI | labeling index |
| LIDAR | Light Detection and Ranging (remote sensing system) |
| LIF | laser-induced fluorescence |
| LINKAGES | individual-based model of forest succession |
| LIS | lateral intercellular space |
| LLJ | low-level jet |
| L/min | liters per minute |
| Ln | Natural logarithm |
| LnRMSSD | natural log of RMSSD; measure of HRV |
| lnSDNN | natural log of the standard deviation of NN intervals in an EKG |
| LOAEL | lowest observed adverse effect level |
| LOD | limit of detection |
| LOEL | lowest-observed-effect level |
| LOESS | locally weighted scatterplot smoothing |
| LOP | lipid ozonation products |
| LOSU | level of scientific understanding |
| LOWESS | locally weighted scatter plot smoother |
| LOX-1 | Lipoxygenase; lectin-like oxidized low density lipoprotein receptor-1 |
| LPS | lipopolysaccharide |
| LRS | lower respiratory symptoms |
| LRT | lower respiratory tract; lower airways; Long range transport |
| LST | local standard time |
| LT | leukotriene (e.g., LTB4, LTC 4, LTD4, LTE4); local time |
| LT-α | lymphotoxin-α |
| LTA | lymphotoxin-alpha |
| LUR | land use regression |
| LVEDD | left ventricular chamber dimensions at end diastole |
| LVEDP | left ventricular end diastolic pressure |
| LWC | liquid water content |
| µ | mu, micro |
| µeq | microequivalent |
| µg | microgram |
| µg/$m^3$ | micrograms per cubic meter |
| µm | micrometer, micron |
| m, cm, µm, nm | meter(s), centimeter(s), micrometer/[micron](s), nanometer(s) |
| M | male |
| M, mM, µM, nM, pM | Molar, milliMolar, microMolar, nanoMolar, picoMolar |
| $m^2$ | square meters |
| $m^3$ | cubic meters |
| M# | Month (M1 Month1; M2 Month2; M3 Month3; M4 Month4) |
| M2 | type of muscarinic receptor |
| M7 | 7-hour seasonal mean |
| M12 | 12-hour seasonal mean of $O_3$ |
| ma | moving average |
| mAOT | modified accumulated exposure over threshold |
| MAP | mitogen-activated protein; mean arterial pressure |
| MAPK | mitogen-activated protein kinase(s), MAP kinase |
| MAQSIP | Multiscale Air Quality Simulation Platform (model) |
| MARAT | Mid-Atlantic Regional Assessment Team |
| MARCO | Macrophage receptor with collagenous structure |

| | |
|---|---|
| max | maximum |
| MBL | marine boundary layer |
| MCA | minimum cross-sectional area |
| MCCP | Mountain Cloud Chemistry Program |
| Mch; MCh | methacholine |
| MCM | master chemical mechanism |
| MCP-1 | monocyte chemotactic protein 1 |
| MDA | malondialdehyde |
| MDAR | monodehydroascorbate reductase |
| MDI | Mediterranean diet index |
| MDL | minimum detection level |
| MED | minimal erythema dose |
| $MEF_{50\%}$ | maximal midexpiratory flow at 50% of forced vital capacity |
| MEGAN | model of emissions of gases and aerosols from nature |
| MeJA | methyl jasmonate |
| MENTOR | Modeling Environment for Total Risk Studies |
| METs | metabolic equivalent unit(s) [of work] |
| MFR | Maximum Feasible Reduction |
| Mg | magnesium |
| MGDG | monogalactosyl diacylglycerol |
| $mg/m^3$ | milligrams per cubic meter |
| MHC | major histocompatibility complex |
| mi | mile(s) |
| MI | myocardial infarction, “heart attack” |
| MIESR | matrix isolation electron spin resonance (spectroscopy) |
| min | minute; minimum |
| MIP | macrophage inflammatory protein |
| MIP-2 | macrophage inflammatory protein 2 |
| mL | milliliter |
| mL/min | milliliter(s) per minute |
| MLN | mediastinal lymph node |
| Mm | megameter |
| mm | millimeter(s) |
| MM Mt. | Mt. Mitchell site |
| MM5 | National Center for Atmospheric Research/Penn State Mesoscale Model (version 5) |
| MMAD | mass median aerodynamic diameter; mass median aerodynamic density |
| MMEF | maximal midexpiratory flow |
| mmHg | millimeters of mercury |
| MMMD | mean maximum mixing height depth |
| MMP-2 | matrix metalloproteinase-2 |
| MMP-3 | matrix metalloproteinase-3 |
| MMP-9 | metalloproteinase-9 |
| MMSP | Mount Mitchell State Park, NC |
| Mn | manganese |
| M/N | pooled data from mouth and nasal exposure |
| MnSOD | Manganese superoxide dismutase |
| mo | month(s) |
| MOA(s) | mode(s) of action |
| MOBILE | (U.S. EPA) mobile vehicle emission factor model (on-road vehicles) |
| MOBILE6 | vehicle emissions modeling software version 6; replaced by MOVES |
| MODNR | Missouri Department of Natural Resources |
| MONICA | Monitoring of Trends and Determinants in Cardiovascular Disease |
| MoOx | molybdenum oxides |
| MOSES | Met Office Surface Exchange Scheme |
| MOVES | Motor Vehicle Emission Simulator (replaced MOBILE6; for estimating emissions from cars, trucks, and motorcycles |
| MOZAIC | Measurement of Ozone and Water Vapor by Airbus In-Service Aircraft |
| MOZART | Model for Ozone and Related chemical Tracers |
| MPAN | peroxymethacryloyl nitrate; peroxy-methacrylic nitric anhydride |
| MPO | myeloperoxidase |
| MQL | Minimum quantification limit |
| MRI | magnetic resonance imaging; Midwest Research Institute; Meteorological Research Institute |
| mRNA | messenger RNA |
| ms | millisecond(s) |
| MS | mass spectrometry; Mt. Moosilauke site |
| MSA | Metropolitan Statistical Area; methane sulfonic acid |
| MSL | mean sea level |
| MS/MS | tandem mass spectrometry |
| MT | million ton(s); metric ton(s) |
| MT, Mt | metallothionein |
| MT1 | mitochondria |
| MTBE | methyl-tertiary-butyl ether |
| mtDNA | mitochondrial DNA |
| Mtn | mountain |
| MW | molecular weight |
| MyD88 | myeloid differentiation primary response gene 88 |
| n, N | number; number of observations |
| N | nitrogen; North; nasal exposure by natural breathing |
| $^{15}N$ | nitrogen-15, stable isotope of nitrogen |
| $N_2$ | molecular nitrogen; nonreactive nitrogen |
| Na | sodium |
| NA | noradrenaline; North American |

| | |
|---|---|
| NA; N/A | not available; not applicable |
| $Na^+$ | sodium ion |
| NAAQS | National Ambient Air Quality Standards |
| NAD | nicotinamide adenine nucleotide |
| NADH | reduced nicotinamide adenine dinucleotide; nicotinamide adenine dinucleotide dehydrogenase |
| NADP | National Atmospheric Deposition Program |
| NADPH | reduced nicotinamide adenine dinucleotide phosphate |
| NADPH-CR | reduced nicotinamide adenine dinucleotide phosphate - cytochrome c reductase |
| NaE | sodium erythorbate |
| NAG | N-acetyl-glucosaminidase |
| Na-K-ATPase | sodium-potassium-dependent adenosine triphosphatase |
| NAMS | National Ambient Monitoring Stations |
| NAPAP | National Acid Precipitation Assessment Program |
| NAPBN | National Air Pollution Background Network |
| NARE | North Atlantic Regional Experiment |
| NARSTO | North American Regional Strategy for Tropospheric Ozone |
| NAS | National Academy of Sciences; Normative Aging Study |
| NASA | National Aeronautics and Space Administration |
| NBS | National Bureau of Standards |
| NBTH | 3-methyl-2-benzothiazolinone acetone azine |
| NCE | net carbon exchange |
| NCEA | National Center for Environmental Assessment |
| NCEA-RTP | NCEA Division in Research Triangle Park, NC |
| NCHS | National Center for Health Statistics |
| NCICAS | National Cooperative Inner-City Asthma Study |
| NCLAN | National Crop Loss Assessment Network |
| NCore | National Core multipollutant monitoring network |
| NC-R | resistant clones of white clover |
| NC-S | sensitive clones of white clover |
| ND; n.d. | not detectable; not detected; no data |
| 2ndHDM | 2nd-highest daily maximum |
| NDF | neutral detergent fiber |
| NEE | net ecosystem exchange (of carbon or $CO_2$) |
| NEI | National Emissions Inventory |
| NEM | National Ambient Air Quality Standards Exposure Model |
| NEP | Net Ecosystem Production |
| NERL | National Exposure Research Laboratory |
| NESCAUM | Northeast States for Coordinated Air Use Management |
| NF | National Forest; non-filtered air |
| NF-κB | nuclear factor kappa B |
| ng | nanogram(s) |
| NGF | nerve growth factor |
| NH | northern hemisphere |
| $NH_3$ | ammonia |
| $NH_4^+$ | ammonium ion |
| $NH_4HSO_4$ | ammonium bisulfate |
| $(NH_4)_2HSO_4$ | ammonium sulfate |
| NHANES | National Health and Nutrition Examination Survey |
| NHANES III | National Health and Nutrition Examination Survey III |
| NHAPS | National Human Activity Pattern Survey |
| NHEERL | (U.S. EPA) National Health and Environmental Effects Research Laboratory |
| NHIS | National Health Interview Survey |
| $(NH_4)_2SO_4$ | ammonium sulfate |
| NIH | National Institutes of Health |
| NIST | National Institute of Standards and Technology |
| NK | natural killer cells; neurokinin |
| NKT | natural killer T-cells |
| NL | nasal lavage |
| NLF | nasal lavage fluid |
| NM | National Monument |
| NMHC(s) | nonmethane hydrocarbon(s) |
| NMMAPS | National Morbidity, Mortality, and Air Pollution Study |
| NMOC(s) | nonmethane organic compound(s) |
| NMVOCs | nonmethane volatile organic compounds |
| NN | normal-to-normal (NN or RR) time interval between each QRS complex in the EKG |
| NNK | 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone |
| nNOS | neuronal nitric oxide synthase (NOS) |
| NO | nitric oxide |
| ·NO | nitric oxide concentration (interpunct NO) |
| $NO_2$ | nitrogen dioxide |
| $NO_3$; $NO_3$• | nitrate, nitrate radical |
| $NO_3^-$ | nitrate, nitrate ion |
| $N_2O$ | nitrous oxide |
| $N_2O_5$ | dinitrogen pentoxide |
| NOAA | National Oceanic and Atmospheric Administration |
| NOAEL | no observed adverse effect level |

| | |
|---|---|
| NOS | nitric oxide synthase (types, NOS-1, NOS-2, NOS-3) |
| $NO_X$ | nitrogen oxides, oxides of nitrogen ($NO + NO_2$) |
| $NO_Y$ | sum of $NO_X$ and $NO_Z$; odd nitrogen species; total oxidized nitrogen |
| $NO_Z$ | sum of all inorganic and organic reaction products of $NO_X$ (HONO, $HNO_3$, $HNO_4$, organic nitrates, particulate nitrate, nitro-PAHs, etc.) |
| NP | National Park |
| NPP | net primary production |
| NPS | National Park Service, U.S. Department of the Interior |
| NQO1 | NAD(P)H-quinone oxidoreductase (genotype) |
| NQO1wt | NAD(P)H-quinone oxidoreductase wild type (genotype) |
| NR | not reported |
| Nr | reactive nitrogen |
| NRC | National Research Council |
| Nrf-2 | nuclear factor erythroid 2-related factor 2 |
| Nrf2-ARE | NF-e2-related factor 2-antioxidant response element |
| NS; n.s. | nonsignificant; non-smoker; national seashore; natural spline |
| NSAID | non-steroidal anti-inflammatory agent |
| NSBR | nonspecific bronchial responsiveness |
| NSF | National Science Foundation |
| NTE | nasal turbinate epithelial (cells) |
| NTN | National Trends Network |
| NTP | National Toxicology Program |
| NTRMs | NIST Traceable Reference Materials |
| NTS | nucleus of the solitary tract (in brainstem) |
| NWR | national wildlife refuge |
| NWS | National Weather Service |
| NZW | New Zealand white (rabbit) |
| O | oxygen; horizon forest floor |
| $^{18}O$ | oxygen-18, stable isotope of oxygen |
| $O_2$ | molecular oxygen |
| $O_2^-$ | superoxide |
| $O_2$• | superoxide radical |
| $^1O_2$ | singlet oxygen |
| $O_3$ | ozone |
| $^{18}O_3$ | (oxygen-18 labeled) ozone |
| $O_3$* | electronically excited ozone |
| OAQPS | Office of Air Quality Planning and Standards |
| OAR | Office of Air and Radiation |
| OBMs | observationally based methods |
| OC | organic carbon |
| OD | outer diameter; optical density |
| $O(^1D)$ | electronically excited oxygen atom |
| OH, OH• | hydroxyl group, hydroxyl radical |
| 8-OHdG | 8-hydroxy-2′-deoxyguanosine |
| OLS | ordinary least squares |
| OMI | Ozone Monitoring Instrument |
| ON | Ontario |
| $ONOO^-$ | peroxynitrate ion |
| $O(^3P)$ | ground-state oxygen atom |
| OPE | ozone production efficiency |
| OPECs | Outdoor Plant Environment Chambers |
| OR | odds ratio |
| ORD | Office of Research and Development |
| OSHA | Occupational Safety and Health Administration |
| OTC | open-top chamber |
| OuJ | $O_3$-sensitive C3H mouse strain (C3H/OuJ) |
| OVA | ovalbumin |
| OX | odd oxygen species; total oxidants |
| OxComp | oxidative capacity of the atmosphere |
| oz | ounce(s) |
| P | pressure in atmospheres; plants grown in pots; phosphorus; penetration fraction of $O_3$ into the microenvironment; pulmonary region |
| p | probability value |
| P450 | cytochrome P450 |
| p53 | cell cycle protein gene |
| P90 | 90th percentile of the absolute difference in concentrations |
| PACF | partial autocorrelation function of the model residuals |
| PAD | peripheral arterial disease; pollutant-applied dose |
| PAF | platelet-activating factor; paroxysmal atrial fibrillation |
| PAH(s) | polycyclic aromatic hydrocarbon(s) |
| PAI-1 | plasminogen activator fibrinogen inhibitor-1 |
| PAL | phenylalanine ammonia lyase |
| PAMS | Photochemical Assessment Monitoring Stations network |
| PAN | peroxyacetyl nitrate |
| $PaO_2$ | arterial oxygen pressure |
| PAPA | Public Health and Air Pollution in Asia |
| PAR | photosynthetically active radiation; proximal alveolar region |
| $P_{atm}$ | Pressure in atmospheres |
| p-ATP | para-acetamidophenol |
| Pb | Lead |
| PBL | planetary boundary layer; peripheral blood lymphocytes |

| | |
|---|---|
| PBM | population-based model or modeling |
| PBN | C-phenyl N-tert-butyl nitrone |
| PBPK | physiologically based pharmacokinetic (model) |
| PBS | phosphate buffered saline |
| PC | phosphatidylchloline |
| $PC_{20}$ | provocative concentration that produces a 20% decrease in forced expiratory volume in 1 second |
| $PC_{20}FEV_1$ | provovative concentration that produces a 20% decrease in $FEV_1$ |
| $PC_{50}$ | provocative concentration that produces a 50% decrease in forced expiratory volume in 1 second |
| PCA | principal component analysis |
| PC-ALF | 1-palmitoyl-2-(9-oxononanoyl)-sn-glycero-3-phosphocholine |
| PCD | programmed cell death |
| PCl | picryl chloride |
| pCNEM | Canadian version of National Ambient Air Quality Standards Exposure Model |
| $PCO_2$ | Average partial pressure of $O_2$ in lung capillaries |
| $pCO_2$ | partial pressure of carbon dioxide |
| PCR | polymerase chain reaction |
| PCR-DGGE | PCR–denaturing gradient gel electrophoresis |
| PD | pregnancy day |
| $PD_{20}$ | provocative dose that produces a 20% decrease in $FEV_1$ |
| $PD_{20}FEV_1$ | provocative dose that produces a 20% decrease in $FEV_1$ |
| $PD_{100}$ | provocative dose that produces a 100% increase in sRAW |
| $PD_{100}S_{Raw}$ | provocative dose that produces a 100% increase in $S_{Raw}$ |
| PDI | pain on deep inspiration |
| PE | postexposure, phosphatidylethanolamine |
| PEF | peak expiratory flow |
| $PEF_{0.75}$ | peak expiratory flow in 0.75 second |
| PEFR | peak expiratory flow rate |
| PEFT | time to peak flow |
| PEG-CAT | polyethylene glycol-catalase |
| PEG-SOD | polyethylene glycol-superoxide dismutase |
| PEM(s) | personal exposure monitor(s) |
| Penh | enhanced pause |
| PEPc | phosphoenolpyruvate carboxylase |
| PFD | photosynthetic flux density |
| PFT | pulmonary function test |
| pg | picogram(s) |
| PG | prostaglandin (e.g., PGE2, PGF2); phosphatidylglycerol |
| 6PGD | 6-phosphogluconate dehydrogenase |
| PGE2 | prostaglandin E2 |
| PGF2α | prostaglandin F2-alpha |
| PGHS-2 | prostaglandin endoperoxide G/H synthase 2 |
| PGP | protein gene product (e.g., PGP9.5) |
| PGSM | Plant Growth Stress Model |
| pH | relative acidity; Log of the reciprocal of the hydrogen ion concentration |
| PHA | phytohemagglutinin A |
| PI | phosphatidylinositol; probability interval; posterior interval |
| PIF | peak inspiratory flow |
| PiZZ | respiratory phenotype |
| PK | pharmacokinetics |
| pKa | dissociation constant |
| PLFA | phospholipid fatty acid |
| PM | particulate matter |
| $PM_X$ | Particulate matter of a specific size range not defined for regulatory use. Usually X refers to the 50% cut point, the aerodynamic diameter at which the sampler collects 50% of the particles and rejects 50% of the particles. The collection efficiency, given by a penetration curve, increases for particles with smaller diameters and decreases for particles with larger diameters. The definition of $PM_X$ is sometimes abbreviated as “particles with a nominal aerodynamic diameter less than or equal to X µm” although X is usually a 50% cut point. |
| $PM_{2.5}$ | In general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 2.5 µm; a measurement of fine particles. In regulatory terms, particles with an upper 50% cut-point of 2.5 µm aerodynamic diameter (the 50% cut point diameter is the diameter at which the sampler collects 50% of the particles and rejects 50% of the particles) and a penetration curve as measured by a reference method based on Appendix L of 40 CFR Part 50 and designated in accordance with 40 CFR Part 53, by an equivalent method designated in accordance with 40 CFR Part 53, or by an approved regional method designated in accordance with Appendix C of 40 CFR Part 58. |

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|---|---|
| $PM_{10}$ | In general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 10 μm; a measurement of thoracic particles (i.e., that subset of inhalable particles thought small enough to penetrate beyond the larynx into the thoracic region of the respiratory tract). In regulatory terms, particles with an upper 50% cut-point of 10 ± 0.5 μm aerodynamic diameter (the 50% cut point diameter is the diameter at which the sampler collects 50% of the particles and rejects 50% of the particles) and a penetration curve as measured by a reference method based on Appendix J of 40 CFR Part 50 and designated in accordance with 40 CFR Part 53 or by an equivalent method designated in accordance with 40 CFR Part 53. |
| $PM_{10-2.5}$ | In general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 10 μm and greater than a nominal 2.5 μm; a measurement of thoracic coarse particulate matter or the coarse fraction of $PM_{10}$. In regulatory terms, particles with an upper 50% cut-point of 10 μm aerodynamic diameter and a lower 50% cut-point of 2.5 μm aerodynamic diameter (the 50% cut point diameter is the diameter at which the sampler collects 50% of the particles and rejects 50% of the particles) as measured by a reference method based on Appendix O of 40 CFR Part 50 and designated in accordance with 40 CFR Part 53 or by an equivalent method designated in accordance with 40 CFR Part 53. |
| $PM_{10C}$ | The $PM_{10-2.5}$ concentration of $PM_{10-2.5}$ measured by the 40 CFR Part 50 Appendix O reference method which consists of currently operated, co-located low-volume (16.7 Lpm) $PM_{10}$ and $PM_{2.5}$ reference method samplers. |
| p38MAPK | p38 mitogen-activated protein kinase(s) |
| PM-CAMx | Comprehensive Air Quality Model with extensions and with particulate matter chemistry |
| PMN(s) | polymorphonuclear leukocyte(s) |
| PMT | photomultiplier tube |
| PND | post natal day |
| pNEM | probabilistic National Exposure Model |
| PnET | Photosynthetic EvapoTranspiration model |
| PNN | proportion of interval differences of successive normal-beat intervals in EKG |
| PNN50 | proportion of interval differences of successive normal-beat intervals greater than 50 ms in EKG |
| $PO_2$ | partial pressure of oxygen |
| POC | particulate organic carbon |
| POD | peroxidase |
| polyADPR | poly(adenosinediphosphate-ribose) |
| POMS | Portable Ozone Monitoring Systems |
| ppb | parts per billion |
| ppb-h | parts per billion per hour |
| ppbv | parts per billion by volume |
| pphm | parts per hundred million |
| ppm | parts per million |
| ppm-h | parts per million hours; weighted concentration values based on hourly concentrations: usually summed over a certain number of hours, day(s), months, and/or season. |
| ppmv | parts per million by volume |
| PPN | peroxypropionyl nitrate; peroxypropionic nitric anhydride |
| PPPs | power plant plumes |
| ppt | parts per trillion |
| pptv | parts per trillion by volume |
| PQH2 | plastoquinone |
| PR | pathogenesis-related (protein) |
| PR-1 | promoter region 1 |
| PRB | policy-relevant background |
| preproET-1 | pre-protein form of ET-1 mRNA |
| PRYL | predicted relative yield (biomass) loss |
| PS | penalized spline |
| PS | paradoxical sleep |
| PS II | Photosystem II: enzyme that uses light to obtain electrons from water (for photosynthesis). |
| PSA | picryl sulfonic acid |
| PSC | polar stratospheric clouds |
| PTB | preterm birth |
| PTR-MS | proton-transfer-reaction mass spectroscopy |
| PU, PUL | pulmonary |
| PUFA(s) | polyunsaturated fatty acid(s) |
| PV | potential vorticity |
| PVCD | peripheral vascular and cerebrovascular disease |
| PVD | peripheral vascular disease |
| PVOCs | photochemical volatile organic compounds |
| PWM | pokeweed mitogen |
| PWTES | (left ventricular) posterior wall thickness at end systole |
| Pxase | peroxidase |
| QA | Quality Assurance |
| QC | quality control |

| | |
|---|---|
| QCE | quasi continuous exercise |
| qNP | non-photochemical quenching |
| $q_{NP}$ | non-photochemical quenching |
| qP | photochemical quenching |
| QRS | A complex of three distinct electrocardiogram waves which represent the beginning of ventricular contraction |
| QT | interval measure of the time interval between the start of the Q wave and the end of the T wave in the heart's electrical cycle |
| QTc | corrected QT interval |
| r | Pearson correlation coefficient |
| R, r | correlation coefficient |
| $r^2$ | correlation coefficient |
| $R^2$ | multiple regression correlation coefficient |
| $R^2$, $r^2$ | coefficient of determination |
| RACM | Regional Atmospheric Chemistry Mechanism |
| RADM | Regional Acid Deposition Model |
| rALP | recombinant antileukoprotease |
| RAMS | Regional Atmospheric Modeling System |
| RANTES | regulated upon activation, normal T-cell expressed and secreted (cells) |
| Raw | airway resistance |
| RB | respiratory bronchiole |
| RBC(s) | red blood cell(s); erythrocyte(s) |
| rbcL | Rubisco large subunit |
| rbcS | Rubisco small subunit |
| R′CO acyl | acyl carrier protein |
| $R'C(O)–O_2$ | acyl peroxy |
| rcd1 | Arabidopsis mutant radical induced cell death |
| RCD3 | rod-cone dysplasia 3 |
| RCP | Representative Concentration Pathways |
| RDBMS | Relational Database Management Systems |
| Re | Reynolds number |
| REHEX | Regional Human Exposure Model |
| RER | rough endoplasmic reticulum; Respiratory exchange ratio |
| RF | radiative forcing |
| RGR | relative growth rate |
| RH | relative humidity |
| RIOPA | Relationship of Indoor, Outdoor, and Personal Air (study) |
| RL | total pulmonary resistance |
| RLKs | receptor-like/Pelle kinase group |
| RMNP | Rocky Mountain National Park, Colorado |
| RMR | resting metabolic rate |
| rMSSD | root mean squared differences between adjacent normal-to-normal heartbeat intervals |
| Rn | nasal resistance |
| RNA | ribonucleic acid |
| $RO_2$ | organic peroxyl; organic peroxy |
| ROG | reactive organic gases |
| ROI | reactive oxygen intermediate/superoxide anion |
| $RONO_2$ | organic nitrate |
| ROOH | organic peroxides |
| $ROONO_2$, $RO_2NO_2$ | peroxy nitrate |
| ROS | reactive oxygen species |
| RPD | relative percent difference |
| RR | normal-to-normal (NN or RR) time interval between each QRS complex in the EKG; risk ratio; relative risk; respiratory rate |
| RRMS | relatively remote monitoring sites |
| RT | respiratory tract |
| RT | transepithelial resistance |
| RTLF | respiratory tract lining fluid |
| RuBisCO; Rubisco | ribulose-1,5-bisphosphate carboxylase/oxygenase |
| RuBP | ribulose bisphosphate |
| σ | sigma, standard deviation |
| σg | sigma-g; (geometric standard deviation) |
| s | second |
| S | Short; smoker; sulfur; South |
| s.c. | subcutaneous (route) |
| SA | salicylic acid |
| SAB | Science Advisory Board |
| SAC | Staphylococcus aureus Cowan 1 strain |
| SAG21 | senescence |
| SAI | Systems Applications International |
| S-allele | short-allele |
| SAMD | S-adenosyl methionine decarboxylase |
| $SaO_2$ | oxygen saturation of arterial blood |
| SAPALDIA | Study of Air Pollution and Lung Diseases in Adults |
| SAPRC | Stratospheric Processes and their Role in Climate; Statewide Air Pollution Research Center, University of California, Riverside |
| SAR | systemic acquired resistance |
| SAROAD | Storage and Retrieval of Aerometric Data (U.S. EPA centralized database; superseded by Aerometric Information Retrieval System [AIRS]) |
| SAWgrp | small airway function group |
| SBNF | San Bernardino National Forest, California |
| SBP | systolic blood pressure |
| SBUV | Solar Backscatter Ultraviolet Spectrometer |
| SC | stratum corneum |
| Sc | scandium |

| | |
|---|---|
| SCAQS | Southern California Air Quality Study |
| SCE(s) | sister chromatid exchange(s) |
| SD | standard deviation; Sprague-Dawley rat |
| SDNN | standard deviation normal-to-normal (NN or RR) time interval between each QRS complex in the EKG |
| SE | standard error |
| SEBAS | Social Environment and Biomarkers of Aging Study |
| sec | second |
| Sess. | session |
| SEM | simultaneously extracted metal; standard error of the mean; scanning electron microscopy |
| SENP | Sequoia National Park, California |
| SES | socioeconomic status |
| SF | San Francisco Bay Area |
| SF6 | sulfur hexafluoride (tracer gas) |
| SGA | small for gestational age |
| sRaw | specific airway conductance |
| SH | Shenandoah National Park site |
| SHEDS | Stochastic Human Exposure and Dose Simulation |
| SHEN | Shenandoah National Park |
| sICAM-1 | soluble intercellular adhesion molecule |
| SIDS | sudden infant death syndrome |
| SIGMOID | sigmoid weighted summed concentration |
| SINIC | Simple Nitrogen Cycle model |
| SIP | State Implementation Plan |
| SIPK | salicylic acid (SA) induced protein kinase |
| SK | shikimate kinase |
| SLA | specific leaf area |
| SLAC1 | (protein) slow anion channel associated 1 |
| SLAMS | State and Local Air Monitoring Stations |
| SM | smooth muscle |
| SMD | soil moisture deficit |
| SME | soybean oil methyl ester |
| SMNP | Great Smoky Mountain National Park (North Carolina and Tennessee) |
| SMOKE | Spare-Matrix Operator Kernel Emissions |
| $S_N$ | normalized slope of the alveolar plateau |
| SNAAQS | Secondary National Ambient Air Quality Standards |
| SNP(s) | single-nucleotide polymorphism |
| $SO_2$ | sulfur dioxide |
| $SO_4^{2-}$ | sulfate |
| SOC | soil organic carbon |
| SOCS | Salmeterol Off Corticosteroids Study |
| SOD | superoxide dismutase |
| SOS | Southern Oxidant Study |
| $SO_X$ | sulfur oxides |
| SoyFACE | Soybean Free Air gas Concentration Enrichment (Facility) |
| SP | surfactant protein (e.g., SPA, SPD); substance P |
| SP-A | surfactant protein-A |
| SPF | specific pathogen free |
| SPMs | special purpose monitors |
| SP-NK | substance P – neurokinin receptor complex |
| sRaw, | specific airway resistance |
| SRBC | sheep red blood cell |
| SRES | Special Report on Emissions Scenarios |
| SRM | standard reference method |
| SRP | standard reference photometers |
| SSCP | single-strand conformation polymorphism |
| 129S1/SvlmJ | mouse strain |
| STE | stratosphere-troposphere exchange |
| STEP | Stratospheric-Tropospheric-exchange Project |
| STN | speciation trends network |
| sTNFR1 | soluble tumor necrosis factor receptor 1 |
| STP | standard temperature and pressure |
| STPD | standard temperature and pressure, dry |
| STRF | Spatio-Temporal Random Field (theory) |
| subscript i | Index of indoor microenvironments |
| subscript o | Index of outdoor microenvironments |
| subscript o,i | Index of outdoor microenvironments adjacent to a given indoor microenvironment *i* |
| SUM00 | sum of all hourly average concentrations |
| SUM06 | seasonal sum of all hourly average concentrations ≥ 0.06 ppm |
| SUM07 | seasonal sum of all hourly average concentrations ≥ 0.07 ppm |
| SUM08 | seasonal sum of all hourly average concentrations ≥ 0.08 ppm |
| SURE | Sulfate Regional Experiment Program |
| SVE | supraventricular ectopy |
| S-W | square-wave |
| SWS | slow wave sleep |
| SZA | solar zenith angle |
| τ | tau, photochemical lifetime; atmospheric lifetime |

| | |
|---|---|
| t | t-test statistical value; t statistic |
| T | time; duration of exposure |
| T-cell(s) | T lymphocyte(s), thymus-dependent lymphocytes |
| T1 | first trimester |
| T2 | second trimester |
| $T_3$ | triiodothyronine |
| T3 | third trimester |
| $T_4$ | thyroxine |
| TAR | IPCC Third Assessment Report |
| TAR WGI | IPCC Third Assessment Report of Working Group I |
| TB | tracheobronchial; terminal bronchioles; tuberculosis |
| TBA | thiobarbituric acid |
| TBARS | thiobarbituric acid reactive substances |
| TC | total carbon |
| $^{99m}$Tc | Technetium-99m |
| T-cells | T-lymphocytes, Thymus-derived lymphocytes |
| $^{99m}$Tc-DTPA | 99mTc-diethylenetriaminepentaacetic acid |
| Tco | core temperature |
| TDLAS | Tunable Diode Laser Absorption Spectrometer |
| Te | expiratory time |
| TEM | transmission electron microscopy; Terrestrial Ecosystem Model |
| TES | Tropospheric Emission Spectrometer |
| TexAQS | Texas Air Quality Field Study |
| Tg | teragram(s) |
| TGF | transforming growth factor |
| TGF β | transforming growth factor beta |
| Th | T helper cell type |
| Th2 | T helper cell type 2 |
| THC | Total hydrocarbon content |
| tHcy | total homocysteine |
| Ti | inspiratory time |
| Ti | titanium |
| TIA | transient ischemic attack |
| TIMP-2 | tissue inhibitor of matrix metalloprotease-2 |
| $TiO_2$ | titanium dioxide |
| TLC | total lung capacity |
| TLNISE | two-level normal independent sampling estimation |
| Tlr | Toll-like receptor gene |
| TLR | Toll-like receptor protein (ex., TLR2, TLR4) |
| TMPO | tetramethylphrrolise 1-oxide |
| TNC | total nonstructural carbohydrate |
| TNF | tumor necrosis factor (e.g., TNF-α) |
| TNF-308 | tumor necrosis factor genotype |
| TNF-α | tumor necrosis factor alpha |
| TNFR | tumor necrosis factor receptor |
| TOMS | Total Ozone Mapping/Monitoring Satellite; total ozone mapping spectrometer |
| TOPSE | Tropospheric Ozone Production About the Spring Equinox |
| tPA | tissue plasminogen activator |
| TPLIF | two-photon laser-induced fluorescence |
| TRAMP | TexAQS-II Radical and Aerosol Measurement Project |
| TREGRO | Tree Growth Model |
| TRIFFID | Top-down Representation of Interactive Foliage and Flora Including Dynamics |
| TRIM | Total Risk Integrated Methodology (model) |
| TRIM.Expo | Total Risk Integrated Methodology Exposure Event (model) |
| TRP | transient receptor potential (ion channel[s], ex., TRP-A1, TRP-V1, TRP-M8) |
| TSH | thyroid stimulating hormone |
| TSP | total suspended particles |
| TTFMS | two-tone frequency-modulated spectroscopy |
| TWA | time-weighted average |
| TX | thromboxane (e.g., $TXB_2$) |
| $TXB_2$ | thromboxane B2 |
| UA | uric acid; urate |
| UAM | Urban Airshed Model |
| UCL | upper 95th% confidence limit |
| UDGT | UDP -galactose-1,2,-diacylglycerol galactosyltransferase |
| UDP | uridine diphosphate |
| U.K. | United Kingdom |
| UNECE | United Nations Economic Commission for Europe |
| UNEP | United Nations Environmental Programme |
| UNFCCC | United Nations Framework Convention on Climate Change |
| U-O | epioxides formed from uric acid |
| $U\text{-}O_2^-$ | peroxides formed from uric acid |
| $U\text{-}O_3^-$ | ozonides formed from uric acid |
| URI | upper respiratory infection |
| URS | upper respiratory symptoms |
| URT | upper respiratory tract; upper airways |
| U.S. | United States (of America) |
| USC; U.S.C. | U.S. Code |
| USDA | U.S. Department of Agriculture |
| USFS | U.S. Forest Service |
| USGCRP | U.S. Global Change Research Program |
| USGS | U.S. Geological Survey |
| UV | ultraviolet radiation |
| UV-A | ultraviolet radiation at wavelengths of 320 to 400 nm |

| | |
|---|---|
| UV-B | ultraviolet radiation at wavelengths of 280 to 320 nm |
| UV-C | ultraviolet radiation at wavelengths of 200 to 280 nm |
| UV-DIAL | Ultraviolet Differential Absorption Lidar |
| V | vanadium |
| V, mV, µV | volt, millivolt, microvolt |
| VA | alveolar ventilation |
| Val | valine |
| VC | vital capacity |
| VCAM | vascular cell adhesion molecule |
| $V_d$ | deposition rate, deposition velocity (cm/sec) |
| $V_D$ | volume of the anatomic or physiological dead space |
| $\dot{V}_E$ | ventilation rate; minute ventilation; ventilatory volume |
| VEGF | vascular endothelial growth factor |
| $\dot{V}_E$max | maximum minute ventilation |
| Vmax | maximum velocity |
| $Vmax_{25\%}$ | maximum expiratory flow at 25% of the vital capacity |
| $Vmax_{50\%}$ | maximum expiratory flow at 50% of the vital capacity |
| $Vmax_{75\%}$ | maximum expiratory flow at 75% of the vital capacity |
| VMD | volume median diameter |
| Vn | nasal volume |
| $VO_2$ | oxygen consumption |
| $VO_2$max | maximum volume per time, of oxygen (maximal oxygen consumption, maximal oxygen uptake or aerobic capacity) |
| VOC(s) | volatile organic compound(s) |
| VP | volumetric penetration |
| $VP_{50\%}$ | volume at which 50% of an inhaled bolus is absorbed |
| VPD | vapor pressure deficit; Vehicles per day; Ventricular premature depolarization |
| VT | tidal volume |
| VTB | terminal bronchiole region volume |
| VTmax | maximum tidal volume |
| VUA | volume of the upper airways |
| vWF | von Willebrand factor |
| W | width; wilderness; week(s) |
| W126 | cumulative integrated exposure index with a sigmoidal weighting function |
| W95 | cumulative integrated exposure index with a sigmoidal weighting function |
| WBC | white blood cell |
| WBGT | wet bulb globe temperature |
| wc | sigmoidal weighting of hourly $O_3$ concentration |
| WCB | warm conveyor belt |
| WED | (U.S. EPA NHEERL) Western Ecology Division |
| WF, WFM | White Face Mountain site |
| WHI | Women's Health Initiative |
| WHO | World Health Organization |
| $W/m^2$, $W\ m^{-2}$ | watts per square meter |
| WMO | World Meteorological Organization |
| WMO/UNEP | World Meteorological Organization/United Nations Environment Program |
| WRF | Weather Research and Forecasting model |
| Ws | Wassilewskija Arabidopsis ecotype |
| WS | wood smoke |
| WT | wild type; White Top Mountain site |
| wt % | percent by weight |
| WUS | western U.S. |
| w/v | weight per volume |
| Y | three parameter Weibull model |
| yr | year |
| Z | Airway generation |
| ZAPS | Zonal Air Pollution System |
| ZELIG | a forest succession simulation model |
| Zn | zinc |

# PREAMBLE

## Process of ISA Development

This preamble outlines the general process for developing an Integrated Science Assessment (ISA) including the framework for evaluating weight of evidence and drawing scientific conclusions and causal judgments. The ISA provides a concise review, synthesis, and evaluation of the most policy-relevant science to serve as a scientific foundation for the review of the National Ambient Air Quality Standards (NAAQS). The general process for NAAQS reviews is described at http://www.epa.gov/ttn/naaqs/review.html. Figure I depicts the general NAAQS review process and information for individual NAAQS reviews is available at www.epa.gov/ttn/naaqs. This preamble is a general discussion of the basic steps and criteria used in developing an ISA; for each ISA, specific details and considerations are included in the introductory section for that assessment.

The fundamental process for developing an ISA includes:

- literature searches;
- study selection;
- evaluation and integration of the evidence;
- development of scientific conclusions and causal judgments.

An initial step in this process is publication of a call for information in the Federal Register that invites the public to provide information relevant to the assessment, such as new or recent publications on health or welfare[1] effects of the pollutant, or from atmospheric and exposure sciences fields. EPA maintains an ongoing literature search process for identification of relevant scientific studies published since the last review of the NAAQS. Search strategies are designed for pollutants and scientific disciplines and iteratively modified to optimize identification of pertinent publications. Papers are identified for inclusion in several additional ways: specialized searches on specific topics; independent review of tables of contents for journals in which relevant papers may be published; independent identification of relevant literature by expert scientists; review of citations in previous assessments and identification by the public and the Clean Air Scientific Advisory Committee (CASAC) during the external review process. This literature search and study selection process is depicted in Figure II. Publications considered for inclusion in the ISA are added to the Health and Environmental Research Online (HERO) database developed by EPA (http://hero.epa.gov/); the references in the ISA include a hyperlink to the database.

[1] Welfare effects as defined in Clean Air Act (CAA) section 302(h) [42 U.S.C. 7602(h)] include, but are not limited to, “effects on soils, water, crops, vegetation, man-made materials, animals, wildlife, weather, visibility and climate, damage to and deterioration of property, and hazards to transportation, as well as effects on economic values and on personal comfort and well-being.”

Studies that have undergone scientific peer review and have been published or accepted for publication and reports that have undergone review are considered for inclusion in the ISA. Analyses conducted by EPA using publicly available data are also considered for inclusion in the ISA. All relevant epidemiologic, controlled human exposure, toxicological, and ecological and welfare effects studies published since the last review are considered, including those related to exposure-response relationships, mode(s) of action (MOA), and potentially at-risk populations and lifestages. Studies on atmospheric chemistry, environmental fate and transport, dosimetry, toxicokinetics and exposure are also considered for inclusion in the document, as well as analyses of air quality and emissions data. References that were considered for inclusion in a specific ISA can be found using the HERO website (http://hero.epa.gov).

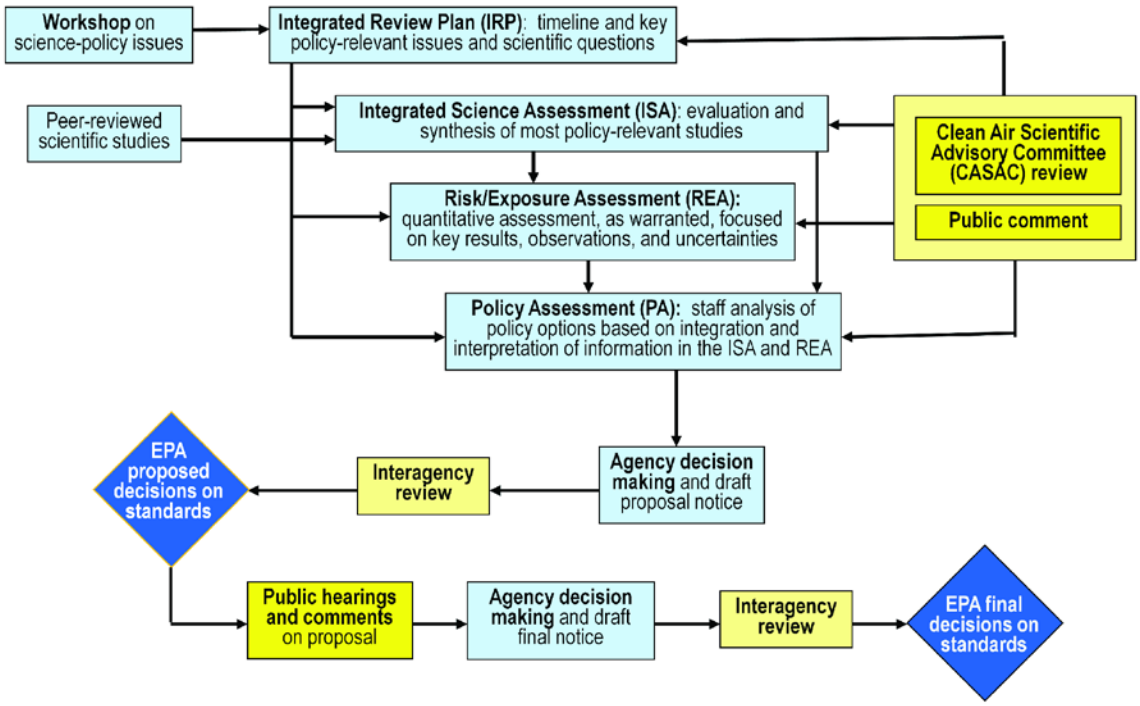
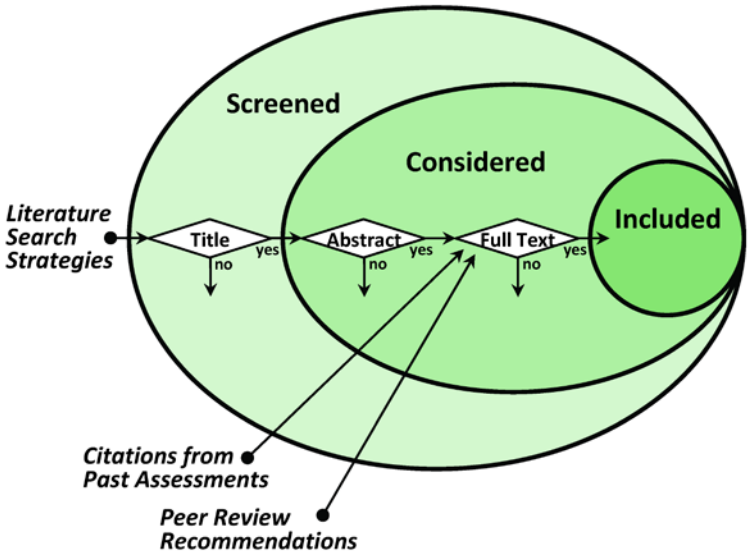



**Figure I** **Illustration of the key steps in the process of the review of National Ambient Air Quality Standards.**




**Criteria for study evaluation include:**

- Are the study populations, subjects, or animal models adequately selected, and are they sufficiently well defined to allow for meaningful comparisons between study or exposure groups?
- Are the statistical analyses appropriate, properly performed, and properly interpreted? Are likely covariates adequately controlled or taken into account in the study design and statistical analysis?
- Are the air quality data, exposure, or dose metrics of adequate quality and sufficiently representative of information regarding ambient conditions?
- Are the health, ecological or welfare effect measurements meaningful, valid and reliable?
- Do the analytical methods provide adequate sensitivity and precision to support conclusions?

**Figure II Illustration of processes for literature search and study selection used for development of ISAs.**

Each ISA builds upon the conclusions of previous assessments for the pollutant under review. EPA focuses on peer reviewed literature published following the completion of the previous review (2006 $O_3$ AQCD) and on any new interpretations of previous literature, integrating the results of recent scientific studies with previous findings. Important earlier studies may be discussed in detail to reinforce key concepts and conclusions or for reinterpretation in light of newer data. Earlier studies also are the primary focus in some areas of the document where research efforts have subsided, or if these earlier studies remain the definitive works available in the literature.

Selection of studies for inclusion in the ISA is based on the general scientific quality of the study, and consideration of the extent to which the study is informative and policy-relevant. Policy relevant and informative studies include those that provide a basis for or describe the relationship between the criteria pollutant and effects, including studies that offer innovation in method or design and studies that reduce uncertainty on critical issues, such as analyses of confounding or effect modification by copollutants or other variables, analyses of concentration-response or dose-

response relationships, or analyses related to time between exposure and response. Emphasis is placed on studies that examine effects associated with pollutant concentrations relevant to current population and ecosystem exposures, and particularly those pertaining to concentrations currently found in ambient air. Other studies are included if they contain unique data, such as a previously unreported effect or MOA for an observed effect, or examine multiple concentrations to elucidate exposure-response relationships. In general, in assessing the scientific quality and relevance of health and welfare effects studies, the following considerations have been taken into account when selecting studies for inclusion in the ISA.

- Are the study populations, subjects, or animal models adequately selected, and are they sufficiently well defined to allow for meaningful comparisons between study or exposure groups?
- Are the statistical analyses appropriate, properly performed, and properly interpreted? Are likely covariates adequately controlled or taken into account in the study design and statistical analysis?
- Are the air quality data, exposure, or dose metrics of adequate quality and sufficiently representative of information regarding ambient conditions?
- Are the health, ecological or welfare effect measurements meaningful, valid and reliable?
- Do the analytical methods provide adequate sensitivity and precision to support conclusions?

Considerations specific to particular disciplines include the following: In selecting epidemiologic studies, EPA considers whether a given study: (1) presents information on associations with short- or long-term pollutant exposures at or near conditions relevant to ambient exposures; (2) addresses potential confounding by other pollutants; (3) assesses potential effect modifiers; (4) evaluates health endpoints and populations not previously extensively researched; and (5) evaluates important methodological issues related to interpretation of the health evidence (e.g., lag or time period between exposure and effects, model specifications, thresholds, mortality displacement).

Considerations for the selection of research evaluating controlled human exposure or animal toxicological studies include a focus on studies conducted using relevant pollutant exposures. For both types of studies, relevant pollutant exposures are considered to be those generally within one or two orders of magnitude of ambient concentrations. Studies in which higher doses were used may also be considered if they provide information relevant to understanding MOA or mechanisms, as noted below.

Evaluation of controlled human exposure studies focuses on those that approximated expected human exposure conditions in terms of concentration and duration. Studies should include control exposures to filtered air, as appropriate. In the selection of controlled human exposure studies, emphasis is placed on studies that: (1) investigate potentially at-risk populations and lifestages such as people with asthma or

cardiovascular diseases, children or older adults; (2) address issues such as concentration-response or time-course of responses; and (3) have sufficient statistical power to assess findings.

Review of the animal toxicological evidence focuses on studies that approximate expected human dose conditions, which vary depending on the dosimetry, toxicokinetics, and biological sensitivity of the particular laboratory animal species or strains studied. Emphasis is placed on studies that: (1) investigate animal models of disease that can provide information on populations potentially at increased risk of effects; (2) address issues such as concentration-response or time-course of responses; and (3) have sufficient statistical power to assess findings. Due to resource constraints on exposure duration and numbers of animals tested, animal studies typically utilize high-concentration exposures to acquire data relating to mechanisms and assure a measurable response. Emphasis is placed on studies using doses or concentrations generally within 1-2 orders of magnitude of current levels. Studies with higher concentration exposures or doses are considered to the extent that they provide useful information to inform understanding of interspecies differences between healthy and at-risk human populations. Results from in vitro studies may also be included if they provide mechanistic insight or further support for results demonstrated in vivo.

These criteria provide benchmarks for evaluating various studies and for focusing on the policy-relevant studies in assessing the body of health, ecological and welfare effects evidence. As stated initially, the intent of the ISA is to provide a concise review, synthesis, and evaluation of the most policy-relevant science to serve as a scientific foundation for the review of the NAAQS, not extensive summaries of all health, ecological and welfare effects studies for a pollutant. Of most relevance for inclusion of studies is whether they provide useful qualitative or quantitative information on exposure-effect or exposure-response relationships for effects associated with pollutant exposures at doses or concentrations relevant to ambient conditions that can inform decisions on whether to retain or revise the standards.

The general process for ISA development is illustrated in Figure III. In developing an ISA, EPA reviews and summarizes the evidence from studies of atmospheric sciences; human exposure, toxicological, controlled human exposure and epidemiologic studies; and studies of ecological and welfare effects. In the process of developing the first draft ISA, EPA may convene a peer input meeting in which EPA the scientific content of preliminary draft materials is reviewed to ensure that the ISA is up to date and focused on the most policy-relevant findings, and to assist EPA with integration of evidence within and across disciplines. EPA integrates the evidence from across scientific disciplines or study types and characterizes the weight of evidence for relationships between the pollutant and various outcomes.
The integration of evidence on health, and ecological or welfare effects, involves collaboration between scientists from various disciplines. As an example, an evaluation of health effects evidence would include the integration of the results from epidemiologic, controlled human exposure, and toxicological studies, and application of the causal framework (described below) to draw conclusions. Integration of results

on health or ecological effects that are logically or mechanistically connected (e.g., a spectrum of effects on the respiratory system) informs judgments of causality. Using the causal framework described in the following section, EPA scientists consider aspects such as strength, consistency, coherence, and biological plausibility of the evidence, and develop causality determinations on the nature of the relationships. Causality determinations often entail an iterative process of review and evaluation of the evidence. Two drafts of the ISA are typically released for review by the CASAC and the public, and comments received on the characterization of the science as well as the implementation of the causal framework are carefully considered in revising and completing the final ISA.

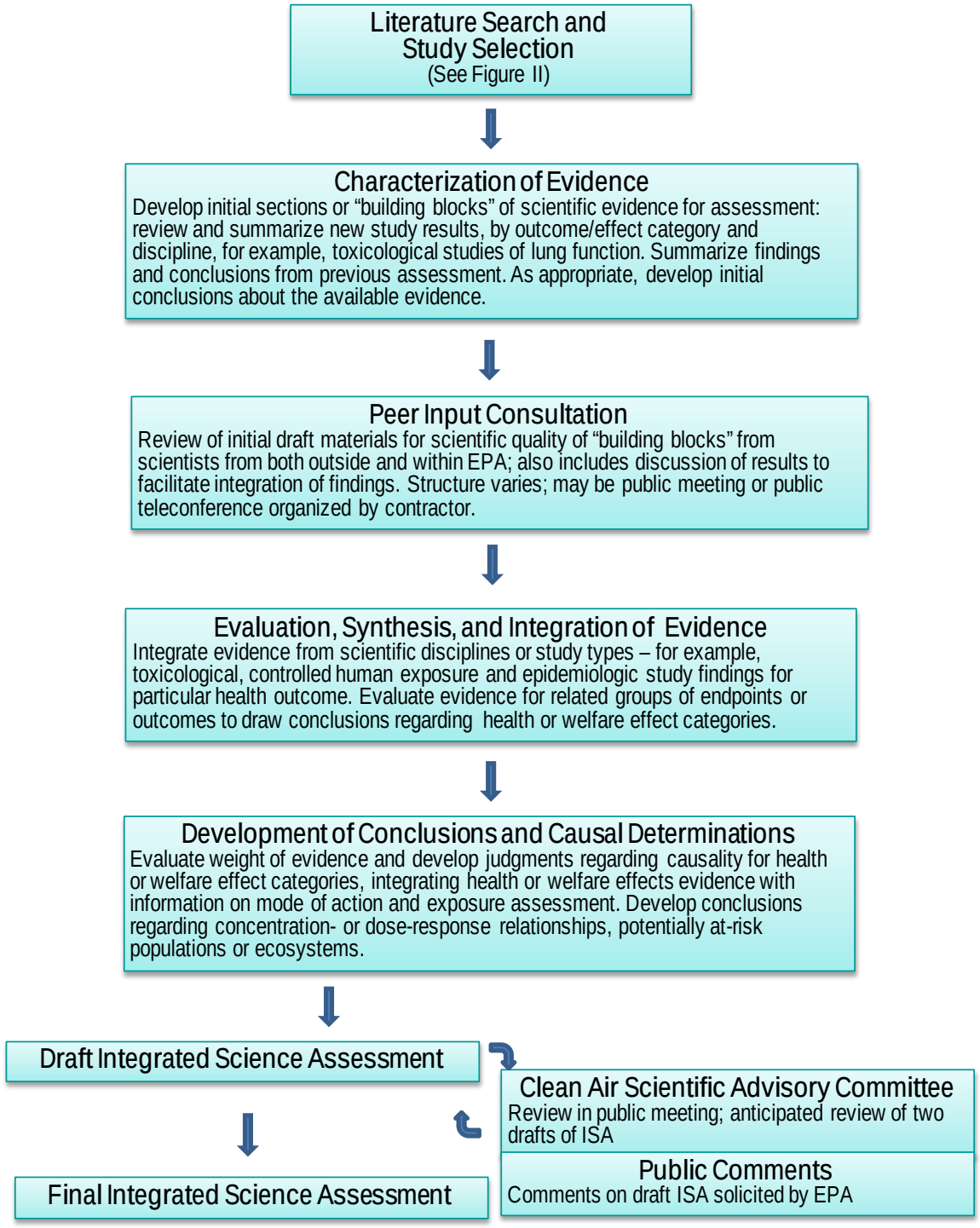



**Figure III Characterization of the general process of ISA development.**

## EPA Framework for Causal Determination

EPA has developed a consistent and transparent basis for integration of scientific evidence and evaluation of the causal nature of air pollution-related health or welfare effects for use in developing ISAs. The framework described below establishes uniform language concerning causality and brings more specificity to the findings. This standardized language was drawn from sources across the federal government and wider scientific community, especially the National Academy of Sciences (NAS) Institute of Medicine (IOM) document, *Improving the Presumptive Disability Decision-Making Process for Veterans* (Samet and Bodurow, 2008), a comprehensive report on evaluating causality. This framework:

- describes the kinds of scientific evidence used in establishing a general causal relationship between exposure and health effects;
- characterizes the process for integration and evaluation of evidence necessary to reach a conclusion about the existence of a causal relationship;
- identifies issues and approaches related to uncertainty;
- provides a framework for classifying and characterizing the weight of evidence in support of a general causal relationship.

Approaches to assessing the separate and combined lines of evidence (e.g., epidemiologic, controlled human exposure, and animal toxicological studies) have been formulated by a number of regulatory and science agencies, including the IOM of the NAS (Samet and Bodurow, 2008), International Agency for Research on Cancer (IARC, 2006), U.S. EPA (2005), and Centers for Disease Control and Prevention (CDC, 2004). Causal inference criteria have also been described for ecological effects evidence (U.S. EPA, 1998; Fox, 1991). These formalized approaches offer guidance for assessing causality. The frameworks are similar in nature, although adapted to different purposes, and have proven effective in providing a uniform structure and language for causal determinations.

## Evaluating Evidence for Inferring Causation

The 1964 Surgeon General's (U.S. Department of Health, Education and Welfare [HEW]) report on tobacco smoking defined "cause" as a "significant, effectual relationship between an agent and an associated disorder or disease in the host" (HEW, 1964). More generally, a cause is defined as an agent that brings about an effect or a result. An association is the statistical relationship among variables; alone, however, it is insufficient proof of a causal relationship between an exposure and a health outcome. Unlike an association, a causal claim supports the creation of counterfactual claims, that is, a claim about what the world would have been like under different or changed circumstances (Samet and Bodurow, 2008).

Many of the health and environmental outcomes reported in these studies have complex etiologies. Diseases such as asthma, coronary heart disease (CHD) or cancer

are typically initiated by multiple agents. Outcomes depend on a variety of factors, such as age, genetic susceptibility, nutritional status, immune competence, and social factors (Samet and Bodurow, 2008; Gee and Payne-Sturges, 2004). Effects on ecosystems are often also multifactorial with a complex web of causation. Further, exposure to a combination of agents could cause synergistic or antagonistic effects. Thus, the observed risk may represent the net effect of many actions and counteractions.

Scientific findings incorporate uncertainty. "Uncertainty" can be defined as having limited knowledge to exactly describe an existing state or future outcome, e.g., the lack of knowledge about the correct value for a specific measure or estimate. Uncertainty analysis may be qualitative or quantitative in nature. In many cases, the analysis is qualitative, and can include professional judgment or inferences based on analogy with similar situations. Quantitative uncertainty analysis may include use of simple measures (e.g., ranges) and analytical techniques. Quantitative uncertainty analysis might progress to more complex measures and techniques, if needed for decision support. Various approaches to evaluating uncertainty include classical statistical methods, sensitivity analysis, or probabilistic uncertainty analysis, in order of increasing complexity and data requirements. However, data may not be available for all aspects of an assessment and those data that are available may be of questionable or unknown quality. Ultimately, the assessment is based on a number of assumptions with varying degrees of uncertainty.

Publication bias is a source of uncertainty regarding the magnitude of health risk estimates. It is well understood that studies reporting non-null findings are more likely to be published than reports of null findings. Publication bias can result in overestimation of effect estimate sizes (Ioannidis, 2008). For example, effect estimates from single-city epidemiologic studies have been found to be generally larger than those from multicity studies which is an indication of publication bias in that null or negative single-city results may be reported in a multicity analyses but might not be published independently (Bell et al., 2005).

## Consideration of Evidence from Scientific Disciplines

Moving from association to causation involves the elimination of alternative explanations for the association. The ISA focuses on evaluation of the findings from the body of evidence, drawing upon the results of all studies determined to meet the criteria described previously. Causality determinations are based on the evaluation, integration, and synthesis of evidence from across scientific disciplines. The relative importance of different types of evidence varies by pollutant or assessment, as does the availability of different types of evidence for causality determination. Three general types of studies inform consideration of human health effects: controlled human exposure, epidemiologic, and toxicological studies. Evidence on ecological or welfare effects may be drawn from a variety of experimental approaches (e.g.,

greenhouse, laboratory, field) and numerous disciplines (e.g., community ecology, biogeochemistry, and paleontological/historical reconstructions).

Direct evidence of a relationship between pollutant exposures and human health effects comes from controlled human exposure studies. Such studies experimentally evaluate the health effects of administered exposures in human volunteers under highly controlled laboratory conditions. Also referred to as human clinical studies, these experiments allow investigators to expose subjects to known concentrations of air pollutants under carefully regulated environmental conditions and activity levels. These studies provide important information on the biological plausibility of associations observed in epidemiologic studies. Essential dose-response profiles and ranges of response severity can be established with these studies. In some instances, controlled human exposure studies can also be used to characterize concentration-response relationships at pollutant concentrations relevant to ambient conditions. Controlled human exposures are typically conducted using a randomized crossover design, with subjects exposed both to the pollutant and a clean air control. In this way, subjects serve as their own controls, effectively controlling for many potential confounders. Considerations for evaluating controlled human study findings include the generally small sample size and short exposure time used in experimental studies, and that severe health outcomes are not assessed. By experimental design, controlled human exposure studies are structured to evaluate physiological or biomolecular outcomes in response to exposure to a specific air pollutant and/or combination of pollutants. In addition, the study design generally precludes inclusion of subjects with serious health conditions, and therefore the results often cannot be generalized to an entire population. Although some controlled human exposure studies have included health-compromised individuals such as those with respiratory or cardiovascular disease, these individuals may also be relatively healthy and may not represent the most sensitive individuals in the population. Thus, observed effects in these studies may underestimate the response in certain populations.

Epidemiologic studies provide important information on the associations between health effects and exposure of human populations to ambient air pollution. In epidemiologic or observational studies of humans, the investigator generally does not control exposures or intervene with the study population. Broadly, observational studies can describe associations between exposures and effects. These studies fall into several categories: e.g., cross-sectional, prospective cohort, panel, and time-series studies. Cross-sectional studies use health outcome, exposure and covariate data available at the community level (e.g., annual mortality rates and pollutant concentrations), but do not have individual-level data. Prospective cohort studies have some data collected at the individual level, generally health outcome data, and in some cases individual-level data on exposure and covariates are collected. Time-series studies evaluate the relationship for changes in a health outcome with changes in exposure indicators, such as an association between daily changes in mortality with air pollution. Panel studies include repeated measurements of health outcomes, such as respiratory symptoms or heart rhythm variable, at the individual level. “Natural experiments” offer the opportunity to investigate changes in health related to a change in exposure, such as closure of a pollution source.

In evaluating epidemiologic studies, consideration of many study design factors and issues must be taken into account to properly inform their interpretation. One key consideration is evaluation of the potential contribution of the pollutant to a health outcome when it is a component of a complex air pollutant mixture. Reported effect estimates in epidemiologic studies may reflect: independent effects on health outcomes; effects of the pollutant acting as an indicator of a copollutant or a complex ambient air pollution mixture; effects resulting from interactions between that pollutant and copollutants.

In the evaluation of epidemiologic evidence, one important consideration is potential confounding. Confounding is "… a confusion of effects. Specifically, the apparent effect of the exposure of interest is distorted because the effect of an extraneous factor is mistaken for or mixed with the actual exposure effect (which may be null)" (Rothman and Greenland, 1998). One approach to remove spurious associations (due to possible confounders); is to control for characteristics that may differ between exposed and unexposed persons; this is frequently termed "adjustment." Scientific judgment is needed to evaluate likely sources and extent of confounding, together with consideration of how well the existing constellation of study designs, results, and analyses address the potential for erroneous inferences. A confounder is associated with both the exposure and the effect; for example, confounding can occur between correlated pollutants that are associated with the same effect.

Several statistical methods are available to detect and control for potential confounders, with none of them being completely satisfactory. Multivariable regression models constitute one tool for estimating the association between exposure and outcome after adjusting for characteristics of participants that might confound the results. The use of multipollutant regression models has been the prevailing approach for controlling potential confounding by copollutants in air pollution health effects studies. Finding the likely causal pollutant from multipollutant regression models is made difficult by the possibility that one or more air pollutants may be acting as a surrogate for an unmeasured or poorly measured pollutant or for a particular mixture of pollutants. In addition, pollutants may independently exert effects on the same system; for example, several pollutants may be associated with respiratory effects through either the same or different modes of action. The number and degree of diversity of covariates, as well as their relevance to the potential confounders, remain matters of scientific judgment. Despite these limitations, the use of multipollutant models is still the prevailing approach employed in most air pollution epidemiologic studies and provides some insight into the potential for confounding or interaction among pollutants.

Confidence that unmeasured confounders are not producing the findings is increased when multiple studies are conducted in various settings using different subjects or exposures, each of which might eliminate another source of confounding from consideration. For example, multicity studies can provide insight on potential confounding through the use of a consistent method to analyze data from across locations with different levels of copollutants and other covariates. Intervention

studies, because of their quasi-experimental nature, can be particularly useful in characterizing causation.

Another important consideration in the evaluation of epidemiologic evidence is effect modification, which occurs when the effect differs between subgroups or strata; for example, effect estimates that vary by age group or potential risk factor. As stated by Rothman and Greenland (1998):

> "Effect-measure modification differs from confounding in several ways. The main difference is that, whereas confounding is a bias that the investigator hopes to prevent or remove from the effect estimate, effect-measure modification is a property of the effect under study …
> In epidemiologic analysis one tries to eliminate confounding but one tries to detect and estimate effect-measure modification."

When a risk factor is a confounder, it is the true cause of the association observed between the exposure and the outcome; when a risk factor is an effect modifier, it changes the magnitude of the association between the exposure and the outcome in stratified analyses. For example, the presence of a pre-existing disease or indicator of low socioeconomic status may act as effect modifiers if they are associated with increased risk of effects related to air pollution exposure. It is often possible to stratify the relationship between health outcome and exposure by one or more of these potential effect modifiers. For variables that modify the association, effect estimates in each stratum will be different from one another and different from the overall estimate, indicating a different exposure-response relationship may exist in populations represented by these variables.

Exposure measurement error, which refers to the uncertainty associated with the exposure metrics used to represent exposure of an individual or population, can be an important contributor to uncertainty in air pollution epidemiologic study results. Exposure error can influence observed epidemiologic associations between ambient pollutant concentrations and health outcomes by biasing effect estimates toward or away from the null and widening confidence intervals around those estimates (Zeger et al., 2000). There are several components that contribute to exposure measurement error in air pollution epidemiologic studies, including the difference between true and measured ambient concentrations, the difference between average personal exposure to ambient pollutants and ambient concentrations at central monitoring sites, and the use of average population exposure rather than individual exposure estimates. Factors that could influence exposure estimates include nonambient sources of exposure, topography of the natural and built environment, meteorology, measurement errors, time-location-activity patterns, and the extent to which ambient pollutants penetrate indoor environments. The importance of exposure error varies with study design and is dependent on the spatial and temporal aspects of the design.

The third main type of health effects evidence, animal toxicological studies, provides information on the pollutant's biological action under controlled and monitored exposure circumstances. Taking into account physiological differences of the experimental species from humans, these studies inform characterization of health

effects of concern, exposure-response relationships and MOAs. Further, animal models can inform determinations of at-risk populations. These studies evaluate the effects of exposures to a variety of pollutants in a highly controlled laboratory setting and allow exploration of toxicological pathways or mechanisms by which a pollutant may cause effects. Understanding the biological mechanisms underlying various health outcomes can prove crucial in establishing or negating causality. In the absence of human studies data, extensive, well-conducted animal toxicological studies can support determinations of causality, if the evidence base indicates that similar responses are expected in humans under ambient exposure conditions.

Interpretations of animal toxicological studies are affected by limitations associated with extrapolation between animal and human responses. The differences between humans and other species have to be taken into consideration, including metabolism, hormonal regulation, breathing pattern, and differences in lung structure and anatomy. Also, in spite of a high degree of homology and the existence of a high percentage of orthologous genes across humans and rodents (particularly mice), extrapolation of molecular alterations at the gene level is complicated by species-specific differences in transcriptional regulation. Given these differences, there are uncertainties associated with quantitative extrapolations of observed pollutant-induced pathophysiological alterations between laboratory animals and humans, as those alterations are under the control of widely varying biochemical, endocrine, and neuronal factors.

For ecological effects assessment, both laboratory and field studies (including field experiments and observational studies) can provide useful data for causality determination. Because conditions can be controlled in laboratory studies, responses may be less variable and smaller differences may be easier to detect. However, the control conditions may limit the range of responses (e.g., animals may not be able to seek alternative food sources) or incompletely reflect pollutant bioavailability, so they may not reflect responses that would occur in the natural environment. In addition, larger-scale processes are difficult to reproduce in the laboratory.

Field observational studies measure biological changes in uncontrolled situations, and describe an association between a disturbance and an ecological effect. Field data can provide important information for assessments of multiple stressors or where site-specific factors significantly influence exposure. They are also often useful for analyses of larger geographic scales and higher levels of biological organization. However, because conditions are not controlled, variability is expected to be higher and differences harder to detect. Field surveys are most useful for linking stressors with effects when stressor and effect levels are measured concurrently. The presence of confounding factors can make it difficult to attribute observed effects to specific stressors.

Intermediate between laboratory and field are studies that use environmental media collected from the field to examine response in the laboratory, and experiments that are performed in the natural environment while controlling for some environmental conditions (i.e., mesocosm studies). This type of study in manipulated natural environments can be considered a hybrid between a field experiment and laboratory

study since some aspects are performed under controlled conditions but others are not. They make it possible to observe community and/or ecosystem dynamics, and provide strong evidence for causality when combined with findings of studies that have been made under more controlled conditions.

## Application of Framework for Causal Determination

In its evaluation and integration of the scientific evidence on health or welfare effects of criteria pollutants, EPA determines the weight of evidence in support of causation and characterizes the strength of any resulting causal classification. EPA also evaluates the quantitative evidence and draws scientific conclusions, to the extent possible, regarding the concentration-response relationships and the loads to ecosystems, exposures, doses or concentrations, exposure duration, and pattern of exposures at which effects are observed.

To aid judgment, various "aspects"[1] of causality have been discussed by many philosophers and scientists. The 1964 Surgeon General's report on tobacco smoking discussed criteria for the evaluation of epidemiologic studies, focusing on consistency, strength, specificity, temporal relationship, and coherence (HEW, 1964). Sir Austin Bradford Hill (Hill, 1965) articulated aspects of causality in epidemiology and public health that have been widely used (Samet and Bodurow, 2008; IARC, 2006; U.S. EPA, 2005; CDC, 2004). These aspects (Hill, 1965) have been modified (Table I) for use in causal determinations specific to health and welfare effects for pollutant exposures (U.S. EPA, 2009d).[2] Although these aspects provide a framework for assessing the evidence, they do not lend themselves to being considered in terms of simple formulas or fixed rules of evidence leading to conclusions about causality (Hill, 1965). For example, one cannot simply count the number of studies reporting statistically significant results or statistically nonsignificant results and reach credible conclusions about the relative weight of the evidence and the likelihood of causality. Rather, these aspects provide a framework for systematic appraisal of the body of evidence, informed by peer and public comment and advice, which includes weighing alternative views on controversial issues. In addition, it is important to note that the aspects in Table I cannot be used as a strict checklist, but rather to determine the weight of the evidence for inferring causality. In particular, not meeting one or more of the principles does not automatically preclude a determination of causality [see discussion in CDC (2004)].

[1] The "aspects" described by Sir Austin Bradford Hill (Hill, 1965) have become, in the subsequent literature, more commonly described as "criteria." The original term "aspects" is used here to avoid confusion with "criteria" as it is used, with different meaning, in the Clean Air Act.

[2] The Hill aspects were developed for interpretation of epidemiologic results. They have been modified here for use with a broader array of data, i.e., epidemiologic, controlled human exposure, ecological, and animal toxicological studies, as well as in vitro data, and to be more consistent with the U.S. EPA (2005) Guidelines for Carcinogen Risk Assessment.

## Table I Aspects to aid in judging causality.

| Aspect | Description |
|---|---|
| Consistency of the observed association | An inference of causality is strengthened when a pattern of elevated risks is observed across several independent studies. The reproducibility of findings constitutes one of the strongest arguments for causality. If there are discordant results among investigations, possible reasons such as differences in exposure, confounding factors, and the power of the study are considered. |
| Coherence | An inference of causality from one line of evidence (e.g., epidemiologic, controlled human exposure [clinical], or animal studies) may be strengthened by other lines of evidence that support a cause-and-effect interpretation of the association. Evidence on ecological or welfare effects may be drawn from a variety of experimental approaches (e.g., greenhouse, laboratory, and field) and subdisciplines of ecology (e.g., community ecology, biogeochemistry, and paleontological/historical reconstructions).<br>The coherence of evidence from various fields greatly adds to the strength of an inference of causality. In addition, there may be coherence in demonstrating effects across multiple study designs or related health endpoints within one scientific line of evidence. |
| Biological plausibility | An inference of causality tends to be strengthened by consistency with data from experimental studies or other sources demonstrating plausible biological mechanisms. A proposed mechanistic linking between an effect and exposure to the agent is an important source of support for causality, especially when data establishing the existence and functioning of those mechanistic links are available. |
| Biological gradient (exposure-response relationship) | A well-characterized exposure-response relationship (e.g., increasing effects associated with greater exposure) strongly suggests cause and effect, especially when such relationships are also observed for duration of exposure (e.g., increasing effects observed following longer exposure times). |
| Strength of the observed association | The finding of large, precise risks increases confidence that the association is not likely due to chance, bias, or other factors. However, it is noted that a small magnitude in an effect estimate may represent a substantial effect in a population. |
| Experimental evidence | Strong evidence for causality can be provided through “natural experiments” when a change in exposure is found to result in a change in occurrence or frequency of health or welfare effects. |
| Temporal relationship of the observed association | Evidence of a temporal sequence between the introduction of an agent, and appearance of the effect, constitutes another argument in favor of causality. |
| Specificity of the observed association | Evidence linking a specific outcome to an exposure can provide a strong argument for causation. However, it must be recognized that rarely, if ever, does exposure to a pollutant invariably predict the occurrence of an outcome, and that a given outcome may have multiple causes. |
| Analogy | Structure activity relationships and information on the agent’s structural analogs can provide insight into whether an association is causal. Similarly, information on mode of action for a chemical, as one of many structural analogs, can inform decisions regarding likely causality. |

## Determination of Causality

In the ISA, EPA assesses the body of relevant literature, building upon evidence available during previous NAAQS reviews, to draw conclusions on the causal relationships between relevant pollutant exposures and health or environmental effects. ISAs use a five-level hierarchy that classifies the weight of evidence for causation[1]. In developing this hierarchy, EPA has drawn on the work of previous evaluations, most prominently the IOM's *Improving the Presumptive Disability Decision-Making Process for Veterans* (Samet and Bodurow, 2008), EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), and the U.S. Surgeon General's smoking report (CDC, 2004). This weight of evidence evaluation is based on integration of findings from various lines of evidence from across the health and environmental effects disciplines. These separate judgments are integrated into a qualitative statement about the overall weight of the evidence and causality. The five descriptors for causal determination are described in Table II.

Determination of causality involves the evaluation and integration of evidence for different types of health, ecological or welfare effects associated with short- and long-term exposure periods. In making determinations of causality, evidence is evaluated for major outcome categories or groups of related endpoints (e.g., respiratory effects, vegetation growth), integrating evidence from across disciplines, and assessing the coherence of evidence across a spectrum of related endpoints to draw conclusions regarding causality. In discussing the causal determination, EPA characterizes the evidence on which the judgment is based, including strength of evidence for individual endpoints within the outcome category or group of related endpoints.

In drawing judgments regarding causality for the criteria air pollutants, the ISA focuses on evidence of effects in the range of relevant pollutant exposures or doses, and not on determination of causality at any dose. Emphasis is placed on evidence of effects at doses (e.g., blood Pb concentration) or exposures (e.g., air concentrations) that are relevant to, or somewhat above, those currently experienced by the population. The extent to which studies of higher concentrations are considered varies by pollutant and major outcome category, but generally includes those with doses or exposures in the range of one to two orders of magnitude above current or ambient conditions. Studies that use higher doses or exposures may also be considered to the extent that they provide useful information to inform understanding of mode of action, interspecies differences, or factors that may increase risk of effects for a population. Thus, a causality determination is based on weight of evidence evaluation for health, ecological or welfare effects, focusing on the evidence from exposures or doses generally ranging from current levels to one or two orders of magnitude above current levels.

[1] Both the CDC and IOM frameworks use a four-category hierarchy for the strength of the evidence. A five-level hierarchy is used here to be consistent with the EPA Guidelines for Carcinogen Risk Assessment and to provide a more nuanced set of categories.

In addition, EPA evaluates evidence relevant to understand the quantitative relationships between pollutant exposures and health, ecological or welfare effects. This includes evaluation of the form of concentration-response or dose-response relationships and, to the extent possible, drawing conclusions on the levels at which effects are observed. The ISA also draws scientific conclusions regarding important exposure conditions for effects and populations that may be at greater risk for effects, as described in the following section.

## Table II Weight of evidence for causal determination.

| | Health Effects | Ecological and Welfare Effects |
|---|---|---|
| Causal relationship | Evidence is sufficient to conclude that there is a causal relationship with relevant pollutant exposures (i.e., doses or exposures generally within one to two orders of magnitude of current levels). That is, the pollutant has been shown to result in health effects in studies in which chance, bias, and confounding could be ruled out with reasonable confidence. For example: a) controlled human exposure studies that demonstrate consistent effects; or b) observational studies that cannot be explained by plausible alternatives or are supported by other lines of evidence (e.g., animal studies or mode of action information). Evidence includes multiple high-quality studies | Evidence is sufficient to conclude that there is a causal relationship with relevant pollutant exposures i.e., doses or exposures generally within one to two orders of magnitude of current levels). That is, the pollutant has been shown to result in effects in studies in which chance, bias, and confounding could be ruled out with reasonable confidence. Controlled exposure studies (laboratory or small- to medium-scale field studies) provide the strongest evidence for causality, but the scope of inference may be limited. Generally, determination is based on multiple studies conducted by multiple research groups, and evidence that is considered sufficient to infer a causal relationship is usually obtained from the joint consideration of many lines of evidence that reinforce each other. |
| Likely to be a causal relationship | Evidence is sufficient to conclude that a causal relationship is likely to exist with relevant pollutant exposures, but important uncertainties remain. That is, the pollutant has been shown to result in health effects in studies in which chance and bias can be ruled out with reasonable confidence but potential issues remain. For example: a) observational studies show an association, but copollutant exposures are difficult to address and/or other lines of evidence (controlled human exposure, animal, or mode of action information) are limited or inconsistent; or b) animal toxicological evidence from multiple studies from different laboratories that demonstrate effects, but limited or no human data are available. Evidence generally includes multiple high-quality studies. | Evidence is sufficient to conclude that there is a likely causal association with relevant pollutant exposures. That is, an association has been observed between the pollutant and the outcome in studies in which chance, bias, and confounding are minimized, but uncertainties remain. For example, field studies show a relationship, but suspected interacting factors cannot be controlled, and other lines of evidence are limited or inconsistent. Generally, determination is based on multiple studies in multiple research groups. |
| Suggestive of a causal relationship | Evidence is suggestive of a causal relationship with relevant pollutant exposures, but is limited. For example, (a) at least one high-quality epidemiologic study shows an association with a given health outcome but the results of other studies are inconsistent; or (b) a well-conducted toxicological study, such as those conducted in the National Toxicology Program (NTP), shows effects in animal species, | Evidence is suggestive of a causal relationship with relevant pollutant exposures, but chance, bias and confounding cannot be ruled out. For example, at least one high-quality study shows an effect, but the results of other studies are inconsistent. |
| Inadequate to infer a causal relationship | Evidence is inadequate to determine that a causal relationship exists with relevant pollutant exposures. The available studies are of insufficient quantity, quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an effect. | The available studies are of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an effect. |
| Not likely to be a causal relationship | Evidence is suggestive of no causal relationship with relevant pollutant exposures. Several adequate studies, covering the full range of levels of exposure that human beings are known to encounter and considering at-risk populations, are mutually consistent in not showing an effect at any level of exposure. | Several adequate studies, examining relationships with relevant exposures, are consistent in failing to show an effect at any level of exposure. |

## Quantitative Relationships: Effects on Human Populations

Once a determination is made regarding the causal relationship between the pollutant and outcome category, important questions regarding quantitative relationships include:

- What is the concentration-response, exposure-response, or dose-response relationship in the human population?
- What is the interrelationship between incidence and severity of effect?
- What exposure conditions (dose or exposure, duration and pattern) are important?
- What populations and lifestages appear to be differentially affected (i.e., more at risk of experiencing effects)?

To address these questions, the entirety of quantitative evidence is evaluated to characterize pollutant concentrations and exposure durations at which effects were observed for exposed populations, including populations and lifestages potentially at increased risk. To accomplish this, evidence is considered from multiple and diverse types of studies, and a study or set of studies that best approximates the concentration-response relationships between health outcomes and the pollutant may be identified. Controlled human exposure studies provide the most direct and quantifiable exposure-response data on the human health effects of pollutant exposures. To the extent available, the ISA evaluates results from across epidemiologic studies that characterize the form of relationships between the pollutant and health outcomes and draws conclusions on the shape of these relationships. Animal data may also inform evaluation of concentration-response relationships, particularly relative to MOAs and characteristics of at-risk populations.

An important consideration in characterizing the public health impacts associated with exposure to a pollutant is whether the concentration-response relationship is linear across the range of concentrations or if nonlinear relationships exist along any part of this range. Of particular interest is the shape of the concentration-response curve at and below the level of the current standards. Various sources of variability and uncertainty, such as low data density in the lower concentration range, possible influence of exposure measurement error, and variability between individuals in susceptibility to air pollution health effects, tend to smooth and "linearize" the concentration-response function, and thus can obscure the existence of a threshold or nonlinear relationship [2006 $O_3$ AQCD (U.S. EPA, 2006b)]. Since individual thresholds vary from person to person due to individual differences such as genetic level susceptibility or pre-existing disease conditions (and even can vary from one time to another for a given person), it can be difficult to demonstrate that a threshold exists in a population study. These sources of variability and uncertainty may explain why the available human data at ambient concentrations for some environmental pollutants (e.g., particulate matter [PM], $O_3$, lead [Pb], environmental tobacco smoke [ETS], radiation) do not exhibit thresholds for cancer or noncancer health effects, even though likely mechanisms include nonlinear processes for some key events.

Finally, identification of the population groups or lifestages that may be at greater risk of health effects from air pollutant exposures contributes to an understanding of the public health impact of pollutant exposures. In the ISA, the term "at-risk population" is used to encompass populations or lifestages that have a greater likelihood of experiencing health effects related to exposure to an air pollutant due to a variety of factors; other terms used in the literature include susceptible, vulnerable, and sensitive. These factors may be intrinsic, such as genetic or developmental factors, race, sex, lifestage, or the presence of pre-existing diseases, or they may be extrinsic, such as socioeconomic status (SES), activity pattern and exercise level, reduced access to health care, low educational attainment, or increased pollutant exposures (e.g., near roadways). Epidemiologic studies can help identify populations potentially at increased risk of effects by evaluating health responses in the study population. Examples include testing for interactions or effect modification by factors such as sex, age group, or health status. Experimental studies using animal models of susceptibility or disease can also inform the extent to which health risks are likely greater in specific population groups.

## Quantitative Relationships: Effects on Ecosystems or Public Welfare

Key questions for understanding the quantitative relationships between exposure (or concentration or deposition) to a pollutant and risk to ecosystems or the public welfare include:

- What elements of the ecosystem (e.g., types, regions, taxonomic groups, populations, functions, etc.) appear to be affected, or are more sensitive to effects? Are there differences between locations or materials in welfare effects responses, such as impaired visibility or materials damage?
- Under what exposure conditions (amount deposited or concentration, duration and pattern) are effects seen?
- What is the shape of the concentration-response or exposure-response relationship?

Evaluations of causality generally consider the probability of quantitative changes in ecological and welfare effects in response to exposure. A challenge to the quantification of exposure-response relationships for ecological effects is the great regional and local spatial variability, as well as temporal variability, in ecosystems. Thus, exposure-response relationships are often determined for a specific ecological system and scale, rather than at the national or even regional scale. Quantitative relationships therefore are estimated site by site and may differ greatly between ecosystems.

## Concepts in Evaluating Adversity of Health Effects

In evaluating health evidence, a number of factors can be considered in delineating between adverse and nonadverse health effects resulting from exposure to air

pollution. Some health outcomes, such as hospitalization for respiratory or cardiovascular diseases, are clearly considered adverse. It is more difficult to determine the extent of change that constitutes adversity in more subtle health measures. These include a wide variety of responses, such as alterations in markers of inflammation or oxidative stress, changes in pulmonary function or heart rate variability, or alterations in neurocognitive function measures. The challenge is determining the magnitude of change in these measures when there is no clear point at which a change becomes adverse. The extent to which a change in health measure constitutes an adverse health effect may vary between populations. Some changes that may not be considered adverse in healthy individuals would be potentially adverse in more at-risk individuals.

The extent to which changes in lung function are adverse has been discussed by the American Thoracic Society (ATS) in an official statement titled *What Constitutes an Adverse Health Effect of Air Pollution?* (ATS, 2000b). An air pollution-induced shift in the population distribution of a given risk factor for a health outcome was viewed as adverse, even though it may not increase the risk of any one individual to an unacceptable level. For example, a population of asthmatics could have a distribution of lung function such that no identifiable individual has a level associated with significant impairment. Exposure to air pollution could shift the distribution such that no identifiable individual experiences any clinically relevant effects. This shift toward decreased lung function, however, would be considered adverse because individuals within the population would have diminished reserve function and therefore would be at increased risk to further environmental insult. The committee also observed that elevations of biomarkers, such as cell number and types, cytokines and reactive oxygen species, may signal risk for ongoing injury and clinical effects or may simply indicate transient responses that can provide insights into mechanisms of injury, thus illustrating the lack of clear boundaries that separate adverse from nonadverse effects.

The more subtle health outcomes may be connected mechanistically to health events that are clearly adverse. For example, air pollution may affect markers of transient myocardial ischemia such as ST-segment abnormalities and onset of exertional angina. These effects may not be apparent to the individual, yet may still increase the risk of a number of cardiac events, including myocardial infarction and sudden death. Thus, small changes in physiological measures may not appear to be clearly adverse when considered alone, but may be a part of a coherent and biologically plausible chain of related health outcomes that range up to responses that are very clearly adverse, such as hospitalization or mortality.

## Concepts in Evaluating Adversity of Ecological Effects

Adversity of ecological effects can be understood in terms ranging in biological level of organization; from the cellular level to the individual organism and to the population, community, and ecosystem levels. In the context of ecology, a population is a group of individuals of the same species, and a community is an assemblage of populations of different species interacting with one another that inhabit an area.

An ecosystem is the interactive system formed from all living organisms and their abiotic (physical and chemical) environment within a given area (IPCC, 2007a). The boundaries of what could be called an ecosystem are somewhat arbitrary, depending on the focus of interest or study. Thus, the extent of an ecosystem may range from very small spatial scales to, ultimately, the entire Earth (IPCC, 2007a).

Effects on an individual organism are generally not considered to be adverse to public welfare. However if effects occur to enough individuals within a population, then communities and ecosystems may be disrupted. Changes to populations, communities, and ecosystems can in turn result in an alteration of ecosystem processes. Ecosystem processes are defined as the metabolic functions of ecosystems including energy flow, elemental cycling, and the production, consumption and decomposition of organic matter (U.S. EPA, 2002). Growth, reproduction, and mortality are species-level endpoints that can be clearly linked to community and ecosystem effects and are considered to be adverse when negatively affected. Other endpoints such as changes in behavior and physiological stress can decrease ecological fitness of an organism, but are harder to link unequivocally to effects at the population, community, and ecosystem level. The degree to which pollutant exposure is considered adverse may also depend on the location and its intended use (i.e., city park, commercial, cropland). Support for consideration of adversity beyond the species level by making explicit the linkages between stress-related effects at the species and effects at the ecosystem level is found in A *Framework for Assessing and Reporting on Ecological Condition: an SAB report* (U.S. EPA, 2002). Additionally, the National Acid Precipitation Assessment Program (NAPAP, 1991) uses the following working definition of "adverse ecological effects" in the preparation of reports to Congress mandated by the Clean Air Act: "any injury (i.e., loss of chemical or physical quality or viability) to any ecological or ecosystem component, up to and including at the regional level, over both long and short terms."

On a broader scale, ecosystem services may provide indicators for ecological impacts. Ecosystem services are the benefits that people obtain from ecosystems (UNEP, 2003). According to the Millennium Ecosystem Assessment, ecosystem services include: "provisioning services such as food and water; regulating services such as regulation of floods, drought, land degradation, and disease; supporting services such as soil formation and nutrient cycling; and cultural services such as recreational, spiritual, religious, and other nonmaterial benefits." For example, a more subtle ecological effect of pollution exposure may result in a clearly adverse impact on ecosystem services if it results in a population decline in a species that is recreationally or culturally important.

## References


ATS (American Thoracic Society). (2000b). What constitutes an adverse health effect of air pollution? This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. Am J Respir Crit Care Med 161: 665-673.

Bell, ML; Dominici, F; Samet, JM. (2005). A meta-analysis of time-series studies of ozone and mortality with comparison to the national morbidity, mortality, and air pollution study. Epidemiology 16: 436-445. http://dx.doi.org/10.1097/01.ede.0000165817.40152.85

CDC (Centers for Disease Control and Prevention). (2004). The health consequences of smoking: A report of the Surgeon General. Washington, DC: U.S. Department of Health and Human Services. http://www.surgeongeneral.gov/library/smokingconsequences/

Fox, GA. (1991). Practical causal inference for ecoepidemiologists. J Toxicol Environ Health A 33: 359-373. http://dx.doi.org/10.1080/15287399109531535

Gee, GC; Payne-Sturges, DC. (2004). Environmental health disparities: A framework integrating psychosocial and environmental concepts. Environ Health Perspect 112: 1645-1653. http://dx.doi.org/10.1289/ehp.7074

HEW (U.S. Department of Health, Education and Welfare). (1964). Smoking and health: Report of the advisory committee to the surgeon general of the public health service. Washington, DC: U.S. Department of Health, Education, and Welfare. http://profiles.nlm.nih.gov/ps/retrieve/ResourceMetadata/NNBBMQ

Hill, AB. (1965). The environment and disease: Association or causation? Proc R Soc Med 58: 295-300.

IARC (International Agency for Research on Cancer). (2006). Preamble to the IARC monographs. Lyon, France. http://monographs.iarc.fr/ENG/Preamble/

Ioannidis, JPA. (2008). Why most discovered true associations are inflated [Review]. Epidemiology 19: 640-648. http://dx.doi.org/10.1097/EDE.0b013e31818131e7

IPCC (Intergovernmental Panel on Climate Change). (2007a). Climate change 2007: Impacts, adaptation and vulnerability. Cambridge, UK: Cambridge University Press.

NAPAP (National Acid Precipitation Assessment Program). (1991). The experience and legacy of NAPAP: Report of the Oversight Review Board of the National Acid Precipitation Assessment Program. Washington, DC.

Rothman, KJ; Greenland, S. (1998). Modern epidemiology (2nd ed.). Philadelphia, PA: Lippincott, Williams, & Wilkins.

Samet, JM; Bodurow, CC. (2008). Improving the presumptive disability decision-making process for veterans. In JM Samet; CC Bodurow (Eds.). Washington, DC: National Academies Press. http://www.nap.edu/openbook.php?record_id=11908

U.S. EPA (U.S. Environmental Protection Agency). (1998). Guidelines for ecological risk assessment [EPA Report]. (EPA/630/R-95/002F). Washington, DC. http://www.epa.gov/raf/publications/guidelines-ecological-risk-assessment.htm

U.S. EPA (U.S. Environmental Protection Agency). (2002). A framework for assessing and reporting on ecological condition: An SAB report [EPA Report]. (EPA-SAB-EPEC-02-009). Washington, DC. http://www.ntis.gov/search/product.aspx?ABBR=PB2004100741

U.S. EPA (U.S. Environmental Protection Agency). (2005). Guidelines for carcinogen risk assessment [EPA Report]. (EPA/630/P-03/001F). Washington, DC. http://www.epa.gov/cancerguidelines/

U.S. EPA (U.S. Environmental Protection Agency). (2006b). Air quality criteria for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-05/004AF). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=149923

U.S. EPA (U.S. Environmental Protection Agency). (2009d). Integrated science assessment for particulate matter [EPA Report]. (EPA/600/R-08/139F). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=216546

UNEP (United Nations Environment Programme). (2003). Ecosystems and human well-being: A framework for assessment. Washington, DC: Island Press.

Zeger, SL; Thomas, D; Dominici, F; Samet, JM; Schwartz, J; Dockery, D; Cohen, A. (2000). Exposure measurement error in time-series studies of air pollution: Concepts and consequences. Environ Health Perspect 108: 419-426.

# LEGISLATIVE AND HISTORICAL BACKGROUND

## Legislative Requirements for the NAAQS Review

Two sections of the Clean Air Act (CAA) govern the establishment and revision of the National Ambient Air Quality Standards (NAAQS). Section 108 (42 USC §7408) directs the Administrator to identify and list certain air pollutants and then to issue air quality criteria for those pollutants. The Administrator is to list those air pollutants that in her "judgement; cause or contribute to air pollution which may reasonably be anticipated to endanger public health or welfare;" … "the presence of which in the ambient air results from numerous or diverse mobile or stationary sources" and "for which … [the Administrator] plans to issue air quality criteria…" (CAA, 1990a). Air quality criteria are intended to "accurately reflect the latest scientific knowledge useful in indicating the kind and extent of identifiable effects on public health or welfare, which may be expected from the presence of [a] pollutant in ambient air …" [42 USC §7408(b)].

Section 109 (CAA, 1990b) directs the Administrator to propose and promulgate "primary" and "secondary" NAAQS for pollutants for which air quality criteria have been issued. Section 109(b)(1) defines a primary standard as one "the attainment and maintenance of which in the judgment of the Administrator, based on such criteria and allowing an adequate margin of safety, are requisite to protect the public health."[1] A 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, based on such criteria, is required to protect the public welfare from any known or anticipated adverse effects associated with the presence of [the] pollutant in the ambient air."[2]

The requirement that primary standards include an adequate margin of safety was intended to address uncertainties associated with inconclusive scientific and technical information available at the time of standard setting. It was also intended to provide a reasonable degree of protection against hazards that research has not yet identified. See Lead Industries Association v. EPA, 647 F.2d 1130, 1154 (D.C. Cir 1980), cert. denied, 449 U.S. 1042 (1980); American Petroleum Institute v. Costle, 665 F.2d 1176, 1186 (D.C. Cir. (1981), cert. denied, 455 U.S. 1034 (1982). Both kinds of uncertainties are components of the risk associated with pollution at levels below those at which human health effects can be said to occur with reasonable scientific certainty. Thus, in selecting primary standards that include an adequate margin of safety, the Administrator is seeking not only to prevent pollution levels that have been demonstrated to be harmful but also to prevent lower pollutant levels that may

[1] The legislative history of section 109 indicates that a primary standard is to be set at "the maximum permissible ambient air level . . . which will protect the health of any [sensitive] group of the population," and that for this purpose "reference should be made to a representative sample of persons comprising the sensitive group rather than to a single person in such a group" [S. Rep. No. 91-1196, 91st Cong., 2d Sess. 10 (1970)].

[2] Welfare effects as defined in section 302(h) include, but are not limited to, "effects on soils, water, crops, vegetation, man-made materials, animals, wildlife, weather, visibility and climate, damage to and deterioration of property, and hazards to transportation, as well as effects on economic values and on personal comfort and well-being" (CAA, 2005).

pose an unacceptable risk of harm, even if the risk is not precisely identified as to nature or degree. The CAA does not require the Administrator to establish a primary NAAQS at a zero-risk level or at background concentration levels, see Lead Industries v. EPA, 647 F.2d at 1156 n.51, but rather at a level that reduces risk sufficiently so as to protect public health with an adequate margin of safety.

In addressing the requirement for a margin of safety, EPA considers such factors as the nature and severity of the health effects involved, the size of the sensitive population(s) at risk, and the kind and degree of the uncertainties that must be addressed. The selection of any particular approach to providing an adequate margin of safety is a policy choice left specifically to the Administrator's judgment. See Lead Industries Association v. EPA, supra, 647 F.2d at 1161-1162; Whitman v. American Trucking Associations, 531 U.S. 457, 495 (2001).

In setting standards that are "requisite" to protect public health and welfare, as provided in Section 109(b), EPA's task is to establish standards that are neither more nor less stringent than necessary for these purposes. In so doing, EPA may not consider the costs of implementing the standards. [See generally, Whitman v. American Trucking Associations, 531 U.S. 457, 465-472, 475-76. (2001)]. Likewise, "[a]ttainability and technological feasibility are not relevant considerations in the promulgation of national ambient air quality standards." American Petroleum Institute v. Costle, 665 F. 2d at 1185.

Section 109(d)(1) requires that "not later than December 31, 1980, and at 5-year intervals thereafter, the Administrator shall complete a thorough review of the criteria published under section 108 and the national ambient air quality standards … and shall make such revisions in such criteria and standards and promulgate such new standards as may be appropriate…" Section 109(d)(2) requires that an independent scientific review committee "shall complete a review of the criteria … and the national primary and secondary ambient air quality standards … and shall recommend to the Administrator any new … standards and revisions of existing criteria and standards as may be appropriate …" Since the early 1980s, this independent review function has been performed by CASAC.

## History of the NAAQS for Ozone

Tropospheric (ground-level) $O_3$ is the indicator for the mix of photochemical oxidants (e.g., peroxyacetyl nitrate, hydrogen peroxide) formed from biogenic and anthropogenic precursor emissions. Naturally occurring $O_3$ in the troposphere can result from biogenic organic precursors reacting with naturally occurring nitrogen oxides ($NO_X$) and by stratospheric $O_3$ intrusion into the troposphere. Anthropogenic precursors of $O_3$, especially $NO_X$, and volatile organic compounds (VOCs), originate from a wide variety of stationary and mobile sources. Ambient $O_3$ concentrations produced by these emissions are directly affected by temperature, solar radiation, wind speed, and other meteorological factors.

NAAQS are comprised of four basic elements: indicator, averaging time, level, and form. The indicator defines the pollutant to be measured in the ambient air for the purpose of determining compliance with the standard. The averaging time defines the time period over which air quality measurements are to be obtained and averaged or cumulated, considering evidence of effects associated with various time periods of exposure. The level of a standard defines the air quality concentration used (i.e., an ambient concentration of the indicator pollutant) in determining whether the standard is achieved. The form of the standard specifies the air quality measurements that are to be used for compliance purposes (e.g., the annual fourth-highest daily maximum 8-h concentration, averaged over 3 years), and whether the statistic is to be averaged across multiple years. These four elements taken together determine the degree of public health and welfare protection afforded by the NAAQS.

**Table III Summary of primary and secondary NAAQS promulgated for $O_3$ during the period 1971-2008.**

| Final Rule | Indicator | Avg Time | Level (ppm) | Form |
|---|---|---|---|---|
| 1971 (36 FR 8186) | Total photochemical oxidants | 1-h | 0.08 | Not to be exceeded more than 1 hour per year |
| 1979 (44 FR 8202) | $O_3$ | 1-h | 0.12 | Attainment is defined when the expected number of days per calendar year, with maximum hourly average concentration greater than 0.12 ppm, is ≤ 1 |
| 1993 (58 FR 13008) | EPA decided that revisions to the standards were not warranted at the time. | | | |
| 1997 (62 FR 38856) | $O_3$ | 8-h | 0.08 | Annual fourth-highest daily maximum 8-h concentration averaged over 3 years |
| 2008 (73 FR 16483) | $O_3$ | 8-h | 0.075 | Form of the standards remained unchanged relative to the 1997 standard |

Table III summarizes the $O_3$ NAAQS that have been promulgated to date. In each review, the secondary standard has been set to be identical to the primary standard. These reviews are briefly described below.

EPA first established primary and secondary NAAQS for photochemical oxidants in 1971 . Both primary and secondary standards were set at a level of 0.08 parts per million (ppm), 1-h avg, total photochemical oxidants, not to be exceeded more than 1 hour per year. The standards were based on scientific information contained in the 1970 $O_3$ AQCD.

In 1977, EPA announced the first periodic review of the 1970 AQCD in accordance with Section 109(d)(1) of the Clean Air Act. In 1978, EPA published an AQCD. Based on the 1978 AQCD, EPA published proposed revisions to the original NAAQS in 1978 (U.S. EPA, 1978b) and final revisions in 1979 (U.S. EPA, 1979a). The level of the primary and secondary standards was revised from 0.08 to 0.12 ppm;

the indicator was revised from photochemical oxidants to $O_3$; and the form of the standards was revised from a deterministic to a statistical form, which defined attainment of the standards as occurring when the expected number of days per calendar year with maximum hourly average concentration greater than 0.12 ppm is equal to or less than one.

In 1982, EPA announced plans to revise the 1978 AQCD (U.S. EPA, 1978a). In 1983, EPA announced that the second periodic review of the primary and secondary standards for $O_3$ had been initiated (U.S. EPA, 1983). EPA subsequently published the 1986 $O_3$ AQCD (U.S. EPA, 1986) and 1989 Staff Paper (U.S. EPA, 1989). Following publication of the 1986 $O_3$ AQCD, a number of scientific abstracts and articles were published that appeared to be of sufficient importance concerning potential health and welfare effects of $O_3$ to warrant preparation of a Supplement to the 1986 $O_3$ AQCD (Costa et al., 1992). Under the terms of a court order, on August 10, 1992, EPA published a proposed decision (U.S. EPA, 1992) stating that revisions to the existing primary and secondary standards were not appropriate at the time (U.S. EPA, 1992). This notice explained that the proposed decision would complete EPA's review of information on health and welfare effects of $O_3$ assembled over a 7-year period and contained in the 1986 $O_3$ AQCD (U.S. EPA, 1986) and its Supplement to the 1986 $O_3$ AQCD (Costa et al., 1992). The proposal also announced EPA's intention to proceed as rapidly as possible with the next review of the air quality criteria and standards for $O_3$ in light of emerging evidence of health effects related to 6- to 8-hour $O_3$ exposures. On March 9, 1993, EPA concluded the review by deciding that revisions to the standards were not warranted at that time (U.S. EPA, 1993).

In August 1992, EPA announced plans to initiate the third periodic review of the air quality criteria and $O_3$ NAAQS (U.S. EPA, 1992). On the basis of the scientific evidence contained in the 1996 $O_3$ AQCD (U.S. EPA, 1996a) and the 1996 Staff Paper (U.S. EPA, 1996e), and related technical support documents, linking exposures to ambient $O_3$ to adverse health and welfare effects at levels allowed by the then existing standards, EPA proposed to revise the primary and secondary $O_3$ standards on December 13, 1996 (U.S. EPA, 1996d). The EPA proposed to replace the then existing 1-hour primary and secondary standards with 8-h avg $O_3$ standards set at a level of 0.08 ppm (equivalent to 0.084 ppm using standard rounding conventions). The EPA also proposed, in the alternative, to establish a new distinct secondary standard using a biologically based cumulative seasonal form. The EPA completed the review on July 18, 1997 by setting the primary standard at a level of 0.08 ppm, based on the annual fourth-highest daily maximum 8-h avg concentration, averaged over 3 years, and setting the secondary standard identical to the revised primary standard (U.S. EPA, 1997).

On May 14, 1999, in response to challenges to EPA's 1997 decision by industry and others, the U.S. Court of Appeals for the District of Columbia Circuit (D.C. Cir.) remanded the $O_3$ NAAQS to EPA, finding that Section 109 of the CAA, as interpreted by EPA, effected an unconstitutional delegation of legislative authority. In addition, the D.C. Cir. directed that, in responding to the remand, EPA should

consider the potential beneficial health effects of $O_3$ pollution in shielding the public from the effects of solar ultraviolet (UV) radiation, as well as adverse health effects. On January 27, 2000, EPA petitioned the U.S. Supreme Court for certiorari on the constitutional issue (and two other issues) but did not request review of the D.C. Cir., ruling regarding the potential beneficial health effects of $O_3$. On February 27, 2001, the U.S. Supreme Court unanimously reversed the judgment of the D.C. Cir. on the constitutional issue, holding that Section 109 of the CAA does not delegate legislative power to the EPA in contravention of the Constitution, and remanded the case to the D.C. Cir. to consider challenges to the $O_3$ NAAQS that had not been addressed by that Court's earlier decisions. On March 26, 2002, the D.C. Cir. issued its final decision, finding the 1997 $O_3$ NAAQS to be "neither arbitrary nor capricious," and denied the remaining petitions for review. On November 14, 2001, in response to the D.C. Cir. remand to consider the potential beneficial health effects of $O_3$ pollution in shielding the public from effects of solar (UV) radiation, EPA proposed to leave the 1997 8-h $O_3$ NAAQS unchanged (U.S. EPA, 2001). After considering public comment on the proposed decision, EPA published its final response to this remand on January 6, 2003, reaffirming the 8-h $O_3$ NAAQS set in 1997 (U.S. EPA, 2003). On April 30, 2004, EPA announced the decision to make the 1-h $O_3$ NAAQS no longer applicable to areas 1 year after the effective date of the designation of those areas for the 8-h NAAQS (U.S. EPA, 2004). For most areas, the date that the 1-h NAAQS no longer applied was June 15, 2005.

EPA initiated the next periodic review of the air quality criteria and $O_3$ standards in September 2000 with a call for information (U.S. EPA, 2000). The schedule for completion of that rulemaking later became governed by a consent decree resolving a lawsuit filed in March 2003 by a group of plaintiffs representing national environmental and public health organizations. Based on the 2006 $O_3$ AQCD (U.S. EPA, 2006b) published in March 2006, the Staff Paper (U.S. EPA, 2007b) and related technical support documents, the proposed decision was published in the Federal Register on July 11, 2007 (U.S. EPA, 2007a). The EPA proposed to revise the level of the primary standard to a level within the range of 0.075 to 0.070 ppm. Two options were proposed for the secondary standard: (1) replacing the current standard with a cumulative, seasonal standard, expressed as an index of the annual sum of weighted hourly concentrations cumulated over 12 daylight hours during the consecutive 3-month period within the $O_3$ season with the maximum index value, set at a level within the range of 7 to 21 ppm-h; and (2) setting the secondary standard identical to the revised primary standard. The EPA completed the rulemaking with publication of a final decision on March 27, 2008 (U.S. EPA, 2008f), revising the level of the 8-hour primary $O_3$ standard from 0.08 ppm to 0.075 ppm and revising the secondary standard to be identical to the primary standard.

In May 2008, state, public health, environmental, and industry petitioners filed suit against EPA regarding that final decision. At EPA's request the consolidated cases were held in abeyance pending EPA's reconsideration of the 2008 decision. A notice of proposed rulemaking to reconsider the 2008 final decision was issued by the Administrator on January 6, 2010. Three public hearings were held. The Agency solicited CASAC review of the proposed rule on January 25, 2010 and additional

CASAC advice on January 26, 2011. On September 2, 2011, the Office of Management and Budget returned the draft final rule on reconsideration to EPA for further consideration. EPA decided to coordinate further proceedings on its voluntary rulemaking on reconsideration with the ongoing periodic review, by deferring the completion of its voluntary rulemaking on reconsideration until it completes its statutorily-required periodic review. In light of that, the litigation on the 2008 final decision is no longer being held in abeyance and is proceeding. The 2008 $O_3$ standards remain in effect.

## References


CAA. Clean Air Act, as amended by Pub. L. No. 101-549, section 108: Air quality criteria and control techniques, § 7408 (1990a). http://www.law.cornell.edu/uscode/text/42/7408

CAA. Clean Air Act, as amended by Pub. L. No. 101-549, section 109: National primary and secondary ambient air quality standards, § 7409 (1990b). http://www.epa.gov/air/caa/title1.html#ia

CAA. Clean Air Act, section 302: Definitions, § 7602 (2005). http://www.gpo.gov/fdsys/pkg/USCODE-2005-title42/pdf/USCODE-2005-title42-chap85-subchapIII-sec7602.pdf

Costa, DL; Folinsbee, LJ; Raub, JA; Tilton, B; Tingey, DT. (1992). Summary of selected new information on effects of ozone on health and vegetation: Supplement to 1986 air quality criteria for ozone and other photochemical oxidants. (EPA/600/8-88/105F). Research Triangle Park, NC: U.S. Environmental Protection Agency. http://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=31093

U.S. EPA (U.S. Environmental Protection Agency). (1971). National primary and secondary ambient air quality standards. Fed Reg 36: 8186-8201.

U.S. EPA (U.S. Environmental Protection Agency). (1978a). Air quality criteria for ozone and other photochemical oxidants [EPA Report]. (EPA/600/8-78/004). Washington, DC.

U.S. EPA (U.S. Environmental Protection Agency). (1978b). Photochemical oxidants: Proposed revisions to the national ambient air quality standards. Fed Reg 43: 26962-26971.

U.S. EPA (U.S. Environmental Protection Agency). (1979a). National primary and secondary ambient air quality standards: Revisions to the national ambient air quality standards for photochemical oxidants. Fed Reg 44: 8202-8237.

U.S. EPA (U.S. Environmental Protection Agency). (1982). Air quality criteria document for ozone and other photochemical oxidants. Fed Reg 47: 11561.

U.S. EPA (U.S. Environmental Protection Agency). (1983). Review of the national ambient air quality standards for ozone. Fed Reg 48: 38009.

U.S. EPA (U.S. Environmental Protection Agency). (1986). Air quality criteria for ozone and other photochemical oxidants [EPA Report]. (EPA-600/8-84-020aF - EPA-600/8-84-020eF). Research Triangle Park, NC. http://www.ntis.gov/search/product.aspx?ABBR=PB87142949

U.S. EPA (U.S. Environmental Protection Agency). (1989). Review of the national ambient air quality standards for ozone: Assessment of scientific and technical information: OAQPS staff report [EPA Report]. (EPA-450/2-92-001). Research Triangle Park, NC. http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=2000LOW6.txt

U.S. EPA (U.S. Environmental Protection Agency). (1992). National ambient air quality standards for ozone; Proposed decision. Fed Reg 57: 35542-35557.

U.S. EPA (U.S. Environmental Protection Agency). (1993). National ambient air quality standards for ozone - Final decision. Fed Reg 58: 13008-13019.

U.S. EPA (U.S. Environmental Protection Agency). (1996d). National ambient air quality standards for ozone: Proposed decision. Fed Reg 61: 65716-65750.

U.S. EPA (U.S. Environmental Protection Agency). (1996e). Review of national ambient air quality standards for ozone: Assessment of scientific and technical information: OAQPS staff paper [EPA Report]. (EPA/452/R-96/007). Research Triangle Park, NC. http://www.ntis.gov/search/product.aspx?ABBR=PB96203435

U.S. EPA (U.S. Environmental Protection Agency). (1997). National ambient air quality standards for ozone; final rule. Fed Reg 62: 38856-38896.

U.S. EPA (U.S. Environmental Protection Agency). (2000). Air quality criteria for ozone and related photochemical oxidants. Fed Reg 65: 57810.

U.S. EPA (U.S. Environmental Protection Agency). (2001). National ambient air quality standards for ozone: Proposed response to remand. Fed Reg 66: 57268-57292.

U.S. EPA (U.S. Environmental Protection Agency). (2003). National ambient air quality standards for ozone: Final response to remand. Fed Reg 68: 614-645.

U.S. EPA (U.S. Environmental Protection Agency). (2004). Final rule to implement the 8-hour ozone national ambient air quality standard-phase 1. Fed Reg 69: 23951-24000.

U.S. EPA (U.S. Environmental Protection Agency). (2006b). Air quality criteria for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-05/004AF). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=149923

U.S. EPA (U.S. Environmental Protection Agency). (2007a). National ambient air quality standards for ozone. Fed Reg 72: 37818-37919.

U.S. EPA (U.S. Environmental Protection Agency). (2007b). Review of the national ambient air quality standards for ozone: Policy assessment of scientific and technical information: OAQPS staff paper [EPA Report]. (EPA/452/R-07/003). Research Triangle Park, NC. http://www.epa.gov/ttn/naaqs/standards/ozone/data/2007_01_ozone_staff_paper

U.S. EPA (U.S. Environmental Protection Agency). (2008f). National ambient air quality standards for ozone. Fed Reg 73: 16436-16514.

# 1 EXECUTIVE SUMMARY

## Introduction and Purpose

The purpose of this Integrated Science Assessment (ISA) is to provide a synthesis and evaluation of the most policy-relevant science that builds the scientific foundation for the periodic review of the primary (health-based) and secondary (welfare-based) national ambient air quality standard (NAAQS) for ozone ($O_3$) and related photochemical oxidants required by the Clean Air Act. The primary NAAQS protects against respiratory health effects incurred after short-term exposure to $O_3$, while the secondary NAAQS protects against damage to vegetation and ecosystems, generally referred to as "welfare effects." The ISA is intended to inform both EPA's Risk and Exposure Assessment and Policy Assessment, and decisions by the EPA Administrator on the NAAQS for $O_3$ (see Figure I in Preamble). Set in 2008, the current primary $O_3$ standard is an 8-hour average standard of 75 parts per billion (ppb). The secondary standard for $O_3$ is equal to the primary standard.

## Scope and Methods

EPA has an extensive, robust process for evaluating the latest scientific evidence and drawing conclusions regarding air pollution-related health and welfare effects. Building upon the findings of previous assessments, this review includes identification, selection, evaluation, and integration of the relevant results pertaining to the atmospheric science of $O_3$; short- and long-term exposure to ambient $O_3$; health effects due to relevant $O_3$ concentrations as characterized in epidemiologic or controlled human exposure and toxicological studies; and ecological and other welfare effects. Additionally, this review will characterize $O_3$ concentration-response relationships, mode(s) of action, and populations at increased risk for $O_3$-related health effects. The conclusions and key findings from previous reviews provide the basis for the consideration of evidence from recent studies (i.e., studies published since the completion of the 2006 $O_3$ Air Quailty Criteria Document [AQCD]). Conclusions are drawn based on the synthesis of evidence across scientific disciplines.

EPA assesses the body of peer-reviewed literature to draw conclusions on the causal relationships between relevant pollutant concentrations and health or welfare effects. EPA specifically evaluates the quantitative evidence and draws scientific conclusions, to the extent possible, regarding the concentration-response relationships and the loads to ecosystems, exposure doses or concentrations, and duration and pattern of exposures at which effects are observed.

EPA uses a consistent and transparent approach to evaluate the causal nature of air pollution-related health and environmental effects for use in developing ISAs; the framework for causal determinations is described in the Preamble to this document. Causality determinations are based on the evaluation and synthesis of evidence

across scientific disciplines. A five-level hierarchy is used to classify the weight of evidence for causation, not just association. This weight of evidence evaluation is based on various lines of evidence from across the health and environmental effects disciplines. These separate conclusions are integrated into a qualitative statement about the overall weight of the evidence and causality. The causal determinations are:

- Causal relationship
- Likely to be a causal relationship
- Suggestive of a causal relationship
- Inadequate to infer a causal relationship
- Not likely to be a causal relationship

## Ambient Ozone Concentrations

Ozone is naturally present in the stratosphere (an elevated layer of the Earth's atmospehre) where it serves the beneficial role of absorbing harmful ultraviolet radiation from the Sun, preventing the majority of this radiation from reaching the surface of the Earth. However, in the troposphere (the layer of the atmosphere extending from the stratosphere down to the Earth's surface), $O_3$ acts as a powerful oxidizing agent, which can harm living organisms and materials. Tropospheric $O_3$ is present not only in polluted urban air, but across the globe. Ozone concentrations can be influenced by local meteorological conditions, circulation patterns, emissions, and topographic barriers, resulting in heterogeneous concentrations across an individual urban area. On a larger scale, $O_3$ can last in the atmosphere long enough that it can be transported from continent to continent.

Ozone in the troposphere originates from both anthropogenic (i.e., man-made) and natural sources. Ozone attributed to anthropogenic sources is formed by photochemical reactions involving sunlight and precursor pollutants, including volatile organic compounds (VOCs), nitrogen oxides ($NO_X$), and carbon monoxide (CO). Ozone attributed to natural sources is formed through the same photochemical reactions involving natural emissions of precursor pollutants from vegetation, microbes, animals, burning biomass (e.g., forest fires), and lightning. Ozone is lost through deposition to surfaces and chemical reactions occurring in the atmosphere. The highest $O_3$ concentrations are not found in urban areas close to the concentrated sources of its precursors such as traffic, but rather in suburban and rural areas downwind of these sources. Reaction of $O_3$ with NO in fresh motor vehicle exhaust depletes $O_3$ in urban cores; but $O_3$ can be regenerated during transport downwind of urban source areas. Also $O_3$ tends to be more uniform in rural than in urban areas because $O_3$ production occurs over large areas and because the concentrated sources of NO depleting $O_3$ in urban cores are generally lacking (except near power plants and other strong sources of NO).

In the context of a review of the NAAQS, it is useful to define background $O_3$ concentrations in a way that distinguishes between concentrations that result from precursor emissions that are relatively less controllable (including natural sources

and foreign anthropogenic sources) from those that are relatively more controllable through U.S. policies. For example, U.S. background $O_3$ concentrations can be defined as those concentrations resulting from natural sources everywhere in the world plus anthropogenic sources outside the U.S.; North American (NA) background $O_3$ concentrations can be defined as those concentrations resulting from natural sources everywhere in the world plus anthropogenic sources outside the U.S., Canada and Mexico. Since background defined either of these ways is a hypothetical construct that cannot be measured, NA background $O_3$ concentrations are estimated using chemistry transport models (see Sections 2.2 and 3.4).

## Human Exposure to Ozone

The widespread presence of $O_3$ in the environment results in exposure as people participate in normal daily activities. Exposure may occur indoors, where people spend most of their time, as well as outdoors, where $O_3$ concentrations are highest. Evaluating relationships among outdoor concentration, indoor concentration, and personal exposure is useful for determining how well ambient monitors represent exposure. The relationship between personal exposure and ambient concentration measured at fixed-site monitors can be described in terms of correlation (how they covary over time) and ratio, which describes their relative magnitude. High correlations imply that changes in ambient concentrations are reflected in personal exposure, while high ratios imply that the magnitude of exposure is similar to the magnitude of concentrations. Personal-ambient $O_3$ correlations are generally moderate (0.3-0.8), although low correlations have been observed with increased time spent indoors, low indoor-outdoor air exchange rates, and concentrations below personal sampler detection limits (see Section 4.3). Ratios of 0.1-0.3 between personal exposure and ambient concentration have been observed for the general population, with ratios of up to 0.9 observed for outdoor workers. Evidence suggests that some groups, particularly children, older adults, and those with respiratory problems, change their behavior on high-$O_3$ days when $O_3$ warnings are issued with advice to reduce exposure (see Section 4.4.2). Such behavioral changes may result in reduced effect estimates in epidemiologic studies that do not account for averting behavior on high-$O_3$ days. Variation in $O_3$ concentrations occurs over multiple spatial and temporal scales, and this introduces exposure error into epidemiologic results (see Section 4.6.2). However, epidemiologic studies evaluating the influence of spatial scale and monitor selection find little difference among effect estimates, and comparable risk estimates have been reported in studies using a variety of exposure assessment techniques expected to produce different levels of personal-ambient associations. This suggests that there is no clear indication that any particular method of exposure assessment produces stronger epidemiologic results than any other method.

## Dosimetry and Modes of Action

When $O_3$ is inhaled, the amount of $O_3$ that is absorbed is affected by the shape and size of the respiratory tract, and the route of breathing (nose or mouth), as well as how quickly and deeply a person is breathing. The amount of $O_3$ that is removed from the air stream during breathing is referred to as uptake. The primary site of $O_3$ uptake moves deeper into the respiratory tract during exercise when breathing becomes faster and the breathing route changes from the nose only to oronasal breathing (i.e., through the nose and mouth) (see Section 5.2). Tissue dose refers to the amount of $O_3$ that diffuses through the lining of the lung and reaches the underlying tissues. The site of the greatest $O_3$ dose to the lung tissue is the junction of the conducting airway and the gas exchange region in the deeper portion of the lung.

Once $O_3$ has been absorbed, there are several key events in the toxicity pathway of $O_3$ in the respiratory tract that lead to $O_3$-induced health effects (see Section 5.3). These include formation of secondary oxidation products in the lung, activation of neural reflexes, initiation of inflammation, alterations of epithelial barrier function, sensitization of bronchial smooth muscle, modification of innate and adaptive immunity, and airway remodeling. Another key event, systemic inflammation and vascular oxidative/nitrosative stress, may be critical to the extrapulmonary effects of $O_3$.

Overall, biological responses to $O_3$ exposure are similar across many species (see Section 5.5). Thus, animal studies are used to add to the understanding of the full range of potential $O_3$-mediated health effects.

## Integration of Ozone Health Effects

In this ISA, the body of evidence from short-term (i.e., hours, days, weeks) or long-term (i.e., months to years) exposure studies is evaluated and integrated across relevant scientific disciplines (i.e., controlled human exposure studies, toxicology, and epidemiology) for health effects that vary in severity from minor subclinical effects to death. The results from the health studies, supported by the evidence from atmospheric chemistry and exposure assessment studies, contribute to the causal determinations made for the various health outcomes. Both the conclusions from the 2006 $O_3$ AQCD and the causality determinations from this review are summarized in Table 1-1. Additional details are provided here for respiratory and cardiovascular health effects and mortality, for which there is the strongest evidence of an effect from $O_3$; details for a wider range of health effects are provided in subsequent chapters[1].

[1] Detailed information $O_3$ concentrations at which health effects are observed can be found in later chapters. For an overview, please see Table 2-2.

## Table 1-1 Summary of $O_3$ causal determinations by exposure duration and health outcome.

| Health Outcome[a] | Conclusions from 2006 $O_3$ AQCD | Conclusions from this ISA |
|---|---|---|
| **Short-term Exposure to $O_3$** | | |
| Respiratory effects | The overall evidence supports a causal relationship between acute ambient $O_3$ exposures and increased respiratory morbidity outcomes. | Causal Relationship |
| Cardiovascular effects | The limited evidence is highly suggestive that $O_3$ directly and/or indirectly contributes to cardiovascular-related morbidity, but much remains to be done to more fully substantiate the association. | Likely to be a Causal Relationship |
| Central nervous system effects | Toxicological studies report that acute exposures to $O_3$ are associated with alterations in neurotransmitters, motor activity, short and long term memory, sleep patterns, and histological signs of neurodegeneration. | Suggestive of a Causal Relationship |
| Total Mortality | The evidence is highly suggestive that $O_3$ directly or indirectly contributes to non-accidental and cardiopulmonary-related mortality. | Likely to be a Causal Relationship |
| **Long-term Exposure to $O_3$** | | |
| Respiratory effects | The current evidence is suggestive but inconclusive for respiratory health effects from long-term $O_3$ exposure. | Likely to be a Causal Relationship |
| Cardiovascular effects | No conclusions in the 2006 $O_3$ AQCD. | Suggestive of a Causal Relationship |
| Reproductive and developmental effects | Limited evidence for a relationship between air pollution and birth-related health outcomes, including mortality, premature births, low birth weights, and birth defects, with little evidence being found for $O_3$ effects. | Suggestive of a Causal Relationship |
| Central nervous system effects | Evidence regarding chronic exposure and neurobehavioral effects was not available. | Suggestive of a Causal Relationship |
| Cancer | Little evidence for a relationship between chronic $O_3$ exposure and increased risk of lung cancer. | Inadequate to Infer a Causal Relationship |
| Total Mortality | There is little evidence to suggest a causal relationship between chronic $O_3$ exposure and increased risk for mortality in humans. | Suggestive of a Causal Relationship |

[a]Health effects (e.g., respiratory effects, cardiovascular effects) include a spectrum of outcomes, from measureable subclinical effects (e.g., blood pressure), to more obvious effects (e.g., medication use, hospital admissions), and cause-specific mortality. Total mortality includes all-cause (non-accidental) mortality, as well as cause-specific mortality (e.g., deaths due to heart attacks).

## Respiratory Effects

The clearest evidence for health effects associated with exposure to $O_3$ is provided by studies of respiratory effects. Collectively, a very large amount of evidence spanning several decades supports a relationship between exposure to $O_3$ and a broad range of respiratory effects (see Section 6.2.9 and Section 7.2.8). The majority of this evidence is derived from studies investigating short-term exposure (i.e., hours to weeks) to $O_3$, although animal toxicological studies and recent epidemiologic evidence demonstrate that long-term exposure (i.e., months to years) may also harm the respiratory system.

The 2006 $O_3$ AQCD concluded that there was clear, consistent evidence of a causal relationship between short-term exposure to $O_3$ and respiratory health effects. This causal association is more substantiated now by the effects observed across recent controlled human exposure, epidemiologic, and toxicological studies indicating associations between short-term $O_3$ exposures and a range of respiratory health endpoints from respiratory tract inflammation to respiratory-related emergency department (ED) visits and hospital admissions. Short-term $O_3$ exposures induced (or were associated with) statistically significant declines in lung function. An equally strong body of evidence from controlled human exposure and toxicological studies demonstrated reversible $O_3$-induced increases in inflammatory responses, epithelial permeability, and airway hyperresponsiveness that were found to last for 18-24 hours after $O_3$ exposure. Toxicological studies in animals provided additional evidence for $O_3$-induced impairment of host defenses. Combined, these findings from experimental studies provided support for epidemiologic evidence, in which short-term increases in $O_3$ concentration were consistently associated with increases in respiratory symptoms and asthma medication use in children with asthma, respiratory-related hospital admissions, and ED visits for chronic obstructive pulmonary disease (COPD) and asthma. Additionally, recent epidemiologic evidence supports the range of respiratory effects induced by $O_3$ by demonstrating that short-term increases in ambient $O_3$ concentrations can lead to respiratory mortality. The combined evidence from these disciplines supports the conclusion that there **is a causal relationship between short-term $O_3$ exposure and respiratory effects.**

Epidemiologic evidence for a relationship between long-term $O_3$ exposure and respiratory effects includes recent studies that evaluate the associations between long-term exposure to $O_3$ and respiratory effects that demonstrate interactions between exercise or different genetic variants and both new-onset asthma in children and increased respiratory symptom effects in individuals with asthma. Additional studies of respiratory health effects and a study of respiratory mortality provide a collective body of evidence supporting this relationship. Studies evaluating other pollutants provide data suggesting that the effects related to $O_3$ are independent from the effects of the other pollutants. Short-term studies provide supportive evidence with increases in respiratory symptoms and asthma medication use, hospital admissions and ED visits for all respiratory outcomes and asthma, and decreased lung function in children. Taken together, the recent epidemiologic studies of

respiratory health effects (including symptoms, new-onset asthma and mortality) combined with toxicological studies in rodents and nonhuman primates, provide biologically plausible evidence that there **is likely to be a causal relationship between long-term exposure to $O_3$ and respiratory effects.**

## Mortality Effects

The last review concluded that the overall body of evidence was highly suggestive that short-term exposure to $O_3$ directly or indirectly contributes to non-accidental and cardiopulmonary-related mortality, but that additional research was needed to more fully establish the underlying mechanisms by which such effects occur. Recent multicity studies and a multicontinent study have reported associations between short-term $O_3$ exposure and mortality, expanding upon evidence available in the last review (see Section 6.6). These recent studies reported consistent positive associations between short-term $O_3$ exposure and total (nonaccidental) mortality, with associations being stronger during the warm season, when $O_3$ concentrations were higher. They also observed associations between $O_3$ exposure and cardiovascular and respiratory mortality. These recent studies also examined previously identified areas of uncertainty in the $O_3$-mortality relationship, and provided additional evidence supporting an association between short-term $O_3$ exposure and mortality. As a result, the current body of evidence indicates that there **is likely to be a causal relationship between short-term exposures to $O_3$ and total mortality.**

## Cardiovascular Effects

In previous $O_3$ reviews, very few studies were available which examined the effect of short-term $O_3$ exposure on the cardiovascular system. New toxicological studies, although limited in number, have provided evidence of $O_3$-induced cardiovascular effects. These effects may, in part, correspond to changes in the autonomic nervous system or to the development and maintenance of oxidative stress and inflammation throughout the body that resulted from inflammation in the lungs. Controlled human exposure studies also suggest cardiovascular effects in response to short-term $O_3$ exposure, including changes in heart rate variability and blood markers of systemic inflammation and oxidative stress, which provide some coherence with the effects observed in animal toxicology studies. Collectively, the experimental studies provide initial biological plausibility for the consistently positive associations observed in epidemiologic studies of short-term $O_3$ exposure and cardiovascular mortality. However, studies in the epidemiologic literature generally have not observed a relationship between short-term exposure to $O_3$ and cardiovascular morbidity including studies that examined the association between short-term $O_3$ exposure and cardiovascular-related hospital admissions and ED visits and other various cardiovascular effects. The lack of coherence between the results from studies that examined associations between short-term $O_3$ exposure and cardiovascular morbidity and cardiovascular mortality complicate the interpretation of the overall evidence for

$O_3$-induced cardiovascular effects. Although there is a lack of coherence with epidemiologic studies of cardiovascular morbidity, animal toxicological studies demonstrate $O_3$-induced cardiovascular effects, and provide support to the strong body of evidence indicating $O_3$-induced cardiovascular mortality. Overall, the body of evidence indicates that there **is likely to be a causal relationship between short-term exposures to $O_3$ and cardiovascular effects, including cardiovascular mortality.**

### Populations Potentially at Increased Risk

The examination of populations and lifestages potentially at increased risk for $O_3$ exposure identifies populations that are at increased risk for $O_3$-related health effects; these studies do so by examining groups within the study population, such as those with an underlying health condition or genetic variant, categories of age, race, or sex, or by developing animal models that mimic the conditions associated with a health effect. Such studies have identified a multitude of factors that could potentially contribute to whether a population is at increased risk for $O_3$-related health effects (see Chapter 8). The populations and lifestages identified as having adequate evidence for being at increased risk of $O_3$-related health effects are individuals with asthma, younger and older age groups, individuals with reduced intake of certain nutrients (i.e., Vitamins C and E), outdoor workers, and individuals having variants (including variations in multiple genes related to oxidative metabolism or inflammation). The evidence for other potential factors; sex, socioeconomic status, and obesity, is suggestive of an increased risk, but further evidence is needed.

## Integration of Effects on Vegetation and Ecosystems

The most policy-relevant information pertaining to the review of the NAAQS for the effects of $O_3$ on vegetation and ecosystems has been evaluated and synthesized, integrating key findings about plant physiology, biochemistry, whole plant biology, ecosystems and exposure-response relationships. The welfare effects of $O_3$ can be observed across spatial scales, starting at the subcellular and cellular level, then the whole plant and finally, ecosystem-level processes. Ozone effects at small spatial scales, such as the leaf of an individual plant, can result in effects along a continuum of larger spatial scales. These effects include altered rates of leaf gas exchange, growth, and reproduction at the individual plant level, and can result in broad changes in ecosystems, such as productivity, carbon storage, water cycling, nutrient cycling, and community composition. The conclusions from the previous NAAQS review and the causality determinations from this review are summarized in Table 1-2 below. Further discussion of these is provided after Table 1-2 for: visible foliar injury; growth, productivity, and carbon storage; yield and quality of agricultural crops; water cycling; below-ground processes; community composition; and $O_3$ exposure-response relationships. Discussion of all relevant welfare effects is provided in Chapter 9.

## Table 1-2 Summary of $O_3$ causal determination for welfare effects.

| Vegetation and Ecosystem Effects | Conclusions from 2006 $O_3$ AQCD | Conclusions from this ISA |
|---|---|---|
| Visible Foliar Injury Effects on Vegetation | Data published since the 1996 $O_3$ AQCD strengthen previous conclusions that there **is strong evidence that current ambient $O_3$ concentrations cause impaired aesthetic quality** of many native plants and trees by increasing foliar injury. | Causal Relationship |
| Reduced Vegetation Growth | Data published since the 1996 $O_3$ AQCD strengthen previous conclusions that there **is strong evidence that current ambient $O_3$ concentrations cause decreased growth and biomass accumulation** in annual, perennial and woody plants, including agronomic crops, annuals, shrubs, grasses, and trees. | Causal Relationship |
| Reduced Productivity in Terrestrial Ecosystems | There **is evidence that $O_3$ is an important stressor of ecosystems** and that the effects of $O_3$ on individual plants and processes are scaled up through the ecosystem, affecting net primary productivity. | Causal Relationship |
| Reduced Carbon (C) Sequestration in Terrestrial Ecosystems | **Limited studies** from the 2006 $O_3$ AQCD. | Likely to be a Causal Relationship |
| Reduced Yield and Quality of Agricultural Crops | Data published since the 1996 $O_3$ AQCD strengthen previous conclusions that there **is strong evidence that current ambient $O_3$ concentrations cause decreased yield and/or nutritive quality** in a large number of agronomic and forage crops. | Causal Relationship |
| Alteration of Terrestrial Ecosystem Water Cycling | Ecosystem **water quantity may be affected by $O_3$ exposure** at the landscape level. | Likely to be a Causal Relationship |
| Alteration of Below-ground Biogeochemical Cycles | Ozone-sensitive species have **well known responses to $O_3$ exposure**, including altered carbon (C) allocation to below-ground tissues, and also altered rates of leaf and root production, turnover, and decomposition. These shifts can affect overall C and nitrogen (N) loss from the ecosystem in terms of respired C, and leached aqueous dissolved organic and inorganic C and N. | Causal Relationship |
| Alteration of Terrestrial Community Composition | Ozone may be affecting above- and below -ground community composition through impacts on both growth and reproduction. **Significant changes in plant community composition resulting directly from $O_3$ exposure** have been demonstrated. | Likely to be a Causal Relationship |

## Visible Foliar Injury

Visible foliar injury resulting from exposure to $O_3$ has been well characterized and documented over several decades on many tree, shrub, herbaceous and crop species. In addition, $O_3$-induced visible foliar injury symptoms on certain plant species (e.g., black cherry, yellow-poplar, and common milkweed, among others) are considered diagnostic of exposure to $O_3$, as experimental evidence has clearly established a consistent association, with greater exposure generally resulting in greater and more prevalent injury. Additional sensitive species showing visible foliar injury continue to be identified from field surveys and verified in controlled exposure studies (see Section 9.4.2). Overall, evidence is sufficient to conclude that there **is a causal relationship between ambient $O_3$ exposure and the occurrence of $O_3$-induced visible foliar injury on sensitive vegetation across the U.S.**

## Growth, Productivity, Carbon Storage and Agriculture

Ambient $O_3$ concentrations have long been known to cause decreases in photosynthetic rates and plant growth. The $O_3$-induced effects at the plant scale may translate to the ecosystem scale, with changes in productivity and carbon (C) storage. Studies demonstrating the effects of $O_3$ exposure on photosynthesis, growth, biomass allocation, ecosystem production and ecosystem C sequestration were reviewed for natural ecosystems (see Section 9.4.3), and crop productivity and crop quality were reviewed for agricultural ecosystems (see Section 9.4.4). There is strong and consistent evidence that current ambient concentrations of $O_3$ decrease plant photosynthesis and growth in numerous plant species across the U.S.

Studies conducted during the past four decades have also demonstrated unequivocally that $O_3$ alters biomass allocation and plant reproduction. Studies at the leaf and plant scales showed that $O_3$ reduced photosynthesis and plant growth, providing coherence and biological plausibility for the reported decreases in ecosystem productivity. In addition to primary productivity, other indicators such as net ecosystem $CO_2$ exchange and C sequestration were often assessed by modeling studies. Model simulations consistently found that $O_3$ exposure caused negative impacts on those indicators, but the severity of these impacts was influenced by multiple interactions of biological and environmental factors. Although $O_3$ generally causes negative effects on ecosystem productivity, the magnitude of the response varies among plant communities. Overall, evidence is sufficient to conclude that there **is a causal relationship between ambient $O_3$ exposure and reduced native plant growth and productivity,** and that there **is a likely causal relationship between $O_3$ exposure and reduced carbon sequestration in terrestrial ecosystems.**

The detrimental effect of $O_3$ on crop production has been recognized since the 1960s, and current $O_3$ concentrations in many areas across the U.S. are high enough to cause yield loss in a variety of agricultural crops including, but not limited to, soybeans, wheat, potatoes, watermelons, beans, turnips, onions, lettuces, and tomatoes.

Continued increases in $O_3$ concentration may further decrease yield in these sensitive crops while also causing yield losses in less sensitive crops. Research has linked increasing $O_3$ concentration to decreased photosynthetic rates and accelerated aging in leaves, which are related to yield (see Section 9.4.4). Recent research has highlighted the effects of $O_3$ on crop quality. Increasing $O_3$ concentration decreases nutritive quality of grasses, decreases macro- and micro-nutrient concentrations in fruits and vegetable crops, and decreases cotton fiber quality. Evidence is sufficient to conclude that there **is a causal relationship between $O_3$ exposure and reduced yield and quality of agricultural crops.**

### Water Cycling

Ozone can affect water use in plants and ecosystems through several mechanisms, including damage to stomatal functioning and loss of leaf area. Possible mechanisms for $O_3$ exposure effects on stomatal functioning include the build-up of $CO_2$ in the substomatal cavity, impacts on signal transduction pathways, and direct $O_3$ impact on guard cells. Regardless of the mechanism, $O_3$ exposure has been shown to alter stomatal performance, which can affect plant and stand transpiration and therefore has the potential to affect hydrological cycling (see Section 9.4.5). Although the direction of the response differed among studies, the evidence is sufficient to conclude that there **is likely to be a causal relationship between $O_3$ exposure and the alteration of ecosystem water cycling.**

### Below Ground Processes

Below-ground processes are tightly linked with above-ground processes. The responses of above-ground process to $O_3$ exposure, such as reduced photosynthetic rates, increased metabolic cost, and reduced allocation of C to the roots, have provided biologically plausible mechanisms for the alteration of below-ground processes. These include altered quality and quantity of C input to soil, changes in microbial community composition, and effects on C and nutrient cycling (see Section 9.4.6). The evidence is sufficient to conclude that there **is a causal relationship between $O_3$ exposure and the alteration of below-ground biogeochemical cycles.**

### Community Composition

Ozone exposure changes competitive interactions and leads to loss of $O_3$-sensitive species or genotypes. Studies at the plant level found that the severity of $O_3$ damage to growth, reproduction, and foliar injury varied among species, which provide the biological plausibility for the alteration of community composition (see Section 9.4.3 and Section 9.4.7). For example, there is a tendency for $O_3$ exposure to shift the biomass of grass-legume mixtures in favor of grass species. In addition, research since the last review has shown that $O_3$ can also alter community composition and diversity of soil microbial communities. Shifts in community composition of bacteria

and fungi have been observed in both natural and agricultural ecosystems, although no general patterns could be identified. The evidence is sufficient to conclude that there **is likely to be a causal relationship between $O_3$ exposure and the alteration of community composition of some ecosystems.**

### Air Quality Indices and Exposure-Response

Exposure indices are metrics that quantify exposure as it relates to measured plant response (e.g., reduced growth). They are summary measures of monitored $O_3$ concentrations over time intended to provide a consistent metric for reviewing and comparing exposure-response effects obtained from various studies. Given the current state of knowledge and the best available data, exposure indices that cumulate and differentially weight the higher hourly average concentrations and also include the mid-level values (e.g., the W126 metric, see Section 9.5) continue to offer the most defensible approach for use in developing response functions and comparing studies, as well as for defining future indices for vegetation protection.

Previous reviews of the NAAQs have included exposure-response functions for the yield of many crop species, and for the biomass accumulation of tree species. They were based on large-scale experiments designed to obtain clear exposure-response data, and are updated by using the W126 metric to quantify exposure. In recent years, extensive exposure-response data obtained in more naturalistic settings have become available for yield of soybean and growth of aspen. The exposure-response median functions are validated based on previous data by comparing their predictions with the newer observations (see Section 9.6). The functions supply very accurate predictions of effects in naturalistic settings. Recent meta-analyses of large sets of crop and tree studies do not give rise to exposure-response functions, but their results are consistent with the functions presented in Section 9.6. Although these median functions provide reliable models for groups of species or group of genotypes within a species, the original data and recent results consistently show that some species, and some genotypes within species are much more severely affected by exposure to $O_3$.

## The Role of Tropospheric Ozone in Climate Change and UV-B Shielding Effects

Atmospheric $O_3$ as a whole plays an important role in the Earth's energy budget by interacting with incoming solar radiation and outgoing infrared radiation. Though tropospheric $O_3$ makes up only a small portion of the total amount of $O_3$ in the atmosphere, it has important incremental effects on the overall radiation budget. Perturbations to tropospheric $O_3$ concentrations can have direct effects on climate and indirect effects on health, ecology, and welfare by changing the shielding of the earth's surface from solar ultraviolet (UV) radiation.

## Radiative Forcing and Climate Change

Radiative forcing by a greenhouse gas or aerosol is a metric used to quantify the change in balance between radiation coming into and going out of the atmosphere caused by the presence of that substance. Tropospheric $O_3$ is a major greenhouse gas and radiative forcing agent; evidence from satellite data shows a sharp dip in the outgoing infrared radiation in the 9.6 μm $O_3$ absorption band. Models calculate that the global average concentration of tropospheric $O_3$ has doubled since the pre-industrial era, while observations indicate that in some regions $O_3$ may have increased by factors as great as 4 or 5. These increases are tied to the rise in emissions of $O_3$ precursors from human activity, mainly fossil fuel consumption and agricultural processes. Overall, the evidence supports a **causal relationship between changes in tropospheric $O_3$ concentrations and radiative forcing.**

The impact of the tropospheric $O_3$ change since pre-industrial times on climate has been estimated to be about 25-40% of the anthropogenic $CO_2$ impact and about 75% of the anthropogenic $CH_4$ impact according to the Intergovernmental Panel on Climate Change (IPCC), ranking it third in importance after $CO_2$ and $CH_4$ according to the IPCC (see Section 10.3). There are large uncertainties in the magnitude of the radiative forcing estimate attributed to tropospheric $O_3$, making the effect of tropospheric $O_3$ on climate more uncertain than the effect of the longer-lived greenhouse gases. Furthermore, radiative forcing does not take into account climate feedbacks that could amplify or dampen the actual surface temperature response. Quantifying the change in surface temperature requires a complex climate simulation in which all important feedbacks and interactions are accounted for. The modeled surface temperature response to a given radiative forcing is highly uncertain and can vary greatly among models and from region to region within the same model. Even with these uncertainties, global climate models indicate that tropospheric $O_3$ has contributed to observed changes in global mean and regional surface temperatures. As a result of such evidence presented in climate modeling studies, there **is likely to be a causal relationship between changes in tropospheric $O_3$ concentrations and effects on climate.**

## UV-B Shielding Effects

UV radiation emitted from the Sun contains sufficient energy when it reaches the Earth to have damaging effects on living organisms and materials (see Section 10.4). Atmospheric $O_3$ plays a crucial role in reducing the amount of UV radiation reaching the Earth's surface. Ozone in the stratosphere is responsible for the majority of this shielding, but $O_3$ in the troposphere provides supplemental shielding of UV-B radiation in the mid-wavelength band (280-315 nm), thereby potentially reducing UV-B related human and ecosystem health effects and materials damage. EPA has found no published studies that adequately examine the incremental health or welfare effects (adverse or beneficial) attributable specifically to changes in UV-B exposure resulting from perturbations in tropospheric $O_3$ concentrations. While the effects are expected to be small, they cannot yet be critically assessed within reasonable

uncertainty. Overall, the evidence **is inadequate to determine if a causal relationship exists between changes in tropospheric $O_3$ concentrations and effects on health and welfare related to UV-B shielding.**

The conclusions from the previous NAAQS review and the causality determinations from this review relating climate change and UV-B shielding effects are summarized in the table below (Table 1-3), with details provided in Chapter 10.

**Table 1-3 Summary of $O_3$ causal determination for climate change and UV-B shielding effects.**

| Effects | Conclusions from 2006 $O_3$ AQCD | Conclusions from this ISA |
|---|---|---|
| Radiative Forcing | The 2006 $O_3$ AQCD concluded that climate forcing by $O_3$ at the regional scale may be its most important impact on climate. | Causal Relationship |
| Climate Change | While more certain estimates of the overall importance of global-scale forcing due to tropospheric $O_3$ await further advances in monitoring and chemical transport modeling, the overall body of scientific evidence reviewed in the 2006 $O_3$ AQCD **suggests** that high concentrations of $O_3$ on the regional scale **could have a discernible** influence on climate, leading to surface temperature and hydrological cycle changes. | Likely to be a Causal Relationship |
| Health and Welfare Effects Related to UV-B Shielding | UV-B has **not been studied in sufficient detail** to allow for a credible health benefits assessment. In conclusion, the effect of changes in surface-level $O_3$ concentrations on UV-induced health outcomes cannot yet be critically assessed within reasonable certainty. | Inadequate to Determine if a Causal Relationship Exists |

## Conclusion

The clearest evidence for human health effects associated with exposure to $O_3$ is provided by studies of respiratory effects. Collectively, there is a very large amount of evidence spanning several decades in support of a causal association between exposure to $O_3$ and a broad range of respiratory effects, indicating that there **is a causal relationship between short-term exposures to $O_3$ and respiratory effects.** The majority of this evidence is derived from studies investigating short-term $O_3$ exposure (i.e., hours to weeks), although animal toxicological studies and recent epidemiologic evidence demonstrate that long-term exposure (i.e., months to years) are likely to be detrimental to the respiratory system. Additionally, consistent positive associations between short-term $O_3$ exposure and total (nonaccidental) mortality have helped to resolve previously identified areas of uncertainty in the $O_3$-mortality relationship, indicating that there **is likely to be a causal relationship between short-term exposures to $O_3$ and total mortality**. Taken together, the recent epidemiologic studies of respiratory health effects (including respiratory symptoms, new-onset asthma and respiratory mortality) combined with toxicological

studies in rodents and nonhuman primates, provide biologically plausible evidence that there **is likely to be a causal relationship between long-term exposure to $O_3$ and respiratory effects**. Animal toxicological studies demonstrate $O_3$-induced cardiovascular effects, and provide support to the strong body of evidence indicating $O_3$-induced cardiovascular mortality, which together indicate that there **is likely to be a causal relationship between short-term exposure to $O_3$ and cardiovascular effects.** Recent evidence **is suggestive of a causal relationship between long-term $O_3$ exposures and total mortality.** The evidence for these health effects indicates that the relationship between concentration and response is linear along the range of $O_3$ concentrations observed in the U.S., with no indication of a threshold within that range. However, there is less certainty in the shape of the concentration-response curve at $O_3$ concentrations generally below 20 ppb. The populations identified as having increased risk of $O_3$-related health effects are individuals with asthma, younger and older age groups, individuals with certain dietary deficiencies, and outdoor workers.

There has been over 40 years of research on the effects of $O_3$ exposure on vegetation and ecosystems. The best evidence for effects is from controlled exposure studies. These studies have clearly shown that **exposure to $O_3$ is causally linked to visible foliar injury, decreased photosynthesis, changes in reproduction, and decreased growth**. Recently, studies at larger spatial scales support the results from controlled studies and indicate that ambient $O_3$ exposures can affect ecosystem productivity, crop yield, water cycling, and ecosystem community composition. And on a global scale, tropospheric $O_3$ is the third most important greenhouse gas, making it likely to play an important role in climate change.

# 2 INTEGRATIVE SUMMARY

This Integrated Science Assessment (ISA) forms the scientific foundation for the review of the current national ambient air quality standards (NAAQS) for ozone ($O_3$). The ISA is a concise evaluation and synthesis of the most policy-relevant science—and it communicates critical science judgments relevant to the review of the NAAQS for $O_3$. The ISA accurately reflects "the latest scientific knowledge useful in indicating the kind and extent of identifiable effects on public health or welfare which may be expected from the presence of [a] pollutant in ambient air" (CAA, 1990a). Key information and judgments contained in prior Air Quality Criteria Documents (AQCD) for $O_3$ are incorporated into this assessment. Additional details of the pertinent scientific literature published since the last review, as well as selected earlier studies of particular interest, are included. This ISA thus serves to update and revise the evaluation of the scientific evidence available at the time of the completion of the 2006 $O_3$ AQCD (U.S. EPA, 2006b). The current primary $O_3$ standard includes an 8-hour (h) average (avg) standard set at 75 parts per billion (ppb). The secondary standard for $O_3$ is set equal to the primary standard. Further information on the legislative and historical background for the $O_3$ NAAQS is contained in the Preface to this ISA.

This chapter summarizes and synthesizes the available scientific evidence and is intended to provide a concise synopsis of the ISA conclusions and findings that best inform consideration of the policy-relevant questions that frame this assessment (U.S. EPA, 2009c). It includes:

- An integration of the evidence on the health effects associated with short- and long-term exposure to $O_3$, discussion of important uncertainties identified in the interpretation of the scientific evidence, and an integration of health evidence from the different scientific disciplines and exposure durations.
- An integration of the evidence on the welfare effects associated with exposure to $O_3$, including those associated with vegetation and ecosystems, and discussion of important uncertainties identified in the interpretation of the scientific evidence.
- Discussion of policy-relevant considerations, such as potentially at-risk populations and concentration-response relationships and how they inform selection of appropriate exposure metrics/indices.

## 2.1 ISA Development and Scope

EPA has a developed a robust, consistent, and transparent process for evaluating the scientific evidence and drawing conclusions and causal judgments regarding air pollution-related health and environmental effects. The ISA development process includes literature search strategies, criteria for selecting and evaluating studies,

approaches for evaluating the weight of the evidence, and a framework for making causality determinations. The process and causality framework are described in more detail in the Preamble to the ISA. This section provides a brief overview of the process for development of this ISA.

EPA initiated the current review of the NAAQS for $O_3$ on September 29, 2008, with a call for information from the public (U.S. EPA, 2008g). Literature searches have been conducted routinely since then to identify studies published since the last review, focusing on studies published from 2005 (closing date for the previous scientific assessment) through July 2011. References that were considered for inclusion in this ISA can be found using the HERO website (http://hero.epa.gov/ozone). This site contains HERO links to lists of references that are cited in the ISA, as well as those that were considered for inclusion but not cited in the ISA, with bibliographic information and abstracts.

This review has endeavored to evaluate all relevant data published since the last review; this includes studies pertaining to the atmospheric science of $O_3$, human exposure to ambient $O_3$, and health, ecological, climate and UV-B shielding effects studies. These include studies that are related to concentration-response relationships, mode(s) of action (MOA), and understanding of at-risk populations for effects of $O_3$ exposure. Added to the body of research were EPA's analyses of air quality and emissions data, and studies on atmospheric chemistry, transport, and fate of these emissions using measurements and chemistry-transport models (CTMs).

Previous $O_3$ AQCDs (U.S. EPA, 2006b, 1996a, b, 1984, 1978a) have included an extensive body of evidence on both health and welfare effects of $O_3$ exposure, as well as an understanding of the atmospheric chemistry of $O_3$ (U.S. EPA, 2006b). In this ISA, the conclusions and key findings from previous reviews are summarized at the beginning of each section, to provide the foundation for consideration of evidence from recent studies. Results of key studies from previous reviews are included in discussions or tables and figures, as appropriate, and conclusions are drawn based on the synthesis of evidence from recent studies with the extensive literature summarized in previous reviews.

The Preamble discusses the general framework for conducting the science assessment and developing an ISA, including criteria for evaluating studies and developing scientific conclusions. For selection of epidemiologic studies in the $O_3$ ISA, particular emphasis is placed on those studies most relevant to the review of the NAAQS. Studies conducted in the United States (U.S.) or Canada are discussed in more detail than those from other geographical regions, and in regard to human health, particular emphasis is placed on: (1) recent multi-city studies that employ standardized analysis methods for evaluating effects of $O_3$ and that provide overall estimates for effects, based on combined analyses of information pooled across multiple cities; (2) studies that help understand quantitative relationships between exposure concentrations and effects; (3) new studies that provide evidence on effects in at-risk populations; and (4) studies that consider and report $O_3$ as a component of a complex mixture of air pollutants. In evaluating toxicological and controlled human exposure studies, emphasis is placed on studies using concentrations that are

generally no greater than about an order of magnitude higher than ambient $O_3$ concentrations currently found in many parts of the United States. Consideration of studies important for evaluation of human exposure to ambient $O_3$ places emphasis on those evaluating the relationship between $O_3$ measured at central site monitors and personal exposure to ambient $O_3$. Important factors affecting this relationship include spatial and temporal variations in ambient $O_3$ concentration, and time spent outdoors, since penetrations of $O_3$ into indoor environments may be limited.

This ISA uses a five-level hierarchy that classifies the weight of evidence for causation:

- Causal relationship
- Likely to be a causal relationship
- Suggestive of a causal relationship
- Inadequate to infer a causal relationship, or
- Not likely to be a causal relationship

Beyond judgments regarding causality are questions relevant to quantifying health or environmental risks based on the understanding of the quantitative relationships between pollutant exposures and health or welfare effects. Once a determination is made regarding the causal relationship between the pollutant and outcome category, important questions regarding quantitative relationships include:

- What is the concentration-response, exposure-response, or dose-response relationship?
- Under what exposure conditions (dose or concentration, duration, and pattern) are effects observed?
- What populations or lifestages appear to be differentially affected, i.e., at increased risk of $O_3$-related health effects?
- What elements of the ecosystem (e.g., types, regions, taxonomic groups, populations, functions, etc.) appear to be affected or are more sensitive to effects?

This chapter summarizes and integrates the newly available scientific evidence that best informs consideration of the policy-relevant questions that frame this assessment. Section 2.2 discusses the trends in ambient concentrations and sources of $O_3$ and provides a brief summary of ambient air quality for past and current short- and long-term exposure durations. Section 2.3 presents the evidence regarding personal exposure to ambient $O_3$ in outdoor and indoor microenvironments, and it discusses the relationship between ambient $O_3$ concentrations and personal exposure to ambient $O_3$. Section 2.4 provides a discussion of the dosimetry and modes of action evidence for $O_3$ exposure. Section 2.5 integrates the evidence for studies that examine the health effects associated with short- and long-term exposure to $O_3$ and discusses important uncertainties identified in the interpretation of the scientific evidence. A discussion of policy-relevant considerations, such as potentially at-risk populations, and the $O_3$ concentration-response relationship is also included in

Section 2.5. Finally, Section 2.6 summarizes the evidence for welfare effects related to $O_3$ exposure, and Section 2.7 reviews the literature on the influence of tropospheric $O_3$ on climate and exposure to solar ultraviolet radiation.

## 2.2 Atmospheric Chemistry and Ambient Concentrations

### 2.2.1 Physical and Chemical Processes

Ozone in the troposphere is a secondary pollutant; it is formed by reactions of precursor gases and is not directly emitted from specific sources. Ozone precursor gases originate from both anthropogenic (i.e., man-made) and natural source categories. Ozone attributed to anthropogenic sources is formed in the atmosphere by photochemical reactions involving sunlight and precursor pollutants including volatile organic compounds (VOCs), nitrogen oxides ($NO_X$), and carbon monoxide (CO). Ozone attributed to natural sources is formed through similar photochemical reactions involving natural emissions of precursor pollutants from vegetation, microbes, animals, biomass burning and lightning. An absolute distinction between natural and anthropogenic sources of $O_3$ precursors is often difficult to make in practice, as human activities affect directly or indirectly emissions from what are considered to be natural sources.

Ozone is present not only in polluted urban atmospheres but throughout the troposphere, even in remote areas of the globe. The same basic processes involving sunlight-driven reactions of $NO_X$, VOCs and CO that occur in polluted urban air also contribute to $O_3$ formation throughout the troposphere[1]. In urban areas, $NO_X$, VOCs, and CO are all important precursors to $O_3$ formation. In non-urban areas, biogenic VOCs emitted from vegetation tend to be the most important precursors to $O_3$ formation. In remote areas with little or no vegetation, and in general above the planetary boundary layer (PBL, extending typically from 1 to 3 km above the surface), methane—structurally the simplest VOC—and CO are the main carbon-containing precursors to $O_3$ formation. Ozone is subsequently removed from the atmosphere through a number of gas phase reactions and deposition to surfaces.

Convective processes and turbulence transport $O_3$ and other pollutants both upward and downward throughout the PBL and the free troposphere above. If pollutants are transported into the free troposphere above the PBL where winds are generally much stronger than in the PBL, they can be transported over longer distances than they can if they remained near the surface. Conversely, pollutants transported downward into the PBL can add to pollution burdens there. The transport of pollutants downwind of major urban centers is characterized by the development of urban plumes. Meteorological conditions, small-scale circulation patterns induced by surface characteristics and structures, localized chemistry, and topographic barriers can

[1] These processes also lead to the formation of other photochemical products, such as peroxyacetyl nitrate, nitric acid, and sulfuric acid, and to other compounds, such as formaldehyde and other carbonyl compounds.

influence mixing on the intra-urban scale, resulting in variability in $O_3$ concentrations across individual urban areas.

Apart from issues in understanding issues in $O_3$ formation in areas such as the Houston-Galveston-Brazoria airshed and the Northeast Corridor that are largely the result of local pollution sources during summer, other issues have become apparent in the past several years. Photochemically produced $O_3$ concentrations that exceed the level of the NAAQS have been observed in the Intermountain West in oil and gas fields during specific meteorological conditions (i.e., fresh snow cover, low mixing layer height trapping emissions) during winter. Because the mean tropospheric lifetime of $O_3$ is a few weeks, $O_3$ can be transported from continent to continent. Locations at high elevations are most susceptible to the intercontinental transport of pollution, particularly during spring. Intrusions of stratospheric air containing high $O_3$ may also cause, or contribute significantly to, exceedances of levels of the NAAQS for $O_3$. These events occur mainly in the West during spring.

### 2.2.2 Background $O_3$ Concentrations

In the context of a review of the NAAQS, it is useful to define background $O_3$ concentrations in a way that distinguishes between concentrations that result from precursor emissions that are relatively less controllable from those that are relatively more controllable through U.S. policies. For this assessment, three definitions of background $O_3$ concentrations are considered, including (1) United States (U.S.) background (simulated $O_3$ concentrations that would exist in the absence of anthropogenic emissions from the U.S.), (2) North American (NA) background (simulated $O_3$ concentrations that would exist in the absence of anthropogenic emissions from the U.S., Canada and Mexico), and (3) natural background (simulated $O_3$ concentrations in the absence of all anthropogenic emissions globally). Each definition of background $O_3$ includes contributions resulting from emissions from natural sources (e.g., stratospheric intrusions, wildfires, biogenic methane and more short-lived VOC emissions) throughout the globe. Differences among these definitions reflect differences in the inclusion of geographic regions that are sources of anthropogenic precursors. These definitions are used to inform policy considerations regarding the current or potential alternative standards. Note also there is no chemical difference between background $O_3$, and $O_3$ attributable to U.S. or North American anthropogenic sources.

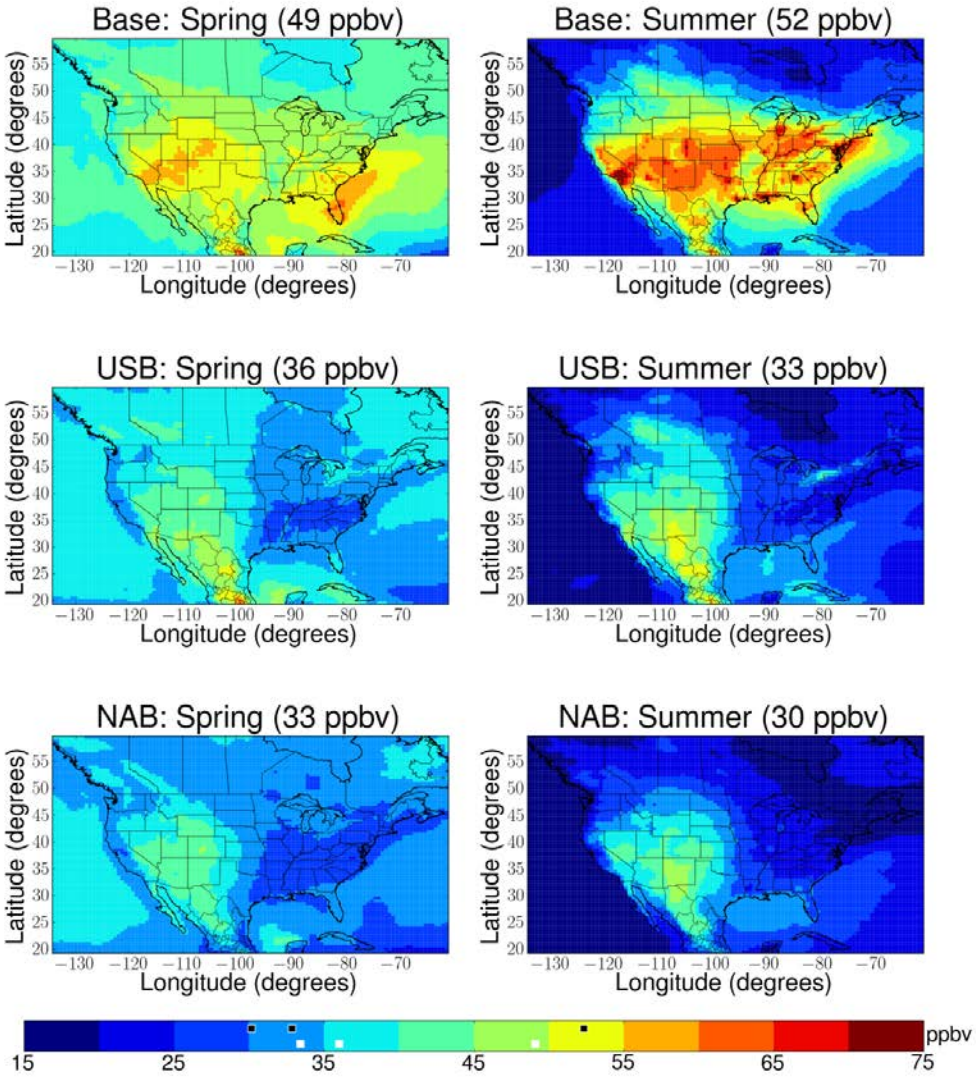
It is important to note that since background $O_3$ concentrations as defined above are a hypothetical construct that cannot be directly measured, the range of background $O_3$ concentrations must be estimated using CTMs. This is because observations within the U.S.—even at relatively remote monitoring sites—can be impacted by transport from anthropogenic sources within the U.S. or within the rest of North America (if a North American background is adopted).

Figure 2-1 shows spring and summer mean, maximum daily 8-h average $O_3$ concentrations simulated by the GEOS-Chem global CTM for the base case (top

panel), which includes all sources of $O_3$ precursors and can be compared to observations; the U.S. background (middle panel) and the North American (NA) background (bottom panel). Seasonal mean values for the entire continental United States are shown above each panel. In general, for most areas of the United States simulated seasonal mean 8-h max $O_3$ concentrations in the base case (top panel) are within a few ppb of those observed at rural Clean Air Status and Trends Network (CASTNET) monitoring sites, for which details are given in the next section. In moving from the top to the bottom panel, it can be seen that calculated concentrations decrease. Note also that mean base case concentrations (including U.S., Canadian and Mexican sources) are higher in the summer than in the spring, but that mean U.S. background and NA background concentrations are higher in spring than in summer, reflecting the increased importance of sources such as intercontinental transport and stratospheric intrusions.

As can be seen from the middle and bottom panels in Figure 2-1, estimated U.S. background and NA background concentrations tend to be higher in the West (particularly in the Intermountain West) and in the Southwest compared to the East in both spring and summer. U.S. background and NA background concentrations tend to be highest in the Southwest during summer in the GEOS-Chem model, and are driven in large part by lightning $NO_X$. As can be seen from Figure 2-1 (middle panel), highest U.S. background concentrations (in the U.S.) are found over the Northern Tier of New York State. High values are also found in other areas bordering Canada and Mexico. NA background concentrations (bottom panel) are on average ~3 ppb higher than U.S. background concentrations (middle panel) during spring and summer across the United States. For March through August 2006, mean NA background $O_3$ concentrations of 29 ± 8 ppb at low elevation (<1,500 meters) and 40 ± 8 ppb at high elevation (>1,500 meters) were calculated. Corresponding natural background concentrations (not shown) range from 18 ± 6 ppb to 27 ± 6 ppb. It should also be noted that methane is an important contributor to NA background $O_3$, accounting for slightly less than half of the increase in background since the pre-industrial era and whose relative contribution is projected to grow in the future.

Note that the calculations of background concentrations presented in Chapter 3 were formulated to answer the question, “what would $O_3$ concentrations be if there were no anthropogenic sources.” This is different from asking, “how much of the $O_3$ measured or simulated in a given area is due to background contributions.” Because of potentially strong non-linearities—particularly in many urban areas—these estimates should not be used by themselves to answer the second question posed above. The extent of these non-linearities will generally depend on location and time, the strength of concentrated sources, and the nature of the chemical regime.




Note: Seasonal mean daily maximum 8-h avg $O_3$ concentrations were calculated by GEOS-Chem for the base case (top, Base), United States background (middle, USB) and North American Background (bottom, NAB).
Values in parentheses (above each map) refer to continental U.S. means, and are shown in the color bar as black squares for summer and white squares for spring.

Source: Adapted from Zhang et al. (2011).

**Figure 2-1 Mean daily average maximum 8-h avg $O_3$ concentrations in surface air, for spring and summer 2006.**

### 2.2.3 Monitoring

The federal reference method (FRM) for $O_3$ measurement is based on the detection of chemiluminescence resulting from the reaction of $O_3$ with ethylene gas. However, almost all of the state and local air monitoring stations (SLAMS) that reported data to the EPA's Air Quality System (AQS) database from 2005 to 2009 used the federal equivalence method (FEM) UV absorption photometer. Relative to FRMs, FEMs must satisfy precision and bias requirements to be accepted as alternative methods for sampling and analyzing ambient air. More than 96% of $O_3$ monitors met these precision and bias requirements for designation as an FEM during this period.

In 2010, there were 1,250 SLAMS $O_3$ monitors reporting data to AQS. Ozone monitoring is required at SLAMS sites during the local "ozone season" which varies by state. In addition, National Core (NCore) is a new multipollutant monitoring network implemented to meet multiple monitoring objectives and each state is required to operate at least one NCore site. The NCore network consists of 60 urban and 20 rural sites nationwide (see Figure 3-21 and Figure 3-22). The densest concentrations of $O_3$ sites are located in California and the eastern half of the U.S.

The Clean Air Status and Trends Network (CASTNET) is a regional monitoring network established to assess trends in acidic deposition and also provides concentration measurements of $O_3$. CASTNET $O_3$ monitors operate year round and are primarily located in rural areas; in 2010, there were 80 CASTNET sites reporting $O_3$ data to AQS. The National Park Service (NPS) operates 23 CASTNET sites in national parks and other Class-i areas, and provided data to AQS from 20 additional Portable Ozone Monitoring Systems (POMS) in 2010 (see Figure 3-22). Compared to urban-focused monitors, rural-focused monitors are relatively scarce across the U.S.

### 2.2.4 Ambient Concentrations

Ozone is the only photochemical oxidant other than $NO_2$ that is routinely monitored and for which a comprehensive database exists. Other photochemical oxidants are typically only measured during special field studies. The concentration analyses in Chapter 3 are limited to widely available $O_3$ data obtained directly from AQS for the period from 2007 to 2009. The median 24-h average, 8-h daily max, and 1-h daily max $O_3$ concentrations across all U.S. sites reporting data to AQS between 2007 and 2009 were 29, 40, and 44 ppb, respectively.

To investigate $O_3$ variability in urban areas across the U.S., 20 combined statistical areas (CSAs) were selected for closer analysis based on their importance in $O_3$ epidemiology studies and on their location. Several CSAs had relatively little spatial variability in daily maximum 8-h avg $O_3$ concentrations (e.g., inter-monitor correlations ranging from 0.61 to 0.96 in the Atlanta, GA, CSA) while other CSAs exhibited considerably more variability in $O_3$ concentrations (e.g., inter-monitor correlations ranging from -0.06 to 0.97 in the Los Angeles, CA, CSA). Uncertainties

resulting from the spatial variability in $O_3$ concentration fields should be considered when using data from the network of ambient $O_3$ monitors to approximate community-scale exposures, since community exposure may not be well-represented when monitors cover large areas with multiple subcommunities having different sources and topographies. However, studies evaluating the influence of monitor selection on epidemiologic study results have found that $O_3$ effect estimates are similar across different spatial scales and monitoring sites (Section 4.6.2.1).

To investigate $O_3$ variability in rural settings across the U.S., six focus areas were selected for closer analysis based on the impact of $O_3$ or $O_3$ precursor transport from upwind urban areas. The selected rural focus area with the largest number of available AQS monitors was Great Smoky Mountains National Park where the May-September median 8-h daily max $O_3$ concentration ranged from 47 ppb at the lowest elevation (564 meters) site to 60 ppb at the highest elevation (2,021 meters) site. Correlations between sites within each rural focus area ranged from 0.78 to 0.92. Ozone in rural areas is produced from emissions of $O_3$ precursors emitted directly within the rural areas, from emissions in urban areas that react and chemically transform during transport, and from occasional stratospheric intrusions. Factors contributing to variations observed within these rural focus areas include proximity to local $O_3$ precursor emissions, local scale circulations related to topography, and possibly stratospheric intrusions as a function of elevation. In addition, $O_3$ tends to persist longer in rural than in urban areas as a result of less chemical scavenging from other primary pollutants. This results in a more uniform $O_3$ concentration throughout the day and night without the typical nocturnal decrease in $O_3$ concentration observed in urban areas. Persistently high $O_3$ concentrations observed at many of the rural sites investigated here indicate that cumulative exposures for humans and vegetation in rural areas can frequently exceed cumulative exposures in urban areas.

Nation-wide surface-level $O_3$ concentrations have declined over the last decade, with a particularly noticeable decrease between 2003 and 2004 coinciding with $NO_X$ emissions reductions resulting from implementation of the $NO_X$ State Implementation Plan (SIP) Call rule, which began in 2003 and was fully implemented in 2004. This rule was designed to reduce $NO_X$ emissions from power plants and other large combustion sources in the eastern United States. The largest density of individual monitors showing downward trends in $O_3$ concentrations over the last decade occur in the Northeast where this rule was focused. In addition to a downward trend, the nation-wide surface-level $O_3$ concentration data also show a general tightening of the distribution across sites. In contrast to the majority of U.S. surface-level monitors reporting downward trends, a few surface-level monitors and elevated observations along the Pacific Coast have shown increases in $O_3$ concentrations in recent years, possibly resulting from intercontinental transport from Asia. As noted in the 2006 $O_3$ AQCD (U.S. EPA, 2006b), trends in national parks and rural areas are similar to nearby urban areas, reflecting the regional nature of $O_3$ pollution.

Since $O_3$ is a secondary pollutant, it is not expected to be highly correlated with primary pollutants such as CO and $NO_X$. Furthermore, $O_3$ formation is strongly influenced by meteorology, entrainment, and transport of both $O_3$ and $O_3$ precursors, resulting in a broad range in correlations with other pollutants which can vary substantially with season. Temporal relationships between 8-h daily max $O_3$ and other criteria pollutants exhibit mostly negative correlations in the winter and mostly positive correlations in the summer. As a result, statistical analyses that may be sensitive to correlations between copollutants need to take seasonality into consideration, especially when $O_3$ is being investigated.

## 2.3 Human Exposure

The widespread presence of $O_3$ in the environment results in exposure as people participate in normal daily activities. Personal exposure measurements have been found to be moderately associated with fixed-site ambient $O_3$ concentrations, although a number of factors affect the relationship between ambient concentration and personal exposure. These include: infiltration of ambient $O_3$ into indoor microenvironments, which is driven by air exchange rate; time spent outdoors and activity pattern, which includes changes in personal behavior by some populations to avoid exposure to $O_3$, and influences of lifestage; and the variation in $O_3$ concentrations at various spatial and temporal scales. Personal exposure to $O_3$ is moderately correlated with ambient $O_3$ concentration, as indicated by studies reporting correlations generally in the range of 0.3-0.8 (Table 4-2). This suggests that ambient monitor concentrations are representative of day-to-day changes in personal exposure to ambient $O_3$. Some studies report lower personal-ambient correlations, a result attributable in part to low building air exchange rates and $O_3$ concentrations below the personal sampler detection limit. Low correlations can also occur for individuals or populations spending increased time indoors. In contrast to correlation, which represents the temporal association between exposure and concentration, the magnitude of exposure can be represented as the ratio between personal exposure and ambient concentration. This ratio varies widely depending on activity patterns, housing characteristics, and season. Personal-ambient ratios are typically 0.1-0.3 for sampling durations of several hours to several days, although individuals spending substantial time outdoors (e.g., outdoor workers) have shown much higher ratios (0.5-0.9) (Table 4-3). Since there are relatively few indoor sources of $O_3$, and because of reactions of $O_3$ with indoor surfaces and airborne constituents, indoor $O_3$ concentrations are often substantially lower than outdoor concentrations (Section 4.3.2). The lack of indoor sources also suggests that fluctuations in ambient $O_3$ may be primarily responsible for changes in personal exposure, even under low-ventilation, low-concentration conditions.

Another factor that can influence the pattern of exposure is the tendency for people to avoid $O_3$ exposure by altering their behavior (e.g., reducing outdoor activity levels or time spent being active outdoors) on high-$O_3$ days. Activity pattern has a substantial effect on ambient $O_3$ exposure, with time spent outdoors contributing to increased

exposure (Section 4.4.2). Air quality alerts and public health recommendations induce reductions in time spent outdoors on high-$O_3$ days among some lifestages and populations, particularly for children, older adults, and people with respiratory problems. Such effects are less pronounced in the general population. Limited evidence from an epidemiologic study conducted in the 1990s in Los Angeles, CA reports increased asthma hospital admissions among children and older adults when $O_3$ alert days (1-h max $O_3$ concentration >200 ppb) were excluded from the analysis of daily hospital admissions and $O_3$ concentrations (presumably thereby eliminating averting behavior based on high $O_3$ forecasts). The lower rate of admissions observed when alert days were included in the analysis suggests that estimates of health effects based on concentration-response functions that do not account for averting behavior may be biased toward the null.

Variations in $O_3$ concentrations occur over multiple spatial and temporal scales. Near roadways, $O_3$ concentrations are reduced due to reaction with NO and other species (Section 4.3.4.2). Over spatial scales of a few kilometers and away from roads, $O_3$ can be somewhat more homogeneous due to its formation as a secondary pollutant, while over scales of tens of kilometers, additional atmospheric processing can result in higher concentrations downwind of an urban area. Although local-scale variability impacts the magnitude of $O_3$ concentrations, $O_3$ formation rates are influenced by factors that vary over larger spatial scales, such as temperature (Section 3.2), suggesting that urban monitors may track one another temporally, but miss small-scale variability. This variation in concentrations changes the pattern of exposure people experience as they move through different microenvironments and affects the magnitude of exposures in different locations within an urban area. The various factors affecting exposure patterns and quantification of exposure result in uncertainty which can contribute to exposure measurement error in epidemiologic studies, which typically use fixed-site monitor data as an indicator of exposure. Low personal-ambient ratios result in attenuation of the magnitude of the exposure-based effect estimate or response function relative to the concentration-based response function, although the statistical association is similar for concentration- and exposure-based effect estimates if the ratio is approximately constant over time. Low personal-ambient correlations are a source of exposure error for epidemiologic studies, tending to obscure the presence of potential thresholds, bias effect estimates toward the null, and widen confidence intervals, and this impact may be more pronounced among populations spending substantial time indoors. The impact of this exposure error may tend more toward widening confidence intervals than biasing effect estimates, since epidemiologic studies evaluating the influence of monitor selection indicate that effect estimates are similar across different spatial averaging scales and monitoring sites. In addition, in examinations of respiratory endpoints in epidemiologic studies, associations were similar in magnitude across analyses using several different concentration metrics to estimate exposure, such as on-site measurements, closest-site measurements, and multi-site averages. These exposure estimation methods likely vary in how well ambient $O_3$ concentrations represent personal exposures and between-subject variability in exposures. Respiratory effects were observed with ambient $O_3$ concentrations found to have stronger personal-ambient relationships, including those measured on-site during long periods of

outdoor activity. However, such effects were also found with ambient $O_3$ measurements expected to have weaker personal-ambient relationships, including those measured at home or school, measured at the closest site, averaged from multiple community sites, and measured at a single site. Overall, there was no clear indication that a particular method of exposure estimation produced stronger findings.

## 2.4 Dosimetry and Mode of Action

Upon inspiration, $O_3$ uptake in the respiratory tract is affected by a number of factors including respiratory tract morphology, and breathing route, frequency, and volume. Additionally, physicochemical properties of $O_3$ itself and how it is transported, as well as the physical and chemical properties of the extracellular lining fluid (ELF) and tissue layers in the respiratory tract can influence $O_3$ uptake. Experimental studies and models have suggested that there are differences between the total absorption of $O_3$ from the inhaled air and the $O_3$ dose reaching the respiratory tract tissues. The total $O_3$ absorption gradually decreases with distal progression into the respiratory tract allowing for a proportionally large concentration of $O_3$ to be absorbed in the upper respiratory tract; thus, causing the nasal membranes to be a potential target site of $O_3$-induced injury. In contrast, the primary site of $O_3$ delivery to the lung epithelium is believed to be the centriacinar region or the junction of the conducting airways with the gas exchange region. In addition, as a large concentration of the $O_3$ absorbed by the respiratory tract is absorbed in the upper respiratory tract, the nasal membranes are another potential site of $O_3$-induced injury.

Ozone uptake is sensitive to a number of factors including tidal volume, breathing frequency, $O_3$ concentration, and exposure time. Interindividual variability also accounts for a large amount of the variability in local dose due to differences in pulmonary physiology, anatomy, and biochemistry. An increase in tidal volume and breathing frequency are both associated with increased physical activity. These changes and a switch to oronasal breathing during exercise result in deeper penetration of $O_3$ into the lower respiratory tract in part due to less oral versus nasal uptake efficiency. For these reasons, increased physical activity acts to move the maximum tissue dose of $O_3$ distally in the respiratory tract and more into the alveolar region.

The ELF is a complex mixture of lipids, proteins, and antioxidants that serves as the first barrier and target for inhaled $O_3$ (see Figure 5-7). Distinct products with diverse reactivity (i.e., secondary oxidation products), are mainly formed by reactions of $O_3$ with soluble ELF components. The thickness of the ELF is an important determinant of the dose of $O_3$ to the tissues; a thicker ELF generally results in a lower dose of $O_3$ to the tissues. Additionally, the quenching ability and the concentrations of antioxidants and other ELF components are determinants of the formation of secondary oxidation products. These reactions appear to limit interaction of $O_3$ with underlying tissues and to reduce penetration of $O_3$ distally into the respiratory tract.

In addition to contributing to the driving force for $O_3$ uptake, formation of secondary oxidation products contributes to oxidative stress which can lead to cellular injury and altered cell signaling in the respiratory tract. Secondary oxidation products initiate pathways (see Figure 5-8) that provide the mechanistic basis for short- and long-term health effects described in detail in Chapters 6 and 7. Other key events involved in the mode of action of $O_3$ in the respiratory tract include the activation of neural reflexes, initiation of inflammation, alterations of epithelial barrier function, sensitization of bronchial smooth muscle, modification of innate and adaptive immunity, and airways remodeling. Another key event, systemic inflammation and vascular oxidative/nitrosative stress, may be critical to the extrapulmonary effects of $O_3$.

Secondary oxidation products can transmit signals to respiratory tract cells resulting in the activation of neural reflexes. Nociceptive sensory nerves mediate the involuntary truncation of respiration, resulting in decreases in lung function (i.e., FVC, $FEV_1$, and tidal volume), and pain upon deep inspiration. Studies implicate TRPA1 receptors on bronchial C-fibers in this reflex. Another neural reflex involves vagal sensory nerves, which mediate a mild increase in airways obstruction (i.e., bronchoconstriction) following exposure to $O_3$ via parasympathetic pathways. Substance P release from bronchial C-fibers and the SP-NK receptor pathway can also contribute to this response.

Secondary oxidation products also initiate the inflammatory cascade following exposure to $O_3$. Studies have implicated eicosanoids, chemokines and cytokines, vascular endothelial adhesion molecules, and tachykinins in mediating this response. Inflammation is characterized by airways neutrophilia as well as the influx of other inflammatory cell types. Recent studies demonstrate a later phase of inflammation characterized by increased numbers of macrophages, which is mediated by hyaluronan. Inflammation further contributes to $O_3$-induced oxidative stress.

Alteration of the epithelial barrier function of the respiratory tract also occurs as a result of $O_3$-induced secondary oxidation product formation. Increased epithelial permeability may lead to enhanced sensitization of bronchial smooth muscle, resulting in airways hyperresponsiveness (AHR). Neurally-mediated sensitization also occurs and is mediated by cholinergic postganglionic pathways and bronchial C-fiber release of substance P. Recent studies implicate hyaluronan and Toll-like receptor 4 (TLR4) signaling in bronchial smooth muscle sensitization, while earlier studies demonstrate roles for eicosanoids, cytokines, and chemokines.

Evidence is accumulating that exposure to $O_3$ modifies innate and adaptive immunity through effects on macrophages, monocytes, and dendritic cells. Enhanced antigen presentation, adjuvant activity, and altered responses to endotoxin have been demonstrated. TLR4 signaling contributes to some of these responses. Effects on innate and adaptive immunity can result in both short- and longer-term consequences related to the exacerbation and/or induction of asthma and to alterations in host defense.

Airways remodeling has been demonstrated following chronic and/or intermittent exposure to $O_3$ by mechanisms that are not well understood. However, the TGF-β signaling pathway has recently been implicated in $O_3$-induced deposition of collagen in the airways wall. These studies were conducted in adult animal models and their relevance to effects in humans is unknown.

Evidence is also accumulating that $O_3$ exposure results in systemic inflammation and vascular oxidative/nitrosative stress. The release of diffusible mediators from the $O_3$-exposed lung into the circulation can initiate or propagate inflammatory responses in the vascular or in systemic compartments. This may provide a mechanistic basis for extrapulmonary effects, such as vascular dysfunction.

Both dosimetric and mechanistic factors contribute to the understanding of inter-individual variability in response. Inter-individual variability is influenced by variability in respiratory tract volume and thus surface area, breathing route, certain genetic polymorphisms, pre-existing conditions and disease, nutritional status, lifestages, attenuation, and co-exposures. In particular, very young individuals may be sensitive to developmental effects of $O_3$ since studies in animal models demonstrated altered development of lung and immune system.

Some of these factors are also influential in understanding species homology and sensitivity. Qualitatively, animal models exhibit a similar pattern of tissue dose distribution for $O_3$ with the largest tissue dose delivered to the centriacinar region. However, due to anatomical and biochemical respiratory tract differences, the actual $O_3$ dose delivered differs between humans and animal models. Animal data obtained in resting conditions underestimates the dose to the respiratory tract tissue relative to exercising humans. Further, it should be noted that, with the exception of airways remodeling, the mechanistic pathways discussed above have been demonstrated in both animals and human subjects in response to the inhalation of $O_3$. Even though interspecies differences limit quantitative comparison between species, the short- and long-term functional responses of laboratory animals to $O_3$ appear qualitatively homologous to those of the human making them a useful tool in determining mechanistic and cause-effect relationships with $O_3$ exposure. Furthermore, animal studies add to a better understanding of the full range of potential $O_3$-mediated effects.

## 2.5 Integration of Ozone Health Effects

This section evaluates the evidence from toxicological, controlled human exposure, and epidemiologic studies (which examined the health effects associated with short- and long-term exposure to $O_3$,) and summarizes the main conclusions of this assessment regarding the health effects of $O_3$ and the concentrations at which those effects are observed. The results from the health studies, supported by the synthesis of atmospheric chemistry (see Section 2.2) and exposure assessment (see Section 2.3) studies, contribute to the causal determinations made for the health

outcomes discussed in this assessment (see Preamble to this document for details on the causal framework).

Epidemiologic studies generally present $O_3$-related effect estimates for mortality and morbidity health outcomes based on an incremental change in exposure, traditionally equal to the interquartile range in $O_3$ concentrations or some other arbitrary value (e.g., 10 ppb). Additionally, various averaging times are used in $O_3$ epidemiologic studies, with the three most common being the maximum 1-hour average within a 24-hour period (1-h max), the maximum 8-hour average within a 24-hour period (8-h max), and 24-hour average (24-h avg). For the purpose of presenting results from studies that use different exposure metrics, EPA consistently applies the same $O_3$ increments to facilitate comparisons between the results of various studies that may use different indices. These increments were derived using the nationwide distributional data for $O_3$ monitors in U.S. Metropolitan Statistical Areas. They are representative of a low-to-high change in $O_3$ concentrations and were approximated on the basis of annual mean to 95th percentile differences (Langstaff, 2003). Therefore, throughout Chapter 6, efforts were made to standardize $O_3$-related effect estimates using the increments of 20 ppb for 24-h avg, 30 ppb for 8-h max, and 40 ppb for 1-h max $O_3$ concentrations, except as noted. In long-term exposure studies, typically, $O_3$ concentrations are lower and less variable when averaged across longer exposure periods, and differences due to the use of varying averaging times (e.g., 1-h max, 24-h avg) become less apparent. As such, in the long-term exposure chapter (Chapter 7) an increment of 10 ppb was consistently applied across studies, regardless of averaging time, to facilitate comparisons between the results from these studies.

### 2.5.1 Conclusions from Previous $O_3$ AQCDs

The 2006 $O_3$ AQCD concluded that there was clear, consistent evidence of a causal relationship between short-term $O_3$ exposure and respiratory health effects (U.S. EPA, 2006b). The causal relationship for respiratory health effects was substantiated by the coherence of effects observed across controlled human exposure, epidemiologic, and toxicological studies indicating effects of short-term $O_3$ exposures on a range of respiratory health endpoints from respiratory tract inflammation to respiratory-related emergency department (ED) visits and hospital admissions.

Across disciplines, short-term $O_3$ exposures induced or were associated with statistically significant declines in lung function. An equally strong body of evidence from controlled human exposure and toxicological studies demonstrated $O_3$-induced inflammatory responses, increased epithelial permeability, and airway hyperresponsiveness (both specific and nonspecific). Toxicological studies provided additional evidence for $O_3$-induced impairment of host defenses. Combined, these findings from experimental studies provided support for epidemiologic evidence, in which short-term increases in ambient $O_3$ concentration were consistently associated

with increases in respiratory symptoms and asthma medication use in children with asthma, respiratory-related hospital admissions, and asthma-related ED visits. Although $O_3$ was consistently associated with nonaccidental and cardiopulmonary mortality, the contribution of respiratory causes to these findings was uncertain.

Collectively, there is a vast amount of evidence spanning several decades that demonstrated that exposure to $O_3$ induces a range of respiratory effects. The majority of this evidence was derived from studies investigating short-term exposure (i.e., hours to weeks) to $O_3$. The combined evidence across disciplines led to the causal relationship between short-term $O_3$ exposure and respiratory effects reported in the 2006 $O_3$ AQCD.

Mechanistic evidence for the effects of $O_3$ on the respiratory system was characterized in the 1996 $O_3$ AQCD (U.S. EPA, 1996a), which identified $O_3$-induced changes in a variety of lung lipid species whose numerous biologically active metabolites, in turn, can affect host defenses, lung function, and the immune system. As summarized in Section 2.4 and fully characterized in Chapter 5, key events in the toxicity pathway of $O_3$ have been identified in humans and animal models. They include formation of secondary oxidation products, activation of neural reflexes, initiation of inflammation, alteration of epithelial barrier function, sensitization of bronchial smooth muscle, modification of innate/adaptive immunity, airways remodeling, and systemic inflammation and oxidative/nitrosative stress.

### 2.5.2 Summary of Causal Determinations

Recent studies support or build upon the strong body of evidence presented in the 1996 and 2006 $O_3$ AQCDs that *short-term $O_3$ exposure is causally associated with respiratory health effects*. Recent controlled human exposure studies demonstrate statistically significant group mean decreases in pulmonary function to exposures as low as 60-70 ppb $O_3$ in young, healthy adults, and are supported by the strong, cumulative evidence from epidemiologic studies. Equally strong evidence demonstrated associations of ambient $O_3$ with respiratory hospital admissions and ED visits across the U.S., Europe, and Canada. Most effect estimates ranged from a 1.6 to 5.4% increase in daily respiratory-related ED visits or hospital admissions in all-year analyses for unit increases[1] in ambient $O_3$ concentrations. Several multicity studies and a multicontinent study reported associations between short-term increases in ambient $O_3$ concentrations and increases in respiratory mortality. This evidence is supported by a large body of individual-level epidemiologic panel studies that demonstrate associations of ambient $O_3$ with respiratory symptoms in children with asthma. Further support is provided by recent studies that found $O_3$-associated increases in indicators of airway inflammation and oxidative stress in children with asthma. Across respiratory endpoints, evidence indicates antioxidant capacity may modify the risk of respiratory morbidity associated with $O_3$ exposure. The potentially elevated risk of populations with diminished antioxidant capacity and the reduced

[1] Effect estimates were standardized to a 40-, 30-, and 20-ppb unit increase for 1-h max, 8-h max, and 24-h avg $O_3$.

risk of populations with enhanced antioxidant capacity identified in epidemiologic studies is strongly supported by similar findings from controlled human exposure studies and by evidence that characterizes $O_3$-induced decreases in intracellular antioxidant levels as a mode of action for downstream effects. By demonstrating $O_3$-induced airway hyperresponsiveness, decreased pulmonary function, allergic responses, lung injury, impaired host defense, and airway inflammation, toxicological studies have characterized $O_3$ modes of action and provided biological plausibility for epidemiologic associations of ambient $O_3$ concentrations with lung function and respiratory symptoms, hospital admissions, ED visits, and mortality. Together, the evidence integrated across controlled human exposure, epidemiologic, and toxicological studies and across the spectrum of respiratory health endpoints continues to demonstrate that there **is a causal relationship between short-term $O_3$ exposure and respiratory health effects**.

The epidemiologic evidence for a relationship between *long-term $O_3$ exposure and respiratory health effects* (including respiratory symptoms, new-onset asthma, and respiratory mortality) is contributed by recent studies that evaluate the associations between long-term exposure to $O_3$ and respiratory effects that demonstrate interactions between exercise or different genetic variants and both new-onset asthma in children and increased respiratory symptom effects in individuals with asthma. While the evidence is limited, a U.S. multicommunity prospective cohort demonstrates that asthma risk is affected by interactions among genetic variability, environmental $O_3$ exposure, and behavior. The evidence relating new-onset asthma to long-term $O_3$ exposure is supported by toxicological studies of asthma in monkeys. This nonhuman primate evidence of $O_3$-induced changes supports the biologic plausibility of long-term exposure to $O_3$ contributing to the effects of asthma in children. Early life $O_3$ exposure can alter airway development and lead to the development of asthma. Other recent epidemiologic studies provide coherent evidence for long-term $O_3$ exposure and respiratory effects such as first asthma hospitalization, respiratory symptoms in asthmatics, and respiratory mortality. Generally, the epidemiologic and toxicological evidence provides a compelling case that supports the hypothesis that a relationship exists between long-term exposure to ambient $O_3$ and measures of respiratory health effects and mortality. The evidence for short-term exposure to $O_3$ and effects on respiratory endpoints provides coherence and biological plausibility for the effects of long-term exposure to $O_3$. Building upon that evidence, the more recent epidemiologic evidence, combined with toxicological studies in rodents and nonhuman primates, provides biologically plausible evidence that there **is likely to be a causal relationship between long-term exposure to $O_3$ and respiratory health effects.**

In past $O_3$ AQCDs the effects of *short- term exposure to $O_3$ on the cardiovascular system* could not be thoroughly evaluated due to the paucity of information available. However, studies investigating $O_3$-induced cardiovascular events have advanced in the last two decades. Animal toxicological studies, although limited in number, demonstrate $O_3$-induced cardiovascular effects; specifically enhanced ischemia/reperfusion (I/R) injury, disrupted NO-induced vascular reactivity, decreased cardiac function, and increased heart rate variability (HRV). These effects

are consistent with cardiovascular system effects observed after long-term $O_3$ exposure, such as increased vascular disease. These effects may, in part, correspond to the alteration of the autonomic nervous system or to the development and maintenance of systemic oxidative stress and a proinflammatory environment that can result from pulmonary inflammation. Controlled human exposure studies provide some coherence with the evidence from animal toxicological studies, by demonstrating increases and decreases in HRV following relatively low (120 ppb during rest) and high (300 ppb with exercise) $O_3$ exposures, respectively. Controlled human exposure studies also support the animal toxicology studies by demonstrating $O_3$-induced effects on blood biomarkers of systemic inflammation and oxidative stress as well as changes in biomarkers suggestive of a pro-thrombogenic response to $O_3$. The experimental evidence provides initial biological plausibility for the consistently positive associations observed across multiple epidemiologic studies of short-term $O_3$ exposure and cardiovascular mortality. However, epidemiologic studies generally do not observe associations between short-term exposure to $O_3$ and cardiovascular morbidity; studies of cardiovascular-related hospital admissions and ED visits and other various cardiovascular effects did not find consistent evidence of a relationship with $O_3$ exposure. The lack of coherence between the results from studies that examined associations between short-term $O_3$ exposure and cardiovascular morbidity and subsequently cardiovascular mortality complicate the interpretation of the overall evidence for $O_3$-induced cardiovascular effects. Overall, animal toxicological studies demonstrate $O_3$-induced cardiovascular effects, and support to the strong body of evidence indicating $O_3$-induced cardiovascular mortality. Animal toxicological and controlled human exposure studies provide evidence for biologically plausible mechanisms underlying these $O_3$-induced cardiovascular effects. However, a lack of coherence with epidemiologic studies of cardiovascular morbidity remains an important uncertainty. Taken together, the overall body of evidence across disciplines indicates that **there is likely to be a causal relationship between short-term exposures to $O_3$ and cardiovascular effects.**

The 2006 $O_3$ AQCD concluded that the overall body of evidence was highly suggestive that short-term exposure to $O_3$ directly or indirectly contributes to nonaccidental and cardiopulmonary-related mortality, but additional research was needed to more fully establish underlying mechanisms by which such effects occur. The evaluation of recent multicity studies and a multicontinent study that examined the association between *short-term increases in ambient $O_3$ concentration and mortality* found evidence that supports the conclusions of the 2006 $O_3$ AQCD. These recent studies reported consistent positive associations between short-term increases in ambient $O_3$ concentration and total (nonaccidental) mortality, with associations being stronger during the warm season, as well as provided additional support for associations between $O_3$ concentrations and cardiovascular mortality being similar or larger in magnitude compared to respiratory mortality. Additionally, these new studies examined previously identified areas of uncertainty in the $O_3$-mortality relationship, and provide additional evidence supporting an association between short-term $O_3$ exposure and mortality. Taken together, the body of evidence indicates

that there **is likely to be a causal relationship between short-term $O_3$ exposures and total mortality.**

The 2006 $O_3$ AQCD concluded that an insufficient amount of evidence existed to suggest a causal relationship between *long-term $O_3$ exposure and mortality* (U.S. EPA, 2006b). A synthesis of the recent and earlier evidence reveals that the strongest evidence for an association between long-term exposure to ambient $O_3$ concentrations and mortality is derived from associations for respiratory mortality that remained robust after adjusting for $PM_{2.5}$ concentrations. There is inconsistent evidence for an association between long-term exposure to ambient $O_3$ and cardiopulomary mortality, with several analyses from the American Cancer Society (ACS) cohort reporting some positive associations, while other studies reported no association. There is generally limited evidence for an association with long-term exposure to ambient $O_3$ and total mortality. The findings for respiratory mortality are consistent and coherent with the evidence from epidemiologic, controlled human exposure, and animal toxicological studies for the effects of short- and long-term exposure to $O_3$ on respiratory effects. Respiratory mortality is a relatively small portion of total mortality [about 7.6% of all deaths in 2010 were due to respiratory causes (Murphy et al., 2012)], thus it is not surprising that the respiratory mortality signal may be difficult to detect in studies of cardiopulmonary or total mortality. Based on the recent evidence for respiratory mortality along with limited evidence for total and cardiopulmonary mortality, the evidence **is suggestive of a causal relationship between long-term $O_3$ exposures and total mortality.**

In past $O_3$ AQCDs the effects of *long-term exposure to $O_3$ on the cardiovascular system* could not be thoroughly evaluated due to the paucity of information available. However, studies investigating $O_3$-induced cardiovascular events have advanced in the last two decades. Animal toxicological studies provide evidence for long-term $O_3$ exposure leading to cardiovascular morbidity, including increased vascular disease. There is limited, inconsistent evidence for cardiovascular morbidity in epidemiologic studies examining long-term exposure to $O_3$. Overall, animal toxicological studies provide some evidence for $O_3$-induced cardiovascular effects, but the effects observed were not consistently supported by controlled human exposure studies or epidemiologic studies. Thus, the overall body of evidence across disciplines **is suggestive of a causal relationship between long-term exposures to $O_3$ and cardiovascular effects.**

In the 2006 $O_3$ AQCD, there were a number of health effects for which an insufficient amount of evidence existed to adequately characterize the relationships with exposure to $O_3$. However, recent evidence suggests that $O_3$ may impart health effects through exposure durations and biological mechanisms not previously considered. For example, recent toxicological studies add to earlier evidence that *short- and long-term exposures to $O_3$ can produce a range of effects on the central nervous system and behavior*. Additionally, an epidemiologic study demonstrated that long-term exposure to $O_3$ affects memory in humans as well. Together the evidence from studies of short- and long-term exposure to $O_3$ **is suggestive of a causal relationship between $O_3$ exposure and central nervous system effects.** There is also limited though positive toxicological evidence for *$O_3$-induced*

*developmental effects*. Limited epidemiologic evidence exists for an association of $O_3$ concentration with decreased sperm concentration and associations with reduced birth weight and restricted fetal growth. Overall, the evidence **is suggestive of a causal relationship between long-term exposures to $O_3$ and reproductive and developmental effects.**

These causal determinations are summarized in Table 2-1, along with the conclusions from the previous NAAQS review. Special emphasis and additional details are provided in Table 2-1 for respiratory health outcomes, for which there is the strongest body of evidence.

**Table 2-1 Summary of evidence from epidemiologic, controlled human exposure, and animal toxicological studies on the health effects associated with short- and long-term exposure to $O_3$.**

| Health Outcome | Conclusions from 2006 $O_3$ AQCD | Conclusions from this ISA |
|---|---|---|
| **Short-Term Exposure to $O_3$** | | |
| Respiratory effects | The overall evidence supports a causal relationship between acute ambient $O_3$ exposures and increased respiratory morbidity outcomes. | Evidence integrated across controlled human exposure, epidemiologic, and toxicological studies and across the spectrum of respiratory health endpoints continues to demonstrate that there **is a causal relationship between short-term $O_3$ exposure and respiratory health effects.** |
| Lung function | Results from controlled human exposure studies and animal toxicological studies provide clear evidence of causality for the associations observed between acute (≤ 24 h) $O_3$ exposure and relatively small, but statistically significant declines in lung function observed in numerous recent epidemiologic studies. Declines in lung function are particularly noted in children, asthmatics, and adults who work or exercise outdoors. | Recent controlled human exposure studies demonstrate group mean decreases in $FEV_1$ in the range of 2 to 3% with 6.6 hour exposures to as low as **60 ppb** $O_3$. The collective body of epidemiologic evidence demonstrates associations between short-term ambient $O_3$ exposure and decrements in lung function, particularly in children with asthma, children, and adults who work or exercise outdoors. |
| Airway hyperresponsiveness | Evidence from human clinical and animal toxicological studies clearly indicate that acute exposure to $O_3$ can induce airway hyperreactivity, thus likely placing atopic asthmatics at greater risk for more prolonged bouts of breathing difficulties due to airway constriction in response to various airborne allergens or other triggering stimuli. | A limited number of studies have observed airway hyperresponsiveness in rodents and guinea pigs after exposure to less than **300 ppb** $O_3$. As previously reported in the 2006 $O_3$ AQCD, increased airway responsiveness has been demonstrated at **80 ppb** in young, healthy adults, and at **50 ppb** in certain strains of rats. |

| Health Outcome | Conclusions from 2006 $O_3$ AQCD | Conclusions from this ISA |
|---|---|---|
| Pulmonary inflammation, injury and oxidative stress | The extensive human clinical and animal toxicological evidence, together with the limited available epidemiologic evidence, is clearly indicative of a causal role for $O_3$ in inflammatory responses in the airways. | Epidemiologic studies provided new evidence for associations of ambient $O_3$ with mediators of airway inflammation and oxidative stress and indicate that higher antioxidant levels may reduce pulmonary inflammation associated with $O_3$ exposure. Generally, these studies had mean 8-h max $O_3$ concentrations less than **73 ppb**. Recent controlled human exposure studies show $O_3$-induced inflammatory responses at 60 ppb, the lowest concentration evaluated. |
| Respiratory symptoms and medication use | Young healthy adult subjects exposed in clinical studies to $O_3$ concentrations ≥ 80 ppb for 6 to 8 h during moderate exercise exhibit symptoms of cough and pain on deep inspiration. The epidemiologic evidence shows significant associations between acute exposure to ambient $O_3$ and increases in a wide variety of respiratory symptoms (e.g., cough, wheeze, production of phlegm, and shortness of breath) and medication use in asthmatic children. | The collective body of epidemiologic evidence demonstrates positive associations between short-term exposure to ambient $O_3$ and respiratory symptoms (e.g., cough, wheeze, and shortness of breath) in children with asthma. Generally, these studies had mean 8-h max $O_3$ concentrations less than **69 ppb**. |
| Lung host defenses | Toxicological studies provided extensive evidence that acute $O_3$ exposures as low as 80 to 500 ppb can cause increases in susceptibility to infectious diseases due to modulation of lung host defenses. A single controlled human exposure study found decrements in the ability of alveolar macrophages to phagocytize microorganisms upon exposure to 80 to 100 ppb $O_3$. | Recent controlled human exposure studies demonstrate the increased expression of cell surface markers and alterations in sputum leukocyte markers related to innate adaptive immunity with short-term $O_3$ exposures of **80-400 ppb**. Recent studies demonstrating altered immune responses and natural killer cell function build on prior evidence that $O_3$ can affect multiple aspects of innate and acquired immunity with short-term $O_3$ exposures as low as **80 ppb**. |
| Allergic and asthma related responses | Previous toxicological evidence indicated that $O_3$ exposure skews immune responses toward an allergic phenotype, and enhances the development and severity of asthma-related responses such as AHR. | Recent controlled human exposure studies demonstrate enhanced allergic cytokine production in atopic individuals and asthmatics, increased IgE receptors in atopic asthmatics, and enhanced markers of innate immunity and antigen presentation in health subjects or atopic asthmatics with short-term exposure to **80-400 ppb** $O_3$, all of which may enhance allergy and/or asthma. Further evidence for $O_3$-induced allergic skewing is provided by a few recent studies in rodents using exposure concentrations as low as **200 ppb**. |

| Health Outcome | Conclusions from 2006 $O_3$ AQCD | Conclusions from this ISA |
|---|---|---|
| Respiratory Hospital admissions, ED visits, and physician visits | Aggregate population time-series studies observed that ambient $O_3$ concentrations are positively and robustly associated with respiratory-related hospitalizations and asthma ED visits during the warm season. | Consistent, positive associations of ambient $O_3$ with respiratory hospital admissions and ED visits in the U.S., Europe, and Canada with supporting evidence from single city studies. Generally, these studies had mean 8-h max $O_3$ concentrations less than **60 ppb**. |
| Respiratory Mortality | Aggregate population time-series studies specifically examining mortality from respiratory causes were limited in number and showed inconsistent associations between acute exposure to ambient $O_3$ exposure and respiratory mortality. | Recent multicity time-series studies and a multicontinent study consistently demonstrated associations between ambient $O_3$ and respiratory-related mortality visits across the U.S., Europe, and Canada with supporting evidence from single city studies. Generally, these studies had mean 8-h max $O_3$ concentrations less than **63 ppb**. |
| Cardiovascular effects | The limited evidence is highly suggestive that $O_3$ directly and/or indirectly contributes to cardiovascular-related morbidity, but much remains to be done to more fully substantiate the association. | The overall body of evidence across disciplines indicates that there **is likely to be a causal relationship for short-term exposures to $O_3$ and cardiovascular effects.** |
| Central nervous system effects | Toxicological studies report that acute exposures to $O_3$ are associated with alterations in neurotransmitters, motor activity, short- and long-term memory, sleep patterns, and histological signs of neurodegeneration. | Together the evidence from studies of short-term exposure to $O_3$ **is suggestive of a causal relationship between $O_3$ exposure and CNS effects.** |
| Total Mortality | The evidence is highly suggestive that $O_3$ directly or indirectly contributes to non-accidental and cardiopulmonary-related mortality. | Taken together, the body of evidence indicates that there **is likely to be a causal relationship between short-term exposures to $O_3$ and total mortality.** |
| **Long-term Exposure to $O_3$** | | |
| Respiratory effects | The current evidence is suggestive but inconclusive for respiratory health effects from long-term $O_3$ exposure. | Recent epidemiologic evidence, combined with toxicological studies in rodents and non-human primates, provides biologically plausible evidence that there **is likely to be a causal relationship between long-term exposure to $O_3$ and respiratory health effects.** |
| New onset asthma | No studies examining this outcome were evaluated in the 2006 $O_3$ AQCD. | Evidence that different genetic variants (HMOX, GST, ARG), in combination with $O_3$ exposure, are related to new onset asthma. These associations were observed when subjects living in areas where the mean annual 8-h max $O_3$ concentration was **55.2 ppb**, compared to those who lived where it was **38.4 ppb**. |

| Health Outcome | Conclusions from 2006 $O_3$ AQCD | Conclusions from this ISA |
|---|---|---|
| Asthma hospital admissions | No studies examining this outcome were evaluated in the 2006 $O_3$ AQCD. | Chronic $O_3$ exposure was related to first childhood asthma hospital admissions in a positive concentration-response relationship. Generally, these studies had mean annual 8-h max $O_3$ concentrations less than **41 ppb**. |
| Pulmonary structure and function | Epidemiologic studies observed that reduced lung function growth in children was associated with seasonal exposure to $O_3$; however, cohort studies of annual or multiyear $O_3$ exposure observed little clear evidence for impacts of longer-term, relatively low-level $O_3$ exposure on lung function development in children. Animal toxicological studies reported chronic $O_3$-induced structural alterations, some of which were irreversible, in several regions of the respiratory tract including the centriacinar region. Morphologic evidence from studies using exposure regimens that mimic seasonal exposure patterns report increased lung injury compared to conventional chronic stable exposures. | Evidence for pulmonary function effects is inconclusive, with some new epidemiologic studies observing positive associations (mean annual 8-h max $O_3$ concentrations less than **65 ppb**). Information from toxicological studies indicates that long-term exposure during development among infant monkeys (**500 ppb**) and adult rodents (**>120 ppb**) can result in irreversible morphological changes in the lung, which in turn can influence pulmonary function. |
| Pulmonary inflammation, injury and oxidative stress | Extensive human clinical and animal toxicological evidence, together with limited epidemiologic evidence available, suggests a causal role for $O_3$ in inflammatory responses in the airways. | Several epidemiologic studies (mean 8-h max $O_3$ concentrations less than **69 ppb**) and toxicology studies (as low as **500 ppb**) add to observations of $O_3$-induced inflammation and injury. |
| Lung host defenses | Toxicological studies provided evidence that chronic $O_3$ exposure as low as 100 ppb can cause increases in susceptibility to infectious diseases due to modulation of lung host defenses, but do not cause greater effects on infectivity than short exposures. | Consistent with decrements in host defenses observed in rodents exposed to **100 ppb** $O_3$, recent evidence demonstrates a decreased ability to respond to pathogenic signals in infant monkeys exposed to **500 ppb** $O_3$. |
| Allergic responses | Limited epidemiologic evidence supported an association between ambient $O_3$ and allergic symptoms. Little if any information was available from toxicological studies. | Evidence relates positive outcomes of allergic response and $O_3$ exposure but with variable strength for the effect estimates; exposure to $O_3$ may increase total IgE in adult asthmatics. Allergic indicators in monkeys were increased by exposure to $O_3$ concentrations of **500 ppb**. |
| Respiratory mortality | Studies of cardio-pulmonary mortality were insufficient to suggest a causal relationship between chronic $O_3$ exposure and increased risk for mortality in humans. | A single study demonstrated that exposure to $O_3$ (long-term mean $O_3$ less than **104 ppb**) elevated the risk of death from respiratory causes and this effect was robust to the inclusion of $PM_{2.5}$. |

| Health Outcome | Conclusions from 2006 $O_3$ AQCD | Conclusions from this ISA |
|---|---|---|
| Cardiovascular Effects | No studies examining this outcome were evaluated in the 2006 $O_3$ AQCD. | The overall body of evidence across disciplines **is suggestive of a causal relationship for long-term exposures to $O_3$ and cardiovascular effects.** |
| Reproductive and developmental effects | Limited evidence for a relationship between air pollution and birth-related health outcomes, including mortality, premature births, low birth weights, and birth defects, with little evidence being found for $O_3$ effects. | Overall, the evidence **is suggestive of a causal relationship between long-term exposures to $O_3$ and reproductive and developmental effects.** |
| Central nervous system effects | Toxicological studies reported that acute exposures to $O_3$ are associated with alterations in neurotransmitters, motor activity, short and long term memory, sleep patterns, and histological signs of neurodegeneration. Evidence regarding chronic exposure and neurobehavioral effects was not available. | Together the evidence from studies of long-term exposure to $O_3$ **is suggestive of a causal relationship between $O_3$ exposure and CNS effects.** |
| Cancer | Little evidence for a relationship between chronic $O_3$ exposure and increased risk of lung cancer. | Overall, the evidence **is inadequate to determine if a causal relationship exists between ambient $O_3$ exposures and cancer.** |
| Total Mortality | There is little evidence to suggest a causal relationship between chronic $O_3$ exposure and increased risk for mortality in humans. | Collectively, the evidence **is suggestive of a causal relationship between long-term $O_3$ exposures and total mortality.** |

### 2.5.3 Integrated Synthesis of Evidence for Health Effects

This section integrates the evidence for respiratory and cardiovascular effects (including mortality) across scientific disciplines and both short- and long-term exposure periods. Here, the complete body of evidence from both previous and the current NAAQS reviews is synthesized for the broad range of respiratory and cardiovascular effects associated with exposure to $O_3$.

#### 2.5.3.1 Respiratory Effects

Building on evidence evaluated in previous $O_3$ AQCDs, recent evidence confirms and extends that $O_3$ is associated with a *broad range of respiratory effects, including altered development of the respiratory tract*. Recent animal toxicological studies of long-term exposure to $O_3$ occurring throughout various lifestages in monkeys, beginning with prenatal and early life exposures, provide novel evidence for effects on the development of the respiratory system, including ultrastructural changes in bronchiole development, increased offspring airway hyper-reactivity (Section 7.4.8),

as well as effects on the developing immune system. The strongest evidence for $O_3$-induced effects on the developing lung comes from a series of experiments using infant rhesus monkeys episodically exposed to 500 ppb $O_3$ for approximately 5 months, starting at one month of age. Functional changes in the conducting airways of infant rhesus monkeys exposed to either $O_3$ alone or $O_3$ + antigen were accompanied by a number of cellular and morphological changes. In addition to these functional and cellular changes, substantial structural changes in the respiratory tract were observed. Importantly, the $O_3$-induced structural pathway changes persisted after recovery in filtered air for six months after cessation of the $O_3$ exposures. Exposure to $O_3$ has also been associated with similar types of alterations in pulmonary structure, including airways remodeling and pulmonary injury and increased permeability, in all adult laboratory animal species studied, from rats to monkeys (U.S. EPA, 1996a).

In addition to effects on the development and structure of the respiratory tract, there is extensive evidence for the effects of *short-term exposure to $O_3$ on pulmonary inflammation and oxidative stress*. Previous evidence from controlled human exposure studies indicated that $O_3$ causes an inflammatory response in the lungs (U.S. EPA, 1996a). This inflammatory response to $O_3$ was detected after a single 1-h exposure with exercise to $O_3$ concentrations of 300 ppb; the increased levels of some inflammatory cells and mediators persisted for at least 18 hours. Toxicological studies provided additional evidence for increases in permeability and inflammation in rabbits at levels as low as 100 ppb $O_3$. Evidence summarized in the 2006 $O_3$ AQCD demonstrated that inflammatory responses were observed subsequent to 6.6 hours $O_3$ exposure to the lowest tested level of 80 ppb in healthy human adults, while toxicological studies provided extensive evidence that short-term (1-3 hours) $O_3$ exposure in the range of 100-500 ppb could cause lung inflammatory responses. The limited epidemiologic evidence reviewed in the 2006 $O_3$ AQCD demonstrated an association between short-term increases in ambient $O_3$ concentration and airways inflammation in children (1-h max $O_3$ of approximately 100 ppb). Recent studies in animals and in vitro models described inflammatory and injury responses mediated by Toll-like receptors (e.g., TLR4, TLR2), receptors for TNF or IL-1, multiple signaling pathways (e.g., p38, JNK, NFκB, MAPK/AP-1), and oxidative stress (Section 6.2.3.3). Recent epidemiologic studies provide additional supporting evidence by demonstrating associations of ambient $O_3$ with mediators of airways inflammation and oxidative stress.

The normal inflammatory response in lung tissue is part of host defense that aids in removing microorganisms or particles that have reached the distal airways and alveolar surface. The 1996 $O_3$ AQCD concluded that short-term exposure to elevated concentrations of $O_3$ resulted in *alterations in these host defense mechanisms in the respiratory system*. Specifically, toxicological studies of short-term exposures as low as 100 ppb $O_3$ for 2 hours were shown to decrease the ability of alveolar macrophages to ingest particles, and short-term exposures as low as 80 ppb for 3 hours prevented mice from resisting infection with streptococcal bacteria and resulted in infection-related mortality. Similarly, alveolar macrophages removed from the lungs of human subjects after 6.6 hours of exposure to 80 and 100 ppb $O_3$

had decreased ability to ingest microorganisms, indicating some impairment of host defense capability. These altered host defense mechanisms can lead to increased risk of respiratory infections, which can often predispose individuals to developing asthma when occurring in early life. Despite the strong toxicological evidence, in the limited body of epidemiologic evidence, ambient $O_3$ concentrations have not been consistently associated with hospital admissions or ED visits for respiratory infection, pneumonia, or influenza (Section 6.2.7.2 and Section 6.2.7.3).

The most commonly observed and strongest evidence for respiratory effects associated with short-term exposure to $O_3$ is transient *decrements in pulmonary function*. Controlled human exposure studies reviewed in previous assessments demonstrated $O_3$-induced decrements in pulmonary function, characterized by alterations in lung volumes and flow and airway resistance and responsiveness for multihour exposures (up to 8 hours) to $O_3$ concentrations as low as 80 ppb (U.S. EPA, 1996a). A series of mobile laboratory studies of lung function and respiratory symptoms reported pulmonary function decrements at mean ambient $O_3$ concentrations of 140 ppb in exercising healthy adolescents and increased respiratory symptoms and pulmonary function decrements at 150 ppb in heavily exercising athletes and at 170 ppb in lightly exercising healthy and asthmatic subjects. Epidemiologic and animal toxicological evidence is coherent with the results of the controlled human exposure studies, both indicating decrements in lung function upon $O_3$ exposure. A combined statistical analysis of epidemiologic studies in children at summer camp with particularly strong exposure assessment demonstrated decrements in $FEV_1$ of 0.50 mL/ppb with an increase in previous hour $O_3$ concentration. For preadolescent children exposed to 120 ppb ambient $O_3$, this estimated volume decrease corresponded to an average decrement of 2.4-3.0% in $FEV_1$. Key studies of lung function ($FEV_1$) measured before and after well-defined outdoor exercise events in adults yielded concentration-response slopes of 0.40 and 1.35 mL/ppb ambient $O_3$ after exposure for up to 1 hour. Animal toxicological studies reported similar respiratory effects in rats at exposures as low as 200 ppb $O_3$ for 3 hours. The 2006 $O_3$ AQCD characterized the controlled human exposure and animal toxicological studies as providing clear evidence of causality for the associations observed between short-term ($\leq$ 24 hours) increases in $O_3$ concentration and relatively small, but statistically significant declines in lung function observed in numerous recent epidemiologic studies. In epidemiologic studies, declines in lung function were particularly noted in children with and without asthma, and adults who work or exercise outdoors.

Recent controlled human exposure studies examined lower concentration $O_3$ exposures (40-80 ppb) and demonstrated that *$FEV_1$, respiratory symptoms, and inflammatory responses were affected by $O_3$ exposures* of 6.6 hours as low as 60 to 70 ppb (Section 6.2.1.1 and Section 6.2.3.1). These studies demonstrated average $O_3$-induced decreases in $FEV_1$ in the range of 2.8 to 3.6% with $O_3$ exposures to 60 ppb for 6.6 hours. Further, in the controlled human exposure studies evaluating effects of 60 ppb $O_3$, on average, 10% of the exposed individuals experienced >10% $FEV_1$ decrements following 6.6 hours of exposure. Considerable intersubject variability has also been reported in studies at higher exposure concentrations

(≥ 70 ppb) with some subjects experiencing considerably greater decrements than average. Recent epidemiologic studies provide greater insight into individual- and population-level factors that can increase for the risk of $O_3$-associated respiratory morbidity. In addition to lung function decrements consistently reported in healthy children at summer camp, $O_3$-associated decreases in lung function were consistently observed in epidemiologic studies that included potentially at-risk populations (e.g., individuals with asthma with concurrent respiratory infection, older adults with AHR or elevated body mass index, or groups with diminished antioxidant capacity).

Exposure to $O_3$ can also result in *respiratory symptoms* (e.g., coughing, wheezing, shortness of breath). The 1996 $O_3$ AQCD identified an association between respiratory symptoms and increasing ambient $O_3$, particularly among children with asthma. In the 2006 $O_3$ AQCD, symptoms of cough and pain on deep inspiration were well documented in young healthy adult subjects after exposure of ≥ 80 ppb $O_3$ for 6-8 hours during moderate exercise. Limited data suggested an increase in respiratory symptoms down to 60 ppb. More recently, these effects have been observed at 70 ppb in healthy adults. Controlled human exposure studies of healthy adults, have also reported an increased incidence of cough with $O_3$ exposures as low as 120 ppb and 1-3 hours in duration with very heavy exercise. The controlled human exposure studies also demonstrated lesser respiratory symptom responses in children and older adults relative to young healthy adults. Cumulative epidemiologic evidence adds to the findings from controlled human exposure studies for healthy adults by demonstrating the effects of ambient $O_3$ exposure on respiratory symptoms in children with asthma. Increases in ambient $O_3$ concentration were associated with a wide variety of respiratory symptoms (e.g., cough, wheeze, and shortness of breath) in children with asthma. Epidemiologic studies also indicated that short-term increases in $O_3$ concentration are likely associated with increased asthma medication use in children with asthma. Additionally, epidemiologic studies provide evidence for an association between long-term exposure to $O_3$ and respiratory symptoms (Section 7.2).

Ozone exposure has been shown to result in *both specific and non-specific airway hyperresponsiveness (AHR)*. Increased AHR is an important consequence of exposure to $O_3$ because its presence represents a change in airway smooth muscle reactivity and implies that the airways are predisposed to narrowing on inhalation of a variety of stimuli (e.g., specific allergens, $SO_2$, cold air). Specifically, short-term (2 or 3 hours) exposure to 250 or 400 ppb $O_3$ was found to cause increases in AHR in response to allergen challenges among allergic asthmatic subjects who characteristically already had somewhat increased AHR at baseline. Increased non-specific AHR has been demonstrated in healthy young adults down to 80 ppb $O_3$ following 6.6 hours of exposure during moderate exercise. While AHR has not been widely examined in epidemiologic studies, findings for $O_3$-induced increases in AHR in controlled human exposure (Section 6.2.2.1) and toxicological (Section 6.2.2.2) studies provide biological plausibility for associations observed between increases in ambient $O_3$ concentration and increases in respiratory symptoms in subjects with asthma.

In addition to asthma exacerbations, recent epidemiologic evidence has indicated that *long-term ambient $O_3$ concentrations can contribute to new onset asthma* (Section 7.2.1, Table 7-2). The new epidemiologic evidence base consists of studies using a variety of designs and analysis methods evaluating the relationship between long-term annual measures of exposure to ambient $O_3$ and measures of respiratory morbidity. Studies from two California cohorts have provided evidence for different variants in genes related to oxidative or nitrosative stress (e.g., *HMOX, GSTs, ARG*) that, depending on community long-term $O_3$ concentrations, are related to new onset asthma. These cohorts provide evidence that extends beyond the association of short-term exposure to $O_3$ and asthma exacerbations to suggest that long-term exposure to $O_3$ may play a role in the development of the disease and contribute to incident cases of asthma.

The frequency of *ED visits and hospital admissions* due to respiratory symptoms, asthma exacerbations and other respiratory diseases is associated with *short- and long-term exposure to ambient $O_3$ concentrations*. Summertime daily hospital admissions for respiratory causes in various locations of eastern North America were consistently associated with ambient concentrations of $O_3$ in studies reviewed in the 1996 $O_3$ AQCD. This association remained even with examination of only concentrations below 120 ppb $O_3$. The 2006 $O_3$ AQCD concluded that aggregate population time-series studies demonstrate a positive and robust association between ambient $O_3$ concentrations and respiratory-related hospitalizations and asthma ED visits during the warm season. Recent epidemiologic time-series studies that include additional multicity studies and a multicontinent study further demonstrate that short-term exposures to ambient $O_3$ concentrations are consistently associated with increases in respiratory hospital admissions and ED visits specifically during the warm/summer months across a range of $O_3$ concentrations (Section 6.2.7). There is also recent evidence for an association between respiratory hospital admissions and long-term exposure to $O_3$ (Section 7.2.2).

Finally, $O_3$ exposure can contribute to *death from respiratory causes*. Recent evidence from several multicity studies and a multicontinent study demonstrate consistent positive associations between short-term exposure to ambient $O_3$ concentrations and increases in respiratory mortality (Section 6.2.8). Similarly, a study of long-term exposure to ambient $O_3$ concentrations also demonstrated an association between $O_3$ and increases in respiratory mortality (Section 7.7.1). Evidence from these recent mortality studies is consistent and coherent with the evidence from epidemiologic, controlled human exposure, and animal toxicological studies for the effects of short- and long-term exposure to $O_3$ on respiratory effects. Additionally, the evidence for respiratory morbidity after short- and long-term exposure provides biological plausibility for mortality due to respiratory disease.

### 2.5.3.2 Cardiovascular Effects

There is an emerging body of animal toxicological evidence demonstrating that short-term exposure to $O_3$ can lead to autonomic nervous system alterations (in heart rate and/or heart rate variability) and suggesting that proinflammatory signals may mediate cardiovascular effects. Interactions of $O_3$ with respiratory tract components result in secondary oxidation products and inflammatory mediators that have the potential to penetrate the epithelial barrier and to initiate toxic effects systemically. In addition, animal toxicological studies of long-term exposure to $O_3$ provide evidence of enhanced atherosclerosis and I/R injury, corresponding with development of a systemic oxidative, proinflammatory environment.

Evidence from controlled human exposure studies also demonstrates cardiovascular effects in response to short-term $O_3$ exposure and provides some coherence with evidence from animal toxicology studies. Controlled human exposure studies support the animal toxicological studies by demonstrating *$O_3$-induced effects on blood biomarkers of systemic inflammation and oxidative stress*, preliminary evidence for *$O_3$-induced modulation of the autonomic nervous system*, as well as *changes in biomarkers that can indicate a prothrombogenic response to $O_3$*. However, epidemiologic studies evaluating cardiovascular morbidity and short- and long-term exposure to $O_3$ do not provide consistent evidence for an association. This is evident by the multiple studies that examined the association between (1) short- and long-term $O_3$ concentrations and cardiovascular-related hospital admissions and ED visits, and (2) cardiovascular disease-related biomarkers, and reported inconsistent results.

When examining *mortality due to cardiovascular disease*, epidemiologic studies consistently observe positive associations with *short-term exposure to $O_3$*. Additionally, there is some evidence for an association between long-term exposure to $O_3$ and mortality. However, the association between long-term ambient $O_3$ concentrations and cardiovascular mortality can be confounded by other pollutants as evident by a study of cardiovascular mortality that reported no association after adjustment for $PM_{2.5}$ concentrations.

Overall, animal toxicological studies demonstrate $O_3$-induced cardiovascular effects, and support the strong body of evidence indicating $O_3$-induced cardiovascular mortality. Animal toxicological and controlled human exposure studies provide evidence for biologically plausible mechanisms underlying these $O_3$-induced cardiovascular effects. However, a lack of coherence with epidemiologic studies of cardiovascular morbidity remains an important uncertainty.

## 2.5.4 Policy Relevant Considerations

This ISA summarizes and integrates the available scientific evidence that best informs consideration of the policy-relevant questions that frame this assessment, presented in the Integrated Review Plan (U.S. EPA, 2011d). This includes considering whether the available body of scientific evidence supports or calls into

question the scientific conclusions reached in the last review regarding health effects related to exposure to $O_3$, with particular emphasis on exposures and health risks among populations potentially at increased risk. Additional policy relevant considerations include how the scientific information, when available, informs decisions regarding the basic elements of the NAAQS: indicator, averaging time, level, and form.

#### 2.5.4.1 Populations Potentially at Increased Risk

Studies were reviewed to identify populations that are at increased risk for $O_3$-related health effects. These studies have investigated factors that can cause populations to be at increased risk for $O_3$-related health effects by conducting stratified epidemiologic analyses; by examining individuals with an underlying health condition, genetic polymorphism, or categorized by age, race, or sex in controlled human exposure studies; or by developing animal models that mimic the pathophysiological conditions associated with a health effect. These studies have identified a multitude of factors that could potentially contribute to whether a population is at increased risk for $O_3$-related health effects.

The populations identified in Chapter 8 that were examined for their potential for increased risk of $O_3$-related health effects are listed in Table 8-6 and are classified as providing adequate, suggestive, inadequate, or no evidence of being an at-risk factor. The factors that have adequate evidence to be classified as an at-risk factor for $O_3$-related health effects are individuals with asthma, younger and older age groups, individuals with reduced intake of certain nutrients (i.e., vitamins C and E), and outdoor workers, based on consistency in findings across studies and evidence of coherence in results from different scientific disciplines. Asthma as a factor affecting risk was supported by controlled human exposure and toxicological studies, as well as some evidence from epidemiologic studies. Generally, studies comparing age groups also reported greater associations for respiratory hospital admissions and ED visits among children than for adults. Biological plausibility for this increased risk is supported by toxicological and controlled human exposure studies. Also, children have higher exposure and dose due to increased time spent outdoors and ventilation rate, and childrens' respiratory systems are also still undergoing lung growth. Most studies comparing age groups reported greater effects of short-term $O_3$ exposure on mortality among older adults, although studies of other health outcomes had inconsistent findings regarding whether older adults were at increased risk. Multiple epidemiologic, controlled human exposure, and toxicological studies reported that diets lower in vitamins E and C are associated with increased risk of $O_3$-related health effects. Previous studies have shown that increased exposure to $O_3$ due to outdoor work leads to increased risk of $O_3$-related health effects and it is clear that outdoor workers have higher exposures, and greater internal doses, of $O_3$, which may lead to increased risk of $O_3$-related health effects.

Other potential factors [genetic variants (such as those in *GSTM1*, *HMOX-1*, *NQO1*, and *TNF-α*), obesity, sex, and SES] provided some suggestive evidence of increased risk, but further investigation is needed. Similarly, many factors had inadequate evidence to determine if they increased the risk of $O_3$-related health effects, including influenza/infection, COPD, CVD, diabetes, hyperthyroidism, smoking, race/ethnicity, and air conditioning use.

### 2.5.4.2 Exposure Metrics in Epidemiologic Studies

Some epidemiologic studies have conducted analyses between $O_3$ concentration and health effects (i.e., mortality, respiratory or cardiovascular) using various exposure metrics (i.e., 1-h max, 8-h max, and 24-h avg). No studies of long-term exposure (i.e., months to years) to $O_3$ have compared the use of different exposure metrics on risk estimation.

Among time-series studies, the limited evidence suggests comparable risk estimates across exposure metrics with some evidence for smaller $O_3$ risk estimates when using a 24-hour average exposure metric. Several panel studies examined whether associations of lung function and respiratory symptoms varied depending on the $O_3$ exposure metric used. Although differences in effect estimates across exposure metrics were found within some studies, collectively, there was no indication that the consistency or magnitude of the observed association was stronger for a particular $O_3$ exposure metric. Several studies examining lung function demonstrated that this was true among populations with and without increased outdoor exposures. It is important to note in these studies, the degree of exposure measurement error associated with use of central site ambient $O_3$ concentrations may vary among $O_3$ averaging times, depending on time spent outdoors. Among studies that examined associations of multiple respiratory symptoms in children with multiple $O_3$ exposure metrics, most did not find higher odds ratios for any particular exposure metric. Overall, the evidence from time-series and panel epidemiologic studies does not indicate that one exposure metric is more consistently or strongly associated with mortality or respiratory-related health effects.

### 2.5.4.3 Lag Structure in Epidemiologic Studies

Epidemiologic studies have attempted to identify the time-frame in which exposure to $O_3$ can impart a health effect. The time period between $O_3$ exposure and health effects can potentially be influenced by a multitude of factors, such as age or existence of pre-existing diseases. Different lag times have been evaluated for specific health outcomes.

The epidemiologic evidence evaluated in the 2006 $O_3$ AQCD indicated that one of the remaining uncertainties in characterizing the $O_3$-mortality relationship was identifying the appropriate lag structure (e.g., single-day lags versus distributed lag

model). An examination of lag times used in the epidemiologic studies evaluated in this assessment can provide further insight on the characterization of the relationship between $O_3$ exposure and morbidity and mortality outcomes from epidemiologic studies.

The majority of epidemiologic studies that focused on the association between short-term $O_3$ exposure and mortality (i.e., all-cause, respiratory and cardiovascular) examined the average of multiday lags with some studies examining single-day lags. Across a range of multiday lags (i.e., average of 0-1 to 0-6 days), the studies evaluated consistently demonstrate that the $O_3$ effects on mortality occur within a few days of exposure (Figure 6-27).

Epidemiologic studies of lung function, respiratory symptoms, and biological markers of airway inflammation and oxidative stress examined associations with single-day ambient $O_3$ concentrations (using various averaging times) lagged from 0 to 7 days as well as concentrations averaged over 2 to 19 days. Lags of 0 and 1 day ambient $O_3$ concentrations were associated with decreases in lung function and increases in respiratory symptoms, airway inflammation, and oxidative stress. Additionally, several studies found that multiday averages of $O_3$ concentration were associated with these endpoints, indicating that not only single day, but exposures accumulated over several days led to a respiratory health effect. In studies of respiratory hospital admissions and ED visits, investigators either examined the lag structure of associations by including both single-day and the average of multiday lags, or selecting lags a priori. The collective evidence indicates a rather immediate response within the first few days of $O_3$ exposure (i.e., for lags days averaged at 0-1, 0-2, and 0-3 days) for hospital admissions and ED visits for all respiratory outcomes, asthma, and chronic obstructive pulmonary disease in all-year and seasonal analyses.

#### 2.5.4.4 Ozone Concentration-Response Relationship

An important consideration in characterizing the $O_3$-morbidity and mortality association is whether the concentration-response (C-R) relationship is linear across the full concentration range that is encountered or if there are concentration ranges where there are departures from linearity (i.e., nonlinearity). In this ISA studies have been identified that attempt to characterize the shape of the $O_3$ C-R curve along with possible $O_3$ "thresholds" (i.e., $O_3$ concentrations which must be exceeded in order to elicit an observable health response). The epidemiologic studies that examined the shape of the C-R curve and the potential presence of a threshold have indicated a generally linear C-R function with no indication of a threshold in analyses that have examined 8-h max and 24-h avg $O_3$ concentrations. However, there is less certainty in the shape of the C-R curve at the lower end of the distribution of $O_3$ concentrations (below NA background concentrations, 29-40 ppb) due to the low density of data in this range.

Controlled human exposure studies have provided strong and quantifiable C-R data on the human health effects of $O_3$. The magnitude of respiratory effects in these

studies is generally a function of $O_3$ exposure, i.e., the product of concentration (C), minute ventilation ($\dot{V}_E$), and exposure duration. Several studies provide evidence for a smooth C-R curve in young healthy adults exposed during moderate exercise for 6.6 hours to $O_3$ concentrations between 40 and 120 ppb (Figure 6-1). It is difficult to characterize the C-R relationship at and below 40 ppb due to uncertainty associated with the sparse data at these lower concentrations.

Although relatively few epidemiologic studies have examined the $O_3$-health effects C-R relationship, the C-R relationship has been examined across multiple health endpoints and exposure durations. Some studies of populations engaged in outdoor activity found that associations between $O_3$ and lung function decrements persisted at lower $O_3$ concentrations with some studies showing larger negative associations in analyses limited to lower $O_3$ concentrations (e.g., 60-80 ppb; Table 6-6) and shorter exposure durations (i.e., in the range of 30 minutes to less than 8 hours; Table 6-6). A study examining the C-R relationship between short-term $O_3$ exposure and pediatric asthma ED visits found no evidence of a threshold with a linear relationship evident down to 8-h max $O_3$ concentrations as low as 30 ppb (Figure 6-17). In an additional study, authors used a smooth function while also accounting for the potential confounding effects of $PM_{2.5}$, to examine whether the shape of the C-R curve for short-term exposure to $O_3$ and asthma hospital admissions is linear. When comparing the curve to a linear fit, the authors found that the linear fit is a reasonable approximation of the C-R relationship between $O_3$ and asthma hospital admissions in the mid-range of the data though it can be seen that there is greater uncertainty at the lower end of the distribution of ambient $O_3$ concentrations, generally below 20 ppb (Figure 6-16) due to sparse data at these lower concentrations.

Several recent studies applied a variety of statistical approaches to examine the shape of the $O_3$-mortality C-R relationship and existence of a threshold (Section 6.6.2.4). These studies suggest that the shape of the $O_3$-mortality C-R curve is linear across the range of $O_3$ concentrations though uncertainty in the relationship increases at the lower end of the distribution (Figure 6-36). Generally, the epidemiologic studies that examined the $O_3$-mortality C-R relationship do not provide evidence for the existence of a threshold within the range of 24-h average (24-h avg) $O_3$ concentrations most commonly observed in the U.S. during the $O_3$ season (i.e., above 20 ppb). However, the evaluation of the C-R relationship for short-term exposure to $O_3$ and mortality is difficult due to the evidence from multicity studies indicating heterogeneity in $O_3$-mortality associations across regions of the United States. In addition, there are numerous issues that can influence the shape of the $O_3$-mortality C-R relationship that need to be taken into consideration including: multiday effects (distributed lags), and potential adaptation and mortality displacement (i.e., hastening of death by a short period). Additionally, given the effect modifiers identified in mortality analyses that are also expected to vary regionally (e.g., temperature, air conditioning prevalence), a national or combined analysis may not be appropriate to identify whether a threshold exists in the $O_3$-mortality C-R relationship.

In addition, the C-R relationship of long-term exposure to $O_3$ and birth outcomes has been evaluated. Evidence from the southern California Children's Health Study identified a C-R relationship of birth weight with 24-h avg $O_3$ concentrations averaged over the entire pregnancy that was clearest above the 30 ppb level (Figure 7-4).

Generally, both short- and long-term exposure studies indicate a linear, no threshold C-R relationship when examining the association between $O_3$ exposure and multiple health effects across the range of 8-h max and 24-h avg $O_3$ concentrations most commonly observed in the U.S. during the $O_3$ season (i.e., greater than 20 ppb). However, evidence from studies of respiratory health effects and mortality indicates less certainty in the shape of the C-R curve at the lower end of the distribution of $O_3$ data, which corresponds to 8-h max and 24-h avg $O_3$ concentrations generally below 20 ppb.

#### 2.5.4.5 Regional Heterogeneity in Risk Estimates

Multicity epidemiologic studies that have examined the relationship between short-term $O_3$ exposures and mortality have provided evidence of city-to-city and regional heterogeneity in $O_3$-mortality risk estimates. A possible explanation for this heterogeneity may be differences in community characteristics (individual- or community-level) across cities that could modify the $O_3$ effect (e.g., activity patterns, housing type and age distribution, prevalence and use of air conditioning). Another possible explanation for the observed heterogeneity could be effect modification by concentrations of other air pollutants or interactions with temperature or other meteorological factors that vary regionally in the U.S.

An examination of community characteristics measured at the individual level that may contribute to the observed heterogeneity in $O_3$-mortality risk estimates indicates increased risk in older adults (i.e., $\geq$ 65 years of age), women, African American individuals, individuals with pre-existing diseases/conditions (e.g., diabetes, atrial fibrillation), and lower SES. Furthermore, studies have examined community characteristics measured at the community level and found that higher $O_3$-mortality risk estimates were associated with higher: percent unemployment, fraction of the population Black/African-American, percent of the population that take public transportation to work; and with lower: temperatures and percent of households with central air conditioning. There is also evidence of greater effects in cities with lower mean $O_3$ concentrations. Additionally, there is evidence of increased risk of $O_3$-related mortality as percentage unemployed increases and a reduction in $O_3$-related mortality as mean temperature increased (i.e., a surrogate for air conditioning rate) in the United States. The lack of a consistent reduction in $O_3$-risk estimates in cities with a higher percentage of central air conditioning across health outcomes complicates the interpretation of the potential modifying effects of air conditioning use.

Overall, the epidemiologic studies that have examined the city-to-city and regional heterogeneity observed in multicity studies have identified a variety of factors that may modify the $O_3$-mortality or -respiratory hospital admission relationship. Some studies have also examined the correlation with other air pollutants or the potential interactive effects between $O_3$ and temperature to explain city-to-city heterogeneity in $O_3$-mortality risk estimates. This includes evidence that $O_3$-mortality risk estimates in the U.S. varied by mean $SO_2$ concentrations, the ratio between mean $NO_2/PM_{10}$ concentrations, and temperatures. However, studies have not consistently identified specific community characteristics that explain the observed heterogeneity.

## 2.6 Integration of Effects on Vegetation and Ecosystems

Chapter 9 presents the most policy-relevant information related to this review of the NAAQS for the welfare effects of $O_3$ on vegetation and ecosystems. This section integrates the key findings from the disciplines evaluated in this assessment of the $O_3$ scientific literature, which includes plant physiology, whole plant biology, ecosystems, and exposure-response.

Overall, exposure to $O_3$ is causally related or likely to be causally related to effects observed on vegetation and ecosystems. These effects are observed across the entire continuum of biological organization; from the cellular and subcellular level to the whole plant level, and up to ecosystem-level processes. Furthermore, there is evidence that the effects observed across this continuum are related to one another; effects of $O_3$ at lower levels of organization, such as the leaf of an individual plant, can result in effects at higher levels. Ozone enters leaves through stomata, and can alter stomatal conductance and disrupt $CO_2$ fixation (Section 9.3). These effects can change rates of leaf gas exchange, growth and reproduction at the individual plant level and result in changes in ecosystems, such as productivity, C storage, water cycling, nutrient cycling, and community composition (Section 9.4). Figure 2-2 is a simplified illustrative diagram of the major endpoints that $O_3$ may affect in vegetation and ecosystems.

The framework for causal determinations (see Preamble) has been applied to the body of scientific evidence to examine effects attributed to $O_3$ exposure (Table 2-2). The summary below provides brief integrated summaries of the evidence that supports the causal determinations. The detailed discussion of the underlying evidence used to formulate each causal determination can be found in Chapter 9. This summary ends with a short discussion of policy relevant considerations.

### 2.6.1 Visible Foliar Injury

Visible foliar injury resulting from exposure to $O_3$ has been well characterized and documented over several decades of research on many tree, shrub, herbaceous, and crop species (U.S. EPA, 2006b, 1996b, 1984, 1978a) (Section 9.4.2). Ozone-induced

visible foliar injury symptoms on certain bioindicator plant species are considered diagnostic as they have been verified experimentally in exposure-response studies, using exposure methodologies such as continuous stirred tank reactors (CSTRs), open-top chambers (OTCs), and free-air fumigation. Experimental evidence has clearly established a consistent association of visible injury with $O_3$ exposure, with greater exposure often resulting in greater and more prevalent injury. Since publication of the 2006 $O_3$ AQCD, the results of several multiple-year field surveys of $O_3$-induced visible foliar injury at National Wildlife Refuges in Maine, Michigan, New Jersey, and South Carolina have been published. New sensitive species showing visible foliar injury continue to be identified from field surveys and verified in controlled exposure studies.

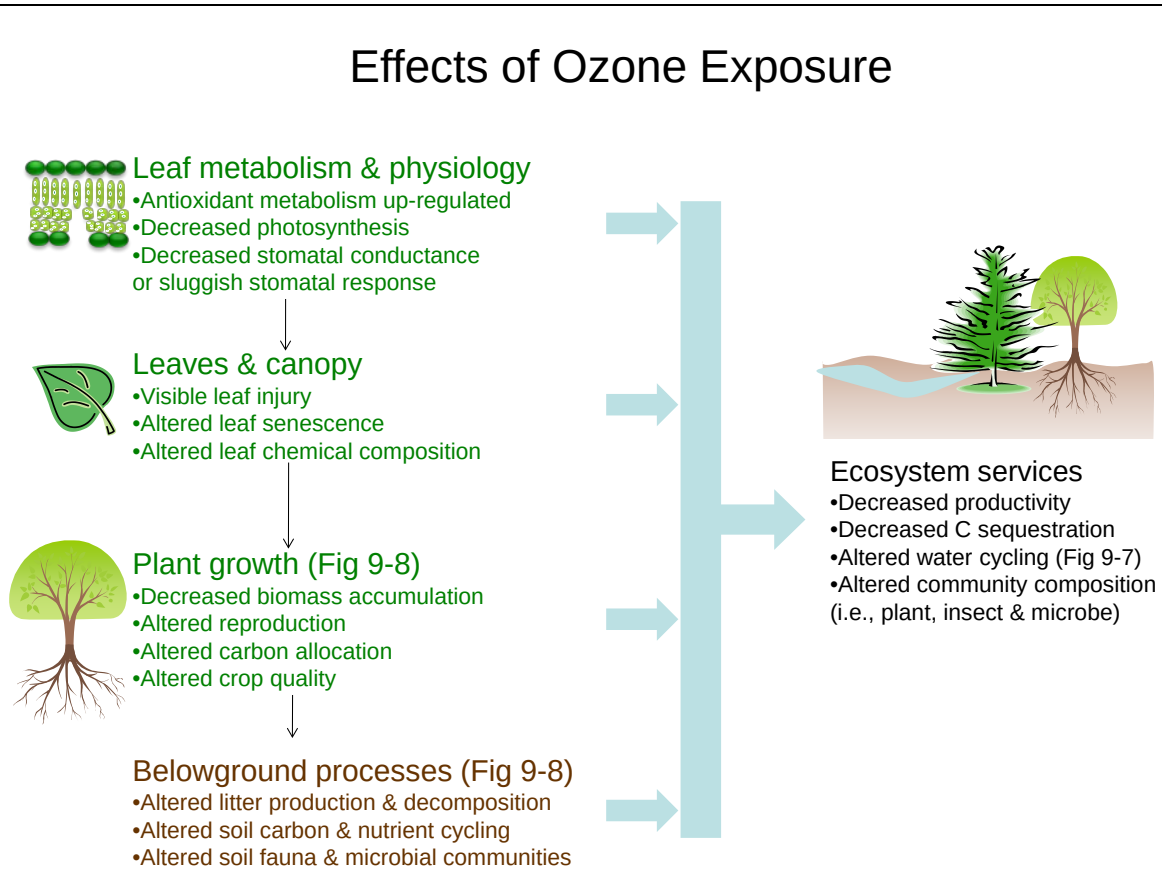



**Figure 2-2 An illustrative diagram of the major endpoints that $O_3$ may affect in plants and ecosystems.**

## Table 2-2 Summary of $O_3$ causal determinations for vegetation and ecosystem effects.

| Vegetation and Ecosystem Effects | Conclusions from 2006 $O_3$ AQCD | Conclusions from this ISA |
|---|---|---|
| Visible Foliar Injury Effects on Vegetation | Data published since the 1996 $O_3$ AQCD strengthen previous conclusions that there is strong evidence that current ambient $O_3$ concentrations cause impaired aesthetic quality of many native plants and trees by increasing foliar injury. | Causal Relationship |
| Reduced Vegetation Growth | Data published since the 1996 $O_3$ AQCD strengthen previous conclusions that there is strong evidence that current ambient $O_3$ concentrations cause decreased growth and biomass accumulation in annual, perennial and woody plants, including agronomic crops, annuals, shrubs, grasses, and trees. | Causal Relationship |
| Reduced Productivity in Terrestrial Ecosystems | There is evidence that $O_3$ is an important stressor of ecosystems and that the effects of $O_3$ on individual plants and processes are scaled up through the ecosystem, affecting net primary productivity. | Causal Relationship |
| Reduced Carbon (C) Sequestration in Terrestrial Ecosystems | Limited studies from the 2006 $O_3$ AQCD. | Likely to be a Causal Relationship |
| Reduced Yield and Quality of Agricultural Crops | Data published since the 1996 $O_3$ AQCD strengthen previous conclusions that there is strong evidence that current ambient $O_3$ concentrations cause decreased yield and/or nutritive quality in a large number of agronomic and forage crops. | Causal Relationship |
| Alteration of Terrestrial Ecosystem Water Cycling | Ecosystem water quantity may be affected by $O_3$ exposure at the landscape level. | Likely to be a Causal Relationship |
| Alteration of Below-ground Biogeochemical Cycles | Ozone-sensitive species have well known responses to $O_3$ exposure, including altered C allocation to below-ground tissues; and also altered rates of leaf and root production, turnover, and decomposition. These shifts can affect overall C loss and nitrogen (N) loss from the ecosystem in terms of respired C, and leached aqueous dissolved organic and inorganic C and N. | Causal Relationship |
| Alteration of Terrestrial Community Composition | Ozone may be affecting above- and below -ground community composition through impacts on both growth and reproduction. Significant changes in plant community composition resulting directly from $O_3$ exposure have been demonstrated. | Likely to be a Causal Relationship |

The use of biological indicators in field surveys to detect phytotoxic levels of $O_3$ is a longstanding and effective methodology. The USDA Forest Service through the Forest Health Monitoring (FHM) Program (1990-2001) and currently the Forest Inventory and Analysis (FIA) Program has been collecting data regarding the incidence and severity of visible foliar injury on a variety of $O_3$ sensitive plant species throughout the United States. The network has provided evidence that $O_3$ concentrations were high enough to induce visible symptoms on sensitive vegetation.

From repeated observations and measurements made over a number of years, specific geographical patterns of visible $O_3$ injury symptoms can be identified. In addition, a study assessed the risk of $O_3$-induced visible foliar injury on bioindicator plants in 244 national parks in support of the National Park Service's Vital Signs Monitoring Network. The results of the study demonstrated that the estimated risk of visible foliar injury was high in 65 parks (27%), moderate in 46 parks (19%), and low in 131 parks (54%). Some of the well-known parks with a high risk of $O_3$-induced visible foliar injury include Gettysburg, Valley Forge, Delaware Water Gap, Cape Cod, Fire Island, Antietam, Harpers Ferry, Manassas, Wolf Trap Farm Park, Mammoth Cave, Shiloh, Sleeping Bear Dunes, Great Smoky Mountains, Joshua Tree, Sequoia and Kings Canyon, and Yosemite. Overall, evidence is sufficient to conclude that there **is a causal relationship between ambient $O_3$ exposure and the occurrence of $O_3$-induced visible foliar injury on sensitive vegetation across the U.S.**

## 2.6.2 Growth, Productivity, Carbon Storage and Agriculture

Ambient $O_3$ concentrations have long been known to cause decreases in photosynthetic rates and plant growth. The $O_3$-induced damages at the plant scale may translate to damages at the stand, then ecosystem scales, and cause changes in productivity and C storage. The effects of $O_3$ exposure on photosynthesis, growth, biomass allocation, ecosystem production, and ecosystem C sequestration were reviewed for the natural ecosystems, and crop productivity and crop quality were reviewed for the agricultural ecosystems.

### 2.6.2.1 Natural Ecosystems

The previous $O_3$ AQCDs concluded that there is strong and consistent evidence that ambient concentrations of $O_3$ decrease plant photosynthesis and growth in numerous plant species across the United States. Studies published since the last review continue to support that conclusion (Section 9.4.3.1). Recent studies, based on the Aspen free-air carbon-dioxide/ozone enrichment (FACE) experiment, found that $O_3$ caused reductions in total biomass relative to the control in aspen, paper birch, and sugar maple communities during the first seven years of stand development. Overall, the studies at the Aspen FACE experiment were consistent with the open-top chamber (OTC) studies that were the foundation of previous $O_3$ NAAQS reviews. These results strengthen the understanding of $O_3$ effects on forests and demonstrate the relevance of the knowledge gained from trees grown in OTC studies.

A set of meta-analyses assessed the effects of $O_3$ on plant photosynthesis and growth across different species and fumigation methods (such as OTC and FACE). Those studies reported that current $O_3$ concentrations in the northern hemisphere are decreasing photosynthesis (~11%) across tree species, and the decreases in photosynthesis are consistent with cumulative uptake of $O_3$ into the leaf. The current ambient $O_3$ concentrations (~40 ppb averaged across all hours of exposure)

decreased annual total biomass growth of forest species by an average of 7%, with potentially greater decreases (11-17%) with elevated $O_3$ exposures (Section 9.4.3.1). The meta-analyses further confirmed that reduction of plant photosynthesis and growth under $O_3$ exposure are coherent across numerous species and various experimental techniques.

Studies during recent decades have also demonstrated $O_3$ alters biomass allocation and plant reproduction (Section 9.4.3). Recent meta-analyses have generally indicated that $O_3$ reduced C allocated to roots. Several recent studies published since the 2006 $O_3$ AQCD further demonstrate that $O_3$ altered reproductive processes, such as timing of flowering, number of flowers, fruits and seeds, in herbaceous and woody plant species. However, a knowledge gap still exists pertaining to the exact mechanism of the responses of reproductive processes to $O_3$ exposure (Section 9.4.3.3).

Studies at the leaf and plant scales show that $O_3$ decreased photosynthesis and plant growth, providing coherence and biological plausibility for the reported decreases in ecosystem productivity. During the previous NAAQS reviews, there were very few studies that investigated the effect of $O_3$ exposure on ecosystem productivity and C sequestration. Recent studies from long-term FACE experiments and ecosystem models provided evidence of the association of $O_3$ exposure and reduced productivity at the ecosystem scale. Elevated $O_3$ reduced stand biomass at Aspen FACE after 7 years of $O_3$ exposure, and annual volume growth at the Kranzberg Forest in Germany. Results across different ecosystem models were consistent with the FACE experimental evidence, which showed that $O_3$ reduced ecosystem productivity (Section 9.4.3.4). In addition to primary productivity, other indicators such as net ecosystem productivity (NEP), net ecosystem $CO_2$ exchange (NEE) and C sequestration were often assessed by model studies. Model simulations consistently found that $O_3$ exposure caused negative impacts on these indicators (Section 9.4.3.4, Table 9-3), but the severity of these impacts was influenced by multiple interactions of biological and environmental factors. The suppression of ecosystem C sinks results in more $CO_2$ accumulation in the atmosphere. A recent study suggested that the indirect radiative forcing caused by $O_3$ exposure through lowering the ecosystem C sink could have an even greater impact on global warming than the direct radiative forcing of $O_3$.

Although $O_3$ generally causes negative effects on ecosystem productivity, the magnitude of the response varies among plant communities (Section 9.4.3.4). For example, $O_3$ had little impact on white fir, but greatly reduced growth of ponderosa pine in southern California. Ozone decreased net primary production (NPP) of most forest types in the Mid-Atlantic region, but had small impacts on spruce-fir forest. Ozone could also affect regional C budgets through interacting with multiple factors, such as N deposition, elevated $CO_2$ and land use history. Model simulations suggested that $O_3$ partially offset the growth stimulation caused by elevated $CO_2$ and N deposition in both Northeast- and Mid-Atlantic-region forest ecosystems of the U.S.

Overall, evidence is sufficient to conclude that there **is a causal relationship between ambient $O_3$ exposure and reduced native plant growth and productivity,** and **a likely causal relationship between $O_3$ exposure and reduced carbon sequestration in terrestrial ecosystems.**

#### 2.6.2.2 Agricultural Crops

The detrimental effect of $O_3$ on crop production has been recognized since the 1960s and a large body of research has subsequently stemmed from those initial findings. Previous $O_3$ AQCDs have extensively reviewed this body of literature. Current $O_3$ concentrations across the U.S. are high enough to cause yield loss for a variety of agricultural crops including, but not limited to, soybean, wheat, potato, watermelon, beans, turnip, onion, lettuce, and tomato (Section 9.4.4.1). Continued increases in $O_3$ concentration may further decrease yield in these sensitive crops. Despite the well-documented yield losses due to increasing $O_3$ concentration, there is still a knowledge gap pertaining to the exact mechanism of $O_3$-induced yield loss. Research has linked increasing $O_3$ concentration to decreased photosynthetic rates and accelerated senescence, which are related to yield.

In addition, recent research has highlighted the effects of $O_3$ on crop quality. Increasing $O_3$ concentration decreases nutritive quality of grasses, decreases macro- and micro-nutrient concentrations in fruits and vegetable crops, and decreases cotton fiber quality. These areas of research require further investigation to determine the mechanism and dose-responses (Section 9.4.4.2).

During the previous NAAQS reviews, there were very few studies that estimated $O_3$ impacts on crop yields at large geographical scales (i.e., regional, national or global). Recent modeling studies found that $O_3$ generally reduced crop yield, but the impacts varied across regions and crop species (Section 9.4.4.1). For example, the largest $O_3$-induced crop yield losses occurred in high-production areas exposed to high $O_3$ concentrations, such as the Midwest and the Mississippi Valley regions of the United States. Among crop species, the estimated yield loss for wheat and soybean were higher than rice and maize. Satellite and ground-based $O_3$ measurements have been used to assess yield loss caused by $O_3$ over the continuous tri-state area of Illinois, Iowa, and Wisconsin. The results showed that $O_3$ concentrations reduced soybean yield, which correlates well with the previous results from FACE- and OTC-type experiments (Section 9.4.4.1).

Evidence is sufficient to conclude that there **is a causal relationship between $O_3$ exposure and reduced yield and quality of agricultural crops.**

### 2.6.3 Water Cycling

Ozone can affect water use in plants and ecosystems through several mechanisms including damage to stomatal functioning and loss of leaf area. Section 9.3.6

reviewed possible mechanisms for $O_3$ exposure effects on stomatal functioning. Regardless of the mechanism, $O_3$ exposure has been shown to alter stomatal performance, which may affect plant and stand transpiration and therefore possibly affecting hydrological cycling.

Although the evidence was from a limited number of field and modeling studies, these findings showed an association of $O_3$ exposure and the alteration of water use and cycling in vegetation and ecosystems (Section 9.4.5). There is not a clear consensus on the nature of leaf-level stomatal conductance response to $O_3$ exposure. When measured at steady-state high light conditions, leaf-level stomatal conductance is often found to be reduced when exposed to $O_3$. However, measurements of stomatal conductance under dynamic light and vapor pressure deficit conditions indicate sluggish responses under elevated $O_3$ exposure which could potentially lead to increased water loss from vegetation. In situations where stomata fail to close under low light or water stressed conditions water loss may be greater over time. In other situations it is possible that sluggish stomata may fail to completely open in response to environmental stimuli and result in decreased water loss. Field studies suggested that peak hourly $O_3$ exposure increased the rate of water loss from several tree species, and led to a reduction in the late-season modeled stream flow in three forested watersheds in eastern Tennessee. Sluggish stomatal responses during $O_3$ exposure was suggested as a possible mechanism for increased water loss during peak $O_3$ exposure. Currently, the $O_3$-induced reduction in stomatal aperture is the biological assumption for most process-based models. Therefore, results of those models normally found that $O_3$ reduced water loss. For example, one study found that $O_3$ damage and N limitation together reduced evapotranspiration and increased runoff.

Although the direction of the response differed among studies, the evidence is sufficient to conclude that there **is likely to be a causal relationship between $O_3$ exposure and the alteration of ecosystem water cycling.**

### 2.6.4 Below-ground Processes

Below-ground processes are tightly linked with aboveground processes. The responses of aboveground process to $O_3$ exposure, such as reduced photosynthetic rates, increased metabolic cost, and reduced root C allocation, have provided biologically plausible mechanisms for the alteration of below-ground processes. Since the 2006 $O_3$ AQCD, more evidence has shown that although the responses are often species specific, $O_3$ altered the quality and quantity of C input to soil, microbial community composition, and C and nutrient cycling.

Results from Aspen FACE and other experimental studies consistently found that $O_3$ reduced litter production and altered C chemistry, such as soluble sugars, soluble phenolics, condensed tannins, lignin, and macro/micro nutrient concentration in litter (Section 9.4.6.1). Under elevated $O_3$, the changes in substrate quality and quantity could alter microbial metabolism, and therefore soil C and nutrient cycling. Several

studies indicated that $O_3$ generally suppressed soil enzyme activities (Section 9.4.6.2). However, the impact of $O_3$ on litter decomposition was inconsistent and varied among species, sites, and exposure length. Similarly, $O_3$ had inconsistent impacts on dynamics of micro and macro nutrients (Section 9.4.6.4).

Studies from the Aspen FACE experiment suggested that the response of below-ground C cycle to $O_3$ exposure, such as litter decomposition, soil respiration, and soil C content, changed over time. For example, in the early part of the experiment (1998-2003), $O_3$ had no impact on soil respiration but reduced the formation rates of total soil C under elevated $CO_2$. However, after 10 to 11 years of exposure, $O_3$ was found to increase soil respiration but have no substantial impact on soil C formation under elevated $CO_2$ (Section 9.4.6.3).

The evidence is sufficient to infer that there **is a causal relationship between $O_3$ exposure and the alteration of below-ground biogeochemical cycles.**

### 2.6.5 Community Composition

In the 2006 $O_3$ AQCD, the impact of $O_3$ exposure on species competition and community composition was assessed. Ozone was found to be one of the dominant factors causing a decline in ponderosa and Jeffrey pine in the San Bernardino Mountains in southern California. Ozone exposure also tended to shift the grass-legume mixtures in favor of grass species. Since the 2006 $O_3$ AQCD, more evidence has shown that $O_3$ exposure changed the competitive interactions and led to loss of $O_3$ sensitive species or genotypes. Studies found that the severity of $O_3$ damage on growth, reproduction and foliar injury varied among species (Section 9.4.3), which provided the biological plausibility for the alteration of community composition. Additionally, research since the last review has shown that $O_3$ can alter community composition and diversity of soil microbial communities.

The decline of conifer forests under $O_3$ exposure was continually observed in several regions. Ozone damage was believed to be an important causal factor in the dramatic decline of sacred fir in the valley of Mexico, as well as cembran pine in southern France and the Carpathian Mountains, although several factors, such as drought, insect outbreak and forest management, may also contribute to or even be the dominant factors causing the mortality of the conifer trees. Results from the Aspen FACE site indicated that $O_3$ could alter community composition of broadleaf forests as well. At the Aspen FACE site, $O_3$ reduced growth and increased mortality of a sensitive aspen clone, while the $O_3$ tolerant clone emerged as the dominant clone in the pure aspen community. In the mixed aspen-birch and aspen-maple communities, $O_3$ reduced the competitive capacity of aspen compared to birch and maple (Section 9.4.7.1).

The tendency for $O_3$-exposure to shift the biomass of grass-legume mixtures in favor of grass species was reported in the 2006 $O_3$ AQCD and has been generally confirmed by recent studies. However, in a high elevation mature/species-rich grass-

legume pasture, $O_3$ fumigation showed no substantial impact on community composition (Section 9.4.7.2).

Ozone exposure not only altered community composition of plant species, but also microorganisms. The shift in community composition of bacteria and fungi has been observed in both natural and agricultural ecosystems, although no general patterns could be identified (Section 9.4.7.3).

The evidence is sufficient to conclude that there **is likely to be a causal relationship between $O_3$ exposure and the alteration of community composition of some ecosystems.**

## 2.6.6 Policy Relevant Considerations

### 2.6.6.1 Air Quality Indices

Exposure indices are metrics that quantify exposure as it relates to measured plant response (e.g., reduced growth). They are summary measures of monitored ambient $O_3$ concentrations over time intended to provide a consistent metric for reviewing and comparing exposure-response effects obtained from various studies. No recent information is available since 2006 that alters the basic conclusions put forth in the 2006 and 1996 $O_3$ AQCDs. These AQCDs focused on the research used to develop various exposure indices to help quantify effects on growth and yield in crops, perennials, and trees (primarily seedlings). The performance of indices was compared through regression analyses of earlier studies designed to support the estimation of predictive $O_3$ exposure-response models for growth and/or yield of crops and tree (seedling) species.

Another approach for improving risk assessment of vegetation response to ambient $O_3$ is based on determining the $O_3$ concentration from the atmosphere that enters the leaf (i.e., flux or deposition). Interest has been increasing in recent years, particularly in Europe, in using mathematically tractable flux models for $O_3$ assessments at the regional, national, and European scale. While some efforts have been made in the U.S. to calculate $O_3$ flux into leaves and canopies, little information has been published relating these fluxes to effects on vegetation. There is also concern that not all $O_3$ stomatal uptake results in a yield reduction, which depends to some degree on the amount of internal detoxification occurring with each particular species. Species having high detoxification capacity may show little relationship between $O_3$ stomatal uptake and plant response. The lack of data in the U.S. and the lack of understanding of detoxification processes have made this technique less viable for vulnerability and risk assessments in the U.S.

The main conclusions from the 1996 and 2006 $O_3$ AQCDs regarding indices based on ambient exposure remain valid. These key conclusions can be restated as follows:

- ozone effects in plants are cumulative;
- higher $O_3$ concentrations appear to be more important than lower concentrations in eliciting a response;
- plant sensitivity to $O_3$ varies with time of day and plant development stage;
- quantifying exposure with indices that cumulate hourly $O_3$ concentrations and preferentially weight the higher concentrations improves the explanatory power of exposure/response models for growth and yield, over using indices based on mean and peak exposure values.

Various weighting functions have been used, including threshold-weighted (e.g., SUM06) and continuous sigmoid-weighted (e.g., W126) functions. Based on statistical goodness-of-fit tests, these cumulative, concentration-weighted indices could not be differentiated from one another using data from previous exposure studies. Additional statistical forms for $O_3$ exposure indices are summarized in Section 9.5 of this ISA. The majority of studies published since the 2006 $O_3$ AQCD do not change earlier conclusions, including the importance of peak concentrations, and the duration and occurrence of $O_3$ exposures in altering plant growth and yield.

Given the current state of knowledge and the best available data, exposure indices that cumulate and differentially weight the higher hourly average concentrations and also include the mid-level values continue to offer the most scientifically defensible approach for use in developing response functions and comparing studies, as well as for defining future indices for vegetation protection.

#### 2.6.6.2 Exposure-Response

None of the information on effects of $O_3$ on vegetation published since the 2006 $O_3$ AQCD has modified the assessment of quantitative exposure-response relationships that was presented in that document (U.S. EPA, 2006b). This assessment updates the 2006 exposure-response models by computing them using the W126 metric, cumulated over 90 days. Almost all of the experimental research on the effects of $O_3$ on growth or yield of plants published since 2006 used only two levels of exposure. In addition, hourly $O_3$ concentration data that would allow calculations of exposure using the W126 metric are generally unavailable. However, two long-term experiments, one with a crop species (soybean), one with a tree species (aspen), have produced data that are used in Section 9.6 to validate the exposure-response models presented in the 2006 $O_3$ AQCD, and the methodology used to derive them. EPA compared predictions from the models presented in the 2006 $O_3$ AQCD, updated to use the 90 day 12hr W126 metric, with more recent observations for yield of soybean and biomass growth of trembling aspen. The models were parameterized using data from the National Crop Loss Assessment Network (NCLAN) and EPA's National Health and Environmental Effects Research Laboratory – Western Ecology Division

(NHEERL-WED) projects, which were conducted in OTCs. The more recent observations were from experiments using FACE technology, which is intended to provide conditions closer to natural environments than OTC. Observations from these new experiments were exceptionally close to predictions from the models. The accuracy of model predictions for two widely different plant species, grown under very different conditions, provides support for the validity of the models for crops and trees developed using the same methodology and data for other species. However, variability observed among species in the NCLAN and NHEERL-WED projects indicates that the range of sensitivity between and among species is likely quite wide.

Results from several meta-analyses have provided approximate values for responses of yield of soybean, wheat, rice and other crops under broad categories of exposure, relative to charcoal-filtered air. Additional reports have summarized yield data for six crop species under various broad comparative exposure categories, and reviewed 263 studies that reported effects on tree biomass. However, these analyses have proved difficult to compare with exposure-response models, especially given that exposure was not expressed using a common metric (i.e., W126).

## 2.7 The Role of Tropospheric $O_3$ in Climate Change and UV-B Shielding Effects

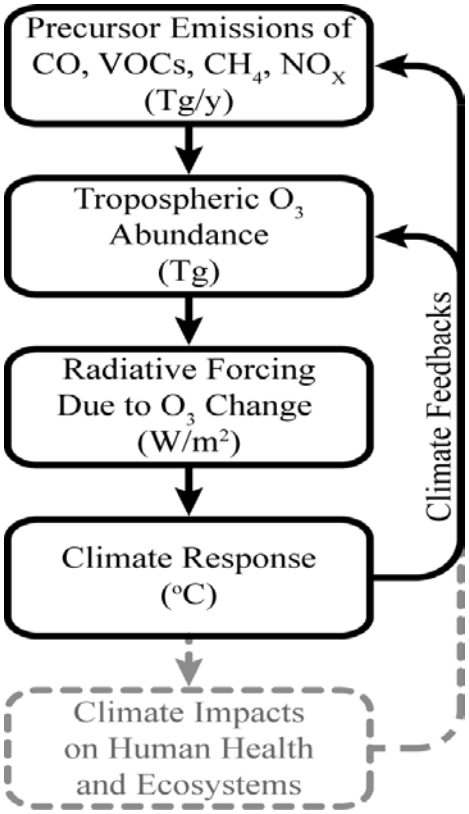
Atmospheric $O_3$ plays an important role in the Earth's energy budget by interacting with incoming solar radiation and outgoing infrared radiation. Tropospheric $O_3$ makes up only a small portion of the total column of $O_3$, but it has important incremental effects on the overall radiation budget. Chapter 10 assesses the specific role of tropospheric $O_3$ in the earth's radiation budget and how perturbations in tropospheric $O_3$ might affect (1) climate through its role as a greenhouse gas, and (2) health, ecology and welfare through its role in shielding the earth's surface from solar ultraviolet (UV) radiation.

### 2.7.1 Tropospheric Ozone as a Greenhouse Gas

Ozone is an important greenhouse gas, and increases in its abundance in the troposphere may contribute to climate change according to the 2007 climate assessment by the Intergovernmental Panel on Climate Change (IPCC). Models calculate that the global burden of tropospheric $O_3$ has doubled since the pre-industrial era, while observations indicate that in some regions $O_3$ may have increased by factors as great as 4 or 5. These increases are tied to the rise in emissions of $O_3$ precursors from human activity, mainly fossil fuel consumption and agricultural processes.

Figure 2-3 shows the main steps involved in the influence of tropospheric $O_3$ on climate. Emissions of $O_3$ precursors including CO, VOCs, $CH_4$, and $NO_X$ lead to

production of tropospheric $O_3$. A change in the abundance of tropospheric $O_3$ perturbs the radiative balance of the atmosphere, an effect quantified by the radiative forcing metric. The earth-atmosphere-ocean system responds to the forcing with a climate response, typically expressed as a change in surface temperature. Finally, the climate response causes downstream climate-related health and ecosystem impacts, such as redistribution of diseases or ecosystem characteristics due to temperature changes. Feedbacks from both the climate response and downstream impacts can, in turn, affect the abundance of tropospheric $O_3$ and $O_3$ precursors through multiple feedback mechanisms as indicated in Figure 2-3. Direct feedbacks are discussed in Section 10.3.2.4 and Section 10.3.3.4, while downstream climate impacts and their feedbacks are extremely complex and outside the scope of this assessment.




Note: Units shown are those typical for each quantity illustrated. Feedbacks from both the climate response and climate impacts can, in turn, affect the abundance of tropospheric $O_3$ and $O_3$ precursors through multiple feedback mechanisms. Climate impacts are deemphasized in the figure since these downstream effects are extremely complex and outside the scope of this assessment.

**Figure 2-3 Schematic illustrating the effects of tropospheric $O_3$ on climate; including the relationship between precursor emissions, tropospheric $O_3$ abundance, radiative forcing, climate response, and climate impacts.**

Radiative forcing by a greenhouse gas or aerosol is a metric used to quantify the change in balance between radiation coming into and going out of the atmosphere caused by the presence of that substance. Tropospheric $O_3$ is a major greenhouse gas and radiative forcing agent; evidence from satellite data shows a sharp dip in the outgoing infrared radiation in the 9.6 μm $O_3$ absorption band. Models calculate that the global average concentration of tropospheric $O_3$ has doubled since the pre-industrial era, while observations indicate that in some regions $O_3$ may have increased by factors as great as 4 or 5. These increases are tied to the rise in emissions of $O_3$ precursors from human activity, mainly fossil fuel consumption and agricultural processes. Overall, the evidence supports **a causal relationship between changes in tropospheric $O_3$ concentrations and radiative forcing.**

The impact of the tropospheric $O_3$ change since pre-industrial times on climate has been estimated to be about 25-40% of the anthropogenic $CO_2$ impact and about 75% of the anthropogenic $CH_4$ impact according to the IPCC, ranking it third in importance after $CO_2$ and $CH_4$ according to the Intergovernmental Panel on Climate Change (IPCC) (see Section 10.3). There are large uncertainties in the magnitude of the radiative forcing estimate attributed to tropospheric $O_3$, making the impact of tropospheric $O_3$ on climate more uncertain than the effect of the longer-lived greenhouse gases. Furthermore, radiative forcing does not take into account the climate feedbacks that could amplify or dampen the actual surface temperature response. Quantifying the change in surface temperature requires a complex climate simulation in which all important feedbacks and interactions are accounted for. The modeled surface temperature response to a given radiative forcing is highly uncertain and can vary greatly among models and from region to region within the same model. Even with these these uncertainties, global climate models indicate that tropospheric $O_3$ has contributed to observed changes in global mean and regional surface temperatures. As a result of such evidence presented in climate modeling studies, there **is likely to be a causal relationship between changes in tropospheric $O_3$ concentrations and effects on climate.**

### 2.7.2 Tropospheric Ozone and UV-B Shielding Effects

UV radiation emitted from the Sun contains sufficient energy when it reaches the Earth to break (photolyze) chemical bonds in molecules, thereby leading to damaging effects on living organisms and materials. Atmospheric $O_3$ plays a crucial role in reducing exposure to solar UV radiation at the Earth's surface. Ozone in the stratosphere is responsible for the majority of this shielding effect, as approximately 90% of total atmospheric $O_3$ is located there over mid-latitudes. Ozone in the troposphere provides supplemental shielding of radiation in the wavelength band from 280-315 nm, referred to as UV-B radiation. UV-B radiation has important effects on human health and ecosystems, and is associated with materials damage.

Human health effects associated with solar UV-B radiation exposure include erythema, skin cancer, ocular damage, and immune system suppression. A potential

human health benefit of increased UV-B exposure involves the UV-induced production of vitamin D which may help reduce the risk of metabolic bone disease, type I diabetes, mellitus, and rheumatoid arthritis, and may provide beneficial immunomodulatory effects on multiple sclerosis, insulin-dependent diabetes mellitus, and rheumatoid arthritis. Ecosystem and materials damage effects associated with solar UV-B radiation exposure include terrestrial and aquatic ecosystem impacts, alteration of biogeochemical cycles, and degradation of man-made materials.

EPA has found no published studies that adequately examine the incremental health or welfare effects (adverse or beneficial) attributable specifically to changes in UV-B exposure resulting from perturbations in tropospheric $O_3$ concentrations. While the effects are expected to be small, they cannot yet be critically assessed within reasonable uncertainty. Overall, the evidence **is inadequate to determine if a causal relationship exists between changes in tropospheric $O_3$ concentrations and effects on health and welfare related to UV-B shielding.**

## 2.8 Summary of Causal Determinations for Health Effects and Welfare Effects

This chapter has provided an overview of the underlying evidence used in making the causal determinations for the health and welfare effects of $O_3$. This review builds upon the conclusions of the previous AQCDs for $O_3$.

The evaluation of the epidemiologic, toxicological, and controlled human exposure studies published since the completion of the 2006 $O_3$ AQCD have provided additional evidence for $O_3$-related health outcomes. Table 2-3provides an overview of the causal determinations for all of the health outcomes evaluated. Causal determinations for $O_3$ and welfare effects are included in Table 2-4, while causal determinations for climate change and UV-B shielding effects are in Table 2-5. Detailed discussions of the scientific evidence and rationale for these causal determinations are provided in subsequent chapters of this ISA.

## Table 2-3 Summary of $O_3$ causal determinations by exposure duration and health outcome.

| Health Outcome | Conclusions from 2006 $O_3$ AQCD | Conclusions from this ISA |
|---|---|---|
| **Short-Term Exposure to $O_3$** | | |
| Respiratory effects | The overall evidence supports a causal relationship between acute ambient $O_3$ exposures and increased respiratory morbidity outcomes. | Causal Relationship |
| Cardiovascular effects | The limited evidence is highly suggestive that $O_3$ directly and/or indirectly contributes to cardiovascular-related morbidity, but much remains to be done to more fully substantiate the association. | Likely to be a Causal Relationship |
| Central nervous system effects | Toxicological studies report that acute exposures to $O_3$ are associated with alterations in neurotransmitters, motor activity, short and long term memory, sleep patterns, and histological signs of neurodegeneration. | Suggestive of a Causal Relationship |
| Total Mortality | The evidence is highly suggestive that $O_3$ directly or indirectly contributes to non-accidental and cardiopulmonary-related mortality. | Likely to be a Causal Relationship |
| **Long-term Exposure to $O_3$** | | |
| Respiratory effects | The current evidence is suggestive but inconclusive for respiratory health effects from long-term $O_3$ exposure. | Likely to be a Causal Relationship |
| Cardiovascular Effects | No studies from previous review | Suggestive of a Causal Relationship |
| Reproductive and developmental effects | Limited evidence for a relationship between air pollution and birth-related health outcomes, including mortality, premature births, low birth weights, and birth defects, with little evidence being found for $O_3$ effects. | Suggestive of a Causal Relationship |
| Central nervous system effects | Evidence regarding chronic exposure and neurobehavioral effects was not available. | Suggestive of a Causal Relationship |
| Cancer | Little evidence for a relationship between chronic $O_3$ exposure and increased risk of lung cancer. | Inadequate to infer a Causal Relationship |
| Total Mortality | There is little evidence to suggest a causal relationship between chronic $O_3$ exposure and increased risk for mortality in humans. | Suggestive of a Causal Relationship |

## Table 2-4 Summary of $O_3$ causal determination for welfare effects.

| Vegetation and Ecosystem Effects | Conclusions from 2006 $O_3$ AQCD | Conclusions from this ISA |
|---|---|---|
| Visible Foliar Injury Effects on Vegetation | Data published since the 1996 $O_3$ AQCD strengthen previous conclusions that there is strong evidence that current ambient $O_3$ concentrations cause impaired aesthetic quality of many native plants and trees by increasing foliar injury. | Causal Relationship |
| Reduced Vegetation Growth | Data published since the 1996 $O_3$ AQCD strengthen previous conclusions that there is strong evidence that current ambient $O_3$ concentrations cause decreased growth and biomass accumulation in annual, perennial and woody plants, including agronomic crops, annuals, shrubs, grasses, and trees. | Causal Relationship |
| Reduced Productivity in Terrestrial Ecosystems | There is evidence that $O_3$ is an important stressor of ecosystems and that the effects of $O_3$ on individual plants and processes are scaled up through the ecosystem, affecting net primary productivity. | Causal Relationship |
| Reduced Carbon (C) Sequestration in Terrestrial Ecosystems | Limited studies from previous review | Likely to be a Causal Relationship |
| Reduced Yield and Quality of Agricultural Crops | Data published since the 1996 $O_3$ AQCD strengthen previous conclusions that there is strong evidence that current ambient $O_3$ concentrations cause decreased yield and/or nutritive quality in a large number of agronomic and forage crops. | Causal Relationship |
| Alteration of Terrestrial Ecosystem Water Cycling | Ecosystem water quantity may be affected by $O_3$ exposure at the landscape level. | Likely to be a Causal Relationship |
| Alteration of Below-ground Biogeochemical Cycles | Ozone-sensitive species have well known responses to $O_3$ exposure, including altered C allocation to below-ground tissues, and altered rates of leaf and root production, turnover, and decomposition. These shifts can affect overall C and N loss from the ecosystem in terms of respired C, and leached aqueous dissolved organic and inorganic C and N. | Causal Relationship |
| Alteration of Terrestrial Community Composition | Ozone may be affecting above- and below -ground community composition through impacts on both growth and reproduction. Significant changes in plant community composition resulting directly from $O_3$ exposure have been demonstrated. | Likely to be a Causal Relationship |

## Table 2-5 Summary of $O_3$ causal determination for climate and UV-B shielding effects.

| Effects | Conclusions from 2006 $O_3$ AQCD | Conclusions from this ISA |
|---|---|---|
| Radiative Forcing | Climate forcing by $O_3$ at the regional scale may be its most important impact on climate. | Causal Relationship |
| Climate Change | While more certain estimates of the overall importance of global-scale forcing due to tropospheric $O_3$ await further advances in monitoring and chemical transport modeling, the overall body of scientific evidence suggests that high concentrations of $O_3$ on the regional scale could have a discernible influence on climate, leading to surface temperature and hydrological cycle changes. | Likely to be a Causal Relationship |
| Health and Welfare Effects Related to UV-B Shielding | UV-B has not been studied in sufficient detail to allow for a credible health benefits assessment. In conclusion, the effect of changes in surface-level $O_3$ concentrations on UV-induced health outcomes cannot yet be critically assessed within reasonable uncertainty. | Inadequate to Determine if a Causal Relationship Exists |

## References


CAA. Clean Air Act, as amended by Pub. L. No. 101-549, section 108: Air quality criteria and control techniques, § 7408 (1990a). http://www.law.cornell.edu/uscode/text/42/7408

Langstaff, J. (2003). Percentiles of 1996-2000 ozone concentrations [memorandum to Joe Pinto]. Available online

Murphy, SL; Xu, JQ; Kochanek, KD. (2012). Deaths: Preliminary data for 2010. In National Vital Statistics Reports. (4). Hyattsville, MD: National Center for Health Statistics. http://www.cdc.gov/nchs/data/nvsr/nvsr60/nvsr60_04.pdf

U.S. EPA (U.S. Environmental Protection Agency). (1978a). Air quality criteria for ozone and other photochemical oxidants [EPA Report]. (EPA/600/8-78/004). Washington, DC.

U.S. EPA (U.S. Environmental Protection Agency). (1984). Air quality criteria for ozone and other photochemical oxidants, volume III of V (review draft) [EPA Report]. (EPA-600/8-84-020A3). Research Triangle Park, NC. http://www.ntis.gov/search/product.aspx?ABBR=PB85126050

U.S. EPA (U.S. Environmental Protection Agency). (1996a). Air quality criteria for ozone and related photochemical oxidants [EPA Report]. (EPA/600/P-93/004AF). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (1996b). Air quality criteria for ozone and related photochemical oxidants, Vol. II of III [EPA Report]. (EPA/600/P-93/004BF). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (2006b). Air quality criteria for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-05/004AF). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=149923

U.S. EPA (U.S. Environmental Protection Agency). (2008g). Notice of workshop and call for information on integrated science assessment for ozone. Fed Reg 73: 56581-56583.

U.S. EPA (U.S. Environmental Protection Agency). (2009c). Integrated review plan for the ozone National Ambient Air Quality Standards review (external review draft) [EPA Report]. (EPA 452/D-09-001). Washington, DC. http://www.epa.gov/ttnnaaqs/standards/ozone/data/externalreviewdraftO3IRP093009.pdf

U.S. EPA (U.S. Environmental Protection Agency). (2011d). Integrated review plan for the ozone National Ambient Air Quality Standards [EPA Report]. (EPA 452/R-11-006). Washington, DC. http://www.epa.gov/ttn/naaqs/standards/ozone/data/2011_04_OzoneIRP.pdf

Zhang, L; Jacob, DJ; Downey, NV; Wood, DA; Blewitt, D; Carouge, CC; Van donkelaar, A; Jones, DBA; Murray, LT; Wang, Y. (2011). Improved estimate of the policy-relevant background ozone in the United States using the GEOS-Chem global model with 1/2 2/3 horizontal resolution over North America. Atmos Environ 45: 6769-6776. http://dx.doi.org/10.1016/j.atmosenv.2011.07.054

# 3 ATMOSPHERIC CHEMISTRY AND AMBIENT CONCENTRATIONS

## 3.1 Introduction

In the stratosphere, ozone ($O_3$) serves the beneficial role of absorbing the Sun's harmful ultraviolet radiation and preventing the majority of this radiation from reaching the Earth's surface. In the troposphere, however, $O_3$ and other photochemical oxidants are air pollutants that can exert harmful effects on humans, animals, and vegetation. This chapter discusses the atmospheric chemistry associated with tropospheric $O_3$ and other related photochemical oxidants and provides a detailed description of their surface-level concentrations. The focus of this chapter is on $O_3$ since it is the NAAQS indicator for all photochemical oxidants. To the extent possible, other photochemical oxidants are discussed, but limited information is currently available. Although $O_3$ is involved in reactions in indoor air, the focus in this chapter will be on chemistry occurring in outdoor, ambient air.

The material in this chapter is organized as follows. Section 3.2 outlines the physical and chemical processes involved in $O_3$ formation and removal. Section 3.3 describes the latest methods used to model global $O_3$ concentrations, and Section 3.4 describes the application of these methods for estimating background concentrations of $O_3$ that are useful for risk and policy assessments informing decisions about the NAAQS. Section 3.5 includes a comprehensive description of available $O_3$ monitoring techniques and monitoring networks, while Section 3.6 presents information on the spatial and temporal variability of $O_3$ concentrations across the U.S. and their associations with other pollutants using available monitoring data. Section 3.7 summarizes the main conclusions from Chapter 3. Finally, Section 3.8 provides supplemental material on atmospheric model simulations of background $O_3$ concentrations (referenced in Section 3.4) and Section 3.9 contains supplemental material on observed ambient $O_3$ concentrations (referenced in Section 3.6).

## 3.2 Physical and Chemical Processes

Ozone in the troposphere is a secondary pollutant formed by photochemical reactions of precursor gases and is not directly emitted from specific sources. Ozone and other oxidants, such as peroxyacetyl nitrate (PAN) and $H_2O_2$ form in polluted areas by atmospheric reactions involving two main classes of precursor pollutants: VOCs and $NO_X$.[1] Carbon monoxide (CO) is also important for $O_3$ formation in polluted areas and in the remote troposphere. The formation of $O_3$, other oxidants and oxidation products from these precursors is a complex, nonlinear function of many factors

[1] The term VOCs refers to all organic gas-phase compounds in the atmosphere, both biogenic and anthropogenic in origin. This definition excludes CO and $CO_2$. $NO_X$, also referred to as nitrogen oxides, is equal to the sum of NO and $NO_2$.

including (1) the intensity and spectral distribution of sunlight; (2) atmospheric mixing; (3) concentrations of precursors in the ambient air and the rates of chemical reactions of these precursors; and (4) processing on cloud and aerosol particles.

Ozone is present not only in polluted urban atmospheres, but throughout the troposphere, even in remote areas of the globe. The same basic processes involving sunlight-driven reactions of $NO_X$, VOCs, and CO contribute to $O_3$ formation throughout the troposphere. These processes also lead to the formation of other photochemical products, such as PAN, $HNO_3$, and $H_2SO_4$, and to other compounds, such as HCHO and other carbonyl compounds, and to secondary components of particulate matter.

A schematic overview of the major photochemical cycles influencing $O_3$ in the troposphere and the stratosphere is given in Figure 3-1. Included in the figure are reactions involving radicals derived from man-made chemicals and that are responsible for depleting stratospheric $O_3$. Most (approximately 90%) of the total $O_3$ column in the earth's atmosphere resides in the stratosphere, and it is responsible for absorbing harmful solar ultraviolet radiation, the harmful effects of which are discussed in Chapter 10. This solar ultraviolet radiation also initiates the photochemical reactions that are responsible for producing $O_3$ in the troposphere. The processes responsible for producing summertime $O_3$ episodes are fairly well understood, and were covered in detail in the 2006 $O_3$ AQCD (U.S. EPA, 2006b). This section focuses on topics that form the basis for discussions in later chapters, and for which there is substantial new information since the previous $O_3$ review.

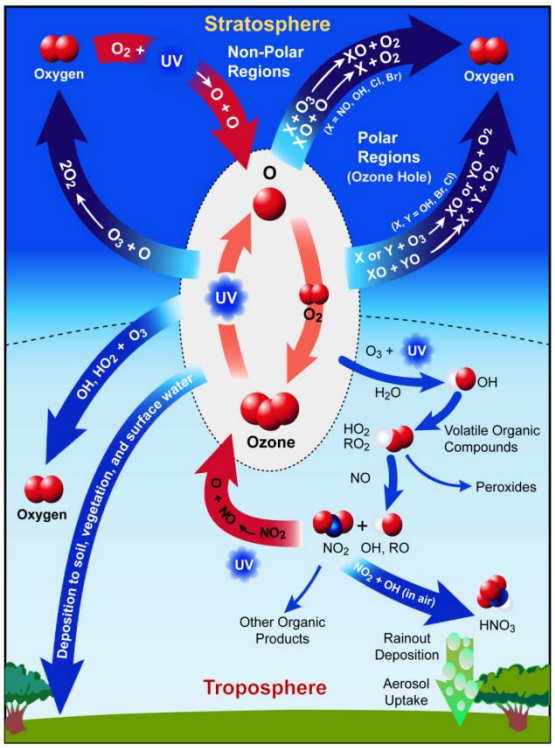



**Figure 3-1 Schematic overview of photochemical processes influencing stratospheric and tropospheric $O_3$.**

Major episodes of high $O_3$ concentrations in the eastern U.S. and in Europe are associated with slow moving high pressure systems. High pressure systems during the warmer seasons are associated with the sinking of air, resulting in warm, generally cloudless skies, with light winds. The sinking of air results in the development of stable conditions near the surface which inhibit or reduce the vertical mixing of $O_3$ precursors. Photochemical activity involving these precursors is enhanced because of higher temperatures and the availability of sunlight during the warmer seasons. In the eastern U.S., concentrations of $O_3$ and other secondary pollutants are determined by meteorological and chemical processes extending typically over areas of several hundred thousand square kilometers (Civerolo et al., 2003; Rao et al., 2003). Ozone episodes are thus best regarded as regional in nature. The conditions conducive to formation of high $O_3$ can persist for several days. These conditions have been described in greater detail in the 1996 and 2006 $O_3$ AQCDs (U.S. EPA, 2006b, 1996a). The transport of pollutants downwind of major urban centers is characterized by the development of urban plumes. Mountain barriers limit mixing (as in Los Angeles and Mexico City) and result in a higher frequency and duration of days with high $O_3$ concentrations. However, orographic lifting over the San Gabriel Mountains results in $O_3$ transport from Los Angeles to areas hundreds of kilometers downwind (e.g., in Colorado and Utah) (Langford et al., 2009). Ozone concentrations in southern urban areas (such as Houston, TX and Atlanta, GA) tend

to decrease with increasing wind speed. In northern U.S. cities (such as Chicago, IL; New York, NY; Boston, MA; and Portland, ME), the average $O_3$ concentrations over the metropolitan areas increase with wind speed, indicating that transport of $O_3$ and its precursors from upwind areas is important (Schichtel and Husar, 2001; Husar and Renard, 1998).

Aircraft observations indicate that there can be substantial differences in mixing ratios of key species between the surface and the overlying atmosphere (Berkowitz and Shaw, 1997; Fehsenfeld et al., 1996). In particular, mixing ratios of $O_3$ can (depending on time and location) be higher in the lower free troposphere (aloft) than in the planetary boundary layer (PBL) during multiday $O_3$ episodes (Taubman et al., 2006; Taubman et al., 2004). Convective processes and turbulence transport $O_3$ and other pollutants both upward and downward throughout the planetary boundary layer and the free troposphere. During the day, convection (driven by heating of the earth's surface) results in a deeper PBL, with vertically well mixed $O_3$ and precursors. As solar heating of the surface decreases going into night, the daytime boundary layer collapses leaving behind $O_3$ and its precursors in a residual layer above a shallow nighttime boundary layer. Pollutants in the residual layer have now become essentially part of the free troposphere, as described in Annex AX2.3.2 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b). Winds in the free troposphere tend to be stronger than those closer to the surface and so are capable of transporting pollutants over long distances. Thus, $O_3$ and its precursors can be transported vertically by convection into the upper part of the mixed layer on one day, then transported overnight as a layer of elevated mixing ratios, and then entrained into a growing convective boundary layer downwind and brought back down to the surface.

High $O_3$ concentrations showing large diurnal variations at the surface in southern New England were associated with the presence of such layers (Berkowitz et al., 1998). Winds several hundred meters above the ground can bring pollutants from the west, even though surface winds are from the southwest during periods of high $O_3$ in the eastern U.S. (Blumenthal et al., 1997). These considerations suggest that in many areas of the U.S., $O_3$ and its precursors can be transported over hundreds if not thousands of kilometers.

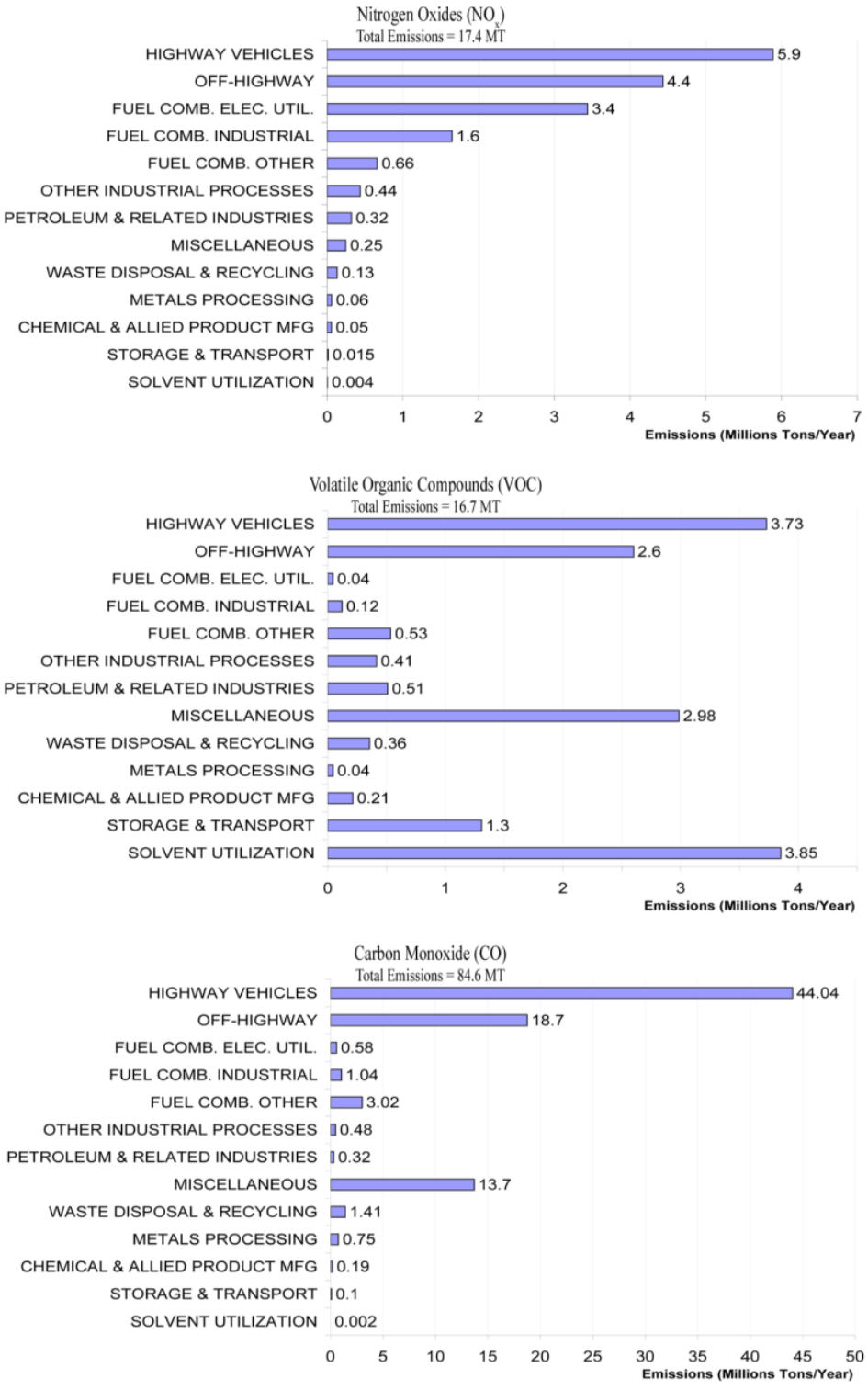
Nocturnal low level jets (LLJs) are an efficient means for transporting pollutants that have been entrained into the residual boundary layer over hundreds of kilometers. LLJs are most prevalent in the central U.S. extending northward from eastern Texas, and along the Atlantic states extending southwest to northeast. LLJs have also been observed off the coast of California. Turbulence induced by wind shear associated with LLJs brings pollutants to the surface and results in secondary $O_3$ maxima during the night and early morning in many locations (Corsmeier et al., 1997). Comparison of observations at low elevation surface sites with those at nearby high elevation sites at night can be used to discern the effects of LLJs. For example, Fischer (2004) found occasions when $O_3$ at the base of Mt. Washington during the night was much higher than typically observed, and closer to those observed at the summit of Mt. Washington. They suggested that mechanically driven turbulence due to wind shear caused $O_3$ from aloft to penetrate the stable nocturnal inversion thus causing $O_3$ to

increase near the base of Mt. Washington. The high wind speeds causing this mechanically driven turbulence could have resulted from the development of a LLJ. Stratospheric intrusions and intercontinental transport of $O_3$ are also important and are covered in Section 3.4 in relation to background concentrations.

### 3.2.1 Sources of Precursors Involved in $O_3$ Formation

Emissions of $O_3$ precursor compounds ($NO_X$, VOCs, and CO) can be divided into natural and anthropogenic source categories. Natural sources can be further divided into biogenic from vegetation, microbes, and animals, and abiotic from biomass combustion, lightning, and geogenic sources. However, the distinction between natural and anthropogenic sources is often difficult to make in practice, as human activities directly or indirectly affect emissions from what would have been considered natural sources during the pre-industrial era. Thus, emissions from plants and animals used in agriculture have been referred to as anthropogenic or biogenic in different applications. Wildfire emissions can be considered natural, except that forest management practices can lead to buildup of fuels on the forest floor, thereby altering the frequency and severity of forest fires.

Estimates of emissions for $NO_X$, VOCs, and CO from the 2005 National Emissions Inventory (NEI) (U.S. EPA, 2008a) are shown in Figure 3-2 to provide a general indication of the relative importance of the different sources in the U.S. as a whole. The magnitudes of the sources are strongly location and time dependent and so should not be used to apportion sources of exposure. Shown in Figure 3-2 are Tier 1 categories. The miscellaneous category can be quite large compared to total emissions, especially for CO and VOCs. The miscellaneous category includes agriculture and forestry, wildfires, prescribed burns, and a much more modest contribution from structural fires.




Note: $NO_X$ (top), VOCs (middle), and CO (bottom) in the U.S. in million metric tons (MT) per year.
Source: U.S. EPA (2008a).

**Figure 3-2 Estimated anthropogenic emissions of $O_3$ precursors for 2005.**

Anthropogenic $NO_X$ emissions are associated with combustion processes. Most emissions are in the form of NO, which is formed at high combustion temperatures from atmospheric nitrogen ($N_2$) and oxygen ($O_2$) and from fuel nitrogen (N). According to the 2005 NEI, the largest sources of $NO_X$ are on- and off-road (such as construction equipment, agricultural equipment, railroad trains, ships, and aircraft) mobile sources and electric power generation plants. Emissions of $NO_X$ therefore are highest in areas having a high density of power plants and in urban regions having high traffic density. Dallmann and Harley (2010) compared $NO_X$ emissions estimates from the 2005 NEI mobile sector data with an alternative method based on fuel consumption and found reasonable agreement in total U.S. anthropogenic emissions between the two techniques (to within about 5%). However, emissions from on-road diesel engines in the fuel based inventory constituted 46% of total mobile source $NO_X$ compared to 35% in the EPA inventory. As a result, emissions from on-road diesel engines in the fuel based approach are even larger than electric power generation as estimated in the 2005 NEI, and on-road diesel engines might represent the largest single $NO_X$ source category. Differences between the two techniques are largely accounted for by differences in emissions from on-road gasoline engines. Uncertainties in the fuel consumption inventory ranged from 3% for on-road gasoline engines to 20% for marine sources, and in the EPA inventory uncertainties ranged from 16% for locomotives to 30% for off-road diesel engines. It should be noted that the on-road diesel engine emissions estimate by Dallmann and Harley (2010) is still within the uncertainty of the EPA estimate (22%). Because of rapid changes to heavy duty diesel $NO_X$ controls, emissions are likely to also rapidly change.

Satellite-based techniques have been used to obtain tropospheric concentrations of $O_3$ precursors (e.g., $NO_2$, VOCs, and CO). Such satellite-based measurements provide a large-scale picture of spatial and temporal distribution of $NO_2$, VOCs, and CO that can be used to evaluate emissions inventories produced using the bottom-up approach and to produce top-down emissions inventories of these species. Although there are uncertainties associated with satellite-based measurements, several studies have shown the utility of top-down constraints on the emissions of $O_3$ precursors (McDonald-Buller et al., 2011 and references therein). Following mobile sources, power plants are considered the second largest anthropogenic source of $NO_X$. Over the past decade, satellite measurements have shown appreciable reductions in $NO_X$ power plant emissions across the U.S. as a result of emission abatement strategies (Stavrakou et al., 2008; Kim et al., 2006). For instance, Kim et al. (2006) observed a 34% reduction in $NO_X$ emission over the Ohio River Valley from 1999-2006 due to such strategies. Based on these results, less than 25% of anthropogenic $NO_X$ emissions were expected to originate from power plants in this region. Uncertainty in $NO_X$ satellite measurements are impacted by several factors, such as cloud and aerosol properties, surface albedo, stratospheric $NO_X$ concentration, and solar zenith angle. Boersma et al. (2004) estimated an overall uncertainty between 35-60% for satellite-retrieved $NO_X$ measurements in urban, polluted regions. Although trends in satellite-retrieved $NO_X$ power plant emissions reported by Kim et al. (2006) are uncertain to some extent, similar reductions were reported by region-wide power plant measurements (e.g., Continuous Emission Monitoring System observations, CEMS).

Major natural sources of $NO_X$ in the U.S. include lightning, soils, and wildfires. Uncertainties in natural $NO_X$ emissions are much larger than for anthropogenic $NO_X$ emissions. Fang et al. (2010) estimated lightning generated $NO_X$ of ~0.6 MT for July 2004. This value is ~40% of the anthropogenic emissions for the same period, but the authors estimated that ~98% is formed in the free troposphere and so contributions to the surface $NO_X$ burden are low because most of this $NO_X$ is oxidized to nitrate containing species during downward transport into the planetary boundary layer. The remaining 2% is formed within the planetary boundary layer. Both nitrifying and denitrifying organisms in the soil can produce $NO_X$, mainly in the form of NO. Emission rates depend mainly on fertilization amount and soil temperature and moisture. Nationwide, about 60% of the total $NO_X$ emitted by soils is estimated to occur in the central corn belt of the United States. Spatial and temporal variability in soil $NO_X$ emissions leads to considerable uncertainty in emissions estimates. However, these emissions are relatively low, only ~0.97 MT/year, or about 6% of anthropogenic $NO_X$ emissions. However, these emissions occur mainly during summer when $O_3$ is of most concern and occur across the entire country including areas where anthropogenic emissions are low.

Hundreds of VOCs, containing mainly 2 to ~12 carbon (C) atoms, are emitted by evaporation and combustion processes from a large number of anthropogenic sources. The two largest anthropogenic source categories in the U.S. EPA's emissions inventories are industrial processes and transportation. Emissions of VOCs from highway vehicles account for roughly two-thirds of the transportation-related emissions. The accuracy of VOC emission estimates is difficult to determine, both for stationary and mobile sources. 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, and age of vehicle), each of which has some degree of uncertainty.

On the U.S. and global scales, emissions of VOCs from vegetation are much larger than those from anthropogenic sources. Emissions of VOCs from anthropogenic sources in the 2005 NEI were ~17 MT/year (wildfires constitute ~1/6 of that total and were included in the 2005 NEI under the anthropogenic category, but see Section 3.4 for how wildfires are treated for background $O_3$ considerations), but were 29 MT/year from biogenic sources. Uncertainties in both biogenic and anthropogenic VOC emission inventories prevent determination of the relative contributions of these two categories, at least in many areas. Vegetation emits substantial quantities of VOCs, such as terpenoid compounds (isoprene, 2-methyl-3-buten-2-ol, monoterpenes), compounds in the hexanal family, alkenes, aldehydes, organic acids, alcohols, ketones, and alkanes. The major chemicals emitted by plants are isoprene (40%), other terpenoid and sesqui-terpenoid compounds (25%) and the remainder consists of assorted oxygenated compounds and hydrocarbons according to the 2005 NEI. Most biogenic emissions occur during the summer because of their dependence on temperature and incident sunlight. Biogenic emissions are also higher in southern states than in northern states for these reasons and because of species variations.

The uncertainty in natural emissions is about 50% for isoprene under midday summer conditions and could be as much as a factor of ten higher for some compounds (Guenther et al., 2000). In EPA's regional modeling efforts, biogenic emissions of VOCs are estimated using the Biogenic Emissions Inventory System (BEIS) model (U.S. EPA, 2010b) with data from the Biogenic Emissions Landuse Database (BELD) and annual meteorological data. However, other emissions models are used such as Model of Emissions of Gases and Aerosols from Nature (MEGAN) (Guenther et al., 2006), especially in global modeling efforts.

Satellite measurements of HCHO, produced by the oxidation of isoprene and other VOCs, have also been used to estimate biogenic VOC emissions attributed to isoprene (Millet et al., 2008; Millet et al., 2006). Millet et al. (2008) demonstrated that both satellite-based and model techniques capture the spatial variability of biogenic isoprene emissions in the U.S. reasonably well (satellite [versus MEGAN] isoprene estimates, $R^2$ = 0.48 or 0.68 depending on vegetation data base used). However, MEGAN tends to overestimate emissions compared to satellite-based measurements. The uncertainty in satellite derived isoprene emissions is roughly 40%, based on combined uncertainty in satellite retrieval and isoprene yield from isoprene oxidation (Millet et al., 2006), which is similar to the error associated with model-based techniques (~50%) (e.g., Millet et al., 2006; Guenther et al., 2000).

Anthropogenic CO is emitted primarily by incomplete combustion of carbon-containing fuels. In general, any increase in fuel oxygen content, burn temperature, or mixing time in the combustion zone will tend to decrease production of CO relative to $CO_2$. However, it should be noted that controls mute the response of CO formation to fuel-oxygen. CO emissions from large fossil-fueled power plants are typically very low since the boilers at these plants are tuned for highly efficient combustion with the lowest possible fuel consumption. Additionally, the CO-to-$CO_2$ ratio in these emissions is shifted toward $CO_2$ by allowing time for the furnace flue gases to mix with air and be oxidized by OH to $CO_2$ in the hot gas stream before the OH concentrations drop as the flue gases cool. Nationally, on-road mobile sources constituted about half of total CO emissions in the 2005 NEI. When emissions from off-highway (non-road) vehicles are included, it can be seen from Figure 3-2 that all mobile sources accounted for about three-quarters of total anthropogenic CO emissions in the U.S.

Analyses by Harley et al. (2005) and Parrish (2006) are consistent with the suggestion in Pollack et al. (2004) that the EPA MOBILE6 vehicle emissions model (U.S. EPA, 2010d) overestimates vehicle CO emissions by a factor of ~2. Field measurements by Bishop and Stedman (2008) were in accord with the Parrish (2006) findings that the measured trends of CO and $NO_X$ concentrations from mobile sources in the U.S. indicated that modeled CO emission estimates were substantially too high. Hudman et al. (2008) found that the NEI overestimated anthropogenic CO emissions by 60% for the eastern U.S. during the period July 1-August 15, 2004 based on comparison of aircraft observations of CO from the International Consortium for Atmospheric Research on Transport and Transformation (ICARTT) campaign (Fehsenfeld et al., 2006) and results from a tropospheric chemistry model

(GEOS-Chem). Improvements in emissions technologies not correctly represented in MOBILE emission models have been suggested as one cause for this discrepancy. For example, Pokharel et al. (2003, 2002) demonstrated substantial decrements in the CO fraction of tailpipe exhaust in several U.S. cities and Burgard et al. (2006) documented improvements in emission from heavy-duty on-road diesel engines. Some of the largest errors in the MOBILE models are addressed in the successor model, MOVES (U.S. EPA, 2011f).

Estimates of biogenic CO emissions in the 2005 NEI are made in a manner similar to that for VOCs. National biogenic emissions, excluding fires, were estimated to contribute ~7% and wildfires added another ~16% to the national CO emissions total. Photodecomposition of organic matter in oceans, rivers, lakes, and other surface waters, and from soil surfaces also releases CO (Goldstein and Galbally, 2007). However, soils can act as a CO source or a sink depending on soil moisture, UV flux reaching the soil surface, and soil temperature (Conrad and Seiler, 1985). Soil uptake of CO is driven by anaerobic bacteria (Inman et al., 1971). Emissions of CO from soils appear to occur by abiotic processes, such as thermodecomposition or photodecomposition of organic matter. In general, warm and moist conditions found in most soils favor CO uptake, whereas hot and dry conditions found in deserts and some savannas favor the release of CO (King, 1999).

### 3.2.2 Gas Phase Reactions Leading to $O_3$ Formation

Photochemical processes involved in $O_3$ formation have been extensively reviewed in a number of books (Jacobson, 2002; Jacob, 1999; Seinfeld and Pandis, 1998; Finlayson-Pitts and Pitts, 1986) and in the 1996 and 2006 $O_3$ AQCDs (U.S. EPA, 2006b, 1996a). The photochemical formation of $O_3$ in the troposphere proceeds through the oxidation of NO to nitrogen dioxide ($NO_2$) by organic-peroxy ($RO_2$) or hydro-peroxy ($HO_2$) radicals. The peroxy radicals oxidizing NO to $NO_2$ are formed during the oxidation of VOCs as presented in Annex AX2.2.2 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b). The photolysis of $NO_2$ yields NO and a ground-state oxygen atom, $O(^3P)$, which then reacts with molecular oxygen to form $O_3$.

VOCs important for the photochemical formation of $O_3$ include alkanes, alkenes, aromatic hydrocarbons, carbonyl compounds (e.g., aldehydes and ketones), alcohols, organic peroxides, and halogenated organic compounds (e.g., alkyl halides). This array of compounds encompasses a wide range of chemical properties and lifetimes: isoprene has an atmospheric lifetime of approximately an hour, whereas methane has an atmospheric lifetime of about a decade.

In urban areas, compounds representing all classes of VOCs and CO are important for $O_3$ formation. In non-urban vegetated areas, biogenic VOCs emitted from vegetation tend to be the most important. In the remote troposphere, methane ($CH_4$) and CO are the main carbon-containing precursors to $O_3$ formation. The oxidation of VOCs is initiated mainly by reaction with hydroxyl (OH) radicals. The primary source of OH radicals in the atmosphere is the reaction of electronically excited

oxygen atoms, $O(^1D)$, with water vapor. $O(^1D)$ is produced by the photolysis of $O_3$ in the Hartley bands. In polluted areas, the photolysis of aldehydes (e.g., HCHO), HONO and $H_2O_2$ can also be appreciable sources of OH, or $HO_2$ radicals that can rapidly be converted to OH (Eisele et al., 1997). Ozone can oxidize alkenes, as can $NO_3$ radicals. $NO_3$ radicals are most effective at night when they are most abundant. In coastal environments and other selected environments, atomic Cl and Br radicals can also initiate the oxidation of VOCs as discussed in Annex AX2.2.3 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b). It was also emphasized in Annex AX2.2.9 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b) that the reactions of oxygenated VOCs are important components of $O_3$ formation. They may be present in ambient air not only as the result of the atmospheric oxidation of hydrocarbons but also by direct emissions. For example, motor vehicles (including compressed natural gas vehicles) and some industrial processes emit formaldehyde (Rappenglück et al., 2009) and vegetation emits methanol.

There are a large number of oxidized N-containing compounds in the atmosphere including NO, $NO_2$, $NO_3$, $HNO_2$, $HNO_3$, $N_2O_5$, $HNO_4$, PAN (and its homologues), and other organic nitrates (such as alkyl nitrates, isoprene nitrates), and particulate nitrate. Collectively these species are referred to as $NO_Y$. Oxidized nitrogen compounds are emitted to the atmosphere mainly as NO, which rapidly interconverts with $NO_2$ and so NO and $NO_2$ are often "lumped" together into their own group or family, which is referred to as $NO_X$. All the other species mentioned above in the definition of $NO_Y$ are products of $NO_X$ reactions are referred to as $NO_Z$, such that $NO_Y = NO_X + NO_Z$. The major reactions involving interconversions of oxidized N species were covered in Annex AX2.2.4 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b). Mollner et al. (2010) identified pernitrous acid (HOONO), an unstable isomer of nitric acid, as a product of the major gas phase reaction forming $HNO_3$. However, since pernitrous acid is unstable, it is not a substantial reservoir for $NO_X$. This finding stresses the importance of identifying products in addition to measuring the rate of disappearance of reactants in kinetic studies.

The photochemical cycles by which the oxidation of hydrocarbons leads to $O_3$ production are best understood by considering the oxidation of methane, structurally the simplest VOC. The $CH_4$ oxidation cycle serves as a model for the chemistry of the relatively clean or unpolluted troposphere (although this is a simplification because vegetation releases large quantities of complex VOCs, such as isoprene, into the atmosphere). In the polluted atmosphere, the underlying chemical principles are the same, as discussed in Annex AX2.2.5 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b). The conversion of NO to $NO_2$ occurring with the oxidation of VOCs is accompanied by the production of $O_3$ and the efficient regeneration of the OH radical, which in turn can react with other VOCs as shown in Figure 3-1.

The oxidation of alkanes and alkenes in the atmosphere has been treated in depth in the 1996 $O_3$ AQCD (U.S. EPA, 1996a) and was updated in Annexes AX2.2.6 and AX2.2.7 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b). In contrast to simple hydrocarbons containing one or two C atoms, detailed kinetic information about the gas phase oxidation pathways of many anthropogenic hydrocarbons (e.g., aromatic

compounds such as benzene and toluene), biogenic hydrocarbons (e.g., isoprene, the monoterpenes), and their intermediate oxidation products (e.g., peroxides, nitrates, carbonyls and epoxides) is lacking. This information is crucial even for compounds formed in low yields, such as isoprene epoxides, as they are major precursors to secondary organic aerosol formation (see, e.g., Surratt et al., 2010). Reaction with OH radicals represents the major loss process for alkanes. Reaction with chlorine (Cl) atoms is an additional sink for alkanes. Stable products of alkane photo-oxidation are known to include a wide range of compounds and concentrations including carbonyl compounds, alkyl nitrates, and d-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.

The reaction of OH radicals with aldehydes produced during the oxidation of alkanes forms acyl (R'CO) radicals, and acyl peroxy radicals (R'C(O)−$O_2$) are formed by the further addition of $O_2$. As an example, the oxidation of ethane ($C_2H_5$−H) yields acetaldehyde ($CH_3$−CHO). The reaction of $CH_3$−CHO with OH radicals yields acetyl radicals ($CH_3$−CO). The acetyl radicals will then participate with $O_2$ in a termolecular recombination reaction to form acetyl peroxy radicals, which can then react with NO to form $CH_3$ + $CO_2$ or they can react with $NO_2$ to form PAN. PAN acts as a temporary reservoir for $NO_2$. Upon the thermal decomposition of PAN, either locally or elsewhere, $NO_2$ is released to participate in the $O_3$ formation process again.

Alkenes react in ambient air with OH, $NO_3$, and Cl radicals and with $O_3$. All of these reactions are important atmospheric transformation processes, and all proceed by initial addition to the carbon double bonds. Major products of alkene photo-oxidation include carbonyl compounds. Hydroxynitrates and nitratocarbonyls, and decomposition products from the energy-rich biradicals formed in alkene-$O_3$ reactions are also produced. Major uncertainties in the atmospheric chemistry of the alkenes concern the products and mechanisms of their reactions with $O_3$, especially the yields of radicals that participate in $O_3$ formation. Examples of oxidation mechanisms of complex alkanes and alkenes can be found in comprehensive texts such as Seinfeld and Pandis (1998).

Although the photochemistry of isoprene is crucial for understanding $O_3$ formation, there are major uncertainties in its oxidation pathways that still need to be addressed. Apart from the effects of the oxidation of isoprene on production of radicals and $O_3$ formation, isoprene nitrates ($RONO_2$) appear to play an important role as $NO_X$ reservoirs over the eastern U.S. (e.g., Perring et al., 2009). Their decomposition leads to the recycling of $NO_X$, which can participate in the $O_3$ formation process. Laboratory and field-based approaches support yields for $RONO_2$ formation from isoprene oxidation ranging from 4% to 12% (see summaries in, Lockwood et al., 2010; Perring et al., 2009; Horowitz et al., 2007; von Kuhlmann et al., 2004). The rate at which $RONO_2$ reacts to recycle $NO_X$ is poorly understood (Archibald et al., 2010; Paulot et al., 2009) with ranges from 0 to 100% in global chemical

transport models. This range affects the sign of the $O_3$ response to changes in biogenic VOC emissions as well as the sensitivity of $O_3$ to changes in $NO_X$ emissions (Archibald et al., 2011; Ito et al., 2009; Weaver et al., 2009; Horowitz et al., 2007; Fiore et al., 2005). In models that assume zero $RONO_2$ recycling (Zhang et al., 2011; Wu et al., 2007; Fiore et al., 2003) $O_3$ production is suppressed relative to a model that recycles $NO_X$ from $RONO_2$ (Kang et al., 2003). A related issue concerns the lack of regeneration of OH + $HO_2$ radicals especially in low $NO_X$ (<~1 ppb) environments. The isomerization of the isoprene peroxy radicals that are formed after initial OH attack and subsequent reactions could help resolve this problem (Peeters and Müller, 2010; Peeters et al., 2009) and result in increases in OH concentrations from 20 to 40% over the southeastern U.S. (Archibald et al., 2011). However, the effectiveness of this pathway is uncertain and depends on the fraction of isoprene-peroxy radicals reacting by isomerization. Crounse et al. (2011) estimated that only 8-11% of the isoprene-peroxy radicals isomerizes to reform $HO_2$ radicals. Hofzumahaus et al. (2009) also found under predictions of OH in the Pearl River Delta and they also note that the sequence of reactions beginning with OH attack on VOCs introduces enormous complexity which is far from being fully understood.

The oxidation of aromatic hydrocarbons constitutes an important component of the chemistry of $O_3$ formation in urban atmospheres as discussed in Annex AX2.2.8 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b). Virtually all of the important aromatic hydrocarbon precursors emitted in urban atmospheres are lost through reaction with the hydroxyl radical. Loss rates for these compounds vary from slow (e.g., benzene) to moderate (e.g., toluene), to very rapid (e.g., xylene and trimethylbenzene isomers). However, the mechanism for the oxidation of aromatic hydrocarbons following reaction with OH is poorly understood, as is evident from the poor mass balance of the reaction products. The mechanism for the oxidation of toluene has been studied most thoroughly, and there is general agreement on the initial steps in the mechanism. However, at present there is no promising approach for resolving the remaining issues concerning the later steps. The oxidation of aromatic hydrocarbons also leads to particle formation that could remove gas-phase constituents that participate in $O_3$ formation.

Adequate analytical techniques needed to identify and quantify key intermediate species are not available for many compounds. In addition, methods to synthesize many of the suspected intermediate compounds are not available so that laboratory studies of their reaction kinetics cannot be performed. Similar considerations apply to the oxidation of biogenic hydrocarbons besides isoprene. These considerations are important because oxidants (other than $O_3$) that are formed from the chemistry described above could exert effects on human health and perhaps also on vegetation (Doyle et al., 2007; Doyle et al., 2004; Sexton et al., 2004). Gas phase oxidants include PAN, $H_2O_2$, $CH_3OOH$, and other organic hydroperoxides.

Ozone is lost through a number of gas phase reactions and deposition to surfaces. The reaction of $O_3$ with NO to produce $NO_2$, for example in urban centers near roads, mainly results in the recycling of $O_3$ downwind via the recombination of

$O(^3P)$ with $O_2$ to re-form $O_3$. By itself, this reaction does not lead to a net loss of $O_3$ unless the $NO_2$ is converted to stable end products such as $HNO_3$. Ozone reacts with unsaturated hydrocarbons and with OH and $HO_2$ radicals.

Perhaps the most recent field study aimed at obtaining a better understanding of atmospheric chemical processes was the Second Texas Air Quality Field Study (TexAQS-II) conducted in Houston in August and September 2006 (Olaguer et al., 2009). The TexAQS-II Radical and Aerosol Measurement Project (TRAMP) found evidence for the importance of short-lived radical sources such as HCHO and HONO in increasing $O_3$ productivity. During TRAMP, daytime HCHO pulses as large as 32 ppb were observed and attributed to industrial activities upwind in the Houston Ship Channel (HSC) and HCHO peaks as large as 52 ppb were detected by in situ surface monitors in the HSC. Primary HCHO produced in flares from local refineries and petrochemical facilities could increase peak $O_3$ by ~30 ppb (Webster et al., 2007). Other findings from TexAQS-II included substantial concentrations of HONO during the day, with peak concentrations approaching 1 ppb at local noon. These concentrations are well in excess of current air quality model predictions using gas phase mechanisms alone (Sarwar et al., 2008) and multiphase processes are needed to account for these observations. Olaguer et al. (2009) also noted that using measured HONO brings modeled $O_3$ concentrations into much better agreement with observations and could result in the production of an additional 10 ppb $O_3$. Large nocturnal vertical gradients indicating a surface or near-surface source of HONO, and large concentrations of night-time radicals (~30 ppt $HO_2$) were also found during TRAMP.

### 3.2.3 Multiphase Processes

In addition to the gas phase, chemical reactions also occur on the surfaces of or within cloud droplets and airborne particles. Their collective surface area is huge, implying that collisions with gas phase species occur on very short time scales. In addition to hydrometeors (e.g., cloud and fog droplets and snow and ice crystals) there are also potential reactions involving atmospheric particles of varying composition (e.g., wet [deliquesced] inorganic particles, mineral dust, carbon chain agglomerates and organic carbon particles). Multiphase reactions are involved in the formation of a number of species such as particulate nitrate, and gas phase HONO that can act to both increase and reduce the rate of $O_3$ formation in the polluted troposphere. Data collected in Houston as part of TexAQS-II summarized by Olaguer et al. (2009) indicate that concentrations of HONO are much higher than can be explained by gas phase chemistry and by tailpipe emissions. Photolysis of HONO formed in multiphase reactions in addition to the other sources can help to reduce the model underestimate of simulated $O_3$ in Houston.

Multiphase processes have been associated with the release of gaseous halogen compounds from marine aerosol, mainly in marine and coastal environments. However, Thornton et al. (2010) found production rates of gaseous nitryl chloride

near Boulder, Colorado, from reaction of $N_2O_5$ with particulate $Cl^-$, similar to those found in coastal and marine environments. $ClNO_2$ readily photolyzes, to yield Cl. They also found that substantial quantities of $N_2O_5$ are recycled through $ClNO_2$ back into $NO_X$ instead of forming $HNO_3$ (a stable reservoir for reactive nitrogen compounds). The oxidation of hydrocarbons by Cl radicals released from the marine aerosol could lead to the rapid formation of peroxy radicals and higher rates of $O_3$ production. It should be noted that in addition to production from marine aerosol, reactive halogen species are also produced by the oxidation of halogenated organic compounds (e.g., $CH_3Cl$, $CH_3Br$, and $CH_3I$). The atmospheric chemistry of halogens is complex because Cl-, Br-, and I-containing species can react among themselves and with hydrocarbons and other species and could also be important for $O_3$ destruction, as has been noted for the lower stratosphere (McElroy et al., 1986; Yung et al., 1980). For example, the reactions of Br- and Cl-containing radicals deplete $O_3$ in selected environments such as the Arctic during the spring (Barrie et al., 1988), the tropical marine boundary layer (Dickerson et al., 1999), and inland salt flats and salt lakes (Stutz et al., 2002). Mahajan et al. (2010) found that I and Br species acting together resulted in $O_3$ depletion that was much larger than would have been expected if they acted individually and did not interact with each other; see Annex AX2.2.10.3 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b).

Multiphase processes have also been associated with the uptake of reactive gas phase species affecting global budgets of $O_3$ and nitrogen oxides among others. The uptake of $N_2O_5$ on aerosols or cloud droplets leads to the loss of $O_3$ and $NO_X$ and the production of aqueous phase nitric acid, aerosol nitrate, and gaseous halogen nitrites. In addition to loss of $HO_2$, the uptake of $HO_2$ radicals on aerosol surfaces potentially reduces $O_3$ concentrations and increases formation of sulfate (if $H_2O_2$ is formed after uptake). Macintyre and Evans (2011) developed a parameterization for uptake of $HO_2$ based on laboratory studies, which were about a factor of seven lower than previously estimated. However they note that some of the earlier studies reporting higher values might have been influenced by transition metal ions (e.g., Cu(II), Fe(II)), which are highly spatially variable and could be important catalysts in areas with high concentrations of these ions. Although the global change in $O_3$ was small (~-0.3%) much larger regional changes were found (e.g., up to -27% at the surface over China).

Uptake coefficients for these species vary widely among laboratory studies. Macintyre and Evans (2010) showed that the sensitivity of key tropospheric species such as $O_3$ varies from very small to significant over the range of uptake coefficients for $N_2O_5$ obtained in laboratory studies. For example, global $O_3$ loss ranges from 0 to over 10%, with large regional variability over the range of $N_2O_5$ uptake coefficients reported. In this regard, it should be stressed that knowledge of multiphase processes is still evolving and there are still many questions that remain to be answered. However, it is becoming clear that multiphase processes are important for $O_3$ chemistry.

Reactions of $O_3$ with monoterpenes have been shown to produce oxidants in the aerosol phase, principally as components of ultrafine particles. Docherty et al. (2005)

found evidence for the substantial production of organic hydroperoxides in secondary organic aerosol (SOA) resulting from the reaction of monoterpenes with $O_3$. Analysis of the SOA formed in their environmental chamber indicated that the SOA consisted mainly of organic hydroperoxides. In particular, they obtained yields of 47% and 85% of organic peroxides from the oxidation of α- and β-pinene. The hydroperoxides then react with aldehydes in particles to form peroxyhemiacetals, which can either rearrange to form other compounds such as alcohols and acids or revert back to the hydroperoxides. The aldehydes are also produced in large measure during the ozonolysis of the monoterpenes. Monoterpenes also react with OH radicals resulting in the production of more lower-molecular-weight products than in the reaction with monoterpenes and $O_3$. Bonn et al. (2004) estimated that hydroperoxides lead to 63% of global SOA formation from the oxidation of terpenes. The oxidation of anthropogenic aromatic hydrocarbons by OH radicals could also produce organic hydroperoxides in SOA (Johnson et al., 2004). Recent measurements show that the abundance of oxidized SOA exceeds that of more reduced hydrocarbon like organic aerosol in Pittsburgh (Zhang et al., 2005) and in about 30 other cities across the Northern Hemisphere (Zhang et al., 2007b). Based on aircraft and ship-based sampling of organic aerosols over coastal waters downwind of northeastern U.S. cities, de Gouw et al. (2008) reported that 40-70% of measured organic mass was water soluble and estimated that approximately 37% of SOA is attributable to aromatic precursors, using PM yields estimated for $NO_X$-limited conditions. Uncertainties still exist as to the pathways by which the oxidation of isoprene leads to the formation of SOA. Nozière et al. (2011) found that a substantial fraction of 2-methyltetrols are primary in origin, although these species have been widely viewed solely as products of the atmospheric oxidation of isoprene. This finding points to lingering uncertainty in reaction pathways in the oxidation of isoprene and in estimates of the yield of SOA from isoprene oxidation.

Reactions of $O_3$ on the surfaces of particles, in particular those with humic acid like composition, are instrumental in the processing of SOA and the release of low molecular weight products such as HCHO (D'Anna et al., 2009). However, direct reactions of $O_3$ and atmospheric particles appear to be too slow to represent a major $O_3$ sink in the troposphere (D'Anna et al., 2009).

#### 3.2.3.1 Indoor Air

Except when activities such as photocopying or welding are occurring, the major source of $O_3$ to indoor air is through infiltration of outdoor air. Reactions involving ambient $O_3$ with NO either from exhaled breath or from gas-fired appliances, surfaces of furnishings and terpenoid compounds from cleaning products, air fresheners and wood products also occur in indoor air as was discussed in the 2006 $O_3$ AQCD (U.S. EPA, 2006b). The previous $O_3$ review also noted that the ozonolysis of terpenoid compounds could be a substantial source of secondary organic aerosol in the ultrafine size fraction. Chen et al. (2011) examined the formation of secondary

organic aerosol from the reaction of $O_3$ that has infiltrated indoors with terpenoid components of commonly used air fresheners. They focused on the formation and decay of particle bound reactive oxygen species (ROS) and on their chemical properties. They found that the ROS content of samples can be decomposed into fractions that differ in terms of reactivity and volatility; however, the overall ROS content of samples decays and over 90% is lost within a day at room temperature. This result also suggests loss of ROS during sampling periods longer than a couple of hours.

### 3.2.4 Temperature and Chemical Precursor Relationships

As might be expected based on the temperature dependence of many reactions involved in the production and destruction of $O_3$ and the temperature dependence of emissions processes such as evaporation of hydrocarbon precursors and the emissions of biogenically important precursors such as isoprene, ambient concentrations of $O_3$ also show temperature dependence. Bloomer et al. (2009) determined the sensitivity of $O_3$ to temperature at rural sites in the eastern United States. They found that $O_3$ increased on average at rural Clean Air Status and Trends Network (CASTNET) sites by ~3.2 ppbv/ºC before 2002; and after 2002 by ~2.2 ppbv/ºC. This change in sensitivity was largely the result of reductions in $NO_X$ emissions from power plants. These results are in accord with model predictions by Wu et al. (2008b) showing that the sensitivity of $O_3$ to temperature decreases with decreases in precursor emissions. Rasmussen et al. (2012) recently extended the work of Bloomer et al. (2009) to quantify seasonal changes in the sensitivity of $O_3$ to temperature as well as regional variability (3-6 ppb/ºC over the Northeast and mid-Atlantic; 3-4 ppb/ºC over the Great Lakes region) and to evaluate the capability of a chemistry-climate model to capture $O_3$ sensitivity to temperature. However, the associations of $O_3$ with temperature are not as clear in the West as they might be in the East. For example, sites downwind of Phoenix, AZ showed basically no sensitivity of $O_3$ to temperature ($R^2$ = 0.02) (U.S. EPA, 2006b). However, Wise and Comrie (2005) did find that meteorological parameters (mixing height and temperature) typically accounts for 40 to 70% of the variability in $O_3$ in the five southwestern cities (including Phoenix) they examined. It is likely that differences in the nature of sites chosen (urban versus rural) accounted for this difference and are at least partially responsible for the difference in results. Jaffe et al. (2008) regressed $O_3$ on temperature at Yellowstone and Rocky Mountain NP and found weak associations ($R^2$ = 0.09 and 0.16). They found that associations with area burned by wildfires are much stronger. Other sources as discussed in Section 3.4 might also be more important in the West than in the East.

The warmer months of the year are generally regarded as being the most conducive to higher $O_3$ concentrations. However, Schnell et al. (2009) reported observations of high $O_3$ concentrations (maximum 1-h avg of 140 ppb; maximum 8-h avg of 120 ppb) in the Jonah-Pinedale gas fields in Wyoming during winter at temperatures of -17ºC. Potential factors contributing to these anomalously high concentrations

include a highly reflective snow surface, emissions of $NO_X$, hydrocarbons and short-lived radical reservoirs (e.g., HONO and HCHO) and a very shallow, stable boundary layer trapping these emissions close to the surface (Schnell et al., 2009). Multiphase processes might also be involved in the production of these short-lived reservoirs. At a temperature of -17ºC, the production of hydroxyl radicals (by the photolysis of $O_3$ yielding $O^1D$ followed by the reaction, $O(^1D) + H_2O$, needed to initiate hydrocarbon oxidation) is severely limited, suggesting that another source of radicals is needed. Radicals can be produced by the photolysis of molecules such as HONO and HCHO which photolyze in optically thin regions of the solar spectrum. A similar issue, in part due to the under-prediction of radicals, has arisen in the Houston airshed where chemistry-transport models (CTMs; discussed further in Section 3.3) under-predict $O_3$ (Olaguer et al., 2009). Carter and Seinfeld (2012) modeled several of the events using the SAPRC-07 chemical mechanism and found that the release of HONO from the snow surface aids in the formation of $O_3$. The chemical mechanism they used—including the temperature dependence of rate coefficients—was developed for application at higher temperatures. They also note that temperature changes will also affect the distribution of products and radicals formed when individual VOCs react, but the current version of the mechanism represents these by lumped overall processes in which the product and radical distributions are treated as if they are temperature independent. It is not clear how this treatment of radical production might affect their results.

Rather than varying directly with emissions of its precursors, $O_3$ changes in a nonlinear fashion with the concentrations of its precursors. At the low $NO_X$ concentrations found in remote continental areas to rural and suburban areas downwind of urban centers (low-$NO_X$ regime), the net production of $O_3$ typically increases with increasing $NO_X$. In the low-$NO_X$ regime, the overall effect of the oxidation of VOCs is to generate (or at least not consume) free radicals, and $O_3$ production varies directly with $NO_X$. In the high-$NO_X$ regime, $NO_2$ reacts with OH radicals to form $HNO_3$ (e.g., Hameed et al., 1979). These OH radicals would otherwise oxidize VOCs to produce peroxy radicals, which in turn would oxidize NO to $NO_2$. In this regime, $O_3$ production is limited by the availability of radicals (Tonnesen and Jeffries, 1994) and $O_3$ shows only a weak dependence on $NO_X$ concentrations. The production of radicals is in turn limited by the availability of solar UV radiation capable of photolyzing $O_3$ (in the Hartley bands) or aldehydes and/or by the abundance of VOCs whose oxidation produce more radicals than they consume. At the even higher $NO_X$ concentrations found in downtown metropolitan areas, especially near busy streets and roads, and in power plant plumes, there is scavenging (sometimes referred to as titration) of $O_3$ by reaction with NO to form $NO_2$ leading to depletion of $O_3$. However, as urban plumes are transported and diluted, this $NO_2$ can lead to photochemical production of $O_3$ downwind of the source areas.

The production of radicals can also be limited by the availability of solar UV radiation capable of photolyzing $O_3$ (in the Hartley bands) and aldehydes. When solar radiation is blocked by clouds or reduced during winter, VOC and $NO_X$ may

both be available, but $O_3$ production is limited by the availability of solar radiation, and this has been defined as the "light-limited" regime (Hess et al., 1992).

There are a number of ways to refer to the chemistry of $O_3$ production in these different chemical regimes. Sometimes the terms VOC-limited and $NO_X$-limited are used. However, there are difficulties with this usage because (1) VOC measurements are not as abundant as they are for nitrogen oxides; (2) rate coefficients for reaction of individual VOCs with radicals (e.g., OH, Cl) vary over an extremely wide range; and (3) consideration is not given to $CH_4$ or CO and other reactions that can produce radicals without involving VOCs (e.g., photolysis of HONO). Many of these difficulties are overcome by the terms $NO_X$ –limited and radical-limited (Tonnesen and Dennis, 2000a, b). This usage recognizes that OH radicals are needed to react with VOCs to form $O_3$ and that either low NO or high $NO_2$ can limit the production of OH radicals. This usage also implicitly considers the importance of processes such as the availability of solar radiation and photolysis in generating radicals. The terms $NO_X$-limited and $NO_X$-saturated (Jaegle et al., 2001) have also been used to describe these two regimes. However, the terminology used in original articles will also be used here. In addition, in the remote marine troposphere, $NO_X$ concentrations can be ~20 ppt or less. Under these very low $NO_X$ conditions, which are not likely to be found in the continental U.S., but can characterize inflowing air, $HO_2$ and $CH_3O_2$ radicals react with each other and $HO_2$ radicals undergo self-reaction (to form $H_2O_2$), OH radicals efficiently convert $NO_2$ to $HNO_3$, and OH and $HO_2$ react with $O_3$, leading to net destruction of $O_3$ and inefficient OH radical regeneration. In addition, halogen-containing radicals also react with $O_3$ acting to keep its concentrations very low. This is in contrast to the situation in areas of the U.S. outside of urban cores, where $HO_2$ 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 $O_3$ as noted in Annex AX2.2.5 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b).

There are no definitive rules governing the concentrations of $NO_X$ at which the transition from $NO_X$-limited to $NO_X$-saturated conditions occurs. The transition between these two regimes is highly spatially and temporally dependent and depends also on the nature and abundance of the hydrocarbons that are present. In a $NO_X$-limited (or $NO_X$-sensitive) regime, $O_3$ formation is not completely insensitive to radical production or the flux of solar UV photons, just that $O_3$ formation is more sensitive to $NO_X$. For example, global tropospheric $O_3$ is sensitive to the concentration of $CH_4$ even though the troposphere is predominantly $NO_X$-limited. Likewise, in a $NO_X$-saturated regime there can still be some peroxyl-peroxyl radical interactions depending on $NO_X$ concentrations.

These considerations introduce a high degree of uncertainty into attempts to relate changes in $O_3$ concentrations to emissions of precursors. The chemistry of OH radicals, which are responsible for initiating the oxidation of hydrocarbons, shows behavior similar to that for $O_3$ with respect to $NO_X$ concentrations (Poppe et al., 1993; Zimmermann and Poppe, 1993; Hameed et al., 1979).

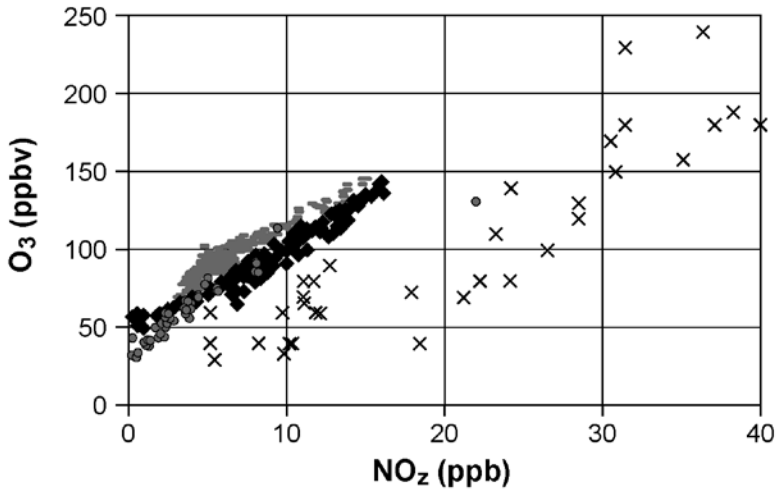
Trainer et al. (1993) and Olszyna et al. (1994) have shown that $O_3$ and $NO_Y$ are highly correlated in rural areas in the eastern United States. Trainer et al. (1993) also

showed that $O_3$ concentrations correlate even better with $NO_Z$ than with $NO_Y$, as may be expected because $NO_Z$ represents the amount of $NO_X$ that has been oxidized, forming $O_3$ in the process. $NO_Z$ is equal to the difference between measured total reactive nitrogen ($NO_Y$) and $NO_X$ and represents the summed products of the oxidation of $NO_X$. $NO_Z$ is composed mainly of $HNO_3$, PAN and other organic nitrates, particulate nitrate, and $HNO_4$. Trainer et al. (1993) also suggested that the slope of the regression line between $O_3$ and $NO_Z$ can be used to estimate the rate of $O_3$ production per $NO_X$ oxidized (also known as the $O_3$ production efficiency [OPE]). Ryerson et al. (2001); Ryerson et al. (1998) used measured correlations between $O_3$ and $NO_Z$ to identify different rates of $O_3$ production in plumes from large point sources. A number of studies in the planetary boundary layer over the continental U.S. have found that the OPE ranges typically from 1 to nearly 10. However, it may be higher in the upper troposphere and in certain areas, such as the Houston-Galveston area in Texas. Observations indicate that the OPE depends mainly on the abundance of $NO_X$ and also on availability of solar UV radiation, VOCs and $O_3$ itself.

Various techniques have been proposed to use ambient $NO_X$ and VOC measurements, i.e., as indicators to derive information about the dependence of $O_3$ production on their concentrations. For example, it has been suggested that $O_3$ formation in individual urban areas could be understood in terms of measurements of ambient $NO_X$ and VOC concentrations during the early morning (NRC, 1991). In this approach, the ratio of summed (unweighted) VOC to $NO_X$ is used to determine whether conditions were $NO_X$-limited or VOC-limited. This approach is inadequate because it does not consider many factors that are important for $O_3$ production such as the impact of biogenic VOCs (which are typically not present in urban centers during early morning); important differences in the ability of individual VOCs to generate radicals (rather than just total VOC) and other differences in $O_3$ forming potential for individual VOCs (Carter, 1995); and changes in the VOC to $NO_X$ ratio due to photochemical reactions and deposition as air moves downwind from urban areas (Milford et al., 1994).

Photochemical production of $O_3$ generally occurs simultaneously with the production of various other species such as $HNO_3$, organic nitrates, and other oxidants such as hydrogen peroxide. The relative rate of production of $O_3$ and other species varies depending on photochemical conditions, and can be used to provide information about $O_3$-precursor sensitivity. Sillman (1995) and Sillman and He (2002) identified several secondary reaction products that show different correlation patterns for $NO_X$-limited and $NO_X$-saturated conditions. The most important correlations are for $O_3$ versus $NO_Y$, $O_3$ versus $NO_Z$, $O_3$ versus $HNO_3$, and $H_2O_2$ versus $HNO_3$. The correlations between $O_3$ and $NO_Y$, and $O_3$ and $NO_Z$ are especially important because measurements of $NO_Y$ and $NO_X$ are more widely available than for VOCs. Measured $O_3$ versus $NO_Z$ (Figure 3-3) shows distinctly different patterns in different locations. In rural areas and in urban areas such as Nashville, TN, $O_3$ is highly correlated with $NO_Z$. By contrast, in Los Angeles, CA, $O_3$ is not as highly correlated with $NO_Z$, and the rate of increase of $O_3$ with $NO_Z$ is lower and the $O_3$ concentrations for a given $NO_Z$ value are generally lower. The different $O_3$ versus

$NO_Z$ relations in Nashville, TN and Los Angeles, CA reflects the difference between $NO_X$-limited conditions in Nashville versus an approach to $NO_X$-saturated conditions in Los Angeles.




Note: ($NO_Y$–$NO_X$) during the afternoon at rural sites in the eastern U.S. (the grey circles) and in urban areas and urban plumes associated with Nashville, TN (the gray dashes); Paris, France (the black diamonds); and Los Angeles, CA (the Xs).

Source: Adapted with permission of American Geophysical Union, (Sillman and He, 2002; Sillman et al., 1998; Trainer et al., 1993).

**Figure 3-3 Measured concentrations of $O_3$ and $NO_Z$.**

The difference between $NO_X$-limited and $NO_X$-saturated regimes is also reflected in measurements of $H_2O_2$. $H_2O_2$ production is highly sensitive to the abundance of radicals and is thus favored in the $NO_X$-limited regime. Measurements in the rural eastern U.S. (Jacob et al., 1995), Nashville, TN (Sillman et al., 1998), and Los Angeles, CA (Sakugawa and Kaplan, 1989), show large differences in $H_2O_2$ concentrations between likely $NO_X$-limited and $NO_X$-saturated locations. Tonnesen and Dennis (2000a) reviewed the use of many indicator ratios and Tonnesen and Dennis (2000b) proposed $HCHO/NO_2$ as an indicator to distinguish between $NO_X$-limited and radical-limited regimes.

The applications of indicator species mentioned above are mainly limited to individual urban areas because these are the areas where it is often not clear which regime is prevalent. Satellites provide a platform for greatly extending the range of applicability of the indicator technique and also have the resolution necessary to examine urban to rural differences. Martin et al. (2004) and Duncan et al. (2010) used satellite data from Ozone Monitoring Instrument (OMI) for HCHO to $NO_2$ column ratios to diagnose $NO_X$-limited and radical-limited ($NO_X$-saturated) regimes. HCHO can be used as an indicator of VOCs as it is a common, short-lived, oxidation

product of many VOCs that is a source of $HO_X$ (Sillman, 1995). In adopting the satellite approach, CTMs are used to estimate the fractional abundance of the indicator species in the planetary boundary layer. Duncan et al. (2010) found that $O_3$ formation over most of the U.S. became more sensitive to $NO_X$ over most of the U.S. from 2005 to 2007 largely because of decreases in $NO_X$ emissions. They also found that surface temperature is correlated with the ratio of HCHO to $NO_2$ especially in cities in the Southeast where emissions of isoprene (a major source of HCHO) are high due to high temperatures in summer.

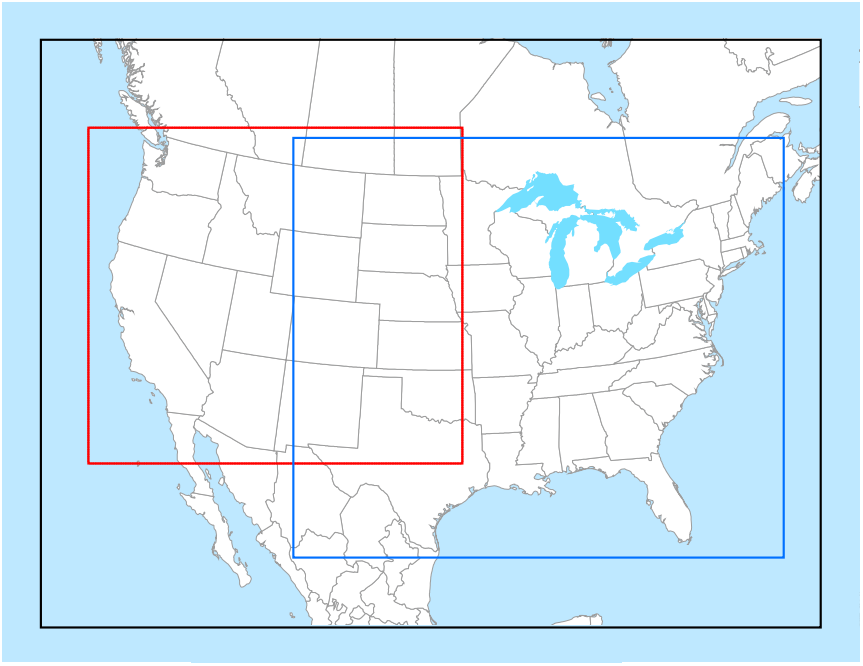
## 3.3 Atmospheric Modeling

CTMs have been widely used to compute the interactions among atmospheric pollutants and their transformation products, and the transport and deposition of pollutants. They have also been widely used to improve basic understanding of atmospheric chemical processes and to develop control strategies. The spatial scales over which pollutant fields are calculated range from intra-urban to regional to global. Generally, these models are applied to problems on different spatial scales but efforts are underway to link across spatial scales for dealing with global scale environmental issues that affect population health within cities. Many features are common to all of these models and hence they share many of the same problems. On the other hand, there are appreciable differences in approaches to parameterizing physical and chemical processes that must be addressed in applying these models across spatial scales.

CTMs solve a set of coupled, non-linear partial differential equations, or continuity equations, for relevant chemical species. Jacobson (2005) described the governing partial differential equations, and the methods that are used to solve them. Because of limitations imposed by the complexity and spatial-temporal scales of relevant physical and chemical processes, the CTMs must include parameterizations of these processes, which include atmospheric transport; the transfer of solar radiation through the atmosphere; chemical reactions; and removal to the surface by turbulent motions and precipitation. Development of parameterizations for use in CTMs requires data for three dimensional wind fields, temperatures, humidity, cloudiness, and solar radiation; emissions data for primary (i.e., directly emitted from sources) species such as $NO_X$, $SO_2$, $NH_3$, VOCs, and primary PM; and chemical reactions.

The domains of CTMs extend from a few hundred kilometers on a side to the entire globe. Most major regional (i.e., sub-continental) scale air-related modeling efforts at EPA rely on the Community Multi-scale Air Quality (CMAQ) modeling system (Byun and Schere, 2006; Byun and Ching, 1999). CMAQ's horizontal domain typically extends over North America with efforts underway to extend it over the entire Northern Hemisphere. Note that CTMs can be 'nested' within each other as shown in Figure 3-4 which shows domains for CMAQ (Version 4.6.1); additional details on the model configuration and application are found elsewhere (U.S. EPA, 2009e). The figure shows the outer domain (36 km horizontal grid spacing) and two

12 km spatial resolution (east and west) sub-domains. The upper boundary for CMAQ is typically set at about 100 hPa, or at about 16 km altitude on average, although in some recent applications the upper boundary has been set at 50 hPa. These domains and grid spacings are quite common and can also be found in a number of other models.



Note: This figure depicts a 36 km grid-spacing outer parent domain in black; 12 km western U.S. domain in red; 12 km eastern U.S. domain in blue.

**Figure 3-4 Sample Community Multi-scale Air Quality (CMAQ) modeling domains.**

The main components of a CTM such as EPA's CMAQ are summarized in Figure 3-5. The capabilities of a number of CTMs designed to study local- and regional-scale air pollution problems were summarized by Russell and Dennis (2000) and in the 2006 $O_3$ AQCD (U.S. EPA, 2006b). Historically, CMAQ has been driven most often by the MM5 mesoscale meteorological model (Seaman, 2000), though it could be driven by other meteorological models including the Weather Research and Forecasting (WRF) model and the Regional Atmospheric Modeling System (RAMS) (ATMET, 2011).

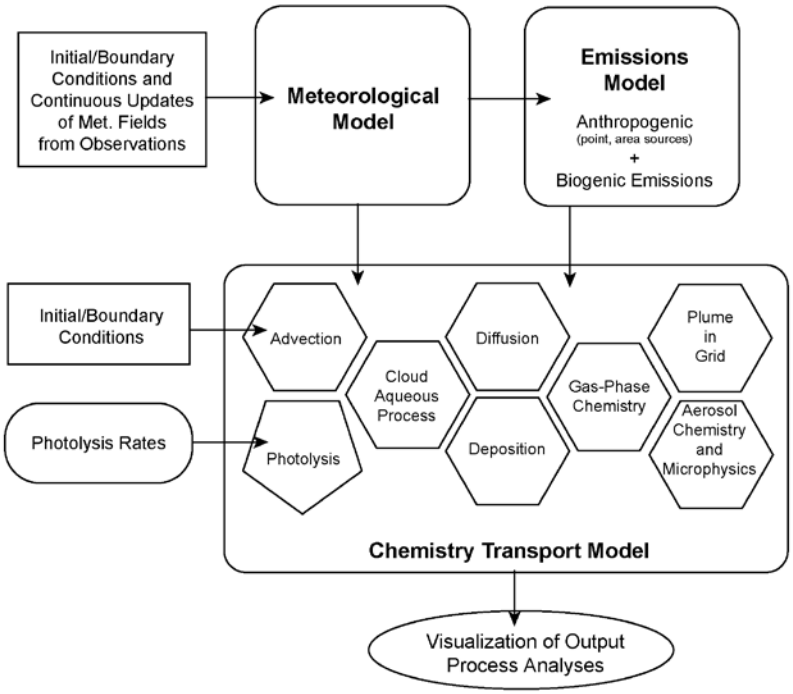



**Figure 3-5 Main components of a comprehensive atmospheric chemistry modeling system, such as the U.S. EPA's Community Multi-scale Air Quality (CMAQ) modeling system.**

Simulations of pollution episodes over regional domains have been performed with a horizontal resolution down to 1 km; see the application and general survey results reported in Ching et al. (2006). However, simulations at such high resolution require better parameterizations of meteorological processes such as boundary layer fluxes, deep convection, and clouds (Seaman, 2000). Finer spatial resolution is necessary to resolve features such as urban heat island circulation; sea, bay, and land breezes; mountain and valley breezes; and the nocturnal low-level jet; all of which can affect pollutant concentrations. Other major air quality systems used for regional scale applications include the Comprehensive Air Quality Model with extensions (CAMx) (ENVIRON, 2005) and the Weather Research and Forecast model with Chemistry (WRF/Chem) (NOAA, 2010).

CMAQ and other grid-based or Eulerian air quality models subdivide the modeling domain into a three-dimensional array of grid cells. The most common approach to setting up the horizontal domain is to nest a finer grid within a larger domain of coarser resolution. The use of finer horizontal resolution in CTMs will necessitate finer-scale inventories of land use and better knowledge of the exact paths of roads, locations of factories, and, in general, better methods for locating sources and estimating their emissions. The vertical resolution of CTMs is variable and usually configured to have more layers in the PBL and fewer in the free troposphere.

The meteorological fields are produced either by other numerical prediction models such as those used for weather forecasting (e.g., MM5, WRF), and/or by assimilation of satellite data. The flow of information shown in Figure 3-5 has most often been unidirectional in the sense that information flows into the CTM (large box) from outside; feedbacks on the meteorological fields and on boundary conditions (i.e., out of the box) have not been included. However, CTMs now have the capability to consider these feedbacks as well; see, for example, Binkowski et al. (2007) and WRF/Chem (NOAA, 2010).

Because of the large number of chemical species and reactions that are involved in the oxidation of realistic mixtures of anthropogenic and biogenic hydrocarbons, condensed mechanisms must be used in atmospheric models. These mechanisms can be tested by comparison with smog chamber data. However, the existing chemical mechanisms often neglect many important processes such as the formation and subsequent reactions of long-lived carbonyl compounds, the incorporation of the most recent information about intermediate compounds, and heterogeneous reactions involving cloud droplets and aerosol particles. To the extent that information is available, models like CMAQ and CAMx do include state-of-the-science parameterization for some of these processes such as heterogeneous $N_2O_5$ chemistry.

The initial conditions, or starting concentration fields of all species computed by a model, and the boundary conditions, or concentrations of species along the horizontal and upper boundaries of the model domain throughout the simulation, must be specified at the beginning of the simulation. Both initial and boundary conditions can be estimated from models or data or, more generally, model plus data hybrids. Because data for vertical profiles of most species of interest are very sparse, results of model simulations over larger, usually global, domains are often used.

Chemical kinetics mechanisms representing the important reactions occurring in the atmosphere are used in CTMs to estimate the rates of chemical formation and destruction of each pollutant simulated as a function of time. The Master Chemical Mechanism (MCM) (Univ of Leeds, 2010) is a comprehensive reaction database providing as near an explicit treatment of chemical reactions in the troposphere as is possible. The MCM currently includes over 12,600 reactions and 4,500 species. However, mechanisms that are this comprehensive are still computationally too demanding to be incorporated into CTMs for regulatory use. Simpler treatments of tropospheric chemistry have been assembled by combining chemical species into mechanisms that group together compounds with similar chemistry. It should be noted that because of different approaches to the lumping of organic compounds into surrogate groups for computational efficiency, chemical mechanisms can produce different results under similar conditions. Jimenez et al. (2003) briefly described the features of the seven main chemical mechanisms in use and compared concentrations of several key species predicted by these mechanisms in a box-model simulation over 24 hours. Several of these mechanisms have been incorporated into CMAQ including extensions of the Carbon Bond (CB) mechanism (Luecken et al., 2008), SAPRC (Luecken et al., 2008), and the Regional Atmospheric Chemistry Mechanism, version 2 (RACM2) (Fuentes et al., 2007). The CB mechanism is currently undergoing

extension (CB06) to include, among other things, longer lived species to better simulate chemistry in the remote and upper troposphere. These mechanisms were developed primarily for homogeneous gas phase reactions and treat multiphase chemical reactions in a very cursory manner, if at all. As a consequence of neglecting multiphase chemical reactions, models such as CMAQ could have difficulties capturing the regional nature of $O_3$ episodes, in part because of uncertainty in the chemical pathways converting $NO_X$ to $HNO_3$ and recycling of $NO_X$ (Godowitch et al., 2008; Hains et al., 2008). Much of this uncertainty also involves multiphase processes as described in Section 3.2.3.

CMAQ and other CTMs incorporate processes and interactions of aerosol-phase chemistry (Zhang and Wexler, 2008; Gaydos et al., 2007; Binkowski and Roselle, 2003). There have also been several attempts to study the feedbacks of chemistry on atmospheric dynamics using meteorological models like MM5 and WRF (Liu et al., 2001; Park et al., 2001; Grell et al., 2000; Lu et al., 1997). This coupling is necessary to accurately simulate feedbacks from PM (Park et al., 2001; Lu et al., 1997) over areas such as Los Angeles or the Mid-Atlantic region. Photolysis rates in CMAQ can now be calculated interactively with model produced $O_3$, $NO_2$, and aerosol fields (Binkowski et al., 2007).

Spatial and temporal characterizations of anthropogenic and biogenic precursor emissions can be specified as inputs to a CTM or these emissions can be calculated in-line in CMAQ. Emissions inventories have been compiled on grids of varying resolution for many hydrocarbons, aldehydes, ketones, CO, $NH_3$, and $NO_X$. Preprocessing of emissions data for CMAQ is done by the Spare-Matrix Operator Kernel Emissions (SMOKE) system (UNC, 2011). For many species, information on temporal variability of emissions is lacking, so long-term annual averages are used in short-term, episodic simulations. Annual emissions estimates can be modified by the model to produce emissions more characteristic of the time of day and season. Appreciable errors in emissions can occur if inappropriate time dependence is applied.

Each of the model components described above has associated uncertainties; and the relative importance of these uncertainties varies with the modeling application. Large errors in photochemical modeling arise from the meteorological, chemical and emissions inputs to the model (Russell and Dennis, 2000). While the effects of poorly specified boundary conditions propagate through the model's domain, the effects of these errors remain undetermined. Because many meteorological processes occur on spatial scales smaller than the model's vertical or horizontal grid spacing and thus are not calculated explicitly, parameterizations of these processes must be used. These parameterizations introduce additional uncertainty.

The performance of CTMs must be evaluated by comparison with field data as part of a cycle of model evaluations and subsequent improvements (NRC, 2007). However, they are too computationally demanding to have the full range of their sensitivities examined using Monte Carlo techniques (NRC, 2007). Models of this complexity are evaluated by comparison with field observations for $O_3$ and other species. Evaluations of the performance of CMAQ are given in Arnold et al. (2003),

Eder and Yu (2005), Appel et al. (2005), and Fuentes and Raftery (2005). Discrepancies between model predictions and observations can be used to point out gaps in current understanding of atmospheric chemistry and to spur improvements in parameterizations of atmospheric chemical and physical processes. Model evaluation does not merely involve a straightforward comparison between model predictions and the concentration field of the pollutant of interest. Such comparisons may not be meaningful because it is difficult to determine if agreement between model predictions and observations truly represents an accurate treatment of physical and chemical processes in the CTM or the effects of compensating errors in complex model routines (in other words, it is important to know if the right answer is obtained for the right reasons). Each of the model components (emissions inventories, chemical mechanism, and meteorological driver) should be evaluated individually as has been done to large extent in some major field studies such as TexAQS I and II and CalNex. Comparisons of correlations between measured and modeled VOCs and $NO_X$ are useful for evaluating results from CTMs and can provide information about the chemical state of the atmosphere. A CTM that accurately computes both VOC and $NO_X$ along with the spatial and temporal relations among the critical secondary species associated with $O_3$ has a higher probability of representing $O_3$-precursor relations correctly than one that does not.

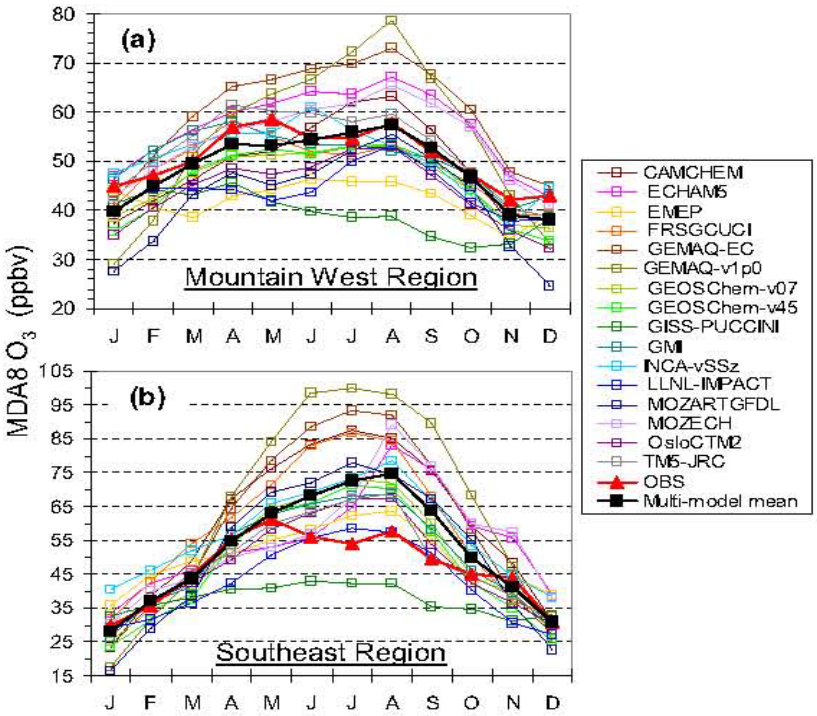
The above evaluation techniques are sometimes referred to as "static" in the sense that individual model variables are compared to observations. It is also crucial to understand the dynamic response to changes in inputs and to compare the model responses to those that are observed. These tests might involve changes in some natural forcing or in emissions from an anthropogenic source. As an example, techniques such as the direct decoupled method (DDM) could be used in CTMs to determine their first order sensitivity to emissions changes (Zhang, 2005; Dunker et al., 2002). However, the observational basis for comparing a model's response is largely unavailable for many problems of interest, in large part because meteorological conditions are also changing while the emissions are changing. As a result, methods such as DDM are used mainly to assess the potential effectiveness of emissions controls (Arunachalam, 2009). Because the chemistry of $O_3$ formation is non-linear near strong sources, and higher order terms taking into account the curvature of the response surface of $O_3$ with respect to changes in sources are needed when using DDM. These additional terms are incorporated in the higher order decoupled method, or HDDM, (see the U.S. EPA technical memorandum on applications of HDDM, particularly with respect to adjusting $O_3$ concentrations in response to emissions reductions [or "roll back"] at http://www.epa.gov/ttnnaaqs/standards/ozone/s_o3_2008_td.html).

### 3.3.1 Global Scale CTMs

With recognition of the global nature of many air pollution problems, global scale CTMs have been applied to regional scale pollution problems (NRC, 2009). Global-scale CTMs are used to address issues associated with global change, to characterize

long-range transport of air pollutants, and to provide boundary conditions for the regional-scale models. The upper boundaries of global scale CTMs extend anywhere from the tropopause (~8 km at the poles to ~16 km in the tropics) to the mesopause at ~80 km, in order to obtain more realistic boundary conditions for problems involving stratospheric dynamics and chemistry. The global-scale CTMs consider the same processes shown in Figure 3-5 for the regional scale models. In addition, many of the same issues that have arisen for the regional models have also arisen for the global scale models (Emmerson and Evans, 2009). For example, after adjusting lightning $NO_X$ to better match observed constraints in the MOZART-4 model, simulated $HNO_3$ was too low and PAN too high in the mid-troposphere, though observations were captured in the upper troposphere, over the U.S. during summer 2004 in the MOZART-4 model (Fang et al., 2010). In contrast, summer 2004 simulations with improved lightning $NO_X$ in GEOS-Chem indicate that PAN is too low but $HNO_3$ is overestimated throughout the mid- and upper troposphere (Hudman et al., 2007). Predictions of $HNO_3$ were too high and PAN too low over the U.S. during summer in the MOZART model (Fang et al., 2010). Similar findings were obtained in a box model of upper tropospheric chemistry (Henderson et al., 2011), indicating a need for improved constraints on processes controlling $NO_Y$ distributions in the free troposphere.

The GEOS-Chem model is a community-owned, global scale CTM that has been widely used to study issues associated with the hemispheric transport of pollution and global change (Harvard University, 2010a). Comparisons of the capabilities of GEOS-Chem and several other models to simulate intra-hemispheric transport of pollutants are given in a number of articles (Fiore et al., 2009; Reidmiller et al., 2009). Reidmiller et al. (2009) compared the ensemble average of 18 global models to spatially and monthly averaged observations of $O_3$ at CASTNET sites in the U.S. (see Figure 3-6). These results show that the multi-model ensemble agrees much better with observations than do most of the individual models. The GEOS-Chem model was run for two grid spacings ($4^o \times 4.5°$ and $2^o \times 2.5°$) over the U.S. with very similar results that lie close to the ensemble average. In general, the model ensemble average and the two GEOS-Chem simulations are much closer to observations in the Intermountain West Region than in the Southeast Region during summer, when most major $O_3$ episodes occur (Note, though, that more current versions of GEOS-Chem are now in use.) However, there are also sizable over-predictions by many models in both regions during summer.




Source: Reprinted with permission of Copernicus Publications, (Reidmiller et al., 2009).

**Figure 3-6 Comparison of global chemical-transport model (CTM) predictions of daily maximum 8-h avg $O_3$ concentrations and multi-model mean with monthly averaged CASTNET observations in the Intermountain West and Southeast Regions of the U.S.**

In their review, McDonald-Buller et al. (2011) noted that global scale chemical transport models exhibit biases in monthly mean of the daily maximum 8-h avg (MDA8) $O_3$ in some regions of the U.S., including the Gulf Coast, regions affected by fires, and regions with complex topography, which have implications for model estimates of background $O_3$; and they also have difficulty representing the fine structures of $O_3$ events at sub-grid scales at relatively remote monitoring sites that include contributions to $O_3$ from background sources.

Global models are not alone in overestimating $O_3$ in the Southeast. Godowitch et al. (2008), Gilliland et al. (2008) and Nolte et al. (2008) found positive $O_3$ biases in regional models over the eastern U.S., as well, which they largely attributed to uncertainties in temperature, relative humidity and planetary boundary layer height. Agreement between monthly average values is expected to be better than with daily values because of a number of factors including the increasing uncertainty of emissions at finer time resolution. Kasibhatla and Chameides (2000) found that the

accuracy of simulations improved as the averaging time of both the simulation and the observations increased.

Simulations of the effects of long-range transport at particular locations must be able to link multiple horizontal resolutions from the global to the local scale. Because of computational limitations, global simulations are not made at the same horizontal resolutions found in the regional scale models, i.e., down to 1-4 $km^2$ horizontal resolution. They are typically conducted with a horizontal grid spacing of 1º-2º of latitude and longitude (or roughly 100-200 km at mid-latitudes). Some models such as GEOS-Chem have the capability to include nested models at a resolution of 0.5º × 0.667º (Wang et al., 2009a) and efforts are underway to achieve even higher spatial resolution. Another approach is to nest regional models within GEOS-Chem. Caution must be exercised with nesting different models because of differences in chemical mechanisms and numerical schemes, and in boundary conditions between the outer and inner models. As an example of these issues, surface $O_3$ concentrations that are too high have been observed in models in which CMAQ was nested inside of GEOS-Chem. The high $O_3$ results in large measure from stratospheric $O_3$ intruding into the CMAQ domain [see (Lam and Fu, 2010) for one way to address this issue]. Large vertical $O_3$ gradients in the upper troposphere must be preserved to accurately represent downward transport of stratospheric $O_3$. This complicates efforts to link global and regional models with different vertical grid spacing. Efforts are also underway to extend the domain of CMAQ over the entire Northern Hemisphere. In this approach, the same numerical schemes are used for transporting species and the same chemistry is used throughout all spatial scales. Finer resolution in models of any scale can only improve scientific understanding to the extent that the governing processes are accurately described. Consequently, there is a crucial need for observations at the appropriate scales to evaluate the scientific understanding represented by the models.

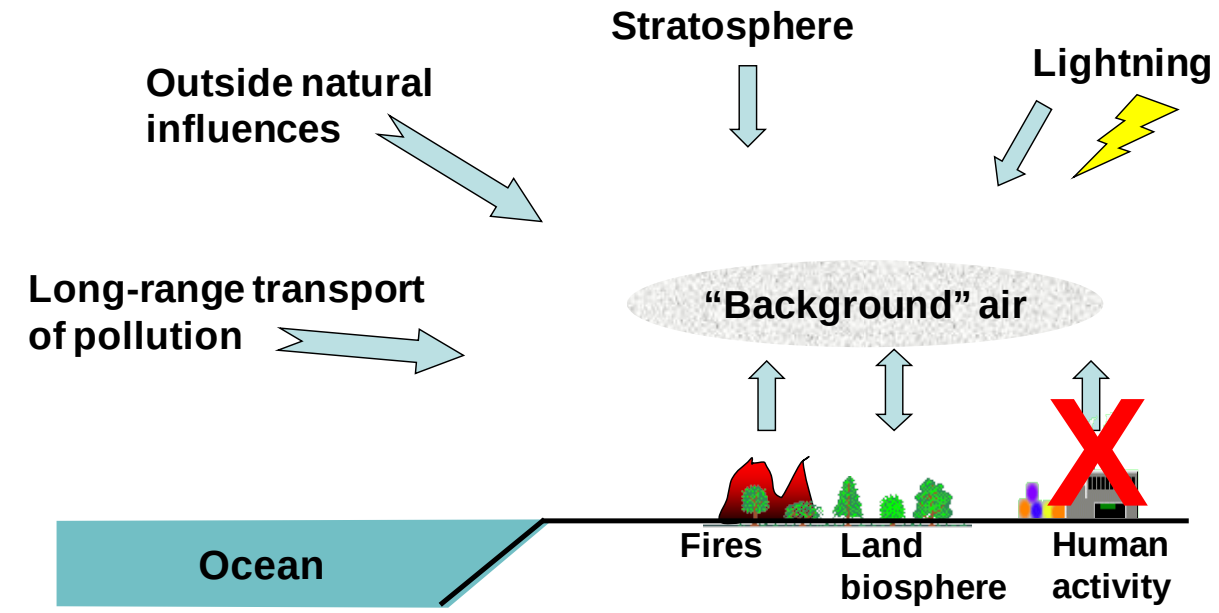
## 3.4 Background $O_3$ Concentrations

Background concentrations of $O_3$ have been given various definitions in the literature over time. An understanding of the sources and contributions of background $O_3$ to $O_3$ concentrations in the U.S. is potentially useful in reviewing the $O_3$ NAAQS, especially related to days at the upper end of the distribution of $O_3$ concentrations. In the context of a review of the NAAQS, it is useful to define background $O_3$ concentrations in a way that distinguishes between concentrations that result from precursor emissions that are relatively less controllable from those that are relatively more controllable through U.S. policies. In previous NAAQS reviews, a specific definition of background concentrations was used and referred to as policy relevant background (PRB). In those previous reviews, PRB concentrations were defined by EPA as those concentrations that would occur in the U.S. in the absence of anthropogenic emissions in continental North America (CNA), defined here as the U.S., Canada, and Mexico. There is no chemical difference between background $O_3$ and $O_3$ attributable to CNA anthropogenic sources. However, to inform policy

considerations regarding the current or potential alternative standards, it is useful to understand how total $O_3$ concentrations can be attributed to different sources.

For this document, EPA has considered background $O_3$ concentrations more broadly by considering three different definitions of background. The first is natural background which includes contributions resulting from emissions from natural sources (e.g., stratospheric intrusion, wildfires, biogenic methane, and more short-lived VOC emissions) throughout the globe simulated in the absence of all anthropogenic emissions. The second is North American background (NA background) which includes contributions from natural background throughout the globe and emissions of anthropogenic pollutants contributing to global concentrations of $O_3$ (e.g., anthropogenic methane) from countries outside North America. The third is United States background (U.S. background) which includes contributions from natural background throughout the globe and emissions from anthropogenic pollutants contributing to global concentrations of $O_3$ from countries outside the United.States. U.S. background differs from NA background in that it includes anthropogenic emissions from neighboring Canada and Mexico. These three definitions have been explored in recent literature and are discussed further below.

Sources included in the definitions of NA-background and U.S.-background $O_3$ are shown schematically in Figure 3-7. Definitions of background and approaches to derive background concentrations were reviewed in the 2006 $O_3$ AQCD (U.S. EPA, 2006b) and in Reid et al. (2008). Further detail about the processes involved in these sources is given in Section 3.4.1 and Section 3.4.2 and application to models calculating background concentrations is presented in Section 3.4.3.




Note: Background concentrations are $O_3$ concentrations that would exist in the absence of anthropogenic emissions from the U.S., Canada, and Mexico. United States (U.S.) background is similarly defined, but includes transport from Canada and Mexico in addition to intercontinental transport.

**Figure 3-7 Schematic overview of contributions to North American (NA) background concentrations of $O_3$.**

### 3.4.1 Contributions from Natural Sources

Natural sources contributing to background $O_3$ include the stratospheric-tropospheric exchange (STE) of $O_3$ and photochemical reactions involving natural $O_3$ precursor emissions of VOCs, $NO_X$, and CO. Natural sources of $O_3$ precursors include biogenic emissions, wildfires, and lightning. Biogenic emissions from agricultural activities in CNA (or the U.S.) are not considered in the formation of NA- (or U.S.)-background $O_3$. Contributions from natural sources are an important component of background concentrations and are discussed in greater detail below.

#### 3.4.1.1 Contributions from the Stratosphere

The basic atmospheric dynamics and thermodynamics of STE were outlined in the 2006 $O_3$ AQCD (U.S. EPA, 2006b); as noted there, stratospheric air rich in $O_3$ is transported into the troposphere. Ozone is produced naturally by photochemical reactions in the stratosphere as shown in Figure 3-1. Some of this $O_3$ is transported downward into the troposphere throughout the year, with maximum contributions at mid-latitudes during late winter and early spring mainly coming from a process known as tropopause folding. These folds occur behind most cold fronts, bringing

stratospheric air with them. The tropopause should not be interpreted as a material surface through which there is no exchange. Rather these folds should be thought of as regions in which mixing of tropospheric and stratospheric air is occurring (Shapiro, 1980). This imported stratospheric air contributes to the natural background of $O_3$ in the troposphere, especially in the free troposphere during winter and spring. Significant intrusions of stratospheric air occur in "ribbons" ~200 to 1000 km in length, 100 to 300 km wide and about 1 to 4 km thick (Wimmers et al., 2003; Hoskins, 1972). Thus, these intrusions are large scale three-dimensional events and should not be thought of as one-dimensional. STE also occurs during other seasons including summer.

Methods for estimating the contribution of stratospheric intrusions rely on the use of tracers of stratospheric origin that can be either dynamical or chemical. Thompson et al. (2007) found that roughly 20-25% of free tropospheric $O_3$ over northeastern North America during July-August 2004 was of stratospheric origin based on an analysis of ozonesonde data. This $O_3$ can be mixed into the PBL where it can either be destroyed or transported to the surface. They relied on the combined use of low relative humidity and high (isentropic) potential vorticity (PV) (>2 PV units) to identify stratospheric contributions. PV has been a widely used tracer for stratospheric air; see the 2006 $O_3$ AQCD (U.S. EPA, 2006b). However, bear in mind that this analysis pertains to a single year only with no indication of interannual variability. Lefohn et al. (2011) used these and additional criteria to assess stratospheric influence on sites in the Intermountain West and in the Northern Tier. Additional criteria include consideration of trajectories originating at altitudes above the 380 K potential temperature surface with a residence time requirement at these heights. Based on these criteria, they identified likely stratospheric influence at the surface sites on a number of days during spring of 2006 to 2008. However, they noted that their analysis of stratospheric intrusions captures only the frequency and vertical penetration of the intrusions but does not provide information about the contribution of the intrusions to the measured $O_3$ concentration. These results are all generally consistent with what was noted in the 2006 $O_3$ AQCD (U.S. EPA, 2006b). Fischer (2004) analyzed the $O_3$ record during summer at Mount Washington and identified a stratospheric contribution to 5% of events during the summers of 1998 - 2003 when $O_3$ was >65 ppb; the air was dry and trajectories originated from altitudes where PV was elevated (PV >1 PV unit). However, this analysis did not quantify the relative contributions of anthropogenic and stratospheric $O_3$ sources more precisely, because as they note identifying stratospheric influences is complicated by transport over industrialized/urban source regions. Stratospheric $O_3$ was hypothesized to influence the summit during conditions also potentially conducive to photochemical $O_3$ production, which make any relative contribution calculations difficult without additional measurements of anthropogenic and stratospheric tracers.

Although most research has been conducted on tropopause folding as a source of stratosphere to troposphere exchange, this is not the only mechanisms by which stratospheric $O_3$ can be brought to lower altitudes. Tang et al. (2011) estimated that deep convection capable of penetrating the tropopause can increase the overall downward flux of $O_3$ by ~20%. This mechanism operates mainly during summer in

contrast with tropopause folding which is at a maximum from late winter through spring and at lower latitudes. Yang et al. (2010) estimated that roughly 20% of free tropospheric $O_3$ above coastal California in 2005 and 2006 was stratospheric in origin. Some of this $O_3$ could also contribute to $O_3$ at the surface.

It should be noted that there is considerable uncertainty in the magnitude and distribution of this potentially important source of tropospheric $O_3$. Stratospheric intrusions that reach the surface are much less frequent than intrusions which penetrate only to the middle and upper troposphere. However, $O_3$ transported to the upper and middle troposphere can still affect surface concentrations through various exchange mechanisms that mix air from the free troposphere with air in the PBL.

Several instances of STE producing high concentrations of $O_3$ around Denver and Boulder, Colorado, were analyzed by Langford et al. (2009) and several likely instances of STE, including one of the cases analyzed by Langford et al. (2009) were also cited in Annex AX2-3 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b). Clear examples of STE have also been observed in southern Quebec province by Hocking et al. (2007), in accord with previous estimates by Wernli and Bourqui (2002) and James et al. (2003). As also noted in the 2006 $O_3$ AQCD (U.S. EPA, 2006b), the identification of stratospheric $O_3$ and the calculation of its contributions to ambient air requires data for other tracers of stratospheric origin. In some cases, stratospheric $O_3$ intrusions can be identified based on measurements of low relative humidity, high potential vorticity and low ratios of $O_3$/PM. Strong stratospheric $O_3$ intrusion events that typically occur during winter or spring have been readily identified using these types of data (Langford et al., 2009). However, it remains challenging to accurately estimate the contributions from smaller direct or indirect (i.e., resulting from shallow intrusions into the mid and upper troposphere that are then mixed downward into the planetary boundary layer) contributions of stratospheric $O_3$ to ambient air.

#### 3.4.1.2 Contributions from Other Natural Sources

Biomass burning consists of wildfires and the intentional burning of vegetation to clear new land for agriculture and for population resettlement; to control the growth of unwanted plants on pasture land; to manage forest resources with prescribed burning; to dispose of agricultural and domestic waste; and as fuel for cooking, heating, and water sterilization. Biomass burning also exhibits strong seasonality and interannual variability (van der Werf et al., 2006), with most biomass burned during the local dry season. This is true for both prescribed burns and wildfires. Globally, most wildfires may be ignited directly as the result of human activities, leaving only 10-30% initiated by lightning (Andreae, 1991). However, because fire management practices suppress natural wildfires, the buildup of fire fuels increases the susceptibility of forests to more severe but less frequent fires. Thus there is considerable uncertainty in attributing the fraction of wildfire emissions to human activities because the emissions from naturally occurring fires that would have been present in the absence of fire suppression practices are not known. Contributions to

$NO_X$, CO, and VOCs from wildfires and prescribed fires are considered as precursors to background $O_3$ formation in this assessment.

Estimating contributions from wildfires is subject to considerable uncertainty. McDonald-Buller et al. (2011) note that "Models generally find little $O_3$ production in wildfire plumes for short aging times (days) because $NO_X$ emissions are low and conversion to peroxyacetylnitrate (PAN) is rapid. In contrast, observations show large $O_3$ production from at least some regional wildfires that may significantly elevate $O_3$ at low altitude sites on a monthly basis, and persist over long distances from the burned region." They also note that fire plumes transported on intercontinental scales can contain very high $O_3$ concentrations. However Singh et al. (2010b) found appreciable increases of $O_3$ in California fire plumes only when they are mixed with urban pollution. Jaffe and Wigder (2012) note that this result could have also been due to suppression of $O_3$ production near the source. Indeed, Singh et al. (2012) note that low $O_3$ found in the California fire plumes of 2008 were associated with low concentrations of $NO_X$ and that $NO_X$ from urban emissions was needed to produce high $O_3$. Factors such as the stage of combustion (smoldering to flaming), fuel nitrogen content, ambient meteorological conditions, and the availability of solar ultraviolet radiation need to be considered when evaluating the potential of fires for producing $O_3$.

Jaffe et al. (2008) examined the effects of wildfires on $O_3$ in the western United States. They found a strong relation ($R^2 = 0.60$) between summer mean $O_3$ measured at various national park and CASTNET sites and area burned in the western United States. They also found generally higher concentrations within surrounding 5° × 5° and 10° × 10° of burned areas. Smaller correlations were found within the surrounding 1°× 1° areas, reflecting near source consumption of $O_3$ and the time necessary for photochemical processing of emissions to form $O_3$. Jaffe et al. (2008) estimate that burning 1 million acres in the western U.S. during summer results in an increase in $O_3$ of 2 ppb across the region; this translates to an average $O_3$ increase across the entire western U.S. of 3.5 and 8.8 ppb during mean and maximum fire years. The unusually warm and dry weather in central Alaska and western Yukon in the summer of 2004 contributed to the burning of 11 million acres there. Subsequent modeling by Pfister et al. (2005) showed that the CO contribution from these fires in July 2004 was 33.1 (± 5.5) MT that summer, roughly comparable to total U.S. anthropogenic CO emissions during the same period.

These results underscore the importance of wildfires as a source of important $O_3$ precursors. In addition to emissions from forest fires in the U.S., emissions from forest fires in other countries can be transported to the U.S., for example from boreal forest fires in Canada (Mathur, 2008), Siberia (Generoso et al., 2007) and tropical forest fires in the Yucatan Peninsula and Central America (Wang et al., 2006). These fires have all resulted in notable increases in $O_3$ concentrations in the U.S.

Estimates of biogenic VOC, NO, and CO emissions can be made using the BEIS model with data from the BELD and annual meteorological data or MEGAN. VOC emissions from vegetation were described in Section 3.2.

As discussed in Section 3.2.1, $NO_X$ is produced by lightning. Kaynak et al. (2008) found lightning contributes 2 to 3 ppb to surface-level background $O_3$ centered mainly over the southeastern U.S. during summer. Although total column estimates of lightning produced $NO_X$ are large compared to anthropogenic $NO_X$ during summer, lightning produced $NO_X$ does not contribute substantially to the $NO_X$ burden in the continental boundary layer. For example, (Fang et al., 2010) estimated that only 2% of $NO_X$ production by lightning occurs within the boundary layer and most occurs in the free troposphere. In addition, much of the $NO_X$ produced in the free troposphere is converted to more oxidized N species during downward transport. Note that contributions of natural sources to North American background arise from everywhere in the world.

### 3.4.2 Contributions from Anthropogenic Emissions

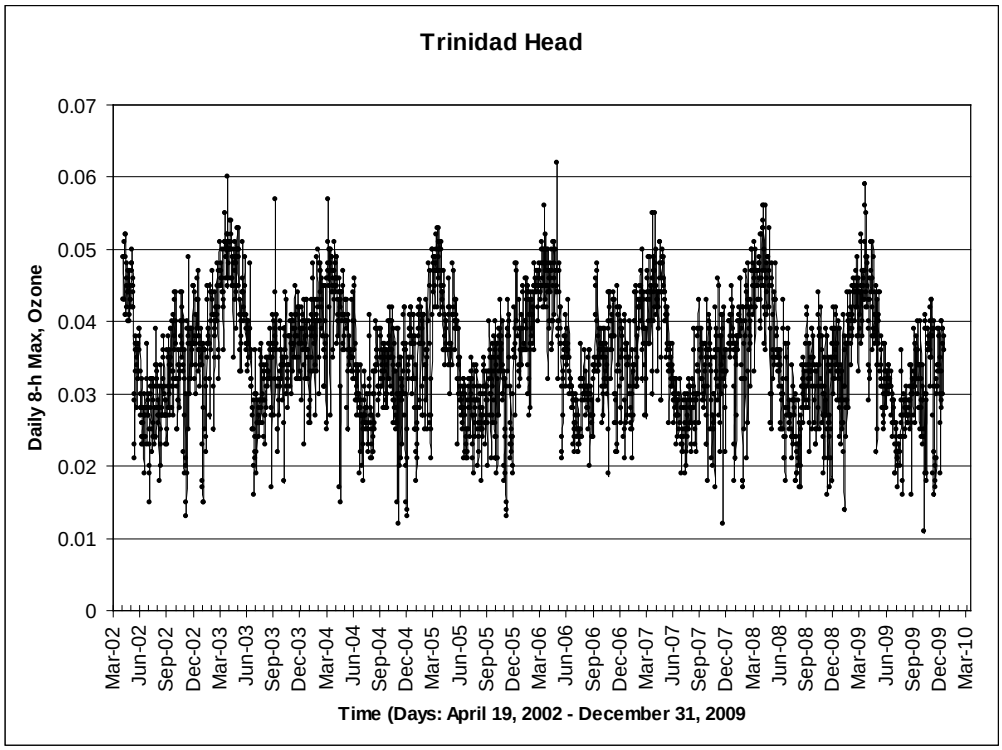
In addition to emissions from North America, anthropogenic emissions from Eurasia have contributed to the global burden of $O_3$ in the atmosphere and to the U.S. (NRC, 2009, and references therein). Because the mean tropospheric lifetime of $O_3$ is on the order of a few weeks (Hsu and Prather, 2009), $O_3$ can be transported from continent to continent and around the globe in the Northern Hemisphere. Ozone produced by U.S. emissions can, therefore, be recirculated around northern mid-latitudes back to the United States. High elevation sites are most susceptible to the intercontinental transport of pollution especially during spring. For example, a number of occurrences of $O_3$>60 ppb from mid-April to mid-May of 2006 were observed at Mt. Bachelor Observatory, OR (elevation 2,700 meters) with a maximum $O_3$ concentration of ~85 ppb observed on April 22, 2006. Calculations using GEOS-Chem, a global-scale CTM, indicate that Asia contributed 9 ± 3 ppb to a modeled mean concentration of 53 ± 9 ppb $O_3$ at Mt. Bachelor during the same period compared to measured concentrations of 54 ± 10 ppb (Zhang et al., 2008). Zhang et al. (2008) also calculated a contribution of 5 to 7 ppb to surface $O_3$ over the western U.S. during that period from Asian anthropogenic emissions. They also estimated an increase in $NO_X$ emissions of ~44% from Asia from 2001 to 2006 resulting in an increase of 1-2 ppb in $O_3$ over North America.

Cooper et al. (2010) analyzed all available $O_3$ measurements in the free troposphere above western North America at altitudes of 3-8 km (above sea level) during April and May of 1995 to 2008 (i.e., times when intercontinental transport is most prominent). They derived a trend of +0.63 ± 0.34 ppb/year in median $O_3$ concentrations with indication of a similar rate of increase since 1984. Back trajectories that were likely to have been strongly and recently influenced by North American emissions were filtered out, resulting in a trend of +0.71 ± 0.45 ppb/year. Considering only trajectories with an Asian origin resulted in a trend of +0.80 ± 0.34 ppb/year. These results suggest that local North American emissions were not responsible for the measured $O_3$ increases. This $O_3$ could have been produced from natural and anthropogenic precursors in Asia and Europe with some contribution from North American emissions that have circled the globe. Cooper et

al. (2010) also found that it is unlikely that the trends in tropospheric $O_3$ are associated with trends in stratospheric intrusions. Note, however, that these results relate to $O_3$ trends above ground level and not to surface $O_3$. Model results (Zhang et al., 2008) show that surface $O_3$ contributions from Asia are much smaller than those derived in the free troposphere because of dilution and chemical destruction during downward transport to the surface. These processes tend to reduce the strength of associations between free tropospheric and surface $O_3$ especially if air from other sources is sampled by the surface monitoring sites.

Trinidad Head, CA is one sampling location at which measurements might be expected to reflect in large measure NA background $O_3$ contributions, at times during the spring (Oltmans et al., 2008; Goldstein et al., 2004). The monitoring station at Trinidad Head is on an elevated peninsula extending out from the mainland of northern California, and so might be expected at times to intercept air flowing in from the Pacific Ocean with little or no influence from sources on the mainland. Figure 3-8 shows the time series of MDA8 $O_3$ concentrations measured at Trinidad Head from April 18, 2002 through December 31, 2009. The data show pronounced seasonal variability with spring maxima and summer minima. Springtime concentrations typically range from 40 to 50 ppb with a number of occurrences >50 ppb. The two highest daily maxima were 60 and 62 ppb. The data also show much lower concentrations during summer, with concentrations typically ranging between 20 and 30 ppb. Oltmans et al. (2008) examined the time series of $O_3$ and back trajectories reaching Trinidad Head. They found that springtime maxima (April-May) were largely associated with back trajectories passing over the Pacific Ocean and most likely entraining emissions from Asia, with minimal interference from local sources. However, Parrish et al. (2009) noted that only considering trajectories coming from a given direction is not sufficient for ruling out local continental influences, as sea breeze circulations are complex phenomena involving vertical mixing and entrainment of long-shore components. They found that using a wind speed threshold in addition to a criterion for wind direction allowed determination of background trajectories not subject to local influence. This was confirmed by measurements of chemical tracers of local influence such as $CO_2$, MTBE and radon. By applying the two criteria for wind speed and direction, they found that Trinidad Head met these criteria only 43% of the time during spring. Goldstein et al. (2004) used $CO_2$ as an indicator of exchange with the local continental environment and found that $O_3$ concentrations were higher by about 2-3 ppb when filtered against local influence indicating higher $O_3$ in air arriving from over the Pacific Ocean. At other times of the year, Trinidad Head is less strongly affected by air passing over Asia and the northern Pacific Ocean; and many trajectories have long residence times over the semi-tropical and tropical Pacific Ocean where $O_3$ concentrations are much lower than they are at mid-latitudes. The use of the Trinidad Head data to derive contributions from background sources requires the use of screening procedures adopted by Parrish et al. (2009) and the application of photochemical models to determine the extent either of titration of $O_3$ by fresh $NO_X$ emissions and the extent of local production of $O_3$ from these emissions. Although $O_3$ concentrations at Trinidad Head might at times be representative of Pacific air arriving over the

U.S., they cannot be viewed as NA background over the continental U.S. because of deposition to the surface and chemical loss over the continental United States. As noted above, anthropogenic emissions from North America also contribute to hemispheric background and must be filtered out from observations at coastal sites such as Trinidad Head even when it is thought that air sampled came directly from over the Pacific Ocean and was not influenced by local pollutant emissions.




Source: Reprinted with permission of Elsevier Ltd., (Oltmans et al., 2008); and NOAA Climate Monitoring Diagnostics Laboratory for data from 2008-2009.

**Figure 3-8 Time series of MDA8 $O_3$ concentrations (ppm) measured at Trinidad Head, CA, from April 18, 2002 through December 31, 2009.**

Parrish et al. (2009) also examined data obtained at other marine boundary layer sites on the Pacific Coast. These include Olympic NP, Redwood NP, Point Arena, and Point Reyes. Using data from these sites, they derived trends in $O_3$ of +0.46 ppb/year (with a 95% confidence interval of 0.13 ppb/year) during spring and +0.34 ppb/year (0.09 ppb/year) for the annual mean $O_3$ increase in air arriving from over the Pacific during the past two decades. Although $O_3$ data are available from the Channel Islands, Parrish et al. (2009) noted that these data are not suitable for determining background influence because of the likelihood of circulating polluted air from the South Coast Basin.

The 2010 Intercontinental Chemical Transport Experiment Ozone Network Study (IONS-2010) and Research at the Nexus of Air Quality and Climate Change (CalNex) study conducted in May through June of 2010 had discerning the contributions of Asian emissions to air quality in California as a major focus. Cooper et al. (2011) examined $O_3$ profiles measured above four coastal sites in California, including Trinidad Head. Based on trajectory analyses coupled with comparison with the $O_3$ profiles, they suggested that Asian pollution, stratospheric intrusions, and international shipping made substantial contributions to lower tropospheric $O_3$ (typically 0 to 3 km above sea level, meant as a rough approximation of planetary boundary layer height) measured at inland California sites. These contributions tended to increase on a relative basis in going from south to north. In particular, no contribution from local pollution was needed to explain lower tropospheric $O_3$ in the northern Central Valley; and the contribution of local pollution to lower tropospheric $O_3$ in the LA basin ranged from 32 to 63% (depending on layer depth; either 0 to 1.5 km or 0 to 3 km). It should be noted that the extent of photochemical production and loss occurring in the descending air masses between the coastal and inland sites remains to be determined. Cooper et al. (2011) also note that very little of the $O_3$ observed above California reaches the eastern United States. However, this does not necessarily mean that the pathways by which Asian $O_3$ could reach the eastern U.S. were fully captured in this analysis.

Lin et al. (2012) used the AM3 model (~50 × 50 km resolution globally) and satellite data to characterize the influence of Asian emissions and stratospheric intrusions on $O_3$ concentrations in southern California and Arizona during CalNex (May-June 2010). The model simulates sharp $O_3$ gradients in the upper troposphere and the interweaving and mixing of stratospheric air and Asian plumes. Similar phenomena were also found during field campaigns conducted in the North Atlantic as noted in Annex AX2.3.1 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b) and introduces uncertainty into attempts to attribute $O_3$ to these sources, based solely on observations, because this mixing will affect relationships between CO (mainly a marker for polluted air that is commonly used to separate air influenced by anthropogenic pollution from stratospheric air) and $O_3$ (a pollutant and a stratospheric component). Lin et al. (2012) found that Asian emissions contributed from 20-30% to $O_3$ in the mid troposphere over the California coast and remnants of stratospheric intrusions contributed from 50 to 60% to $O_3$ in discrete layers in the same altitude range. This $O_3$ then has the potential to mix downward into the planetary boundary layer. Lin et al. (2012) also found evidence of Asian contributions of up to 8 -15 ppb in surface air during strong transport events in southern California. These contributions are in addition to contributions from dominant local pollution sources. Their results suggest that the influence of background sources on high surface $O_3$ concentrations is not always confined to high elevation sites. However, it is not clear to what extent the contributions inferred by Cooper et al. (2011) and Lin et al. (2012) are likely to be found in other years or how long they would extend into summer.

### 3.4.3 Estimating Background Concentrations

Historically, two approaches to estimating NA background concentrations (previously referred to as PRB) have been considered in past $O_3$ assessments. In the 1996 and earlier $O_3$ AQCDs, measurements from remote monitoring sites were used. In the 2006 $O_3$ AQCD, the use of CTMs was adopted, because as noted in Section 3.9 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b), estimates of background concentrations cannot be obtained directly by examining measurements of $O_3$ obtained at relatively remote monitoring sites in the U.S. because of the long-range transport from anthropogenic source regions within North America. The 2006 $O_3$ AQCD (U.S. EPA, 2006b) also noted that it is impossible to determine sources of $O_3$ without ancillary data that could be used as tracers of sources or to calculate photochemical production and loss rates. As further noted by Reid et al. (2008), the use of monitoring data for estimating background concentrations is essentially limited to the edges of the domain of interest. The current definition of NA background implies that only CTMs (see Section 3.3 for description and associated uncertainties) can be used to estimate the range of background concentrations. An advantage to using models is that the entire range of $O_3$ concentrations measured in different environments can be used to evaluate model performance. In this regard, data from the relatively small number of monitoring sites at which large background contributions are expected are best used to evaluate model predictions.

Estimates of NA background concentrations in the 2006 $O_3$ AQCD (U.S. EPA, 2006b) were based on output from the GEOS-Chem (v4.3.3) model (Fiore et al., 2003) with 2° × 2.5° horizontal resolution. The GEOS-Chem model estimates indicated that NA background $O_3$ concentrations in eastern U.S. surface air were 25 ± 10 ppb (or generally 15-35 ppb) from June through August, based on conditions for 2001. Values reported by Fiore et al. (2003) represent averages from 1 p.m. to 5 p.m.; all subsequent values given for background concentrations refer to MDA8 $O_3$ concentrations. Background concentrations decline from spring to summer. Background $O_3$ concentrations may be higher, especially at high altitude sites during the spring, due to enhanced contributions from (1) pollution sources outside North America; and (2) stratospheric $O_3$ exchange. At the time, only the GEOS-Chem model (Harvard University, 2010b) was documented in the literature for calculating background $O_3$ concentrations (Fiore et al., 2003). The simulated monthly mean concentrations in different quadrants of the U.S. were typically within 5 ppbv of observations at CASTNET sites, with no discernible bias, except in the Southeast in summer when the model was 8-12 ppbv too high. This bias was attributed to excessive background $O_3$ transported in from the Gulf of Mexico and the tropical Atlantic Ocean in the model (Fiore et al., 2003).

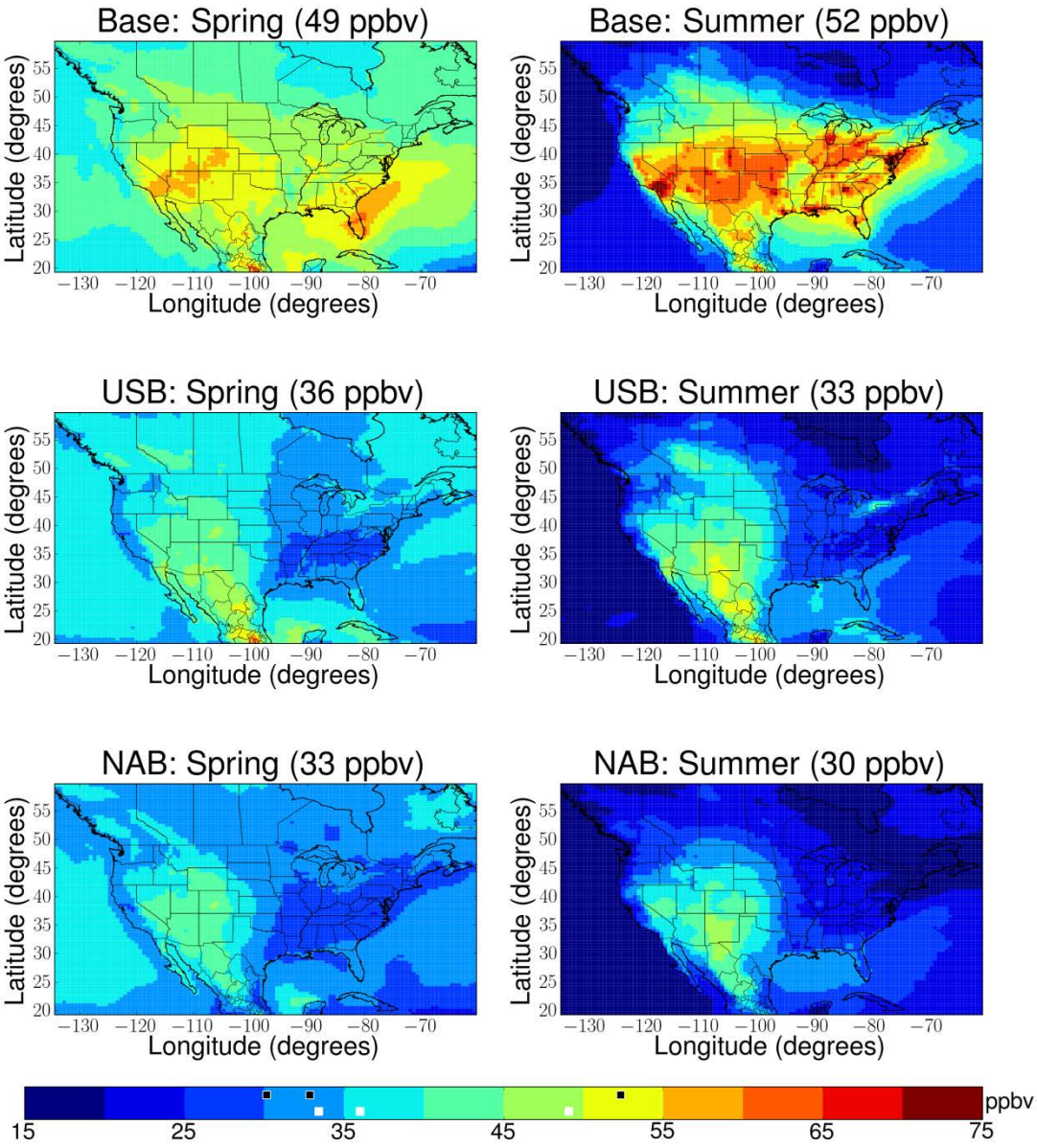
Although many of the features of the day-to-day variability in $O_3$ at relatively remote monitoring sites in the U.S. were simulated reasonably well by GEOS-Chem (Fiore et al., 2003), uncertainties in the calculation of the temporal variability of $O_3$ originating from different sources on shorter time scales must be recognized. The uncertainties stem in part from an underestimate in the seasonal variability in the

STE of $O_3$ (Fusco and Logan, 2003), the geographical variability of this exchange, and the variability in the exchange between the free troposphere and the PBL in the model. In addition, the relatively coarse spatial resolution in that version of GEOS-Chem (2° × 2.5°) limited the ability to provide separate estimates for cities located close to each other, and so only regional estimates were provided for the 2006 $O_3$ AQCD (U.S. EPA, 2006b) based on the results of Fiore et al. (2003).

Wang et al. (2009a) recomputed NA background concentrations for 2001 using GEOS-Chem (v7-01-01) at higher spatial resolution (1° × 1°) over North America and not only for afternoon hours but for the daily maximum 8-h avg $O_3$ concentration. The resulting background concentrations, 26.3 ± 8.3 ppb for summer, are consistent with those of 26 ± 7 ppb for summer reported by Fiore et al. (2003), suggesting horizontal resolution was not a substantial factor limiting the accuracy of the earlier results. In addition to computing NA background concentrations, Wang et al. (2009a) also computed U.S. background concentrations of 29.6 ± 8.3 ppb with higher concentrations in the Northeast (up to 15 ppb higher) and the Southwest (up to 13 ppb higher) for summer means.

#### 3.4.3.1 Updated GEOS-Chem Model Estimates of Background Concentrations

Zhang et al. (2011) computed NA background, U.S. background and natural background $O_3$ concentrations using GEOS-Chem (v8-02-03) at an even finer grid spacing of 0.5° × 0.667° over North America for 2006 through 2008. For March through August 2006, mean NA background $O_3$ concentrations of 29 ± 8 ppb at low elevation (<1,500 meters) and 40 ± 8 ppb at high elevation (>1,500 meters) were predicted. Spring and summer mean $O_3$ concentrations calculated for the base case (i.e., including all natural and anthropogenic sources worldwide), U.S. background, and NA background in surface air for spring and summer 2006 calculated by Zhang et al. (2011) are shown in the upper, middle and lower panels of Figure 3-9.




Note: Values in parentheses refer to continental U.S. means and are shown as black squares in the color bar for summer and white squares for spring.

Source: Adapted from Zhang et al. (2011).

**Figure 3-9 Mean MDA8 $O_3$ concentrations in surface air for spring and summer 2006 calculated by GEOS-Chem for the base case (Base), U.S. background (USB), and NA background (NAB).**

As noted above, Zhang et al. (2011) found increases in Asian emissions only accounted for an average increase of between 1 to 2 ppb in background $O_3$ across the U.S. even though Asian emissions have increased by about 44% from 2001 to 2006. As can be seen from Figure 3-9, U.S. background and NA background concentrations are very similar throughout most of the United States. Zhang et al. (2011) also found that NA background concentrations are ~4 ppb higher, on average, in the 0.5° × 0.667° version than in the coarser 2° × 2.5° version. This difference was partially due to higher resolution (~1 to 2 ppb) and the remainder to the combination of changes in lightning and Asian emission estimates as well as higher model resolution.

As can be seen from the middle and lower panels in Figure 3-9, U.S. background and NA background concentrations tend to be higher in the West, particularly in the Intermountain West and in the Southwest compared to the East in both spring and summer. U.S. background and NA background concentrations tend to be highest in the Southwest during summer in the GEOS-Chem model, driven by lightning $NO_X$.

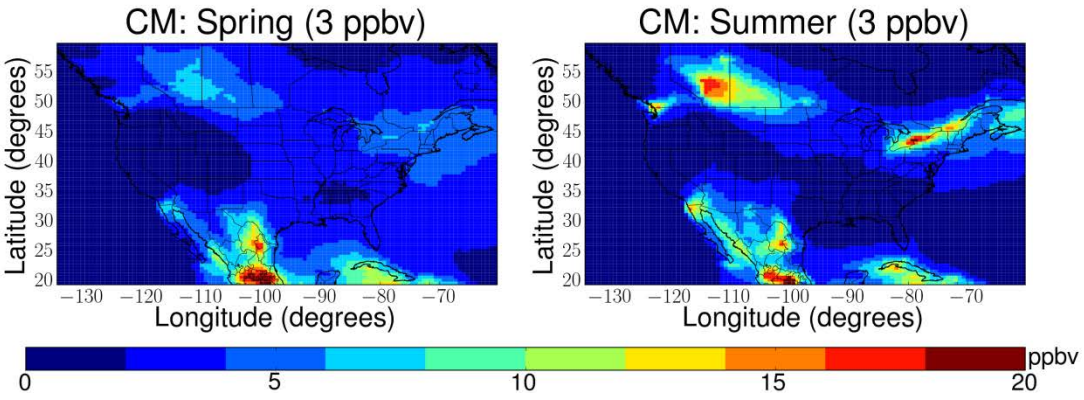
Intercontinental transport and stratospheric intrusions are major contributors to the high elevation, Intermountain West during spring with wildfires becoming more important sources during summer. The base case $O_3$ concentrations (upper panels) show two broad maxima with highest concentrations extending throughout the Southwest, Intermountain West, and the East; in both spring and summer. These maxima extend over many thousands of kilometers demonstrating that $O_3$ is a regional pollutant. Low-level outflow from the Northeast out over the Atlantic Ocean and from the Southeast out over the Gulf of Mexico is also apparent.

Note that background concentrations tend to increase with increasing base model (and measured) concentrations at higher elevation sites, particularly during spring. However, it is not only background $O_3$ that increases with elevation. Convection efficiently lifts precursor emissions and $O_3$ from the polluted boundary layer in North America as well as in Europe and Asia to the mid and upper troposphere. Significant production of $O_3$ from precursor emissions and from lightning can occur aloft, providing a diffuse source in the mid and upper troposphere because the $O_3$ production efficiency with respect to $NO_X$ is much higher aloft than at low elevations. Higher wind speeds aloft, coupled with a lifetime of $O_3$ that increases with height in the troposphere, allow pollutants to be transported much farther and to mix over wider areas than they would at lower elevations. Thus, through these mechanisms, $O_3$ aloft at a given location can be higher than at low elevations and caution should be observed in attempting to ascribe increases in $O_3$ with elevation to particular sources.

Although the results of Zhang et al. (2011) are broadly consistent with results from earlier coarser resolution versions of GEOS-Chem used by Fiore et al. (2003) and Wang et al. (2009a), there are some apparent differences. Concentrations of $O_3$ for both the base case and the NA background case in Zhang et al. (2011) are higher in the Intermountain West than in earlier versions. In addition, background concentrations in many eastern areas tend to be higher on days when predicted total

$O_3$ is > 60 ppb or at least do not decrease with increasing total $O_3$ Zhang et al. (2011).

Figure 3-10 shows seasonal mean estimates of contributions to $O_3$ from Canadian and Mexican emissions calculated by Zhang et al. (2011) as the difference between U.S. background and NA background values and then averaged over spring and summer following the procedure in Wang et al. (2009a). U.S. background concentrations are on average 3 ppb higher than NA background concentrations during spring and summer across the United States. Highest values in Figure 3-10 (in the U.S.) are found over the Northern Tier of New York State (19.1 ppb higher than NA background) in summer. High values are also found in other areas bordering Canada and Mexico. Although the contributions from Canada and Mexico were obtained by differencing, it should be remembered that relations between $O_3$ and precursors are subject to non-linear effects that are strongest near concentrated sources of precursors, as noted in Section 3.2.4. Therefore, the values shown in the figure are only estimates of contributions to total $O_3$ coming from Canada and Mexico.

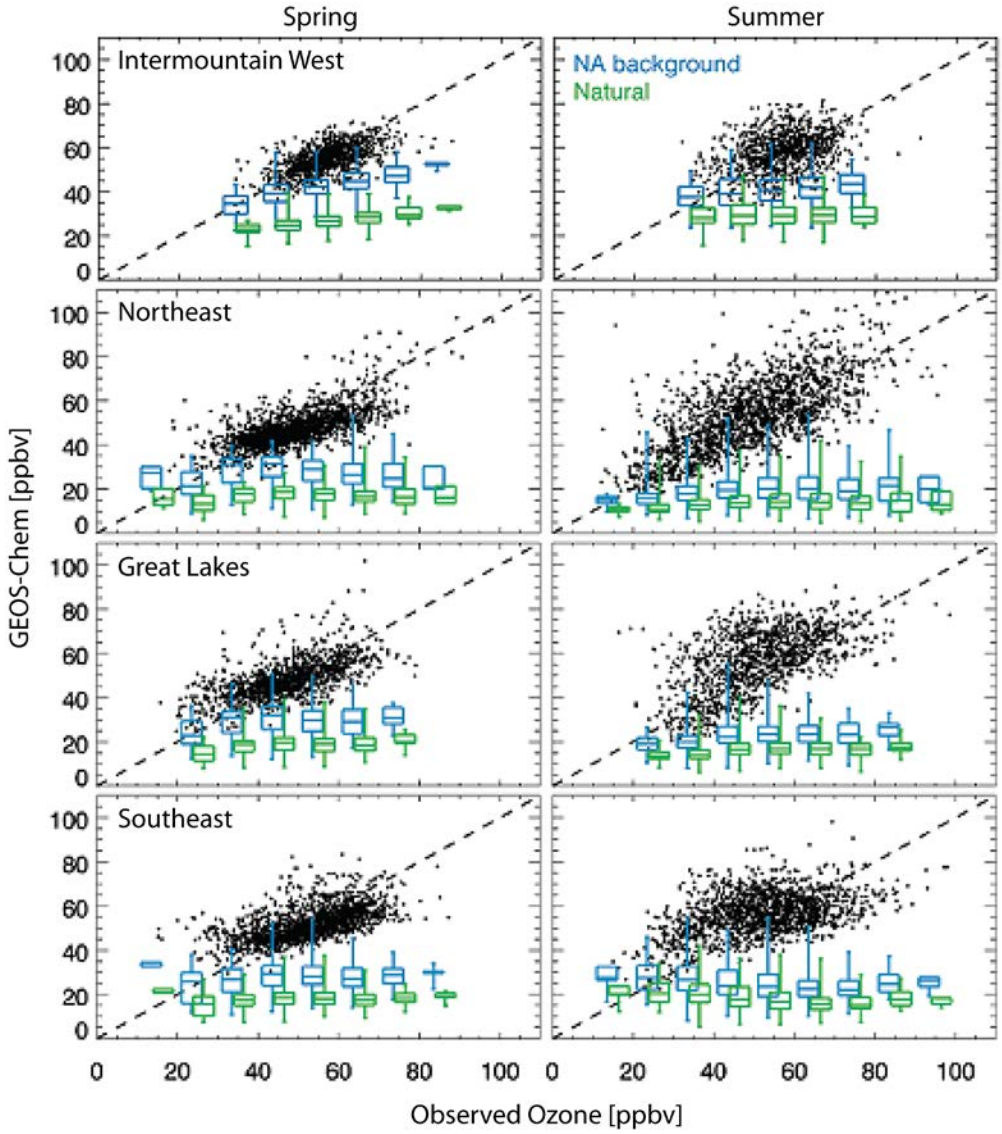



Note: Values in parentheses show mean difference (ppb) across the U.S.
Source: Adapted from Zhang et al. (2011).

**Figure 3-10 Spring and summer mean Canadian and Mexican (CM) contributions to MDA8 $O_3$ determined as the difference between the U.S. background and NA background.**

Figure 3-11 shows MDA8 $O_3$ concentrations for spring (March-May) and summer (June-August) 2006 simulated by GEOS-Chem (versus measured) by the ensemble of CASTNET sites in the Intermountain West, Northeast, Great Lakes, and Southeast (Zhang et al., 2011). Shown is the 1:1 line and NA background (blue) and natural background (green) model statistics as box plots (minimum, 25th, 50th, 75th

percentile, and maximum) for 10-ppbv bins of observed $O_3$ concentrations. These plots show that NA background constitute a larger fraction of modeled base case $O_3$ at the upper end of the concentration distribution for the Intermountain West than for other regions of the country.




Note: Shown is the 1:1 line and North American (NA) background (blue) and natural background (green) model statistics as box plots (minimum, 25th, 50th, 75th percentile, and maximum) for 10-ppbv bins of observed $O_3$ concentrations.

Source: Adapted from Zhang et al. (2011).

**Figure 3-11 MDA8 $O_3$ concentrations for spring (March-May) and summer (June-August) 2006 simulated by GEOS-Chem vs. measured by the ensemble of CASTNET sites in the Intermountain West, Northeast, Great Lakes, and Southeast.**

Comparisons between GEOS-Chem and measurements of the mean MDA8 $O_3$ between March and August at individual CASTNET sites across the country are shown as supplemental material in Section 3.8, Figure 3-58 through Figure 3-64. In general, the GEOS-Chem predictions tend to show better agreement with observations at the high-altitude sites than at the low-altitude sites. Overall agreement between model results for the base case and measurements is within a few parts per billion for spring-summer means in the Northeast (see Figure 3-58 in Section 3.8) and the Southeast (see Figure 3-59 in Section 3.8), except in and around Florida where the base case over-predicts $O_3$ by 10 ppb on average. In the Upper Midwest (Figure 3-60 in Section 3.8), the Intermountain West (Figure 3-61 and Figure 3-62 in Section 3.8), and the West (Figure 3-63 in Section 3.8) including most sites in California (Figure 3-64 in Section 3.8), the model predictions are within 5 ppb of measurements. The model under-predicts $O_3$ by 10 ppb at the Yosemite site (Figure 3-64 in Section 3.8). These results suggest that the model is capable of calculating March to August mean MDA8 $O_3$ to within ~5 ppb at most (26 out of 28) sites chosen.

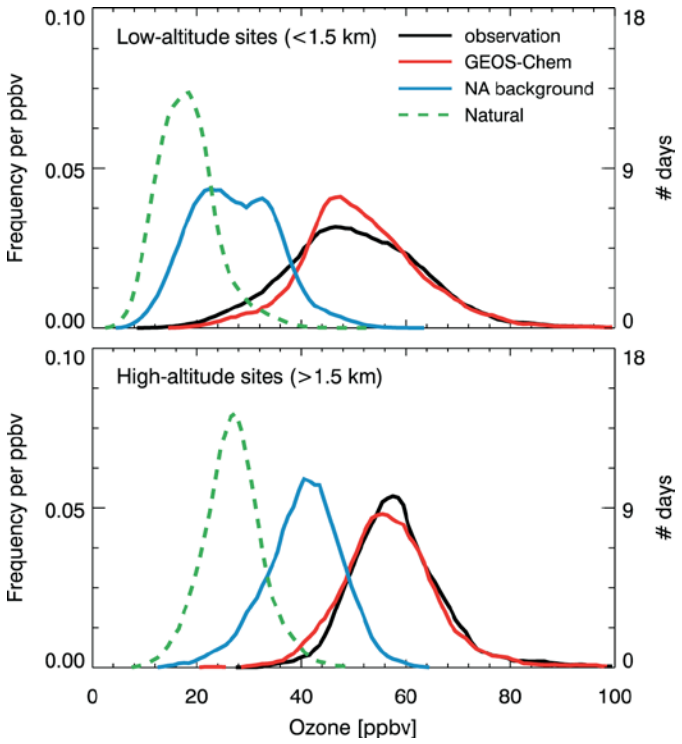
Comparison between results in Wang et al. (2009a) for 2001with data obtained in the Virgin Islands indicate that GEOS-Chem over-predicts summer mean MDA8 $O_3$ concentrations there by 10 ppb (28 vsersus 18 ppb). Ozone concentrations at the Virgin Islands NP site appear not to have been affected by U.S. emissions, based on the close agreement between the base case and the NA background case. Wind roses calculated for the Virgin Islands site indicate that wind patterns affecting this site are predominantly easterly/southeasterly in spring and summer. The over-predictions at the Virgin Islands site imply that modeled $O_3$ over the tropical Atlantic Ocean is too high. As a result, inflow of $O_3$ over Florida and into the Gulf of Mexico is also likely to be too high as winds are predominantly easterly at these low latitudes. Similar considerations apply to the results of Zhang et al. (2011). Possible explanations include deficits in model chemistry (for example, reactions involving halogens are not included) and/or subsidence that is too strong over tropical oceans in the model. No clear explanation can be provided on why the model under-predicts mean $O_3$ at Yosemite (elevation 1,680 meters) by ~10 ppb (see Figure 3-64 in Section 3.8). However, March to August mean MDA8 $O_3$ concentrations are simulated to within a few parts per billion at an even higher elevation site in California (Converse Station, elevation 1,837 meters) and at the low elevation sites.

Figure 3-65 in Section 3.8 shows a comparison of GEOS-Chem output with measurements at Mt. Bachelor, OR and Trinidad Head, CA from March-August, 2006 from Zhang et al. (2011). For the Mt. Bachelor model runs, model estimates are given for both a coarse (2° × 2.5°) and fine (0.5° × 0.667°) resolution model. In general, mean concentrations are simulated reasonably well at both coarse and finer grid resolution versions of the model with mean values 2 ppb higher in the finer resolution model. Although the finer resolution version provides some additional day to day variability and can capture the timing of peaks, it still does not adequately resolve peak concentrations as can be seen for an event in the second half of April.

Figure 3-66 in Section 3.8 shows a comparison of vertical profiles (mean ± 1σ) calculated by GEOS-Chem with ozonesondes launched at Trinidad Head, CA and Boulder, Colorado. As can be seen from the figure, variability in both model and measurements increases with altitude, but variability in the model results is much smaller at high altitudes than seen in the observations. This may be due in part to the inability of grid-point models to capture the fine-scale, layered structure often seen in $O_3$ in the mid and upper troposphere (Rastigejev et al., 2010; Newell et al., 1999) and to inadequacies in parameterizations of relevant chemistry and dynamics. Figure 3-67 and Figure 3-68 in Section 3.8 show a comparison of vertical profiles simulated by AM3 at 50 × 50 km global resolution (Lin et al., 2012) with ozonesondes launched at several locations in California during May-June 2010. Note that in contrast to comparing measured mean monthly $O_3$ profiles to monthly mean profiles calculated by GEOS-CHEM (see, for example, Figure 3-66 in Section 3.8), AM3 is sampled for comparison to individual measurements of $O_3$ profiles. This model has likely had the most success in simulating vertical $O_3$ gradients in the upper troposphere and in capturing layered structures in the mid and upper troposphere.

The natural background for $O_3$ averages 18 ± 6 ppbv at the low elevation sites and 27 ± 6 ppbv at the high elevation sites in the GEOS-Chem model Zhang et al. (2011). In regions where non-linear effects are small, far from concentrated sources of $O_3$ precursors, the difference between NA background and natural background $O_3$ concentrations provides an estimate of contributions from intercontinental pollution including anthropogenic methane (given by the difference between values in 2006 and the pre-industrial era, or 1,760 ppb and 700 ppb). The difference between the two backgrounds averages 9 ppbv at the low elevation sites and 13 ppbv at sites in the Intermountain West. Based on the Zhang et al. (2011) model runs, anthropogenic methane emissions are estimated to contribute ~4-5 ppb to global annual mean $O_3$ surface concentrations. North American emissions of methane are uncertain, but are a small fraction of total anthropogenic input. This suggests that slightly less than half of the difference between North American background and natural background is due to the increase of methane since the beginning of the industrial era and the other half is due to anthropogenic emissions of shorter lived VOCs and $NO_X$. However, the relative importance of methane for $O_3$ production is expected to increase in the future. Indeed, variations in methane concentrations account for approximately 75% of the wide spread (~5 ppb) in tropospheric $O_3$ projections between Representative Concentration Pathway (RCP) scenarios for the next century (Wild et al., 2012); see Section 10.3.6.1 in Chapter 10 for more on the RCP scenarios.

Figure 3-12 shows frequency distributions for observations at low-altitude and high-altitude CASTNET sites along with GEOS-Chem frequency distributions for the base case, NA background, and natural background. Most notable is the shift to higher concentrations and the narrowing of the concentration distributions for all three simulations and the observations in going from low to high altitudes. However, maximum concentrations show little if any dependence on altitude, except for the natural background (which tends to be slightly higher at high altitude sites).




Note: Observations (black) as well as GEOS-Chem estimates for the base case (red), NA background (blue), and natural background (green dashed).

Source: Zhang et al. (2011).

**Figure 3-12 Frequency distributions of MDA8 $O_3$ concentrations in March-August 2006 for the ensemble of low-altitude (<1,500 meters) and high-altitude CASTNET sites (>1,500 meters) in the U.S.**

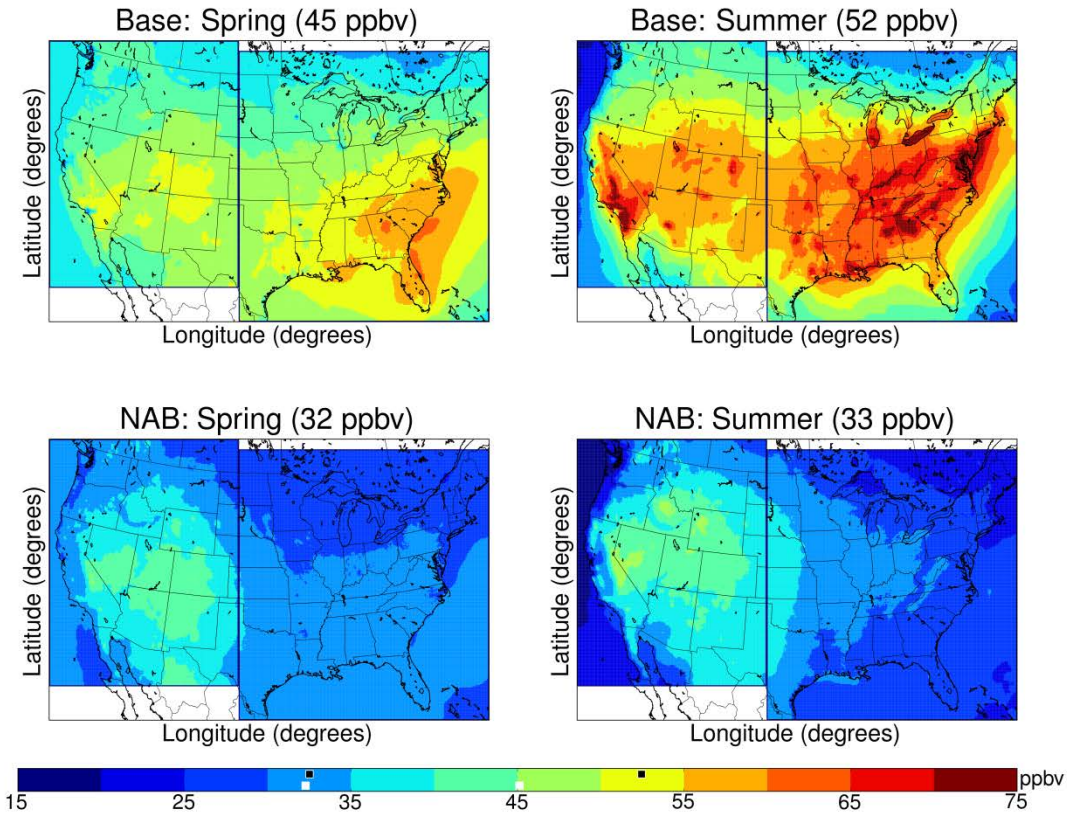
### 3.4.3.2 Using Other Models to Estimate Background Concentrations

Another approach to modeling background concentrations involves using a regional CTM such as CMAQ or CAMx with boundary conditions taken from a global scale CTM such as GEOS-Chem (see Section 3.3 for discussion of this approach). Mueller and Mallard (2011a), while not calculating NA background values exactly as defined here, calculated contributions from natural sources and inflow from the boundaries to $O_3$ for 2002 using MM5 and CMAQ for the outermost domain (36 km resolution) shown in Figure 3-4 with boundary conditions from GEOS-Chem. The overall bias based on comparison with AQS monitors for the base case is about 3 ppb; the annual mean fractional bias and mean fractional error were 7% and 21% for the ozone season across the United States. Note that Figure 2 in their paper is mislabeled, as it

should refer to the case with total emissions - not to natural emissions in North America only (Mueller and Mallard, 2011b). However, boundary conditions are fixed according to monthly averages based on an earlier version of GEOS-Chem and do not reflect shorter term variability or trends in Northern Hemispheric emissions of pollution. In addition, fluxes of $O_3$ from the stratosphere are not included explicitly. Note that their natural background includes North American natural background emissions only and influence from boundary conditions and thus is not a global natural background. Calculated values including natural emissions from North America and from fluxes through the boundaries are somewhat larger than given in Zhang et al. (2011), in large measure because of much larger contributions from wildfires and lightning. Wildfire contributions reach values of ~140 ppb in Redwoods National Park, CA and higher elsewhere in the U.S. and in Quebec in the simulations by Mueller and Mallard (2011a). Lightning contributions (ranging up to ~30 ppb) are substantially larger than estimated by Kaynak et al. (2008) (see Section 3.4.1.2). The reasons for much larger contributions from wildfires and lightning found by Mueller and Mallard (2011a) are not clear and need to be investigated further.

Emery et al. (2012) used CAMx with boundary conditions taken from the coarse resolution version of GEOS-Chem (2 × 2.5° or ~200 km resolution) to derive NA background concentrations of $O_3$. The nested CAMx simulations were run at a horizontal resolution of 12 km separately for the eastern and western United States. The following paragraphs compare results from the Emery et al. (2012) nested CAMx simulations at 12 km resolution, with those obtained by Zhang et al. (2011) using GEOS-Chem simulations at 0.5° × 0.667° (~50 km) resolution. This is in contrast to the comparison reported in Emery et al. (2012) in which results from CAMx at 12 km resolution were compared to results from the 2° × 2.5° (~200 km) resolution version of the GEOS-Chem model over the United States.

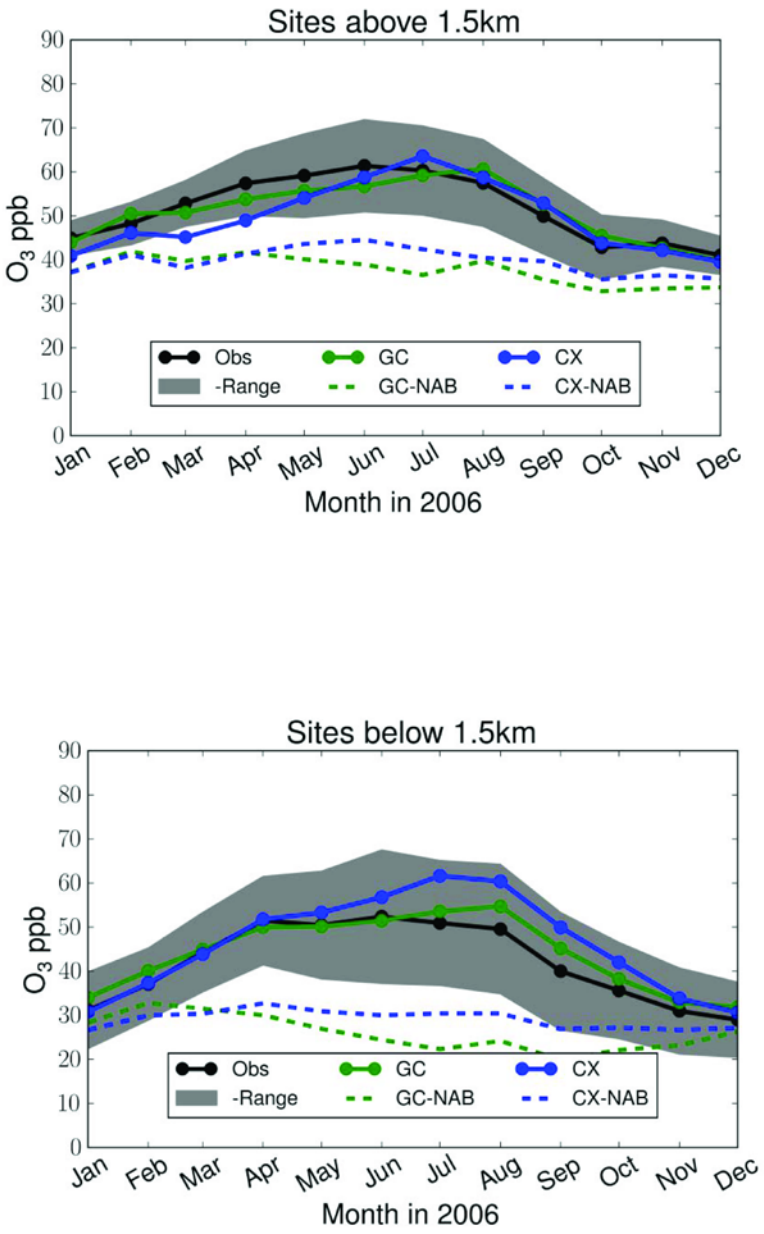
Figure 3-13 shows seasonal mean MDA8 $O_3$ concentrations calculated by Emery et al. (2012) using CAMx for 2006 for the base case and for NA background. Figure 3-14 shows a comparison of monthly average $O_3$ concentrations calculated by GEOS-Chem (Zhang et al., 2011) with those calculated by CAMx (Emery et al., 2012). Comparison of the base case for GEOS-Chem with that for CAMx in Figure 3-14 indicates broad agreement in spatial patterns.




Note: Values in parentheses refer to continental U.S. means and are shown as black squares in the color bar for summer and white squares for spring.

Source: Adapted from Emery et al. (2012).

**Figure 3-13 Mean MDA8 $O_3$ concentrations in surface air during spring and summer 2006 (top) calculated by GEOS-Chem/CAMx for the base case (Base, top) and NA background (NAB, bottom).**




Note: Shaded area shows 1 SD range about the mean of observations.

Source: Adapted [with permission of Elsevier, Emery et al. (2012)] and from Zhang et al. (2011).

**Figure 3-14 Monthly average MDA8 $O_3$ concentrations observed (Obs) and predicted for the base case and NA background (NAB) by GEOS-Chem (GC) and GEOS-Chem/CAMx (CX) at CASTNET sites above 1,500 meters elevation (upper panel) and CASTNET sites below 1,500 meters elevation (lower panel).**

Supplemental figures (Figure 3-69 through Figure 3-74) in Section 3.8 show box plots comparing MDA8 $O_3$ concentrations calculated by GEOS-Chem at 0.5° × 0.667° resolution and CAMx for March-August 2006 at the combined set of CASTNET sites used by both groups for model evaluation. Note that the individual model results and the observations are unpaired in time. At CASTNET sites in the Northern Rockies, both models tend to under-predict maximum $O_3$ concentrations, but they are generally higher in CAMx than in GEOS-Chem (typically by 5-10 ppb). The distribution of MDA8 values from GEOS-Chem is consistent with measured distributions (i.e., cannot be rejected using Mann-Whitney rank sum test, p-value <0.01) at 18 of 39 sites in spring and 21 of 39 sites in summer. The distribution of MDA8 values from CAMx is consistent with measured distributions at 13 of 39 sites in spring and 18 of 39 sites in summer. When spring and summer are pooled, both simulations are consistent with measured distributions at 16 out of 39 sites (but not the same 16 sites). There are examples in which either model over- or under-simulates maximum concentrations. However, over-predictions are made more often by CAMx. At high elevations in the Intermountain West (see Figure 3-72 in Section 3.8), both models tend to under-predict maxima, but their interquartile range agrees much better with observations. As McDonald-Buller et al. (2011) noted, complex topography in some regions of the U.S. could influence surface $O_3$ through fine-scale, orographically induced flow regimes. In addition, numerical diffusion broadly affects the ability of models to capture observed maxima, particularly at mountain sites. McDonald-Buller et al. (2011) also note there are regions in the U.S. where global models show consistent biases. For example, models are generally unable to simulate the low $O_3$ concentrations observed at Gulf Coast sites in summer during onshore flow from the Gulf of Mexico, which could reflect marine boundary layer chemistry and/or stratification that is not properly represented in the model. Both models over-predict $O_3$ at two sites in Florida (Sumatra and Indian River Lagoon). However, further inland, CAMx tended to over-predict $O_3$ at the Coffeeville, MS; Sand Mountain, AL; and Georgia Station, GA sites whereas GEOS-Chem did not. The same is true for higher elevation sites (Great Smoky Mountain, NC-TN; Shenandoah, VA). In the Northeast, there is a general tendency for both models to over-predict the measured distributions with somewhat higher maximum concentrations in CAMx compared to GEOS-Chem and observations (see Figure 3-69 to Figure 3-74 in Section 3.8).

The most readily discernible differences in model formulation are in the model grid spacing and the treatment of wildfires. The finer resolution in CAMx allows for topography to be better-resolved producing higher maximum $O_3$ concentrations in the Intermountain West. For wildfires, treatment differences include emission composition, emission time averaging, and associated chemistry. Wildfires produce more $O_3$ in CAMx simulations than in GEOS-Chem simulations, and Emery et al. (2012) attribute these enhancements to shorter emission time averaging. The CAMx emissions average fire emissions at hourly resolution based on the SmartFire algorithm, whereas GEOS-Chem uses monthly averages from GFED2. Each model representation also uses different emission compositions. The emissions used by Emery et al. (2012) include a larger number of VOCs and additional categories of VOCs than used by Zhang et al. (2011). Following emission, Emery et al. (2012)

note that photochemical aging of wildfire emissions depends on the chemical mechanism. Neither chemical mechanism was designed specifically for these type of events. GEOS-Chem has traditionally focused on the chemistry of the non-urban troposphere and does not represent secondary products of fast reacting VOCs as does CB05. A lack of reactivity of secondary products would cause a dampening of fire contributions to $O_3$. CB05 has traditionally focused on urban chemistry and does not explicitly includes ketones (Henderson et al., 2011), which are among the top ten VOCs emitted from fires (Andreae and Merlet, 2001). The $O_3$ increases seen in Emery et al. (2012) and Mueller and Mallard (2011a), however, are subject to uncertainties in the representation of physics in the wildfire plumes.
The improvements in characterizing emissions would lead to smoke plumes that attenuate light, thereby reducing photolysis and photoreactivity (e.g., Real et al., 2007). The wildfires would also alter temperature and convective activity that influences plume rise and the height of the planetary boundary layer. Emery et al. (2012) note the need for more research to improve simulation of $O_3$ from fires. Using a sensitivity analysis of CAMx, the authors showed that removing wildfires in the West (California, Oregon, and Idaho) resulted in reductions of NA background $O_3$ of 10 to 50 ppb, with smaller reductions elsewhere. Further, Emery et al. (2012) note that their calculated $O_3$ increases in the vicinity of wildfires is consistent with that of Mueller and Mallard (2011a).

Emery et al. (2012) captured the timing of a possible stratospheric intrusion at Gothic, Colorado, on April 19-20, 2006, and predicted an MDA8 $O_3$ value of ~73 ppb using CAMx on April 20, compared to a measured observation value of 87 ppb. GEOS-Chem (at 0.5° × 0.667°) predicted ~65 ppb for this event. The higher spatial resolution in CAMx likely contributed to the improvement in model performance, but this may not be the only factor. AM3, another global scale CTM (Lin et al., 2012) at ~2° × 2.5° resolution predicted ~75 ppb for that event; suggesting that differences in dynamical cores between WRF and AM3, different treatments of the stratospheric $O_3$ source, and perhaps the spatial extent of the intrusion's effect on surface $O_3$ should be considered in addition to model resolution. Typically, these strong intrusions have spatial extent of thousands of kilometers along their axis and hundreds of kilometers transversely to this axis, as noted in the 2006 $O_3$ AQCD (U.S. EPA, 2006b). Note that all three models (CAMx, GEOS-Chem, and AM3) under-predicted the magnitude of this event. These results indicate a need for process-oriented evaluation and targeted measurements that yield insight into both chemical and dynamical processes. The $R^2$ for comparison of AM3 with observations of MDA8 $O_3$ from March-August 2006 was 0.33, with lower $R^2$ for GEOS-Chem and CAMx. All three models predicted very similar means for March to August 2006: 54.9 ppb (simulated by the fine resolution version of GEOS-Chem), 55.0 ppb (simulated by CAMx), and 58.6 ppb (simulated by AM3), compared to 55.9 ppb for observed concentrations. See Figure 3-75 in Section 3.8; however, note that Figure 3-75 does not show the CAMx mean of 55.0 ppb, which was reported in Emery et al. (2012).

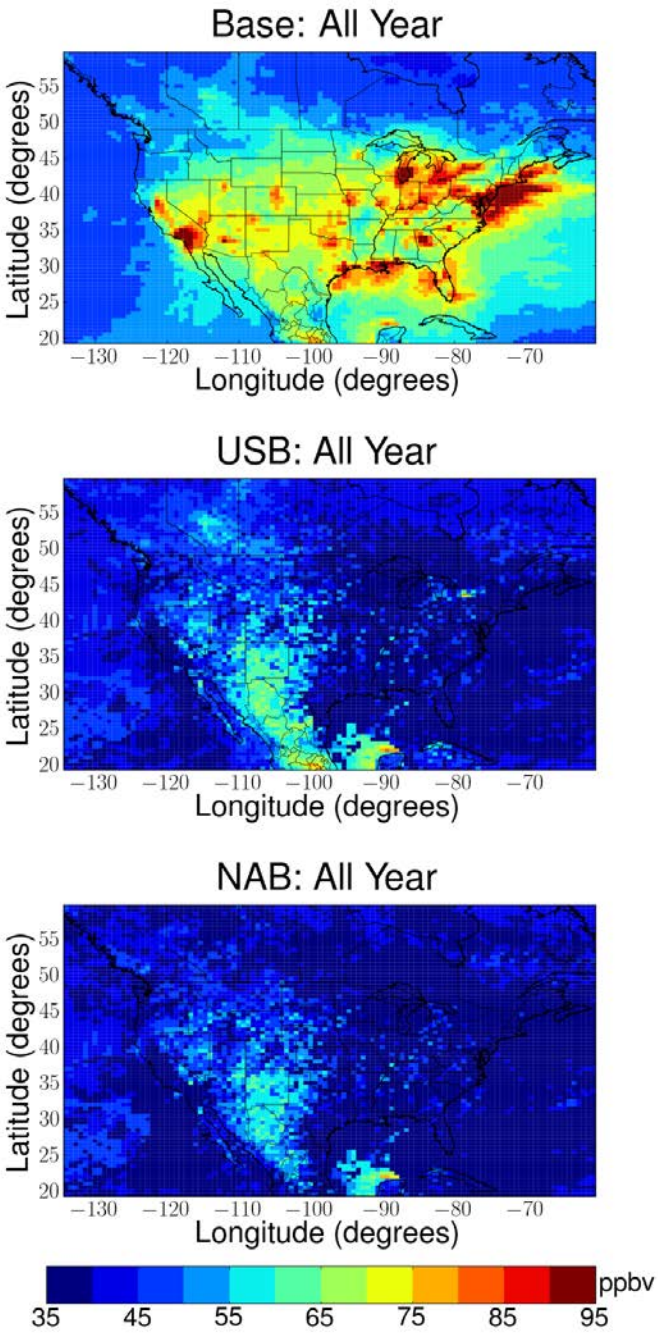
The results from either model have also been compared to more urban oriented sites in the AQS network. As noted earlier, comparisons between model results and

observations become problematic near concentrated sources of $O_3$ precursors ($NO_X$ and VOCs) in urban cores. Emery et al. (2012) note that in coarse resolution models rural biogenic and urban precursor emissions are mixed immediately leading to higher production efficiency for $O_3$. Finer resolution models are better able to separate these two source categories and to resolve features of urban chemistry such as titration of $O_3$ by $NO_X$ emitted by traffic and subsequent processing of $NO_X$ emissions during transport downwind. CAMx at 12 × 12 km resolution is better able to capture these features than GEOS-Chem at 50 × 50 km resolution. Both models tend to over-predict $O_3$ at the low $O_3$ concentrations in areas where $O_3$ scavenging by $NO_X$ is evident. In these situations, NA background $O_3$ concentrations are often higher than in the respective models for the base case. At high $O_3$ concentrations downwind of source areas, both models predict NA background $O_3$ concentrations that are much lower than observed or base case $O_3$. The latter results are in accord with results shown in Figure 3-11 for rural CASTNET sites at low elevations, which show lower ratios between NA background $O_3$ and either observations or base case $O_3$ at high $O_3$ than at low $O_3$ concentrations.

Figure 3-15 shows the annual 4th-highest MDA8 $O_3$ predicted by GEOS-Chem (at 0.5° × 0.667° resolution) for the base case (upper panel), and corresponding U.S. background (middle panel) and NA background (lower panel) MDA8 $O_3$ on the same days for 2006. Figure 3-16 shows corresponding values predicted by CAMx for the base case (upper panel) and NA background (lower panel) MDA8 $O_3$ on the same days for 2006. As can be seen from Figure 3-15 and Figure 3-16, on those days when models predicted their annual 4th-highest MDA8 $O_3$, the corresponding NA background concentrations are 36 ± 9 ppb in the eastern United States. Base case concentrations are much higher indicating that regional pollution is mainly responsible for the models 4th-highest concentrations. In the western U.S. on the other hand, NA background concentrations are generally higher and make up a larger fraction of the calculated 4th-highest MDA8 $O_3$ in both models, but for different reasons. GEOS-Chem predicts highest values in the Southern Rockies because of over-production of $NO_X$ by lightning. CAMx predicts highest values in ID, OR and WA from wildfires. The CAMx run includes day specific values for area burned, but GEOS-Chem uses monthly averages. (A more recent version of GEOS-Chem also incorporates day specific estimates for area burned.) Remaining areas of relatively high background levels (>60 ppb) are due mainly to some combination of stratospheric intrusions and Eurasian emissions. There are a few examples that can be used to give a rough idea of the magnitudes of episodically high background contributions. A comparison of the annual 4th-highest MDA8 $O_3$ concentration simulated by CAMx including wildfires and omitting them indicates that wildfires contributed ~ 30 to 40 ppb in Idaho, Montana, and Washington with a potentially larger contribution in the upper northwestern corner of California. Estimated contributions from strong stratospheric intrusions to surface $O_3$ in AM3 could range up to ~ 55 ppb in the western United States. It should be borne in mind in using these figures, that they are model derived and hence could be model specific. Issues related to calculating $O_3$ formation in wild fire plumes by CAMx were mentioned above. The method for calculating the stratospheric contributions in AM3 is based on the amount of $O_3$ carried downward through a given surface thus raising the possibility

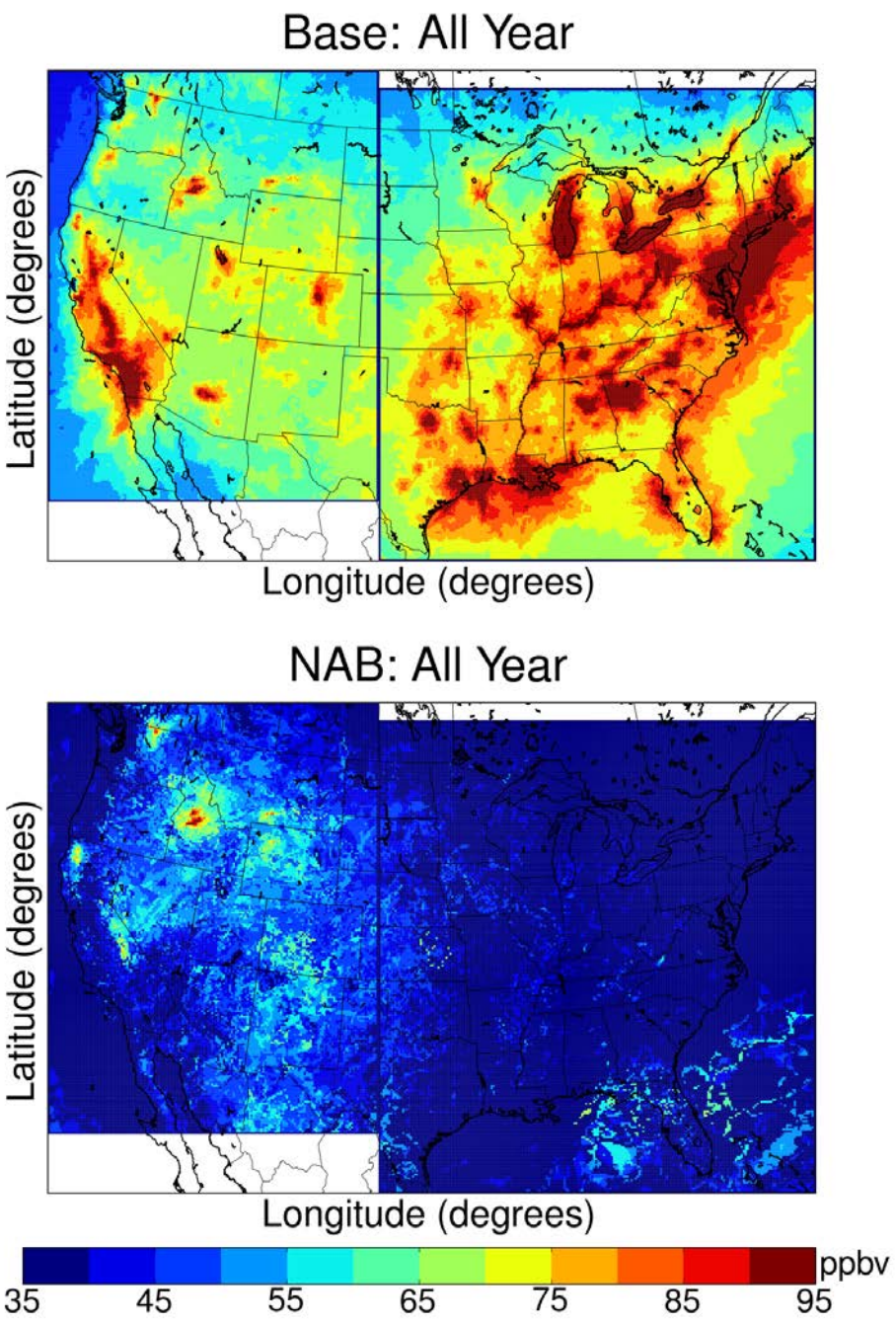
that $O_3$ could be generated in the upper troposphere, transported into the stratosphere and then transported downward again. This could indeed lead to an overestimate of the baseline stratospheric attribution in the model. However, Lin et al. (2012) focus on the "delta during events" rather than the baseline, because during specific intrusion events this recycling of $O_3$ between troposphere and stratosphere is less likely to be a problem. Therefore, there is greater confidence in the anomaly from stratospheric $O_3$ rather than the absolute amount. Tagging $O_3$ according to where it was produced will remove this ambiguity in future model runs.

All models undergo continuous updating of inputs, parameterizations of physical and chemical processes, and improvements in model resolution. Inputs that might be considered most relevant include emissions inventories, chemical reactions, and meteorological fields. This leads to uncertainty in model predictions in part because there is typically a lag between updated information for the above inputs—as outlined in Section 3.2 for chemical processes and emissions and in Section 3.3 for model construction—and their implementation in CTMs including GEOS-Chem or the other models described above. Quantitative estimates of uncertainties from meteorological and emission inputs and chemical mechanisms are problematic because simulations designed to quantify uncertainties from these sources have not been performed for these model runs. At best, these uncertainties can be estimated by comparison with observations while recognizing that compensating errors likely exist.




Source: Adapted from Zhang et al. (2011).

**Figure 3-15 Annual 4th-highest MDA8 $O_3$ predicted by GEOS-Chem (0.5° × 0.667°) for the base case (Base) with corresponding U.S. background (USB) and NA background (NAB) MDA8 $O_3$ for the same days in 2006.**




Source: Adapted from Emery et al. (2012).

**Figure 3-16 Annual 4th-highest MDA8 $O_3$ predicted by CAMx for the base case (Base) and corresponding NA background (NAB) MDA8 $O_3$ for the same days in 2006.**

Since NA background is a construct that cannot be directly measured, the range of background $O_3$ concentrations must be estimated using CTMs. Results from the Zhang et al. (2011) GEOS-Chem and Emery et al. (2012) CAMx model estimates were chosen for further analysis because these models have produced the latest estimates for background $O_3$ concentrations documented in the open literature. The main results from these two modeling efforts can be described as follows:

- Both models show background concentrations vary spatially and temporally;
- Simulated mean background concentrations are highest in the Intermountain West (i.e., at high altitude) in spring and lowest in the Northeast during summer;
- Background concentrations tend to increase with total (i.e., base case) $O_3$ concentrations at high elevation; but that tendency is not as clear at low elevations.

The most pronounced differences between the Zhang et al. (2011) GEOS-Chem and the Emery et al. (2012) CAMx models—when compared with observations—are at the upper end of the concentration distribution. At high elevations, differences are likely to be the result of under-predictions of background contributions which are driven mainly by episodic events such as stratospheric intrusions and wildfires. In general, CAMx predicts higher values at the upper end of the concentration distribution than does GEOS-Chem. At low elevations (<1,500 meters)—located mainly in the East—the reasons for under-predictions at the upper end of the concentration distribution are more complex and likely involve extensive interactions between anthropogenic and natural sources.

Table 3-1 summarizes modeling results for seasonal mean MDA8 $O_3$ by region simulated by the two models. The regions in Table 3-1 are shown in Figure 3-50. As can be seen from the table, seasonal means predicted by GEOS-Chem are within a few parts per billion of measurements in both spring and summer for all regions shown except for California in the spring. Although CASTNET sites are meant to represent regional background air, they can be heavily influenced by polluted air masses, particularly in California where the under-predictions are largest. Seasonal means are simulated by CAMx to within 2-5 ppb except in California in the spring where they are under-predicted by 8 ppb and at sites in the Northeast and Southeast where they are over-predicted by 8-9 ppb in summer. When compared to observations, the mean $R^2$ within each region—except for California in the spring—is higher for CAMx than for GEOS-Chem suggesting better ability to track day-to-day variability by CAMx. It is clear from these results that model resolution (at least for the model resolutions considered here) is not the dominant factor determining agreement of the means between simulations or between simulations and measurements. Differences in model chemistry and physics must also be considered.

Table 3-2 summarizes modeling results for the annual 4th-highest (99th –percentile) MDA8 $O_3$ for the same seasons and regions used in Table 3-1. As can be seen in the table, the GEOS-Chem and the CAMx models both underestimate mean paired (day-specific) 4th-highest values in California by ~20 ppb. In general, CAMx simulates

paired annual 4th-highest MDA8 $O_3$ concentrations that are higher and in better agreement with measurements. Shown alongside the model estimates is the number of days the modeled MDA8 $O_3$ concentrations were within 5 ppb of observed concentrations. The unpaired entries for the models in Table 3-2 show model predicted 4th-highest MDA8 $O_3$ concentrations that are not calculated on the same day as the 4th-highest values observed at CASTNET sites. It can be seen that simulated regional means of the 4th-highest MDA8 $O_3$ are in better agreement with measurements when results are unpaired by date. In other words, the models do not necessarily predict the annual 4th-highest MDA8 $O_3$ concentrations on the same day as they are observed.

These results underscore the uncertainties inherent in any model's attempts to simulate day specific 4th-highest $O_3$ concentrations. As noted earlier, uncertainties in calculating day specific $O_3$ concentrations are especially challenging because of the lack of day specific data for emissions of many species. While progress is being made in obtaining day specific data for lightning strikes and area burned in wildfires, the emission factors for precursors from these episodic sources such as lightning and wildfires are still uncertain. In addition to uncertainty in emissions, uncertainties in the treatment of transport and chemical mechanisms in the models must also be considered.

## Table 3-1 Comparison of Zhang et al. (2011) and Emery et al. (2012) results for MDA8 $O_3$ concentrations (ppbv) with measurements at selected CASTNET sites.

| Region | Observation | Model | Mean MDA8 $O_3$ concentration (ppbv)[a] | | Mean $R^2$ | |
|---|---|---|---|---|---|---|
| | | | Spring | Summer | Spring[b] | Summer[b] |
| California (5 sites) | CASTNET[c] | | 58 ± 12[c] | 69 ± 14[c] | | |
| | | GEOS-Chem[d] | | | | |
| | | Base[d] | 52 ± 11 | 66 ± 18 | 0.52 | 0.22 |
| | | U.S. bkg[e] | 38 ± 7 | 37 ± 9 | | |
| | | NA bkg[f] | 37 ± 6 | 35 ± 9 | | |
| | | CAMx[d] | | | | |
| | | Base[d] | 50 ± 10 | 66 ± 13 | 0.50 | 0.30 |
| | | NA bkg[f] | 39 ± 6 | 42 ± 6 | | |
| West (14 sites) | CASTNET[c] | | 54 ± 9[c] | 55 ± 11[c] | | |
| | | GEOS-Chem[d] | | | | |
| | | Base[d] | 53 ± 7 | 55 ± 11 | 0.30 | 0.12 |
| | | U.S. bkg[e] | 42 ± 6 | 40 ± 9 | | |
| | | NA bkg[f] | 41 ± 6 | 38 ± 9 | | |
| | | CAMx[d] | | | | |
| | | Base[d] | 49 ± 8 | 57 ± 10 | 0.39 | 0.33 |
| | | NA bkg[f] | 40 ± 7 | 41 ± 8 | | |
| North Central (6 sites) | CASTNET[c] | | 47 ± 10[c] | 50 ± 12[c] | | |
| | | GEOS-Chem[d] | | | | |
| | | Base[d] | 47 ± 8 | 51 ± 14 | 0.52 | 0.44 |
| | | U.S. bkg[e] | 33 ± 6 | 27 ± 7 | | |
| | | NA bkg[f] | 30 ± 7 | 24 ± 7 | | |
| | | CAMx[d] | | | | |
| | | Base[d] | 45 ± 11 | 54 ± 13 | 0.63 | 0.48 |
| | | NA bkg[f] | 30 ± 6 | 31 ± 5 | | |
| Northeast (5 sites) | CASTNET[c] | | 48 ± 10[c] | 45 ± 14[c] | | |
| | | GEOS-Chem[d] | | | | |
| | | Base[d] | 45 ± 7 | 45 ± 13 | 0.44 | 0.47 |
| | | U.S. bkg[e] | 33 ± 7 | 24 ± 7 | | |
| | | NA bkg[f] | 29 ± 6 | 18 ± 6 | | |
| | | CAMx[d] | | | | |
| | | Base[d] | 46 ± 11 | 53 ± 14 | 0.53 | 0.54 |
| | | NA bkg[f] | 30 ± 5 | 27 ± 6 | | |
| Southeast (9 sites) | CASTNET[c] | | 52 ± 11[c] | 52 ± 16[c] | | |
| | | GEOS-Chem[d] | | | | |
| | | Base[d] | 51 ± 7 | 54 ± 9 | 0.42 | 0.21 |
| | | U.S. bkg[e] | 32 ± 7 | 29 ± 10 | | |
| | | NA bkg[f] | 29 ± 7 | 28 ± 9 | | |
| | | CAMx[d] | | | | |
| | | Base[d] | 54 ± 9 | 61 ± 12 | 0.56 | 0.45 |
| | | NA bkg[f] | 33 ± 6 | 30 ± 6 | | |

[a]Seasonal (spring, summer) mean MDA8 $O_3$ concentration ± standard deviation;
[b]Mean $R^2$ of all individual model-measurement pairs at individual CASTNET sites;
[c]Observed concentrations at CASTNET sites;
[d]Modeled concentrations at CASTNET sites;
[e]Modeled U.S. background concentrations at CASTNET sites,
[f]Modeled NA background concentrations at CASTNET sites;
Source: Data from Zhang et al. (2011) for GEOS-Chem and Emery et al. (2012) for CAMx.

## Table 3-2 Comparison of annual 4th-highest MDA8 $O_3$ concentrations measured at CASTNET sites in 2006 with MDA8 $O_3$ concentrations simulated by the GEOS-Chem and CAMx base case models.

| Region | Observation | Model | 4th-highest MDA8 $O_3$ concentration (ppbv)[a] | Number of Days within 5 ppb[b] |
|---|---|---|---|---|
| California (5 sites) | CASTNET[c] | | 90 ± 13[c] | |
| | | GEOS-Chem (paired)[d] | 71 ± 15 | 0 |
| | | GEOS-Chem (unpaired)[e] | 85 ± 19 | |
| | | CAMx (paired)[d] | 71 ± 9 | 0 |
| | | CAMx (unpaired)[e] | 85 ± 13 | |
| West (14 sites) | CASTNET[c] | | 70 ± 4[c] | |
| | | GEOS-Chem (paired)[d] | 62 ± 8 | 4 |
| | | GEOS-Chem (unpaired)[e] | 68 ± 7 | |
| | | CAMx (paired)[d] | 63 ± 8 | 6 |
| | | CAMx (unpaired)[e] | 71 ± 7 | |
| North Central (6 sites) | CASTNET[c] | | 71 ± 5[c] | |
| | | GEOS-Chem (paired)[d] | 58 ± 10 | 1 |
| | | GEOS-Chem (unpaired)[e] | 69 ± 10 | |
| | | CAMx (paired)[d] | 63 ± 7 | 1 |
| | | CAMx (unpaired)[e] | 73 ± 8 | |
| Northeast (5 sites) | CASTNET[c] | | 71 ± 4[c] | |
| | | GEOS-Chem (paired)[d] | 61 ± 6 | 0 |
| | | GEOS-Chem (unpaired)[e] | 68 ± 5 | |
| | | CAMx (paired)[d] | 72 ± 7 | 3 |
| | | CAMx (unpaired)[e] | 75 ± 3 | |
| Southeast (9 sites) | CASTNET[c] | | 76 ± 8[c] | |
| | | GEOS-Chem (paired)[d] | 61 ± 6 | 2 |
| | | GEOS-Chem (unpaired)[e] | 71 ± 5 | |
| | | CAMx (paired)[d] | 71 ± 11 | 5 |
| | | CAMx (unpaired)[e] | 79 ± 9 | |

[a]Annual 4th-highest (99th-percentile) MDA8 $O_3$ concentration regional means (ppb) ± standard deviation;

[b]Number of days the model predicted MDA8 $O_3$ concentrations were within 5 ppb of the observed 4th-highest concentrations;

[c]Observed concentrations at CASTNET sites;

[d]Modeled concentrations at CASTNET sites on days when the 4th-highest MDA8 $O_3$ concentration was observed (paired by date);

[e]Model predicted annual 4th-highest MDA8 $O_3$ concentration at CASTNET sites (unpaired by date).

Source: Data from Zhang et al. (2011) for GEOS-Chem and Emery et al. (2012) for CAMx.

Comparison of GEOS-Chem results for natural and NA background indicate that methane is also a major contributor to NA background $O_3$, accounting for slightly less than half of the increase in background since the pre-industrial era and whose relative contribution is projected to grow in the future. U.S. background concentrations are on average 2.6 ppb higher than NA background concentrations during spring and 2.7 ppb during summer across the United States. Highest values for U.S. background (in the U.S.) are found over the Northern Tier of New York State (19.1 ppb higher than local NA background concentrations) in summer. High values are also found in other areas bordering Canada and Mexico.

Analyses of results from GEOS-Chem and CAMx presented here and shown in Table 3-1 and Table 3-2 are in accord with results from Kasibhatla and Chameides (2000) who found that the accuracy of simulations improved as the averaging time of both the simulation and the observations increased (see Section 3.3). Note that any CTM—not just the ones considered here—will have difficulty in predicting day specific quantities. When analyzing results over long time periods (e.g., months), special care should be taken to examine temporal trends in bias because this will improve understanding of the modeling results.

Overall, these results suggest that GEOS-Chem is capable of simulating seasonal or monthly mean MDA8 $O_3$ to within a few parts per billion on a regional basis throughout the U.S., except in California. These results suggest that CAMx is capable of simulating seasonal or monthly mean MDA8 $O_3$ to within a few ppb, though, CAMx also shows relatively large disagreements in California and, in addition, shows relatively large positive bias in seasonal mean MDA8 $O_3$ in the eastern United States. However, differences between the models in the East are likely to narrow with updates to chemistry. Neither model is capable of simulating 4th-highest MDA8 $O_3$ to within suitable bounds on a day-specific basis at all sites, or even most sites. However, agreement between simulated versus observed 4th-highest MDA8 $O_3$ is improved for either model when the models and the measurements are sampled on different days.

Note that the calculations of background concentrations presented in this section were formulated to answer the question, “what would $O_3$ concentrations be if there were no anthropogenic sources.” This is different from asking, “how much of the $O_3$ measured or simulated in a given area is due to background contributions.” Because of potentially strong non-linearities (i.e., the fate, or lifetime, of the background $O_3$ transported into the urban area will depend on the concentration of the background $O_3$ in addition to interactions of background $O_3$ with the local chemical regime) in many urban areas, these estimates by themselves should not be used to answer the second question posed above. The extent of these non-linearities will generally depend on location and time, the strength of concentrated sources and the nature of the chemical regime.

## 3.5 Monitoring

### 3.5.1 Routine Monitoring Techniques

The federal reference method (FRM) for $O_3$ measurement is called the Chemiluminescence Method (CLM) and is based on the detection of chemiluminescence resulting from the reaction of $O_3$ with ethylene gas. The UV absorption photometric analyzers were approved as federal equivalent methods (FEMs) in 1977 and gained rapid acceptance for NAAQS compliance purposes due to ease of operation, relatively low cost, and reliability. The UV absorption method is based on the principle that $O_3$ molecules absorb UV radiation at a wavelength of 254 nm from a mercury lamp. The concentration of $O_3$ is computed from Beer's law using the radiation absorbed across a fixed path length, the absorption coefficient, and the measured pressure and temperature in the detection cell. UV absorption photometry is the predominant method for assessing compliance with the NAAQS for $O_3$. Almost all of the state and local air monitoring stations (SLAMS) that reported data to EPA AQS from 2005 to 2009 used UV absorption photometer FEMs. No CLM monitors, approved as FRMs or FEMs, reported $O_3$ data to AQS from 2005 to 2009 and only one monitor reported data using a long-path or open path Differential Optical Absorption Spectrometer (DOAS) FEM during this period.

The rationale, history, and calibration of $O_3$ measurements were summarized in the 1996 and 2006 $O_3$ AQCDs (U.S. EPA, 2006b, 1996a) and focused on the state of ambient $O_3$ measurements at that time as well as evaluation of interferences and new developments. This discussion will continue with the current state of $O_3$ measurements, interferences, and new developments for the period 2005 to 2010.

UV $O_3$ monitors use mercury lamps as the source of UV radiation and employ an $O_3$ scrubber (typically manganese dioxide) to generate an $O_3$-free air flow to serve as a reference channel for $O_3$ measurements. There are known interferences with UV $O_3$ monitors. The 2006 $O_3$ AQCD (U.S. EPA, 2006b) reported on the investigation of the effects of water vapor, aromatic compounds, ambient particles, mercury vapor and alternative materials in the instrument's $O_3$ scrubber. The overall conclusions from the review of the scientific literature covered in the 2006 $O_3$ AQCD (U.S. EPA, 2006b) are briefly summarized below.

Kleindienst et al. (1993) found water vapor to have no measurable impact and aromatic compounds to have a minor impact (as much as 3% higher than the FRM extrapolated to ambient conditions) on UV absorption measurements. UV $O_3$ monitor response evaluated by chamber testing using cigarette smoke, reported an elimination of the $O_3$ monitor response to the smoke when a particle filter was used that filtered out particles less than 0.2 µm in diameter (Arshinov et al., 2002). One study (Leston et al., 2005) in Mexico City compared a UV $O_3$ FEM to a CLM FRM. The UV FEM reported consistently higher $O_3$ than the CLM FRM. They suggested that $O_3$ measured in ambient air could be too high by 20 to 40 ppb under specific conditions due to positive interference by a number of organic compounds, mainly

those produced during the oxidation of aromatic hydrocarbons and some primary compounds such as styrene and naphthalene. However, the concentrations of these compounds were many times higher in both of these environments than are typically found at ambient air monitoring sites in the United States Although Hg is also potentially a strong interfering agent, because the Hg resonance line is used in this technique, its concentration would also have to be many times higher than is typically found in ambient air, e.g., as might be found in power plant plumes. Thus, it seems unlikely that such interferences would amount to more than one or two ppb (within the design specifications of the FEM), except under conditions conducive to producing high concentrations of the substances they identified as causing interference. Leston et al. (2005) also presented smog chamber data which demonstrated that heated metal and heated silver wool scrubbers perform better in the presence of aromatic hydrocarbon irradiations than manganese dioxide scrubbers when compared to the FRM. They also suggested the use of humidified calibration gas and alternative scrubber materials to improve UV $O_3$ measurements. Some $O_3$ monitor manufacturers now offer heated silver wool scrubbers as an alternative to manganese dioxide. Another possible solution to the $O_3$ scrubber problem may be the use of a gas phase scrubber such as NO. A commercial version of this has recently been introduced by 2B Technologies as an option on their model 202 FEM; however, it has not been field tested or approved for use as an FEM.

Review of the recent literature is summarized below. Study of UV monitors by Williams et al. (2006) concluded that well maintained monitors showed no substantial interferences when operated in locations with high concentrations of potentially interfering VOCs including Nashville, Houston, and the Gulf of Maine. Monitors were tested in urban and suburban environments, as well as on board a ship in both polluted and clean marine air. Comparisons of UV measurements to a non-FRM/FEM NO based CLM demonstrated agreement to within 1%. At the Houston location, they did observe a brief period on one day for about 30 minutes where the UV measurements exceeded the CLM by about 8 ppb (max). This was attributed to probable instrument malfunction.

Wilson and Birks (2006) investigated water vapor interference in $O_3$ measurements by four different UV monitors. In extreme cases where a rapid step change in relative humidity between 0 and 90% was presented, large transitory responses (tens to hundreds of ppb) were found for all monitors tested. Rapid changes in relative humidity such as this would not be expected during typical ambient $O_3$ measurements and could only be expected during measurement of vertical profiles from balloon or aircraft. The magnitude of the interference and the direction (positive or negative) was dependent on the manufacturer and model. Wilson and Birks (2006) also hypothesized that water vapor interference is caused by physical interactions of water vapor on the detection cell. The $O_3$ scrubber was also thought to act as a reservoir for water vapor and either added or removed water vapor from the air stream, subsequently affecting the detector signal and producing either a positive or negative response. They demonstrated that the use of a Nafion permeation membrane just before the $O_3$ detection cell to remove water vapor eliminated this interference.

Dunlea et al. (2006) evaluated multiple UV $O_3$ monitors with two different $O_3$ scrubber types (manganese dioxide and heated metal wool) in Mexico City. Large spikes in $O_3$ concentrations were observed while measuring diesel exhaust where large increases in particle number density were observed. The interference due to small particles passing through the Teflon filter and scattering/absorbing light in the detection cell were estimated to cause at most a 3% increase in measurements in typical ambient air environments. This estimate pertains to measurements in the immediate vicinity of fresh diesel emissions and most monitor siting guidelines would not place the monitor close to such sources, so actual interferences are expected to be much less than 3%. Dunlea et al. (2006) also observed no evidence for either a positive or negative interference or dependence due to variations in aromatics during their field study.

Li et al. (2006c) verified early reports of gas phase mercury interference with the UV $O_3$ measurement. They found that 300 ng/m$^3$ of mercury produced an instrument response of about 35 ppb $O_3$. Background concentrations of mercury are around 1-2 ng/m$^3$ and expected to produce an $O_3$ response that would be <1 ppb.

Spicer et al. (2010) examined potential UV $O_3$ monitor interferences by water vapor, mercury, aromatic compounds, and reaction products from smog chamber simulations. Laboratory tests showed little effect of changing humidity on conventional FEM UV $O_3$ monitors with manganese dioxide or heated metal wool scrubbers in the absence of other interferences. Mercury vapor testing produced an $O_3$ response by the UV monitors that was <1 ppb $O_3$ per 1 ppt (about 8 ng/m$^3$) mercury vapor. Interference by aromatic compounds at low (3% RH) and high (80% RH) humidity showed some positive responses that varied by UV monitor and ranged from 0 to 2.2 ppb apparent $O_3$ response, per ppb of aromatic compound tested. The authors acknowledged that the aromatic compounds most likely to interfere are rarely measured in the atmosphere and therefore, make it difficult to assess the impact of these compounds during ambient air monitoring. Comparison of UV and CLM responses to photochemical reaction products in smog chamber simulations at 74% to 85% RH showed varied responses under low (0.125 ppmv/0.06 ppmv) to high (0.50 ppmv/0.19 ppmv) hydrocarbon/$NO_X$ conditions. The conventional UV monitors were as much as 2 ppb higher than the CLM under low hydrocarbon/$NO_X$ conditions and 6 ppb higher under the high hydrocarbon/$NO_X$ conditions. Two FEM UV monitors were also co-located at six sites in Houston from May to October, 2007 with one UV monitor equipped with Nafion permeation membrane. The average difference between 8-h daily max $O_3$ concentrations using the UV and the UV with Nafion permeation membrane ranged from -4.0 to 4.1 ppb.

### 3.5.2 Precision and Bias

In order to provide decision makers with an assessment of data quality, EPA's Quality Assurance (QA) group derives estimates of both precision and bias for $O_3$ and the other gaseous criteria pollutants from the biweekly single point quality control (QC) checks using calibration gas, performed at each site by the monitoring agency. The single-point QC checks are typically performed at concentrations around 90 ppb. Annual summary reports of precision and bias can be obtained for each monitoring site at http://www.epa.gov/ttn/amtic/qareport.html. The assessment of precision and bias are based on the percent-difference values, calculated from single-point QC checks. The percent difference is based on the difference between the pollutant concentration indicated by monitoring equipment and the known (actual) concentration of the standard used during the QC check. The monitor precision is estimated from the 90% upper confidence limit of the coefficient of variation (CV) of relative percent difference (RPD) values. The bias is estimated from the 95% upper confidence limit on the mean of the absolute values of percent differences. The data quality goal for $O_3$ precision and bias at the 90 and 95% upper confidence limits is 7% (40 CFR Part 58, Appendix A). Table 3-3 presents a summary of the number of monitors that meet the precision and bias goal of 7% for 2005 to 2009. Greater than 96% of $O_3$ monitors met the precision and bias goal between 2005 and 2009. Another way to look at the precision (CV) and bias (percent difference) information using the single-point QC check data from the monitoring network is to present box plots of the monitors' individual precision and percent-difference data; Figure 3-17 and Figure 3-18 include this information for $O_3$ monitors operating from 2005 to 2009.

**Table 3-3 Summary of $O_3$ monitors meeting 40 CFR Part 58, Appendix A Precision and Bias Goals.**

| Year | Number of Monitors | Monitors with Acceptable Precision (%) | Monitors with Acceptable Bias (%) |
|---|---|---|---|
| 2005 | 879 | 96.5 | 96.7 |
| 2006 | 881 | 98.1 | 97.6 |
| 2007 | 935 | 98.1 | 98.1 |
| 2008 | 955 | 97.1 | 96.7 |
| 2009 | 958 | 97.4 | 97.5 |

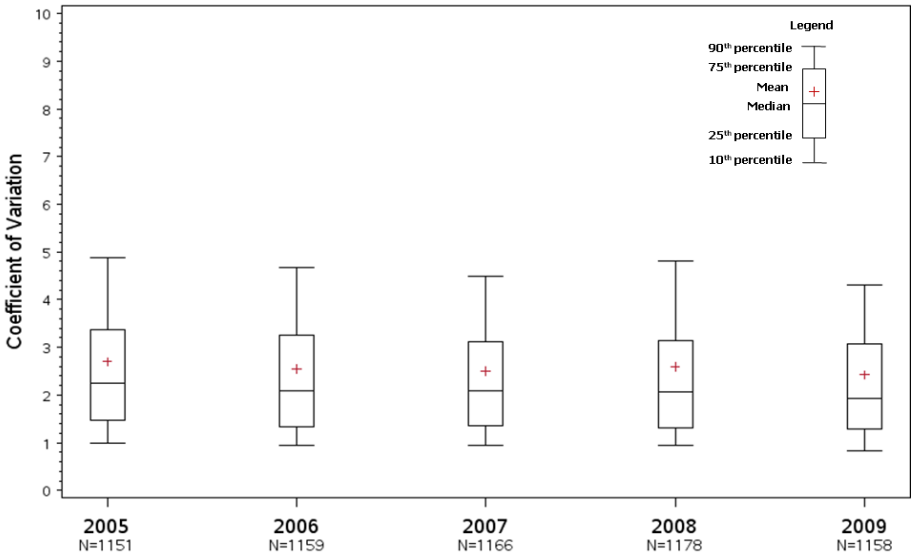



**Figure 3-17 Box plots of precision data by year (2005-2009) for all $O_3$ monitors reporting single-point QC check data to AQS.**

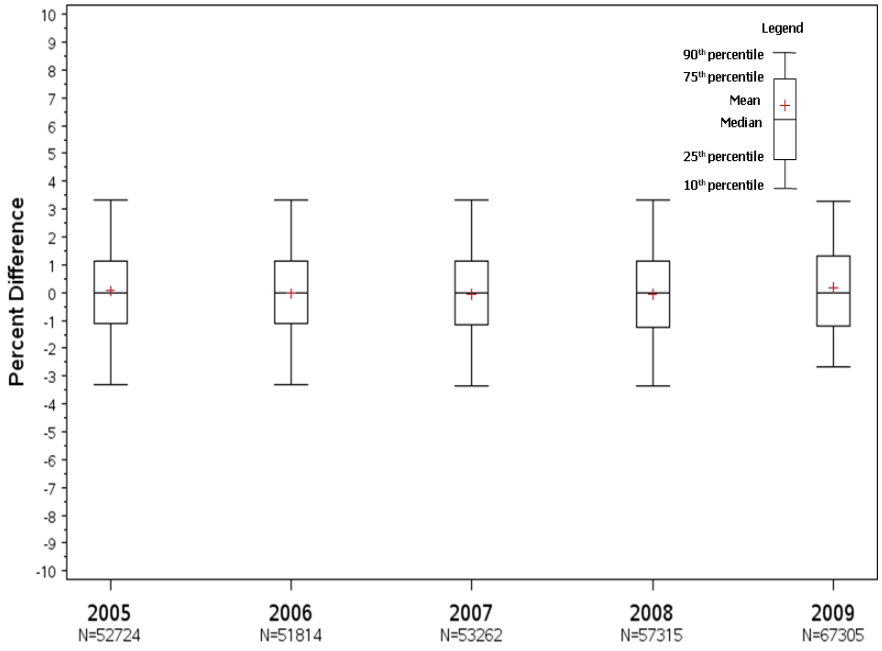



**Figure 3-18 Box plots of percent-difference data by year (2005-2009) for all $O_3$ monitors reporting single-point QC check data to AQS.**

### 3.5.2.1 Precision from Co-located UV $O_3$ Monitors in Missouri

The Missouri Department of Natural Resources (MODNR) maintains a network of co-located UV $O_3$ analyzers. The MODNR provided co-located data from four monitors: two co-located at the same monitoring site in Kansas City (AQS ID 290370003) and two co-located at the same monitoring site in St. Louis (AQS ID 291831002). Hourly observations for the co-located measurements at these two sites between April and October, 2006-2009 were used to evaluate precision from co-located UV monitors. These data were then compared with the precision obtained by the biweekly single point QC checks for all sites reporting single-point QC check data to AQS between 2005 and 2009; the method normally used for assessing precision. Box plots of the RPD between the primary and co-located hourly $O_3$ measurements in Missouri are shown in Figure 3-19 and box plots of the RPD between the actual and indicated QC check for all U.S. sites are shown in Figure 3-20. As mentioned above, the average concentration of the single-point QC check is 90 ppb, whereas the average ambient $O_3$ concentration measured at the two sites in Missouri was 34 ppb. The mean RPD for the co-located monitors in Missouri and the single-point QC check data from all sites were less than 1 percent.

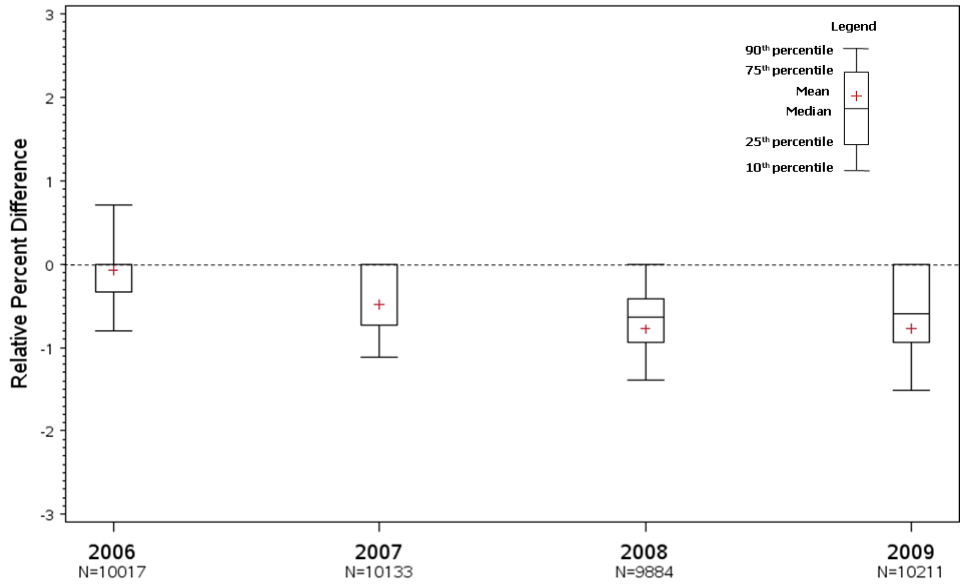



**Figure 3-19 Box plots of RPD data by year for the co-located $O_3$ monitors at two sites in Missouri from 2006-2009.**

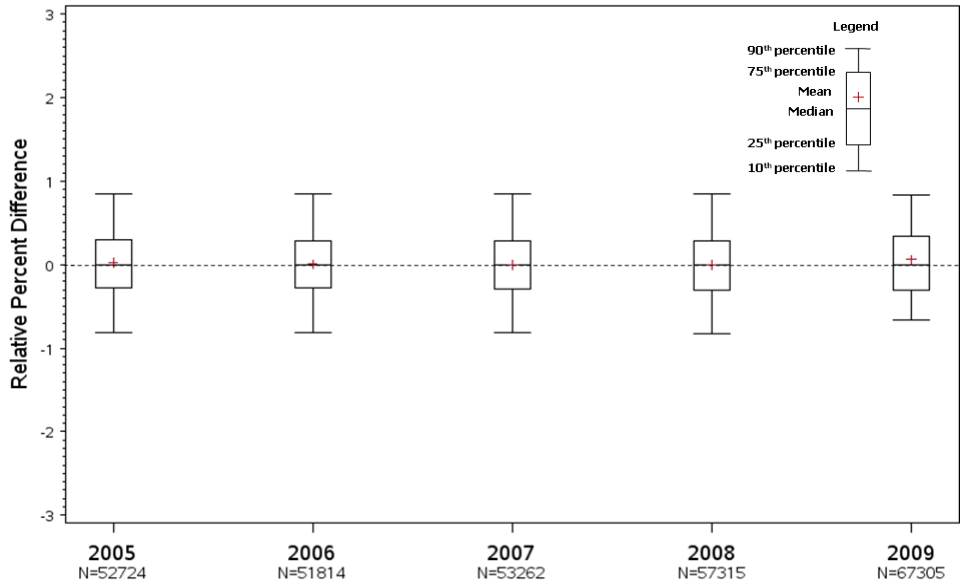



**Figure 3-20 Box plots of RPD data by year for all of the United States. Ozone sites reporting single-point QC check data to AQS from 2005-2009.**

### 3.5.3 Performance Specifications

The performance specifications for evaluating and approving new FEMs in accordance with 40 CFR Part 53 are provided in Table 3-4. These specifications were developed and originally published in the Federal Register in 1975. Modern, commercially-available instruments can now perform much better than the requirements specified below. For example, the lower detectable limit (LDL) performance specification is 10 ppb and the typical vendor-stated performance for the LDL is now less than 0.60 ppb. The amount of allowable interference equivalent for total interference substances is 60 ppb, and the current NAAQS for $O_3$ is 75 ppb, with an averaging time of 8 hours. Improvements in new measurement technology have occurred since these performance specifications were originally developed. These specifications should be revised to more accurately reflect the necessary performance requirements for $O_3$ monitors used to support the current NAAQS.

**Table 3-4 Performance specifications for $O_3$ based in 40 CFR Part 53.**

| Parameter | Specification |
|---|---|
| Range | 0 – 0.5 ppm (500 ppb) |
| Noise | 0.005 ppm (5 ppb) |
| LDL – defined as two times the noise | 0.01 ppm (10 ppb) |
| Interference equivalent | |
| Each interfering substance | ± 0.02 ppm (20 ppb) |
| Total interfering substances | 0.06 ppm (60 ppb) |
| Zero drift | |
| 12 h | ± 0.02 ppm (20 ppb) |
| 24 h | ± 0.02 ppm (20 ppb) |
| Span Drift, 24 h | |
| 20% of upper range limit | ± 20.0% |
| 80% of upper range limit | ± 5.0% |
| Lag time | 20 min |
| Rise time | 15 min |
| Fall time | 15 min |
| Precision | |
| 20% of upper range limit | 0.01 ppm (10 ppb) |
| 80% of upper range limit | 0.01 ppm (10 ppb) |

### 3.5.4 Monitor Calibration

The calibration of $O_3$ monitors was summarized in detail in the 1996 $O_3$ AQCD (U.S. EPA, 1996a). The calibration of $O_3$ monitors is done using an $O_3$ generator and UV photometers. UV photometry is the prescribed procedure for the calibration of reference methods to measure $O_3$ in the atmosphere. Because $O_3$ is unstable and cannot be stored, the $O_3$ calibration procedure specifically allows the use of transfer standards for calibrating ambient $O_3$ monitors. A transfer standard is calibrated against a standard of high authority and traceability and then moved to another location for calibration of $O_3$ monitors. The EPA and the National Institute of Standards and Technology (NIST) have established a network of standard reference photometers (SRPs) that are used to verify transfer standards. The International Bureau of Weights and Measures (BIPM) maintain one NIST SRP (SRP27) as the World's $O_3$ reference standard. NIST maintains two SRPs (SRP0 and SRP2) that are used for comparability to ten other SRPs maintained by the EPA's Regional QA staff.

SRPs have been compared to other reference standards. Tanimoto et al. (2006) compared NIST SRP35, owned by the National Institute for Environmental Studies

in Japan, to gas phase titration (GPT). The SRP was found to be 2% lower than GPT. GPT is no longer used as a primary or transfer standard in the United States. Viallon et al. (2006) compared SRP27 built at BIPM to four other NIST SRPs maintained by BIPM (SRP28, SRP31, SRP32, and SRP33). A minimum bias of +0.5% was found for all SRP measurement results, due to use of the direct cell length measurement for the optical path length; this bias was accounted for by applying the appropriate correction factor. Study of the bias-corrected SRPs showed systematic biases and measurement uncertainties for the BIPM SRPs. A bias of -0.4% in the instrument $O_3$ mole fraction measurement was identified and attributed to non-uniformity of the gas temperature in the instrument gas cells, which was compensated by a bias of +0.5% due to an under-evaluation of the UV light path length in the gas cells. The relative uncertainty of the $O_3$ absorption cross section was 2.1% at 253.65 nm and this was proposed as an internationally accepted consensus value until sufficient experimental data is available to assign a new value.

In November, 2010, the EPA revised the Technical Assistance Document for *Transfer Standards for Calibration of Air Monitoring Analyzers for Ozone* (U.S. EPA, 2010f) that was first finalized in 1979 (U.S. EPA, 1979b). The revision removed methods no longer in use and updated definitions and procedures where appropriate. In the revised document, the discussion of transfer standards for $O_3$ applies to the family of standards that are used beyond SRPs or Level 1 standards. To reduce confusion, EPA reduced the number of common terms that were used in the past such as: primary standard, local primary standard, transfer standard, and working standard. Beyond the SRPs, all other standards are considered transfer standards.

## 3.5.5 Other Monitoring Techniques

### 3.5.5.1 Portable UV $O_3$ Monitors

Small, lightweight, and portable UV $O_3$ monitors with low power consumption are commercially available. These monitors are based on the same principle of UV absorption by $O_3$ at 254 nm. Monitors of this type are typically used for vertical profiling using balloons, kites, or light aircraft where space and weight are limited. They have also been used for monitoring at remote locations such as National Parks. Burley and Ray (2007) compared portable $O_3$ monitor measurements to those from a conventional UV monitor in Yosemite National Park. Calibrations of the portable $O_3$ monitors against a transfer standard resulted in an overall precision of ± 4 ppb and accuracy of ± 6%. Field measurement comparisons between the portable and conventional monitor at Turtleback Dome showed the portable monitor to be 3.4 ppb lower on average, with daytime deviation typically on the order of 0-3 ppb. Agreement between the portable and conventional monitor during daylight hours (9:00 a.m. to 5:00 p.m. PST) resulted in an $R^2$ of 0.95, slope of 0.95, and intercept of 0.36 ppb. Substantial deviations were observed in the predawn hours where the

portable monitor was consistently low. These deviations were attributed to the difference in sampling inlet location. The portable monitor was located at 1.3 meters above ground and the conventional monitor was located at 10 meters above ground. Agreement between the portable and conventional monitors for all hours sampled resulted in an $R^2$ of 0.88, slope of 1.06, and intercept of -6.8 ppb. (Greenberg et al., 2009) also compared a portable UV $O_3$ monitor to a conventional UV monitor in Mexico City and obtained good agreement for a 14 day period with an $R^2$ of 0.97, slope of 0.97, and intercept of 6 ppb. One portable $O_3$ monitor was recently approved as an FEM (EQOA-0410-190) on April 27, 2010 (75 FR 22126).

### 3.5.5.2 NO-based Chemiluminescence Monitors

One commercially available NO-based chemiluminescence monitor has been approved as an FEM (EQOA-0611-199) on October 7, 2011 (75 FR 62402). It may also be designated as a second or replacement FRM since the ethene based FRMs are no longer manufactured. Although this is a relatively new monitor, other NO-based CLM instruments have been custom built for various field studies since the early 1970s. A commercial version that measured both $O_3$ and $NO_X$ was offered in the early 1970s but failed to gain commercial acceptance. Initial testing with $SO_2$, $NO_2$, $Cl_2$, $C_2H_2$, $C_2H_4$ and $C_3H_6$ (Stedman et al., 1972) failed to identify any interferences. In the intervening years, custom built versions have not been found to have any interference; however, they do experience a slight decrease in response with increasing relative humidity (due to quenching of the excited species by the water molecules). The new NO-based CLM solves this problem with the use of a Nafion membrane dryer. A custom built NO-based CLM similar to the FEM was used by Williams et al. (2006) in Houston, TX; Nashville, TN; and aboard ship along the New England coast. It was found to be in good agreement with a standard UV based FEM and with a custom built DOAS.

### 3.5.5.3 Passive Air Sampling Devices and Sensors

A passive $O_3$ sampling device depends on the diffusion of $O_3$ in air to a collecting or indicating medium. In general, passive samplers are not adequate for compliance monitoring because of the limitations in averaging time (typically one week or more), particularly for $O_3$. However, these devices are valuable for personal human exposure estimates and for obtaining long-term data in rural areas where conventional UV monitors are not practical or feasible to deploy. The 1996 $O_3$ AQCD (U.S. EPA, 1996a) provided a detailed discussion of passive samplers, along with the limitations and uncertainties of the samplers evaluated and published in the literature from 1989 to 1995. The 2006 $O_3$ AQCD (U.S. EPA, 2006b) provided a brief update on available passive samplers developed for use in direct measurements of personal exposure published through 2004. The 2006 $O_3$ AQCD (U.S. EPA, 2006b) also noted the sensitivity of these samplers to wind velocity, badge

placement, and interference by other copollutants that may result in measurement error.

Subsequent evaluations of passive diffusion samplers in Europe showed good correlation when compared to conventional UV $O_3$ monitors, but a tendency for the diffusion samplers to overestimate the $O_3$ concentration (Gottardini et al., 2010; Vardoulakis et al., 2009; Buzica et al., 2008). The bias of $O_3$ diffusion tubes were also found to vary with concentration, season, and exposure duration (Vardoulakis et al., 2009). Development of simple, inexpensive, passive $O_3$ measurement devices that rely on $O_3$ detection papers and a variety of sensors with increased time resolution (sampling for hours instead of weeks) and improved sensitivity have been reported (Maruo et al., 2010; Ebeling et al., 2009; Miwa et al., 2009; Ohira et al., 2009; Maruo, 2007; Utembe et al., 2006). Limitations for some of these sensors and detection papers include air flow dependence and relative humidity interference.

#### 3.5.5.4 Differential Optical Absorption Spectrometry

Optical remote sensing methods can provide direct, sensitive, and specific measurements of $O_3$ over a broad area or open path in contrast with conventional single-point UV monitors. The 1996 $O_3$ AQCD (U.S. EPA, 1996a) provided a brief discussion of DOAS for $O_3$ measurements and cited references to document the sensitivity (1.5 ppb for a 1-minute averaging time), correlation (r = 0.89), and agreement (on the order of 10%) with UV $O_3$ monitors (Stevens et al., 1993). The 2006 $O_3$ AQCD (U.S. EPA, 2006b) provided an update on DOAS where a positive interference due to an unidentified absorber was noted (Reisinger, 2000).

More recent study of the accuracy of UV absorbance monitors by Williams et al. (2006) compared UV and DOAS measurements at two urban locations. In order to compare the open path measurements and UV, the data sets were averaged to 30-minute periods and only data when the boundary layer was expected to be well mixed (between 10:00 a.m. and 6:00 p.m. CST) were evaluated. The comparisons showed variations of no more than ± 7% (based on the slope of the linear least squares regression over a concentration range from about 20 to 200 ppb) and good correlation ($R^2$ = 0.96 and 0.98). Lee et al. (2008b) evaluated DOAS and UV $O_3$ measurements in Korea and found the average DOAS concentration to be 8.6% lower than the UV point measurements with a good correlation ($R^2$ = 0.94).

DOAS has also been used for the measurement of $HNO_2$ (or HONO). DOAS was compared to chemical point-measurement methods for HONO. Acker et al. (2006) obtained good results when comparing wet chemical and DOAS during well mixed atmospheric conditions (wet chemical = 0.009 + 0.92 × DOAS; r = 0.7). Kleffmann and Wiesen (2008) noted that interferences with the HONO wet chemical methods can affect results from inter-comparison studies if not addressed. In an earlier study, Kleffmann et al. (2006) demonstrated that when the interferences were addressed, excellent agreement with DOAS can be obtained. Stutz et al. (2010) found good agreement (15% or better) between DOAS and a wet chemical method (Mist

Chamber/Ion Chromatography) in Houston, TX except generally during mid-day when the chemical method showed a positive bias that may have been related to concentrations of $O_3$. DOAS remains attractive due to its sensitivity, speed of response, and ability to simultaneously measure multiple pollutants; however, further inter-comparisons and interference testing are recommended.

### 3.5.5.5 Satellite Remote Sensing

Satellite observations for $O_3$ are growing as a resource for many purposes, including model evaluation, assessing emissions reductions, pollutant transport, and air quality management. Satellite remote sensing instruments do not directly measure the composition of the atmosphere. Satellite retrievals are conducted using the solar backscatter or thermal infrared emission spectra and a variety of algorithms. Most satellite measurement systems have been developed for stratospheric measurement of the total $O_3$ column. Mathematical techniques have been developed and must be applied to derive information from these systems about tropospheric $O_3$ (Tarasick and Slater, 2008; Ziemke et al., 2006). Direct retrieval of global tropospheric $O_3$ distributions from solar backscattered UV spectra have been reported from OMI and the Global Ozone Monitoring Experiment (GOME) (Liu et al., 2006). Another satellite measurement system, Tropospheric Emission Spectrometer (TES), produces global-scale vertical concentration profiles of tropospheric $O_3$ from measurements of thermal infrared emissions. TES has been designed specifically to focus on mapping the global distribution of tropospheric $O_3$ extending from the surface to about 10-15 km altitude (Beer, 2006). Satellite measurements of tropospheric $O_3$ generally require monthly averaging to reduce noise, and thus are of little use for observing synoptic-scale variability.

In order to improve the understanding of the quality and reliability of the data, satellite-based observations of total column and tropospheric $O_3$ have been validated in several studies using a variety of techniques, such as aircraft observations, ozonesondes, CTMs, and ground-based spectroradiometers. Antón et al. (2009) compared satellite data from two different algorithms (OMI-DOAS and OMI-TOMS) with total column $O_3$ data from ground-based spectroradiometers at five locations. The satellite total column $O_3$ data underestimated ground-based measurements by less than 3%. Richards et al. (2008) compared TES tropospheric $O_3$ profiles using airborne differential absorption lidar (DIAL) and found TES to have a 7 ppbv positive bias relative to DIAL throughout the troposphere. Nassar et al. (2008) compared TES $O_3$ profiles and ozonesonde coincidences and found a positive bias of 3-10 ppbv for TES. Worden et al. (2007a) also compared TES with ozonesondes and found TES $O_3$ profiles to be biased high in the upper troposphere (average bias of 16.8 ppbv for mid-latitudes and 9.8 ppbv for the tropics) and biased low in the lower troposphere (average bias of -2.6 ppbv for mid-latitudes and -7.4 ppbv for the tropics). Comparisons of TES and OMI with ozonesondes by Zhang et al. (2010b) showed a mean positive bias if 5.3 ppbv (10%) for TES and 2.8 ppbv (5%) for OMI at 500 hPa. In addition, Zhang et al. (2010b) used a CTM (GEOS-

Chem) to determine global differences between TES and OMI. They found differences between TES and OMI were generally ± 10 ppbv except at northern mid-latitudes in summer and over tropical continents. Satellite observations have also been combined (e.g., OMI and TES) to improve estimates of tropospheric $O_3$ (Worden et al., 2007b).

Satellite measurements are also available for $O_3$ precursors such as CO, $NO_2$, and HCHO. These measurements are useful for constraining model estimates of $O_3$ precursor emissions and long-range transport of pollution (Section 3.4). Zhang et al. (2008) used satellite measurements of CO and $NO_2$ along with $O_3$ to constrain estimates of background $O_3$ precursors in Asia and $O_3$ produced during long range transport.

## 3.5.6 Ambient $O_3$ Network Design

### 3.5.6.1 Monitor Siting Requirements

To monitor compliance with the NAAQS, state and local monitoring agencies operate $O_3$ monitoring sites at various locations depending on the area size (population and geographic characteristics[1]) and typical peak concentrations (expressed in percentages below, or near the $O_3$ NAAQS). SLAMS make up the ambient air quality monitoring sites that are primarily needed for NAAQS comparisons, but may also serve some other basic monitoring objectives that include: providing air pollution data to the general public in a timely manner; emissions strategy development; and support for air pollution research. SLAMS include National Core (NCore), Photochemical Assessment Monitoring Stations (PAMS), and all other State or locally-operated stations except for the monitors designated as special purpose monitors (SPMs).

The SLAMS minimum monitoring requirements to meet the $O_3$ design criteria are specified in 40 CFR Part 58, Appendix D. Although NCore and PAMS are a subset of SLAMS, the monitoring requirements for those networks are separate and discussed below. The minimum number of $O_3$ monitors required in a Metropolitan Statistical Area (MSA) ranges from zero for areas with a population of at least 50,000 and under 350,000 with no recent history of an $O_3$ design value[2] greater than 85 percent of the NAAQS, to four for areas with a population greater than 10 million and an $O_3$ design value greater than 85 percent of the NAAQS. Within an $O_3$ network, at least one site for each MSA, or Combined Statistical Area (CSA) if multiple MSAs are involved, must be designed to record the maximum concentration for that particular metropolitan area. More than one maximum concentration site may

[1] Geographic characteristics such as complexity of terrain, topography, land use, etc.

[2] A design value is a statistic that describes the air quality status of a given area relative to the level of the NAAQS. Design values are typically used to classify nonattainment areas, assess progress toward meeting the NAAQS, and develop control strategies. See http://epa.gov/airtrends/values.html (U.S. EPA, 2010a) for guidance on how these values are defined.

be necessary in some areas. The spatial scales for $O_3$ sites are neighborhood, urban and regional.

- Neighborhood scale: represents concentrations within some extended area of the city that has relatively uniform land use with dimensions in the 0.5-4.0 km range. The neighborhood and urban scales listed below have the potential to overlap in applications that concern secondary or homogeneously distributed primary air pollutants.
- Urban scale: represents concentrations within an area of city-like dimensions, on the order of 4-50 km. Within a city, the geographic placement of sources may result in there being no single site that can be said to represent air quality on an urban scale.
- Regional scale: usually defines a rural area of reasonably homogeneous geography without large sources, and extends from tens to hundreds of kilometers.

Since $O_3$ concentrations decrease appreciably in the colder parts of the year in many areas, $O_3$ is required to be monitored at SLAMS monitoring sites only during the "ozone season." Table D-3 of 40 CFR Part 58, Appendix D lists the beginning and ending month of the ozone season for each U.S. state or territory. Most operate $O_3$ monitors only during the ozone season. Those that operate some or all of their $O_3$ monitors on a year-round basis include Arizona, California, Hawaii, Louisiana, Nevada, New Mexico, Puerto Rico, Texas, American Samoa, Guam and the Virgin Islands.

The total number of SLAMS $O_3$ sites needed to support the basic monitoring objectives includes more sites than the minimum numbers required in 40 CFR Part 58, Appendix D. In 2010, there were 1250 $O_3$ monitoring sites reporting values to the EPA AQS database (Figure 3-21). Monitoring site information for EPA's air quality monitoring networks is available in spreadsheet format (CSV) and keyhole markup language format (KML or KMZ) that is compatible with Google Earth™ and other software applications on the AirExplorer website (U.S. EPA, 2011e). States may operate $O_3$ monitors in non-urban or rural areas to meet other objectives (e.g., support for research studies of atmospheric chemistry or ecosystem impacts). These monitors are often identified as SPMs and can be operated up to 24 months without being considered in NAAQS compliance determinations. The current monitor and probe siting requirements have an urban focus and do not address the siting for SPMs or monitors in non-urban, rural areas to support ecosystem impacts and the secondary standards.

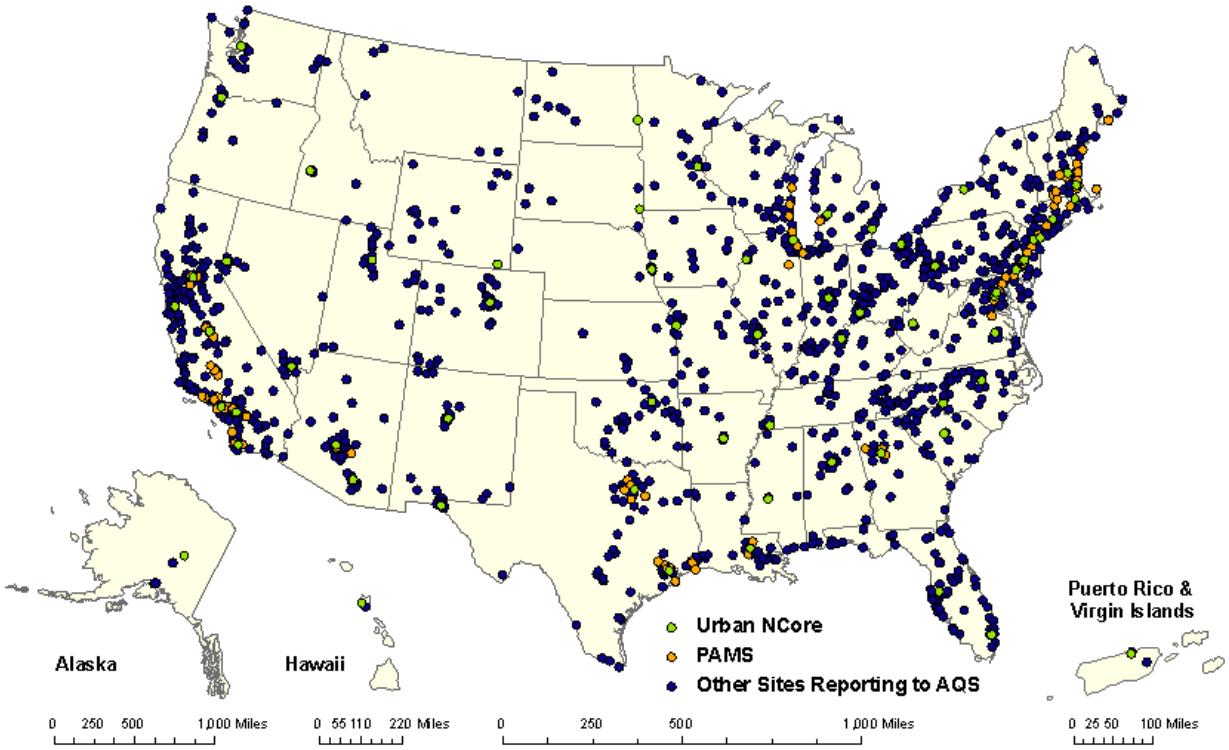



**Figure 3-21 U.S. $O_3$ sites reporting data to AQS in 2010.**

NCore is a new multipollutant monitoring network implemented to meet multiple monitoring objectives. Those objectives include: timely reporting of data to the public through AirNow (U.S. EPA, 2011a); support for the development of emission reduction strategies; tracking long-term trends of criteria pollutants and precursors; support to ongoing reviews of the NAAQS and NAAQS compliance; model evaluation; support for scientific research studies; and support for ecosystem assessments. Each state is required to operate at least one NCore site. The NCore monitoring network began January 1, 2011 at about 80 stations (about 60 urban and 20 rural sites). NCore has leveraged the use of sites in existing networks; for example, some IMPROVE sites also serve as rural NCore sites. In addition to $O_3$, other components including CO, $NO_X$, $NO_Y$, $SO_2$, and basic meteorology are also measured at NCore sites. The spatial scale for urban NCore stations is urban or neighborhood; however, a middle-scale[1] site may be acceptable in cases where the site can represent many such locations throughout a metropolitan area. Rural NCore sites are located at a regional or larger scale, away from any large local emission sources so that they represent ambient concentrations over an extensive area. Ozone monitors at NCore sites are operated year round.

[1] Middle scale defines an area up to several city blocks in size with dimensions ranging from about 100 to 500 meters.

PAMS provides more comprehensive data on $O_3$ in areas classified as serious, severe, or extreme nonattainment for $O_3$. In addition to $O_3$, PAMS provides data for $NO_X$, $NO_Y$, VOCs, carbonyls, and meteorology. The PAMS network design criteria are based on locations relative to $O_3$ precursor source areas and predominant wind directions associated with high $O_3$ concentrations. The overall network design is location specific and geared toward enabling characterization of precursor emission sources in the area, $O_3$ transport, and photochemical processes related to $O_3$ nonattainment. Minimum monitoring for $O_3$ and its precursors is required annually during the months of June, July, and August when peak $O_3$ concentrations are expected. In 2006, the EPA reduced the minimum PAMS monitoring requirements (71 FR 61236). There were a total of 92 PAMS sites reporting values to the AQS data base in 2010.

CASTNET is a regional monitoring network established to assess trends in acidic deposition due to emission reduction regulations. CASTNET also provides concentration measurements of air pollutants involved in acidic deposition, such as sulfate and nitrate, in addition to the measurement of $O_3$. CASTNET $O_3$ monitors operate year round and are primarily located in rural areas. In 2010, there were 80 CASTNET sites located in, or near, rural areas. As part of CASTNET, the National Park Service (NPS) operates 23 sites located in national parks and other Class-I areas. Ozone data collected at the 23 NPS sites is compliant with the SLAMS QA requirements in 40 CFR Part 58, Appendix A. Ozone measurements at the remaining CASTNET sites were not collected with the QA requirements for SLAMS outlined in 40 CFR Part 58, Appendix A, and therefore, these $O_3$ data cannot be used for NAAQS compliance purposes. The SLAMS QA requirements and procedures are currently being implemented at the remaining sites.

The NPS also operates a Portable Ozone Monitoring Systems (POMS) network. The POMS couples the small, low-power $O_3$ monitor with a data logger, meteorological measurements, and solar power in a self contained system for monitoring in remote locations. Typical uses for the POMS data include research projects, survey monitoring, and assessments of spatial $O_3$ distribution. The portable $O_3$ monitor in use by the NPS was recently designated as an equivalent method for $O_3$ (75 FR 22126). Seventeen NPS POMS monitors were operating in 2010 (NPS, 2011). A map of the rural NCore sites, along with the CASTNET, and the NPS POMS sites are shown in Figure 3-22. As can be seen from Figure 3-21 and Figure 3-22, vast rural areas of the country still exist without any monitor coverage. Monitoring opportunities exist in these areas where relatively few and easily characterized precursor sources dominate and could be used to improve understanding of $O_3$ formation.

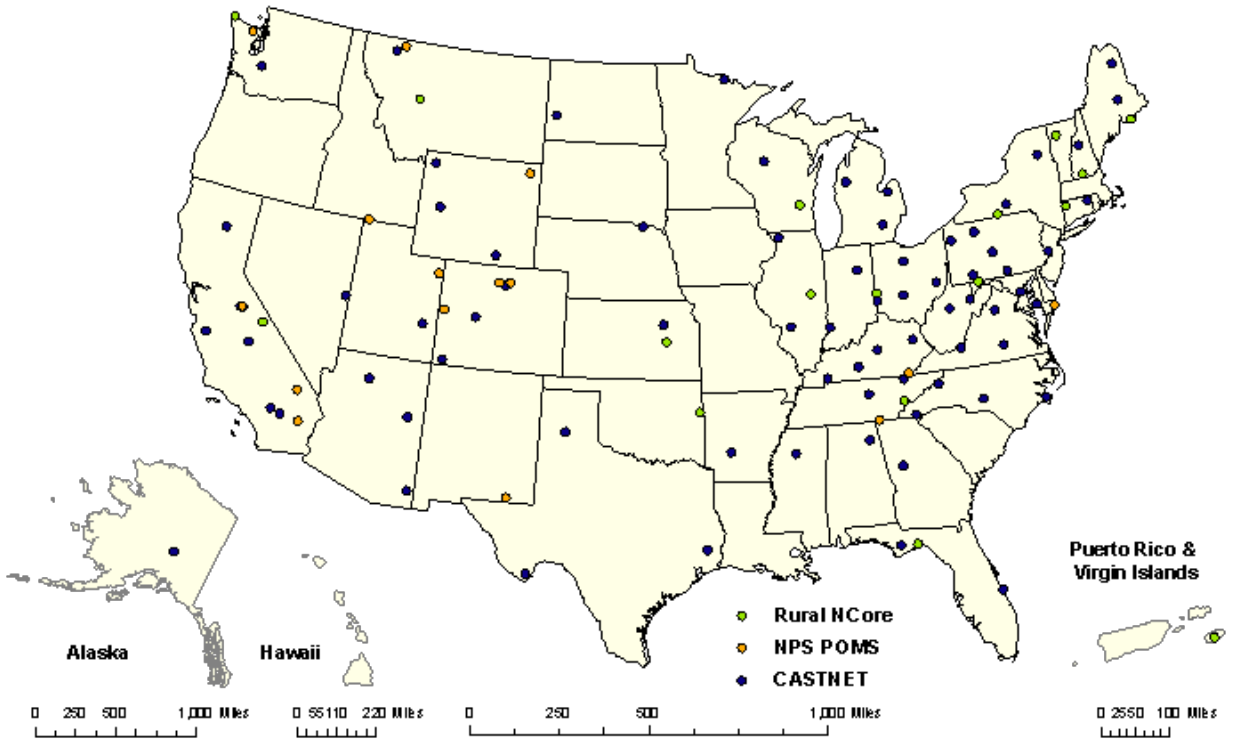



**Figure 3-22 U.S. Rural NCore, CASTNET and NPS POMS $O_3$ sites in 2010.**

### 3.5.6.2 Probe/Inlet Siting Requirements

Probe and monitoring path siting criteria for ambient air quality monitoring are contained in 40 CFR Part 58, Appendix E. For $O_3$, the probe must be located between 2 and 15 meters above ground level and be at least 1 meter away (both in the horizontal and vertical directions) from any supporting structure, walls, etc. If it is located on the side of a building, it must be located on the windward side, relative to prevailing wind direction during the season of highest potential $O_3$ concentration. Ozone monitors are placed to determine air quality in larger areas (neighborhood, urban, or regional scales) and therefore, placement of the monitor probe should not be near local, minor sources of NO, $O_3$-scavenging hydrocarbons, or $O_3$ precursors. The probe or inlet must have unrestricted air flow in an arc of at least 180 degrees and be located away from any building or obstacle at a distance of at least twice the height of the obstacle. The arc of unrestricted air flow must include the predominant wind direction for the season of greatest $O_3$ concentrations. Some exceptions can be made for measurements taken in street canyons or sites where obstruction by buildings or other structures is unavoidable. The scavenging effect of trees on $O_3$ is greater than other pollutants and the probe/inlet must be located at least 10 meters from the tree drip line to minimize interference with normal air flow. When siting $O_3$ monitors near roadways, it is important to minimize the destructive interferences

from sources of NO, since NO reacts readily with $O_3$. For siting neighborhood and urban scale $O_3$ monitors, guidance on the minimum distance from the edge of the nearest traffic lane is based on roadway average daily traffic count (40 CFR Part 58, Appendix E, Table E-1). The minimum distance from roadways is 10 meters (average daily traffic count ≤ 1,000) and increases to a maximum distance of 250 meters (average daily traffic count ≥ 110,000).

## 3.6 Ambient Concentrations

This section investigates spatiotemporal variability in ambient $O_3$ concentrations and associations between $O_3$ and copollutants. To set the stage for the rest of the section, common $O_3$ measurement units, metrics, and averaging times are described and compared in Section 3.6.1. Spatial variability is covered in Section 3.6.2 and is divided into urban-focused variability and rural-focused variability. Urban-focused variability is organized by scale, extending from national-scale down to neighborhood-scale and the near-road environment. Rural-focused variability is organized by region and includes observations of ground-level vertical $O_3$ gradients where available. Temporal variability is covered in Section 3.6.3 and is organized by time, extending from multiyear trends down to hourly (diel) variability. In many instances, spatial and temporal variability are inseparable (e.g., seasonal dependence to spatial variability), resulting in some overlap between Section 3.6.2 and Section 3.6.3. Finally, Section 3.6.4 covers associations between $O_3$ and copollutants including CO, $SO_2$, $NO_2$, $PM_{2.5}$, and $PM_{10}$.

As noted in the 2006 $O_3$ AQCD (U.S. EPA, 2006b), $O_3$ is the only photochemical oxidant other than nitrogen dioxide ($NO_2$) that is routinely monitored and for which a comprehensive database exists. Data for other photochemical oxidants (e.g., PAN, $H_2O_2$, etc.) typically have been obtained only as part of special field studies. Consequently, no data on nationwide patterns of occurrence are available for these other oxidants; nor are extensive data available on the relationships of concentrations and patterns of these oxidants to those of $O_3$. As a result, this section focuses solely on $O_3$, the NAAQS indicator for photochemical oxidants. The majority of ambient $O_3$ data reported in this section were obtained from AQS, EPA's repository for detailed, hourly data that has been subject to EPA quality control and assurance procedures (the AQS network was described in Section 3.5).

### 3.6.1 Measurement Units, Metrics, and Averaging Times

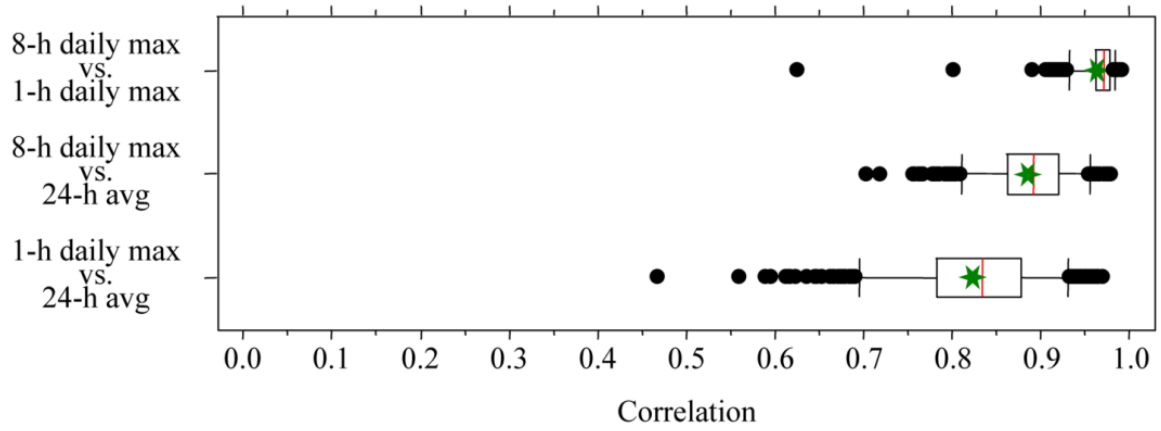
Several approaches are commonly used for reporting $O_3$ data. In atmospheric sciences and epidemiology, $O_3$ is frequently reported as a concentration, expressed as a volume-to-volume mixing ratio, commonly measured in ppm or ppb. In human exposure, $O_3$ is frequently reported as a cumulative exposure, expressed as a mixing ratio times time (e.g., ppm-h). In ecology, cumulative exposure indicators are frequently used that extend over longer time periods, such as growing season or year.

This section focuses on ambient concentrations derived primarily from hourly average $O_3$ measurements and concentrations are reported in ppb wherever possible. Further details on human and ecological exposure metrics can be found in Chapter 4 and Chapter 9, respectively.

As discussed in Section 3.5, most continuous $O_3$ monitors report hourly average concentrations to AQS with a required precision of 10 ppb and LDL of 10 ppb (see Table 3-4). This data can be used as reported (1-h avg), or further summarized in one of several ways to focus on important aspects of the data while simultaneously reducing the volume of information. Three common daily reporting metrics include: (1) the average of the hourly observations over a 24-hour period (24-h avg); (2) the maximum hourly observation occurring in a 24-hour period (1-h daily max); and (3) the maximum 8-h running average of the hourly observations occurring in a 24-hour period (8-h daily max)[1]. Throughout this ISA and the literature, $O_3$ concentrations are reported using different averaging times as appropriate, making it important to recognize the differences between these metrics.

Nation-wide, year-round 1-h avg $O_3$ data reported to AQS from 2007-2009 was used to compare these different daily metrics. Correlations between the 24-h avg, 1-h daily max and 8-h daily max metrics were generated on a site-by-site basis. Figure 3-23 contains box plots of the distribution in correlations from all sites. The top comparison in Figure 3-23 is between 8-h daily max and 1-h daily max $O_3$. Not surprisingly, these two metrics are very highly correlated (median r = 0.97, IQR = 0.96-0.98). There are a couple outlying sites, with correlations between these two metrics as low as 0.63, but 95% of sites have correlations above 0.93. The middle comparison in Figure 3-23 is between 8-h daily max and 24-h avg $O_3$. For these metrics, the distribution in correlations is shifted down and broadened out (median r = 0.89, IQR = 0.86-0.92). Finally, the bottom comparison in Figure 3-23 is between 1-h daily max and 24-h avg $O_3$. Again, for these metrics the distribution in correlations is shifted down and broadened out relative to the other two comparisons (median r = 0.83, IQR = 0.78-0.88). The correlation between the two daily maximum metrics (1-h daily max and 8-h daily max) are quite high for most sites, but correlations between the daily maximum metrics and the daily average metric (24-h avg) are lower. This illustrates the influence of the overnight period on the 24-h avg $O_3$ concentration. In contrast, the 1-h daily max and 8-h daily max are more indicative of the daytime, higher $O_3$ periods. The correlation between these metrics, however, can be very site-specific, as is evident from the broad range in correlations in Figure 3-23 for all three comparisons. Therefore, understanding which $O_3$ metric is being used in a given study is very important since they capture different aspects of $O_3$ temporal variability.

[1] For $O_3$ regulatory monitoring purposes, the 8-h daily max is calculated by first generating all 8-h running averages and storing these averages hourly by the first hour in the 8-hour period. The 8-h daily max is then set equal to the maximum of the 24 individual 8-h averages occurring in a given day.




Note: Shown are the median (red line), mean (green star), inner-quartile range (box), 5th and 95th percentiles (whiskers), and extremes (black circles).

**Figure 3-23 Distribution in nation-wide year-round site-level correlations between daily $O_3$ metrics including 24-h avg, 1-h daily max and 8-h daily max using AQS data, 2007-2009.**

The median 1-h daily max, 8-h daily max, and 24-h avg $O_3$ concentrations across all sites included in the 3-year nation-wide data set were 44, 40, and 29 ppb, respectively. Representing the upper end of the distribution, the 99th percentiles of these same metrics across all sites were 94, 80, and 60 ppb, respectively. While the ratio of these metrics will vary by location, typically the 1-h daily max will be the highest value representing peak concentrations and the 24-h avg will be considerably lower representing daily average concentrations incorporating the overnight period. The 8-h daily max typically represents the higher mid-day concentrations and will generally lie somewhere between the other two metrics[1].

## 3.6.2 Spatial Variability

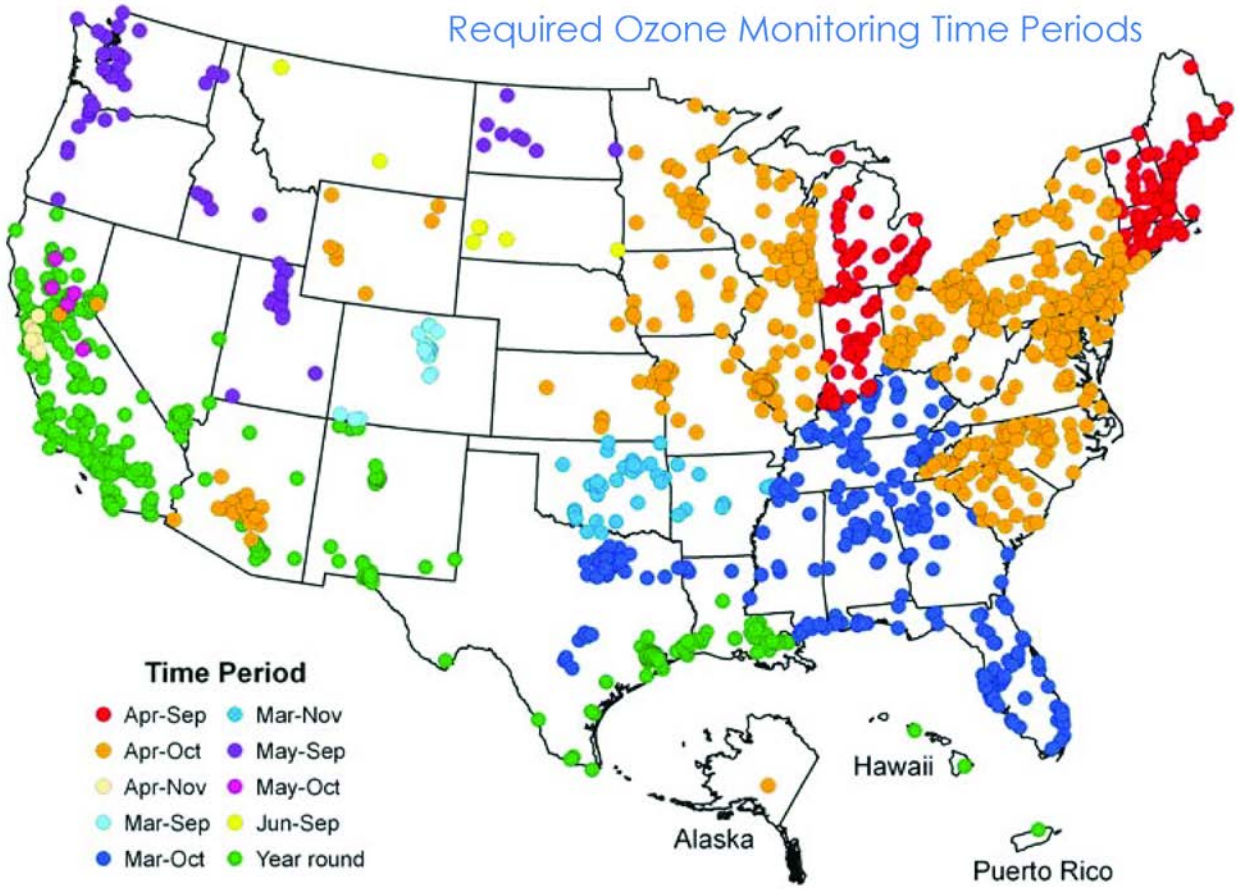
### 3.6.2.1 Urban-Focused Variability

#### National-Scale Variability

AQS contains a large depository of national $O_3$ data collected to meet the monitoring objectives described in Section 3.5.6. In many areas, $O_3$ concentrations decrease appreciably during months with lower temperatures and decreased sunlight. As a

[1] The 8-h daily max is not strictly limited to lie between the 1-h daily max and the 24-h avg since the 8-hour averaging period used to calculate the 8-h daily max can extend into the morning hours of the subsequent day. However, the 8-h daily max typically incorporates the middle of the day when $O_3$ concentrations are at their highest, resulting in an 8-h daily max somewhere between the 1-h daily max and the 24-h avg calculated for that day.

result, year-round $O_3$ monitoring is only required in certain areas. Table D-3 of 40 CFR Part 58, Appendix D lists the beginning and ending month of the ozone season (defined in Section 3.5.6.1) by geographic area and Figure 3-24 illustrates these time periods on a monitor-by-monitor basis. Monitoring is optional outside the ozone season and many states elect to operate their monitors year-round or for time periods outside what is strictly mandated.




Source: U.S. EPA (2008e).

**Figure 3-24 Required $O_3$ monitoring time periods (ozone season) identified by monitoring site.**

Hourly FRM and FEM $O_3$ data reported to AQS for the period 2007-2009 were used to investigate national-scale spatial variability in $O_3$ concentrations. Given the variability in $O_3$ monitoring time periods available in AQS as a result of the regionally-varying ozone seasons, the analyses in this section were based on two distinct data sets:

- a **year-round** data set: data only from monitors reporting year-round;
- a **warm-season** data set: data from all monitors reporting May through September.

The warm-season data set was used to capture the majority of ozone season data while providing a consistent time-frame for comparison across states. All available monitoring data including data from year-round monitors was included in the warm-season data set after removing observations outside the 5-month window. Data were retrieved from AQS on February 25, 2011 for these two data sets, and all validated data was included regardless of flags or regional concurrence[1]. A summary of the two $O_3$ data sets including the applied completeness criteria is provided in Table 3-5. Figure 3-25 and Figure 3-26 show the location of the 457 year-round and 1,064 warm-season monitors meeting the completeness criteria for all three years (2007-2009).

**Table 3-5 Summary of $O_3$ data sets originating from AQS.**

| | Year-Round Data Set | Warm-Season Data Set |
|---|---|---|
| Years | 2007-2009 | 2007-2009 |
| Months | January - December (12 mo) | May - September (5 mo) |
| Completeness Criteria | 75% of hours in a day | 75% of hours in a day |
| | 75% of days in a calendar quarter | 75% of days between May - September |
| | All 4 quarters per year | |
| Number of monitors meeting completeness criteria | 618 containing at least one valid year in 2007-2009 | 1,267 containing at least one valid year in 2007-2009 |
| | 550 containing at least two valid years in 2007-2009 | 1,169 containing at least two valid years in 2007-2009 |
| | 457 containing all three valid years in 2007-2009 | 1,064 containing all three valid years in 2007-2009 |

[1] Concentrations that might have been affected by exceptional events (and contribute to a violation of the NAAQS) can be flagged in the Air Quality System (AQS) by the reporting organization. Exceptional events are defined as unusual or naturally occurring events that can affect air quality but are not reasonably controllable using techniques that tribal, state or local air agencies may implement in order to attain and maintain the National Ambient Air Quality Standards (NAAQS). The corresponding EPA Regional Office is responsible for reviewing the data and evidence of the event, and deciding whether to concur with the flag. Flagged data that has been concurred by the Regional office is typically excluded for regulatory purposes.

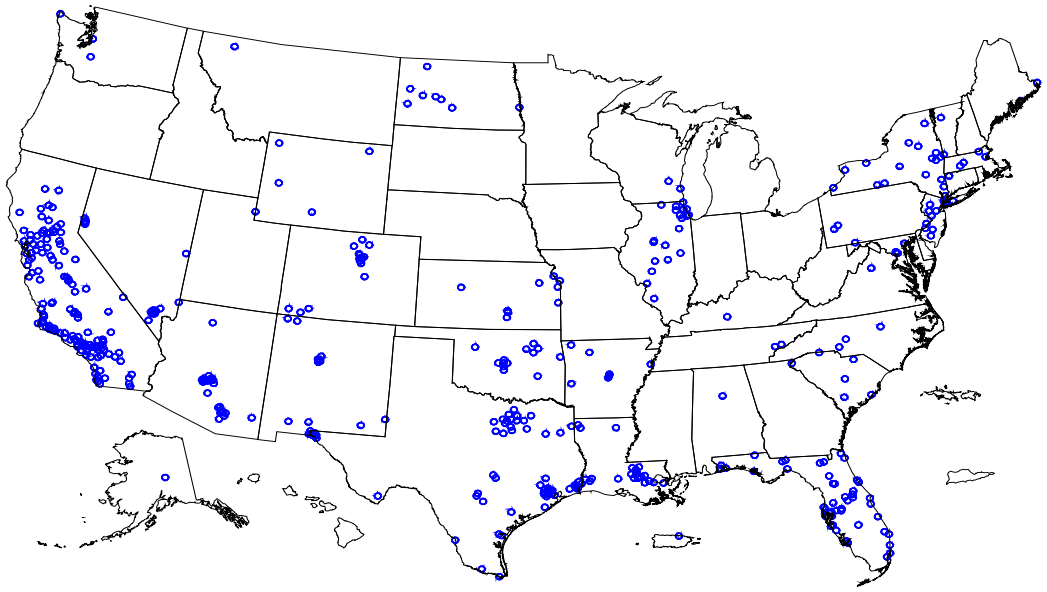


**Figure 3-25 Location of the 457 $O_3$ monitors meeting the year-round data set completeness criterion for all 3 years between 2007 and 2009.**

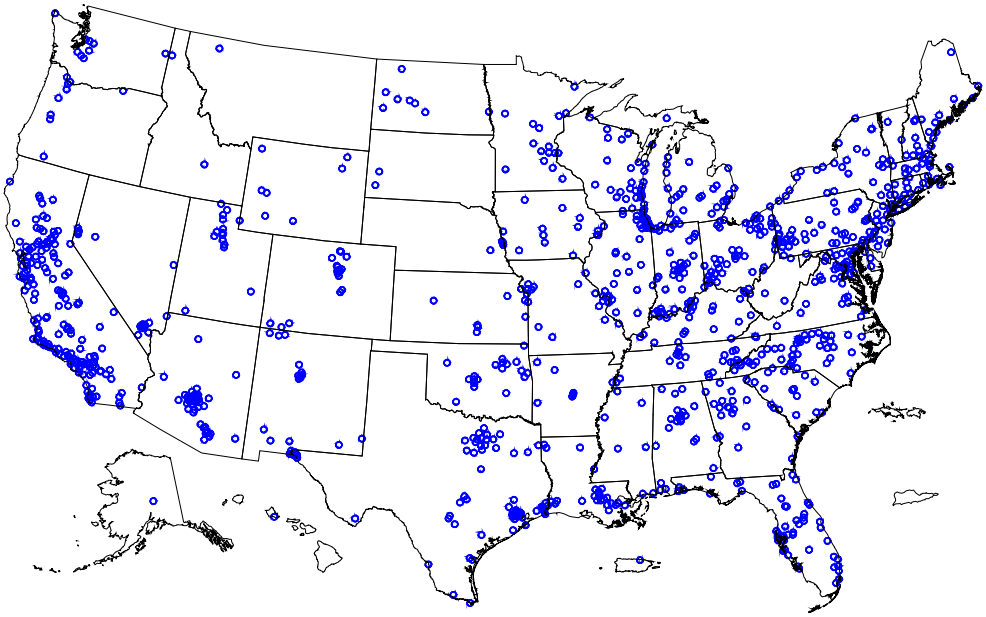


**Figure 3-26 Location of the 1,064 $O_3$ monitors meeting the warm-season data set completeness criteria for all 3 years between 2007 and 2009.**

Tabulated statistics generated from the year-round and warm-season data sets are included in Table 3-6 and Table 3-7, respectively. This information was used to compare (1) the year-round and warm-season data sets; (2) the $O_3$ distribution variability across years (2005-2009); and (3) four different averaging times (1-h avg, 24-h avg, 1-h daily max, and 8-h daily max). Summary statistics for 2005 and 2006 were added to these tables in order to gain a broader view of year-to-year variability, but the year-round and warm-season data sets used for analyses in the rest of this section are limited to 2007-2009 as described above and in Table 3-5.
The 8-h daily max pooled by site was also included in these tables to show the distribution of the annual and 3-year (2007-2009) site-averages of the 8-h daily max statistic.

The year-round data set includes data from roughly half the number of monitors as the warm-season data set and a larger fraction of the year-round monitors are located in the southern half of the U.S. due to extended monitoring requirements in these areas. Despite these differences, the mean, SD and percentiles of the nation-wide $O_3$ concentrations were quite similar for the year-round data presented in Table 3-6 and the warm-season data presented in Table 3-7. In both data sets, there was very little variability across years in the central statistics; for example, the median 1-h avg concentrations between 2005 and 2009 ranged from 28 to 29 ppb for the year-round data and from 29 to 30 ppb for the warm-season data. The 8-h daily max showed similar uniformity in median across the five years, with concentrations ranging from 39 to 41 ppb for the year-round data and from 40 to 43 for the warm-season data. The upper percentiles (95th and above) showed a general downward trend from 2005 to 2009 in both nation-wide data sets. For example, the 99th percentile of the 8-h daily max observed in the warm-season data dropped from 85 ppb in 2005 to 75 ppb in 2009. Trends in $O_3$ concentrations investigated over a longer time period are included in Section 3.6.3.1.

## Table 3-6 Nationwide distributions of $O_3$ concentrations (ppb) from the year-round data set.

| Time Period | N Monitors | N Obs | Mean | SD | Min | 1 | 5 | 10 | 25 | 50 | 75 | 90 | 95 | 98 | 99 | Max | Max Site ID[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1-h avg[a]** | | | | | | | | | | | | | | | | | |
| 2005 | 499 | 4,284,219 | 29 | 18 | 2 | 2 | 2 | 2 | 15 | 28 | 41 | 53 | 61 | 71 | 78 | 182 | 060710005 |
| 2006 | 532 | 4,543,205 | 30 | 18 | 2 | 2 | 2 | 5 | 16 | 29 | 42 | 54 | 61 | 71 | 78 | 175 | 060370016 |
| 2007 | 522 | 4,547,280 | 29 | 18 | 2 | 2 | 2 | 5 | 16 | 29 | 41 | 52 | 60 | 68 | 75 | 237 | 450790021 |
| 2008 | 520 | 4,470,065 | 30 | 17 | 2 | 2 | 2 | 6 | 17 | 29 | 41 | 52 | 59 | 67 | 74 | 222 | 450210002 |
| 2009 | 551 | 4,716,962 | 29 | 16 | 2 | 2 | 2 | 6 | 17 | 29 | 40 | 50 | 56 | 64 | 70 | 188 | 720770001 |
| 2007-2009 | 599 | 13,734,307 | 29 | 17 | 2 | 2 | 2 | 6 | 17 | 29 | 40 | 51 | 58 | 67 | 73 | 237 | 450790021 |
| **24-h avg[a]** | | | | | | | | | | | | | | | | | |
| 2005 | 504 | 183,815 | 29 | 13 | 2 | 4 | 9 | 13 | 20 | 28 | 37 | 46 | 51 | 57 | 61 | 103 | 060719002 |
| 2006 | 536 | 194,884 | 30 | 13 | 2 | 5 | 10 | 14 | 21 | 29 | 38 | 47 | 52 | 58 | 62 | 102 | 061070009 |
| 2007 | 531 | 194,873 | 29 | 12 | 2 | 5 | 11 | 14 | 20 | 29 | 37 | 45 | 50 | 56 | 60 | 96 | 060651016 |
| 2008 | 528 | 191,875 | 30 | 12 | 2 | 5 | 11 | 14 | 21 | 29 | 38 | 46 | 50 | 56 | 61 | 98 | 060710005 |
| 2009 | 556 | 202,142 | 29 | 11 | 2 | 6 | 11 | 14 | 21 | 28 | 37 | 44 | 48 | 53 | 57 | 95 | 060710005 |
| 2007-2009 | 611 | 588,890 | 29 | 12 | 2 | 5 | 11 | 14 | 21 | 29 | 37 | 45 | 49 | 55 | 60 | 98 | 060710005 |
| **1-h daily max[a]** | | | | | | | | | | | | | | | | | |
| 2005 | 504 | 183,815 | 48 | 18 | 2 | 11 | 21 | 26 | 35 | 46 | 58 | 71 | 80 | 91 | 100 | 182 | 060710005 |
| 2006 | 536 | 194,884 | 48 | 18 | 2 | 13 | 23 | 28 | 36 | 46 | 58 | 71 | 80 | 91 | 100 | 175 | 060370016 |
| 2007 | 531 | 194,873 | 47 | 17 | 2 | 14 | 23 | 28 | 36 | 45 | 57 | 69 | 77 | 87 | 94 | 237 | 450790021 |
| 2008 | 528 | 191,875 | 47 | 17 | 2 | 14 | 23 | 27 | 35 | 45 | 56 | 67 | 76 | 87 | 96 | 222 | 450210002 |
| 2009 | 556 | 202,142 | 45 | 15 | 2 | 14 | 22 | 27 | 35 | 44 | 54 | 64 | 72 | 83 | 91 | 188 | 720770001 |
| 2007-2009 | 611 | 588,890 | 46 | 16 | 2 | 14 | 23 | 27 | 35 | 44 | 55 | 67 | 75 | 86 | 94 | 237 | 450790021 |
| **8-h daily max[a]** | | | | | | | | | | | | | | | | | |
| 2005 | 504 | 183,279 | 42 | 16 | 2 | 7 | 16 | 21 | 30 | 40 | 52 | 63 | 70 | 78 | 84 | 145 | 060710005 |
| 2006 | 536 | 194,285 | 42 | 16 | 2 | 9 | 18 | 23 | 31 | 41 | 52 | 63 | 70 | 79 | 85 | 142 | 060710005 |
| 2007 | 528 | 194,266 | 41 | 15 | 2 | 10 | 19 | 23 | 31 | 40 | 51 | 61 | 68 | 75 | 81 | 137 | 060710005 |
| 2008 | 528 | 191,283 | 41 | 15 | 2 | 11 | 19 | 23 | 31 | 40 | 51 | 60 | 66 | 75 | 82 | 172 | 450210002 |
| 2009 | 556 | 201,536 | 40 | 14 | 2 | 11 | 18 | 23 | 30 | 39 | 49 | 57 | 63 | 71 | 77 | 128 | 060712002 |
| 2007-2009 | 608 | 587,085 | 41 | 15 | 2 | 10 | 19 | 23 | 31 | 40 | 50 | 60 | 66 | 74 | 80 | 172 | 450210002 |
| **8-h daily max (pooled by site)[a]** | | | | | | | | | | | | | | | | | |
| 2005 | 508 | 508 | 42 | 6 | 23 | 27 | 32 | 34 | 38 | 42 | 45 | 48 | 51 | 53 | 55 | 61 | 060710005 |
| 2006 | 538 | 538 | 42 | 6 | 12 | 28 | 31 | 34 | 38 | 43 | 46 | 50 | 52 | 54 | 55 | 61 | 060719002 |
| 2007 | 538 | 538 | 41 | 6 | 17 | 27 | 31 | 34 | 38 | 41 | 45 | 49 | 51 | 54 | 55 | 63 | 060719002 |
| 2008 | 529 | 529 | 41 | 6 | 20 | 28 | 31 | 34 | 37 | 40 | 45 | 50 | 52 | 55 | 57 | 61 | 060719002 |
| 2009 | 558 | 558 | 40 | 6 | 20 | 26 | 30 | 33 | 36 | 39 | 44 | 48 | 50 | 53 | 54 | 60 | 060719002 |
| 2007-2009 | 457 | 457 | 41 | 6 | 19 | 29 | 32 | 34 | 38 | 40 | 45 | 49 | 51 | 54 | 55 | 61 | 060719002 |

[a]Includes all validated data regardless of flags or regional concurrence and therefore may differ from data used for regulatory purposes

[b]AQS Site ID corresponding to the observation in the Max column

## Table 3-7 Nationwide distributions of $O_3$ concentrations (ppb) from the warm-season data set.

| Time Period | N Monitors | N Obs | Mean | SD | Min | 1 | 5 | 10 | 25 | 50 | 75 | 90 | 95 | 98 | 99 | Max | Max Site ID[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1-h avg[a]** | | | | | | | | | | | | | | | | | |
| 2005 | 1,023 | 7,455,018 | 30 | 19 | 2 | 2 | 2 | 5 | 16 | 29 | 43 | 55 | 64 | 73 | 79 | 182 | 060710005 |
| 2006 | 1,036 | 7,590,796 | 31 | 18 | 2 | 2 | 2 | 6 | 17 | 30 | 43 | 55 | 62 | 71 | 77 | 175 | 060370016 |
| 2007 | 1,021 | 7,711,463 | 31 | 18 | 2 | 2 | 2 | 6 | 18 | 30 | 43 | 55 | 63 | 71 | 77 | 237 | 450790021 |
| 2008 | 1,034 | 7,701,597 | 31 | 17 | 2 | 2 | 2 | 7 | 18 | 30 | 42 | 53 | 60 | 68 | 74 | 222 | 450210002 |
| 2009 | 1,029 | 7,835,074 | 29 | 16 | 2 | 2 | 2 | 7 | 17 | 29 | 40 | 50 | 56 | 63 | 69 | 259 | 311090016 |
| 2007-2009 | 1,103 | 23,248,134 | 30 | 17 | 2 | 2 | 2 | 7 | 18 | 30 | 42 | 53 | 60 | 68 | 74 | 259 | 311090016 |
| **24-h avg[a]** | | | | | | | | | | | | | | | | | |
| 2005 | 1,103 | 319,410 | 30 | 12 | 2 | 5 | 10 | 14 | 22 | 30 | 39 | 46 | 51 | 57 | 61 | 103 | 060719002 |
| 2006 | 1,110 | 324,993 | 31 | 12 | 2 | 6 | 12 | 15 | 22 | 30 | 39 | 47 | 52 | 58 | 61 | 102 | 061070009 |
| 2007 | 1,100 | 330,197 | 31 | 12 | 2 | 6 | 12 | 16 | 23 | 31 | 39 | 47 | 51 | 57 | 61 | 96 | 060651016 |
| 2008 | 1,120 | 329,918 | 31 | 12 | 2 | 6 | 12 | 16 | 22 | 30 | 38 | 46 | 50 | 56 | 60 | 98 | 060710005 |
| 2009 | 1,141 | 335,669 | 29 | 11 | 2 | 6 | 12 | 15 | 21 | 29 | 37 | 44 | 48 | 53 | 56 | 95 | 060710005 |
| 2007-2009 | 1,197 | 995,784 | 30 | 12 | 2 | 6 | 12 | 16 | 22 | 30 | 38 | 45 | 50 | 55 | 59 | 98 | 060710005 |
| **1-h daily max[a]** | | | | | | | | | | | | | | | | | |
| 2005 | 1,103 | 319,410 | 50 | 18 | 2 | 12 | 23 | 28 | 38 | 49 | 61 | 74 | 81 | 91 | 99 | 182 | 060710005 |
| 2006 | 1,110 | 324,993 | 50 | 17 | 2 | 15 | 25 | 29 | 38 | 48 | 60 | 72 | 80 | 90 | 98 | 175 | 060370016 |
| 2007 | 1,100 | 330,197 | 50 | 17 | 2 | 16 | 25 | 30 | 38 | 48 | 60 | 72 | 80 | 88 | 95 | 237 | 450790021 |
| 2008 | 1,120 | 329,918 | 48 | 16 | 2 | 16 | 25 | 29 | 37 | 47 | 58 | 69 | 76 | 86 | 93 | 222 | 450210002 |
| 2009 | 1,141 | 335,669 | 46 | 15 | 2 | 15 | 23 | 28 | 36 | 45 | 54 | 64 | 71 | 80 | 87 | 259 | 311090016 |
| 2007-2009 | 1,197 | 995,784 | 48 | 16 | 2 | 16 | 24 | 29 | 37 | 47 | 58 | 68 | 76 | 85 | 93 | 259 | 311090016 |
| **8-h daily max[a]** | | | | | | | | | | | | | | | | | |
| 2005 | 1,104 | 318,771 | 44 | 16 | 2 | 9 | 18 | 23 | 32 | 43 | 55 | 66 | 72 | 79 | 85 | 145 | 060710005 |
| 2006 | 1,112 | 324,327 | 44 | 16 | 2 | 11 | 20 | 25 | 33 | 43 | 54 | 64 | 70 | 78 | 84 | 142 | 060710005 |
| 2007 | 1,097 | 329,482 | 44 | 15 | 2 | 12 | 20 | 25 | 33 | 43 | 54 | 65 | 71 | 78 | 82 | 137 | 060710005 |
| 2008 | 1,120 | 329,223 | 43 | 15 | 2 | 12 | 20 | 25 | 33 | 42 | 52 | 61 | 67 | 74 | 80 | 172 | 450210002 |
| 2009 | 1,141 | 334,972 | 40 | 13 | 2 | 12 | 19 | 24 | 31 | 40 | 49 | 57 | 63 | 69 | 75 | 128 | 060712002 |
| 2007-2009 | 1,194 | 993,677 | 42 | 15 | 2 | 12 | 20 | 24 | 32 | 42 | 52 | 61 | 67 | 75 | 80 | 172 | 450210002 |
| **8-h daily max (pooled by site)[a]** | | | | | | | | | | | | | | | | | |
| 2005 | 1,141 | 1,141 | 45 | 6 | 14 | 28 | 34 | 36 | 41 | 46 | 49 | 52 | 54 | 56 | 57 | 61 | 040139508 |
| 2006 | 1,152 | 1,152 | 44 | 6 | 12 | 29 | 34 | 37 | 41 | 45 | 48 | 51 | 54 | 58 | 59 | 65 | 060170020 |
| 2007 | 1,164 | 1,164 | 45 | 7 | 17 | 28 | 34 | 36 | 40 | 45 | 50 | 54 | 56 | 58 | 59 | 64 | 471550102 |
| 2008 | 1,163 | 1,163 | 43 | 6 | 20 | 29 | 33 | 36 | 39 | 44 | 48 | 50 | 53 | 56 | 58 | 61 | 060719002 |
| 2009 | 1,173 | 1,173 | 41 | 5 | 20 | 28 | 32 | 35 | 38 | 41 | 44 | 47 | 50 | 53 | 55 | 63 | 060651016 |
| 2007-2009 | 1,064 | 1,064 | 43 | 6 | 19 | 29 | 34 | 36 | 39 | 43 | 47 | 50 | 52 | 55 | 57 | 61 | 060719002 |

[a]Includes all validated data regardless of flags or regional concurrence and therefore may differ from data used for regulatory purposes.

[b]AQS Site ID corresponding to the observation in the Max column.

Given the strong diurnal pattern in $O_3$ concentrations, the selection of averaging time has a substantial effect on the magnitude of concentration reporting. The nation-wide median 1-h avg, 24-h avg, 1-h daily max, and 8-h daily max concentrations for the year-round data set in 2009 were 29, 28, 44 and 39 ppb, respectively. The median concentrations for the warm-season data set in 2009 were: 29, 29, 45 and 40 ppb, respectively. The 1-h avg and 24-h avg both include the lowest concentrations typically observed in the overnight period which lowers their values relative to the daily maximum statistics.

A strong seasonal pattern in $O_3$ concentrations can also be seen in the year-round data. Table 3-8 shows the 8-h daily max stratified by season, with the seasons defined as:

- winter: December-February;
- spring: March-May;
- summer: June-August;
- fall: September-November.

In addition, warm-season (May-Sept) and cold-season (Oct-Apr) stratifications of the year-round data set are included in the table for comparison with the four seasonal stratifications. Substantial seasonal variability in the 8-h daily max concentration for the period 2007-2009 was evident with lower concentrations present in fall (median = 36 ppb) and winter (median = 32 ppb) and higher concentrations in spring (median = 47 ppb) and summer (median = 46 ppb). The seasonal differences were even more pronounced in the upper percentiles. For example, the 99th percentile in the 8-h daily max over the 2007-09 time period ranged from 52 ppb in winter to 90 ppb in summer. The distribution in 8-h daily max $O_3$ during the warm-season (as defined above) and during summer were very similar, which is not surprising given their close overlap in months. The distribution during the cold-season (as defined above) is shifted toward higher 8-h daily max $O_3$ concentrations compared with the distribution during winter. This is a result of including the four transition months (Oct, Nov, Mar and Apr) in the cold-season when high $O_3$ concentrations can occur. Further investigation of temporal variability including multiyear trends and diel behavior is included in Section 3.6.3.

## Table 3-8 Seasonally stratified distributions of 8-h daily max $O_3$ concentrations (ppb) from the year-round data set (2007-2009).

| Time Period | N Monitors | N Obs | Mean | SD | Min | 1 | 5 | 10 | 25 | 50 | 75 | 90 | 95 | 98 | 99 | Max | Max Site ID[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **8-h daily max (2007-2009)[a]** | | | | | | | | | | | | | | | | | |
| Year-round | 608 | 587,085 | 41 | 15 | 2 | 10 | 19 | 23 | 31 | 40 | 50 | 60 | 66 | 74 | 80 | 172 | 450210002 |
| **8-h daily max by season (2007-2009)[a]** | | | | | | | | | | | | | | | | | |
| Winter (Dec-Feb) | 608 | 143,855 | 31 | 10 | 2 | 6 | 14 | 18 | 25 | 32 | 38 | 43 | 46 | 49 | 52 | 172 | 450210002 |
| Spring (Mar-May) | 612 | 148,409 | 47 | 12 | 2 | 20 | 28 | 33 | 40 | 47 | 55 | 62 | 67 | 72 | 77 | 118 | 060370016 |
| Summer (June-Aug) | 613 | 148,280 | 47 | 16 | 2 | 16 | 22 | 26 | 35 | 46 | 57 | 67 | 75 | 84 | 90 | 137 | 060710005 |
| Fall (Sept-Nov) | 608 | 146,541 | 37 | 13 | 2 | 10 | 17 | 21 | 28 | 36 | 45 | 54 | 61 | 68 | 75 | 116 | 060370016 |
| Warm-season (May-Sept) | 616 | 246,233 | 47 | 16 | 2 | 16 | 22 | 27 | 35 | 46 | 57 | 66 | 73 | 81 | 87 | 137 | 060710005 |
| Cold-season (Oct-Apr) | 608 | 340,852 | 36 | 12 | 2 | 8 | 16 | 21 | 28 | 36 | 44 | 52 | 57 | 63 | 67 | 172 | 450210002 |

[a]Includes all validated data regardless of flags or regional concurrence and therefore may differ from data used for regulatory purposes.

[b]AQS Site ID corresponding to the observation in the Max column.

A national picture of AQS $O_3$ concentrations was generated from the year-round and warm-season data sets by aggregating the 8-h daily max observations by U.S. county. For this purpose, the 8-h daily max concentrations at each site were averaged over one or more calendar years and then the highest site in each county was selected for that county. Figure 3-27 contains the county-scale 8-h daily max $O_3$ concentrations from the year-round data set for 2007-2009 (top map) with seasonal stratification (bottom four maps). Figure 3-28 contains the county-scale 8-h daily max $O_3$ concentrations from the warm-season data set for 2007-2009 (top map) along with individual maps for each calendar year between 2007 and 2009 (bottom three maps). These maps are meant to illustrate the general national-scale distribution in long-term average 8-h daily max $O_3$ concentrations and are not representative of $O_3$ concentrations at all locations or times within the counties shown; considerable spatial variability can exist within a county. This is particularly important in the West where counties are larger on average than in the East. These maps are limited by monitor availability, resulting in the majority of U.S. counties not having available data (the white regions in Figure 3-27 and Figure 3-28).

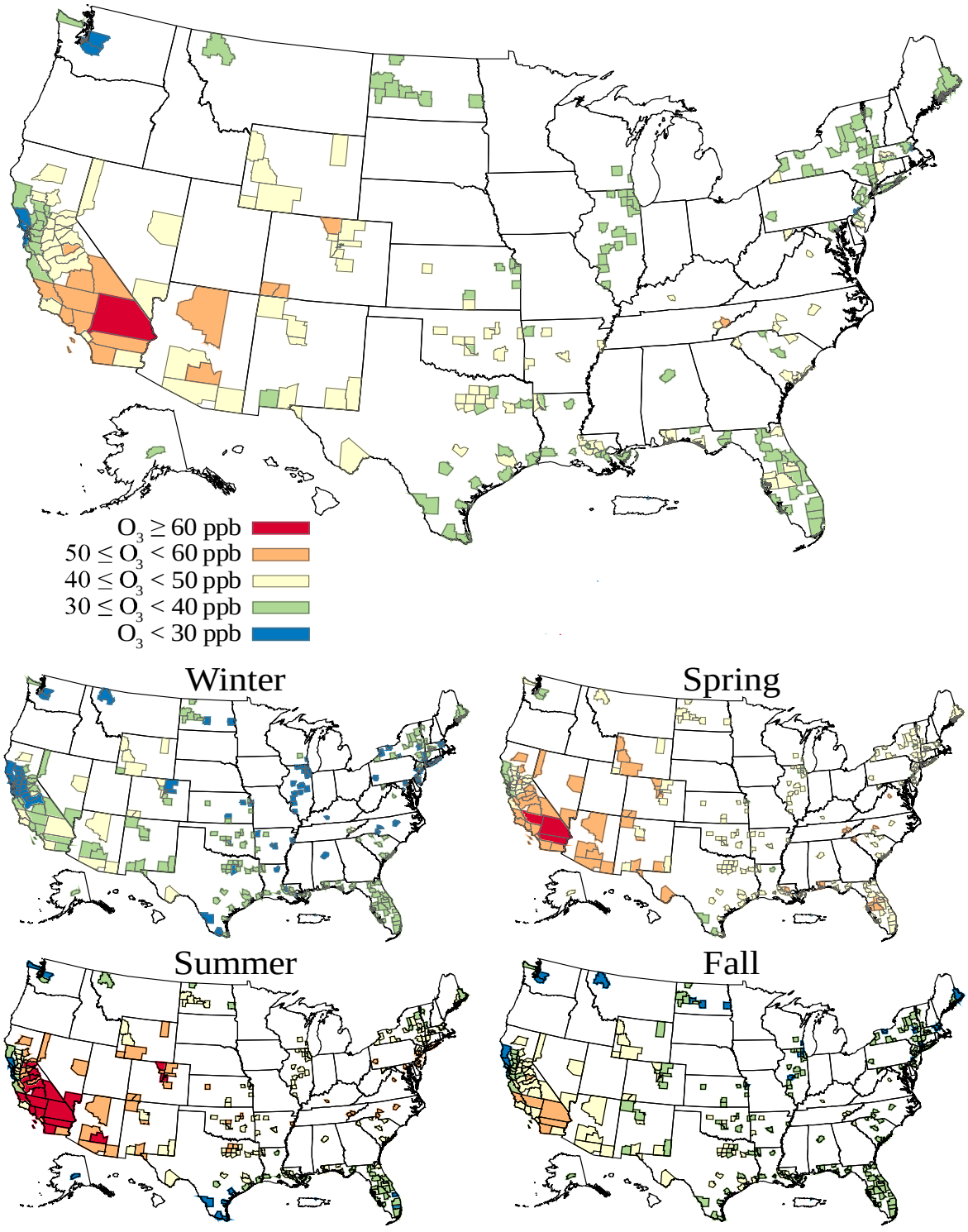



**Figure 3-27 Highest monitor (by county) 3-year avg (2007-2009) of the 8-h daily max $O_3$ concentration based on the year-round data set (the top map) with seasonal stratification (the four bottom maps).**

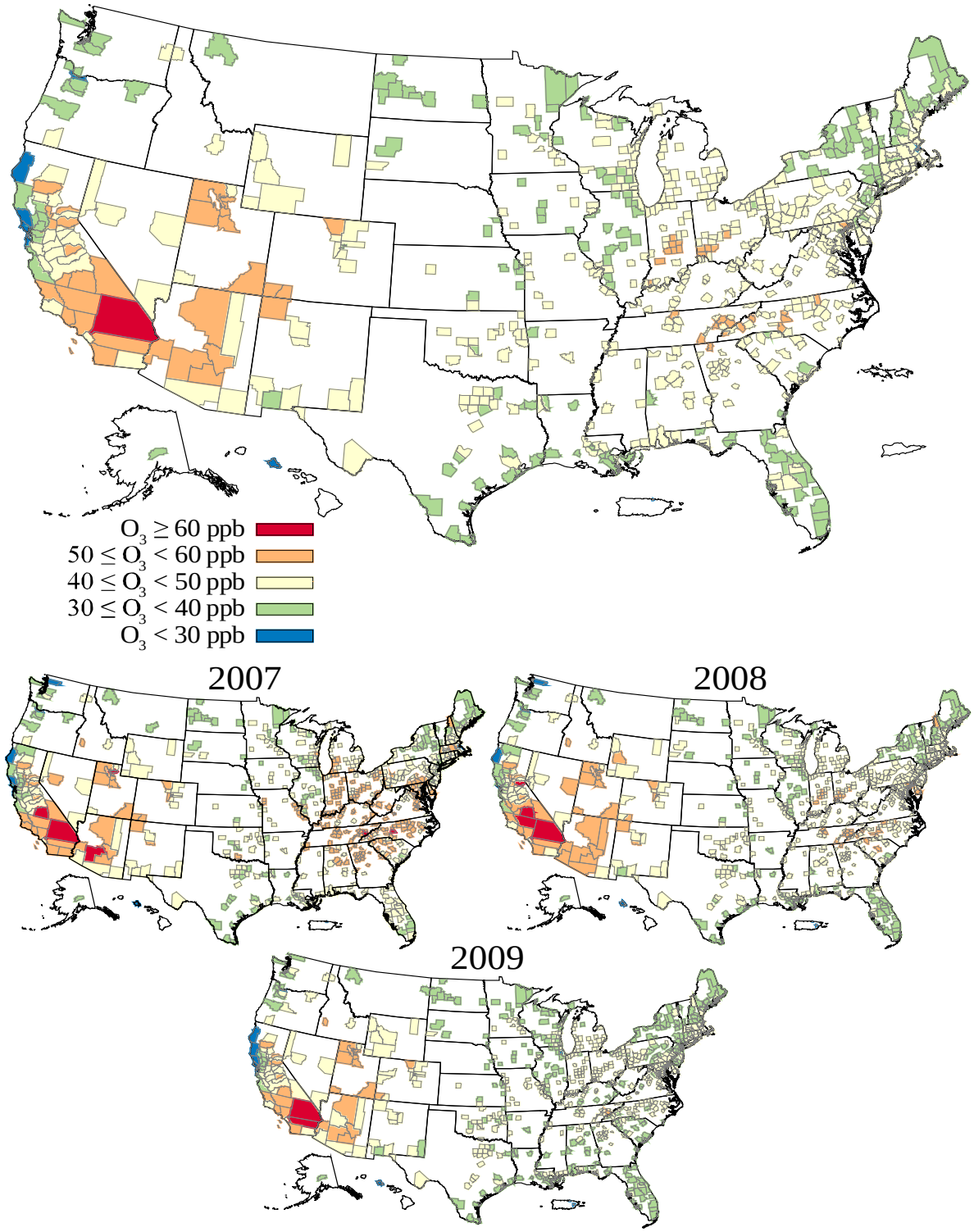



**Figure 3-28 Highest monitor (by county) 3-year avg (2007-2009) of the 8-h daily max $O_3$ concentration based on the warm-season data set (the top map) with annual stratification (the three bottom maps).**

As shown in the top county-scale map generated from the 2007-2009 year-round data set in Figure 3-27, the highest 3-year avg 8-h daily max $O_3$ concentrations (≥ 50 ppb) occur in counties in central and southern California, Arizona, Colorado and high elevation counties in Tennessee. The highest year-round average concentration of 61 ppb over this period comes from Site #060719002 located at an elevation of 1,244 meters in Joshua Tree National Monument, San Bernardino County, CA. The lowest 3-year avg 8-h daily max $O_3$ concentrations (<30 ppb) occur in Pacific Coast counties in northern California and Washington as well as in two northeastern counties in Pennsylvania and Massachusetts. The seasonally-stratified county-scale maps in Figure 3-28 reinforce the strong seasonality in 8-h daily max $O_3$ concentrations shown in Table 3-8. The highest wintertime concentrations (≥ 40 ppb) occur in the West with the highest 3-year wintertime avg of 46 ppb calculated for Site #080690007 located at an elevation of 2,743 meters near Rocky Mountain National Park, Larimer County, Colorado. In spring and summer, the concentrations increase considerably across all counties, with the highest concentrations (≥ 60 ppb) occurring during the summer in 15 counties in California, 3 counties in Colorado and 1 county each in Nevada and Arizona. Many counties in rural Wyoming, Montana, North Dakota, Maine, and along the Gulf Coast peak in the spring instead of the summer. In the fall, 8-h daily max $O_3$ concentrations drop back down below their spring and summer concentrations.

The top county-scale map in Figure 3-28 based on the 2007-2009 warm-season data set looks similar to the corresponding map in Figure 3-27 based on the year-round data set. The warm-season map, however, incorporates approximately twice as many monitors across the U.S., providing more spatial coverage. Several counties in Utah, New Mexico, Indiana, Ohio, Maryland, North Carolina, and Georgia in addition to California, Arizona, Colorado and Tennessee identified above have 3-year avg (2007-2009) 8-h daily max $O_3$ concentrations ≥ 50 ppb based on the warm-season data set. The individual yearly average county-maximum 8-h daily max $O_3$ concentrations in the lower half of Figure 3-27 show a general decrease in most counties from 2007 to 2009. The number of counties containing a monitor reporting an annual average 8-h daily max $O_3$ concentration above 50 ppb dropped from 230 counties in 2007 to 30 counties in 2009. This is consistent with the general decrease across these years shown in Table 3-6 and Table 3-7 for the upper percentiles of the 8-h daily max $O_3$ concentration.

### Urban-Scale Variability

Statistical analysis of the human health effects of airborne pollutants based on aggregate population time-series data have often relied on ambient concentrations of pollutants measured at one or more central monitoring sites in a given metropolitan area. The validity of relying on central monitoring sites is strongly dependent on the spatial variability in concentrations within a given metropolitan area. To investigate urban-scale variability, 20 focus cities were selected for closer analysis of $O_3$ concentration variability; these cities are listed in Table 3-9 and were selected based on their importance in $O_3$ epidemiology studies and on their geographic distribution

across the United States. In order to provide a well-defined boundary around each city, the combined statistical area (CSA) encompassing each city was used. If the city was not within a CSA, the smaller core-based statistical area (CBSA) was selected. The CSAs/CBSAs are defined by the U.S. Census Bureau (2011)[1] and have been used to establish analysis regions around cities in previous ISAs for particulate matter (PM) (U.S. EPA, 2009d) and carbon monoxide (CO) (U.S. EPA, 2010c).

The distribution of the 8-h daily max $O_3$ concentrations from 2007-2009 for each of the 20 focus cities is included in Table 3-10. These city-specific distributions were extracted from the warm-season data set and can be compared to the nationwide warm-season 8-h daily max distribution for 2007-2009 in Table 3-7 (and repeated in the first line of Table 3-10 for reference). The median 8-h daily max concentration in these focus cities was 41 ppb, similar to the nationwide median of 42 ppb. Seattle had the lowest median (31 ppb) and Salt Lake City had the highest median (53 ppb) of the 20 cities investigated. The 99th percentile of the 8-h daily max concentration in the focus cities was 84 ppb; similar once again to the nationwide 99th percentile of 80 ppb. Seattle had the lowest 99th percentile (64 ppb) and Los Angeles had the highest 99th percentile (98 ppb) of the 20 cities investigated. In aggregate, the 20 focus cities selected are similar in distribution to the nationwide data set, but there is substantial city-to-city variability in the individual distributions of the 8-h daily max concentrations based on the warm-season data set.

Maps showing the location of central monitoring sites with $O_3$ monitors reporting to AQS for each of the 20 focus cities are included as supplemental material in Section 3.9.1, Figure 3-76 through Figure 3-95; examples for Atlanta,GA, Boston, MA, and Los Angeles, CA, are shown in Figure 3-29 through Figure 3-31. The sites are delineated in the maps as year-round or warm-season based on their inclusion in the year-round data set and the warm-season data set (the warm-season data set includes May-September data from both the warm-season monitors and the year-round monitors meeting the warm-season data inclusion criteria). The maps also include the CSA/CBSA boundary selected for monitor inclusion, the location of urban areas and water bodies, the major roadway network, as well as the population gravity center based on the entire CSA/CBSA and the individual focus city boundaries. Population gravity center is calculated from the average longitude and latitude values for the input census tract centroids and represents the mean center of the population in a given area. Census tract centroids are weighted by their population during this calculation.

[1]A CBSA represents a county-based region surrounding an urban center of at least 10,000 people determined using 2000 census data and replaces the older Metropolitan Statistical Area (MSA) definition from 1990. The CSA represents an aggregate of adjacent CBSAs tied by specific commuting behaviors. The broader CSA definition was used when selecting monitors for the cities listed above with the exception of Phoenix and San Antonio, which are not contained within a CSA. Therefore, the smaller CBSA definition was used for these metropolitan areas.

## Table 3-9 Focus cities used in this and previous assessments.

| Focus City | Short Name | CSA/CBSA Name[a] | Year-Round $O_3$ Monitoring Sites[b] | Warm-Season $O_3$ Monitoring Sites[c] | Included in Prior ISAs[d] |
|---|---|---|---|---|---|
| Atlanta, GA | Atlanta CSA | Atlanta-Sandy Springs-Gainesville | 0 | 11 | CO, PM, $SO_X$, $NO_X$ |
| Baltimore, MD | Baltimore CSA | Washington-Baltimore-northern VA | 9 | 19 | $NO_X$ |
| Birmingham, AL | Birmingham CSA | Birmingham-Hoover-Cullman | 1 | 9 | PM |
| Boston, MA | Boston CSA | Boston-Worcester-Manchester | 3 | 18 | CO, PM, $NO_X$ |
| Chicago, IL | Chicago CSA | Chicago-Naperville-Michigan City | 11 | 15 | PM, $NO_X$ |
| Dallas, TX | Dallas CSA | Dallas-Fort Worth | 19 | 0 | |
| Denver, CO | Denver CSA | Denver-Aurora-Boulder | 12 | 3 | CO, PM |
| Detroit, MI | Detroit CSA | Detroit-Warren-Flint | 0 | 9 | PM |
| Houston, TX | Houston CSA | Houston-Baytown-Huntsville | 21 | 0 | CO, PM, $NO_X$ |
| Los Angeles, CA | Los Angeles CSA | Los Angeles-Long Beach-Riverside | 47 | 3 | CO, PM, $SO_X$, $NO_X$ |
| Minneapolis, MN | Minneapolis CSA | Minneapolis-St. Paul-St. Cloud | 2 | 6 | |
| New York, NY | New York CSA | New York-Newark-Bridgeport | 20 | 10 | CO, PM, $SO_X$, $NO_X$ |
| Philadelphia, PA | Philadelphia CSA | Philadelphia-Camden-Vineland | 9 | 8 | PM, $NO_X$ |
| Phoenix, AZ | Phoenix CBSA | Phoenix-Mesa-Scottsdale | 14 | 17 | CO, PM |
| Pittsburgh, PA | Pittsburgh CSA | Pittsburgh-New Castle | 2 | 12 | CO, PM |
| Salt Lake City, UT | Salt Lake City CSA | Salt Lake City-Ogden-Clearfield | 2 | 10 | |
| San Antonio, TX | San Antonio CBSA | San Antonio | 5 | 0 | |
| San Francisco, CA | San Francisco CSA | San Jose-San Francisco-Oakland | 25 | 6 | |
| Seattle, WA | Seattle CSA | Seattle-Tacoma-Olympia | 5 | 5 | CO, PM |
| St Louis, MO | St Louis CSA | St. Louis-St. Charles-Farmington | 3 | 13 | CO, PM, $SO_X$ |

[a]Defined based on 2000 Census data from the U.S. Census Bureau (2011).

[b]The number of sites within each CSA/CBSA with AQS monitors meeting the year-round data set inclusion criteria.

[c]The number of sites within each CSA/CBSA with AQS monitors meeting the warm-season data set inclusion criteria; the warm-season data set includes May - September data from both the warm-season and year-round monitors meeting the warm-season data set inclusion criteria.

[d]Boundaries for the 2010 CO ISA (U.S. EPA, 2010c) and 2009 PM ISA (U.S. EPA, 2009d) focus cities were based on CSA/CBSA definitions; boundaries for the 2008 $SO_X$ ISA (U.S. EPA, 2008d) and 2008 $NO_X$ ISA (U.S. EPA, 2008c) focus cities were based on similar metropolitan statistical area (MSA) definitions from the 1990 U.S. Census.

## Table 3-10 City-specific distributions of 8-h daily max $O_3$ concentrations (ppb) from the warm-season data set (2007-2009).

| Time Period | N Monitors | N Obs | Mean | SD | Min | 1 | 5 | 10 | 25 | 50 | 75 | 90 | 95 | 98 | 99 | Max | Max Site ID[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **8-h daily max (2007-2009)[a]** | | | | | | | | | | | | | | | | | |
| Nationwide | 1,194 | 993,677 | 42 | 15 | 2 | 12 | 20 | 24 | 32 | 42 | 52 | 61 | 67 | 75 | 80 | 172 | 450210002 |
| **8-h daily max by CSA/CBSA (2007-2009)[a]** | | | | | | | | | | | | | | | | | |
| Atlanta, GA, CSA | 11 | 7,844 | 47 | 16 | 2 | 15 | 22 | 27 | 36 | 47 | 58 | 67 | 72 | 81 | 87 | 124 | 130890002 |
| Baltimore, MD, CSA | 28 | 20,999 | 43 | 16 | 2 | 9 | 18 | 23 | 31 | 43 | 54 | 64 | 70 | 78 | 83 | 118 | 240030014 |
| Birmingham, AL, CSA | 10 | 7,676 | 44 | 15 | 2 | 14 | 21 | 25 | 34 | 44 | 54 | 63 | 68 | 76 | 83 | 108 | 010732006 |
| Boston, MA, CSA | 21 | 12,603 | 41 | 14 | 2 | 13 | 21 | 25 | 31 | 40 | 49 | 59 | 67 | 75 | 81 | 104 | 250270015 |
| Chicago, IL, CSA | 27 | 20,764 | 37 | 14 | 2 | 9 | 15 | 19 | 27 | 37 | 47 | 57 | 62 | 69 | 74 | 108 | 170310042 |
| Dallas, TX, CSA | 19 | 19,858 | 41 | 15 | 2 | 11 | 20 | 24 | 31 | 39 | 50 | 61 | 67 | 74 | 79 | 121 | 484390075 |
| Denver, CO, CSA | 15 | 12,217 | 44 | 15 | 2 | 8 | 18 | 24 | 34 | 44 | 55 | 63 | 68 | 72 | 76 | 98 | 080590006 |
| Detroit, MI, CSA | 9 | 5,016 | 45 | 14 | 2 | 15 | 23 | 28 | 35 | 44 | 52 | 62 | 69 | 77 | 83 | 100 | 260990009 |
| Houston, TX, CSA | 21 | 22,305 | 36 | 15 | 2 | 8 | 15 | 19 | 25 | 34 | 46 | 57 | 64 | 72 | 78 | 110 | 482011034 |
| Los Angeles, CA, CSA | 49 | 49,295 | 47 | 18 | 2 | 10 | 20 | 26 | 35 | 45 | 58 | 72 | 81 | 91 | 98 | 137 | 060710005 |
| Minneapolis, MN, CSA | 8 | 5,315 | 40 | 12 | 2 | 15 | 21 | 25 | 31 | 40 | 48 | 54 | 58 | 63 | 67 | 86 | 270031002 |
| New York, NY, CSA | 21 | 26,304 | 39 | 16 | 2 | 6 | 15 | 20 | 28 | 37 | 47 | 59 | 68 | 77 | 83 | 123 | 090050005 |
| Philadelphia, PA, CSA | 14 | 12,673 | 41 | 17 | 2 | 8 | 17 | 21 | 29 | 39 | 52 | 64 | 70 | 78 | 83 | 125 | 240150003 |
| Phoenix, AZ, CBSA | 22 | 26,129 | 49 | 12 | 2 | 18 | 27 | 32 | 41 | 50 | 58 | 65 | 68 | 72 | 75 | 85 | 040137021 |
| Pittsburgh, PA, CSA | 13 | 9,814 | 43 | 15 | 2 | 12 | 19 | 24 | 32 | 43 | 53 | 62 | 68 | 74 | 78 | 100 | 420050001 |
| Salt Lake City, UT, CSA | 12 | 5,146 | 51 | 14 | 2 | 8 | 23 | 32 | 44 | 53 | 61 | 67 | 71 | 77 | 80 | 96 | 490353008 |
| San Antonio, TX, CSA | 5 | 4,701 | 39 | 13 | 2 | 13 | 20 | 23 | 29 | 37 | 46 | 56 | 62 | 67 | 72 | 90 | 480290032 |
| San Francisco, CA, CSA | 31 | 28,325 | 34 | 12 | 2 | 8 | 16 | 20 | 26 | 33 | 41 | 48 | 55 | 63 | 68 | 110 | 060010007 |
| Seattle, WA, CSA | 5 | 6,148 | 31 | 12 | 2 | 4 | 12 | 17 | 23 | 31 | 39 | 46 | 51 | 59 | 64 | 91 | 530330023 |
| St Louis, MO, CSA | 19 | 11,569 | 43 | 15 | 2 | 12 | 19 | 23 | 32 | 43 | 53 | 61 | 68 | 76 | 81 | 113 | 295100086 |
| All CSAs/CBSAs listed | 360 | 314,701 | 42 | 16 | 2 | 9 | 18 | 22 | 31 | 41 | 52 | 63 | 69 | 78 | 84 | 137 | 060710005 |

[a]Includes all validated data regardless of flags or regional concurrence and therefore may differ from data used for regulatory purposes.

[b]AQS Site ID corresponding to the observation in the Max column.

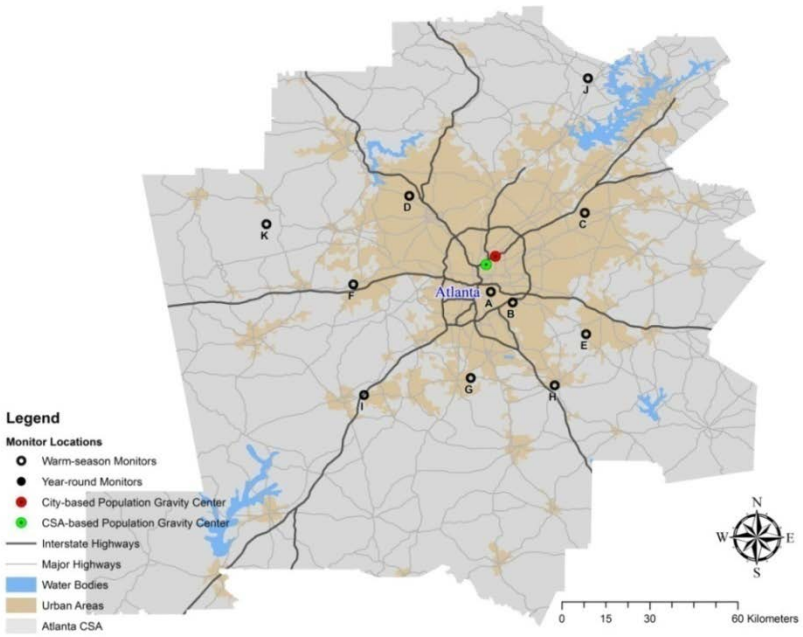



**Figure 3-29 Map of the Atlanta, Georgia, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**




**Figure 3-30 Map of the Boston, Massachusetts, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**

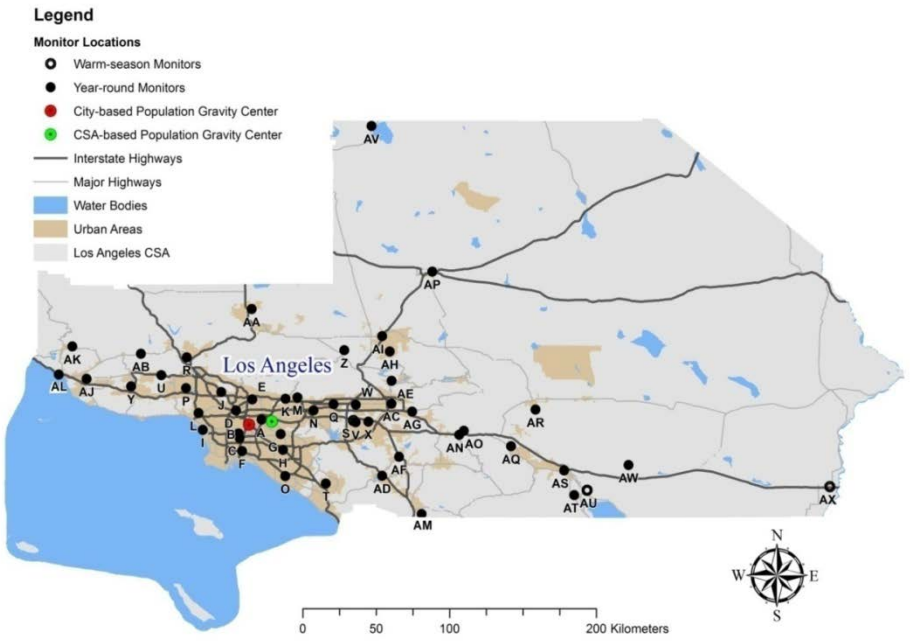



**Figure 3-31 Map of the Los Angeles, California, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**

The Atlanta, GA, CSA contains 11 warm-season monitors distributed evenly yet sparsely around the city center (Figure 3-29). The population gravity center for the city and the larger CSA are only separated by 4 km, indicating that the majority of the population lives within or evenly distributed around the city limits. Atlanta is landlocked with a radial network of interstate highways leading to the city center. The Boston, MA, CSA contains 3-year-round and 18 warm-season monitors spread evenly throughout the CSA. Boston is a harbor city with the Atlantic Ocean to the east, resulting in the city-based population gravity center being located 17 km east of the CSA-based population gravity center. The Los Angeles, CA, CSA contains the largest number of monitors of the 20 CSA/CBSAs investigated with 47 year-round and 3 warm-season monitors. These monitors are primarily concentrated in the Los Angeles urban area with relatively few monitors extending out to the northern and eastern reaches of the CSA. These unmonitored areas are very sparsely populated, resulting in only 15 km separating the city-based and the CSA-based population gravity centers despite the vast area of the Los Angeles CSA.

Other CSAs/CBSAs (see Section 3.9.1) with monitors concentrated within the focus city limits include Birmingham, AL, Chicago, IL, Denver, CO, Houston, TX, Phoenix AZ, San Antonio, TX, and Salt Lake City, UT. The remaining CSAs/CBSAs have monitors distributed more evenly throughout the CSA/CBSA area. Baltimore, MD, is contained within the same CSA as Washington D.C. and suburbs, resulting in

a 50-km separation (the largest of the focus cities investigated) between the city-based population gravity center for Baltimore and the CSA-based population gravity center for the Washington-Baltimore-Northern Virginia CSA.

Box plots depicting the distribution of 2007-2009 warm-season 8-h daily max $O_3$ data from each individual monitor in the 20 focus cities are included as supplemental material in Section 3.9.2, Figure 3-96 through Figure 3-115; examples for Atlanta, GA, Boston, MA, and Los Angeles, CA, are shown in Figure 3-32 through Figure 3-34. The Atlanta CSA has little spatial variability in 8-h daily max $O_3$ concentrations with median concentrations ranging from 47 ppb at Sites I and J located far from the city center to 54 ppb at Site A located closest to the city center. The variation in warm-season 8-h daily max concentrations are also relatively similar across monitors with IQRs ranging from 17 ppb at Site J to 23 ppb at Site B. The Boston CSA has more spatial variability in 8-h daily max $O_3$ concentrations than the Atlanta CSA with median concentrations ranging from 33 ppb at Site A nearest to the city center to 46 ppb at Site L located 84 km west of the city center. For monitors located within and just adjacent to the Boston city limits (Sites A-D), the $O_3$ concentrations can vary over relatively short distances owing to differing degrees of $NO_X$ titration and influence from the local topography. Like the Atlanta CSA, the variation in warm-season 8-h daily max concentrations are relatively similar across monitors within the Boston CSA with IQRs ranging from 15 ppb at Site U to 21 ppb at Site K. The Los Angeles CSA exhibits the most variability in $O_3$ concentrations between monitors of all the CSAs/CBSAs investigated. The median 8-h daily max $O_3$ concentration in the Los Angeles CSA ranged from 20 ppb at Site AM in the south-central extreme of the CSA to 80 ppb at Site AE near Crestline, CA in the San Bernardino National Forest just north of San Bernardino, CA. These two sites are at approximately the same longitude and are separated by only 85 km, but the Crestline site is downwind of the Los Angeles basin, resulting in substantially higher $O_3$ concentrations. Site AM also contains data for only 2009, which could explain some of the deviation when comparing this site with others in the Los Angeles CSA. Sites AM and AE also had the lowest (8 ppb) and highest (28 ppb) IQR, respectively. The remaining focus cities exhibited spatial variability ranging from uniform as in the Atlanta CSA to non-uniform as observed in the Los Angeles CSA (see supplemental figures in Section 3.9.2).

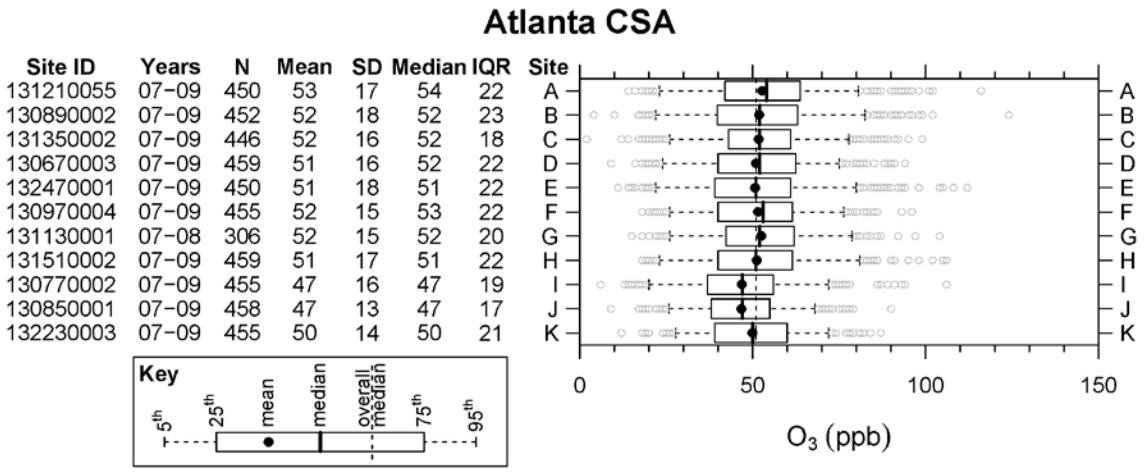



**Figure 3-32 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Atlanta CSA.**

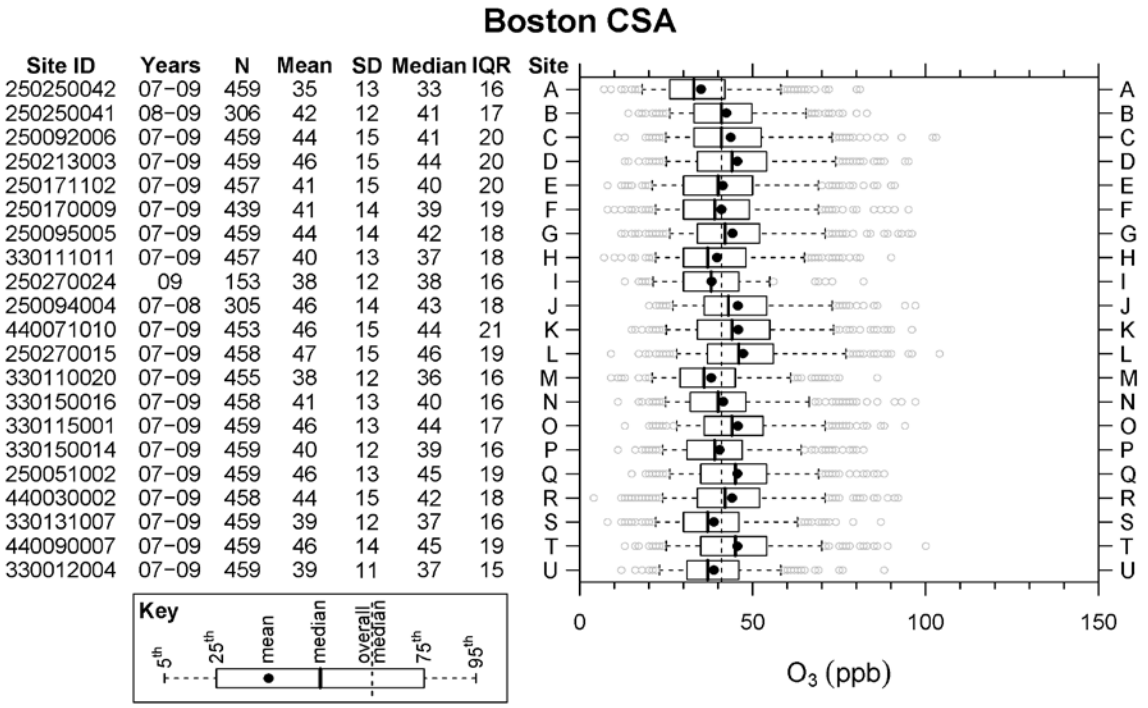



**Figure 3-33 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Boston CSA.**

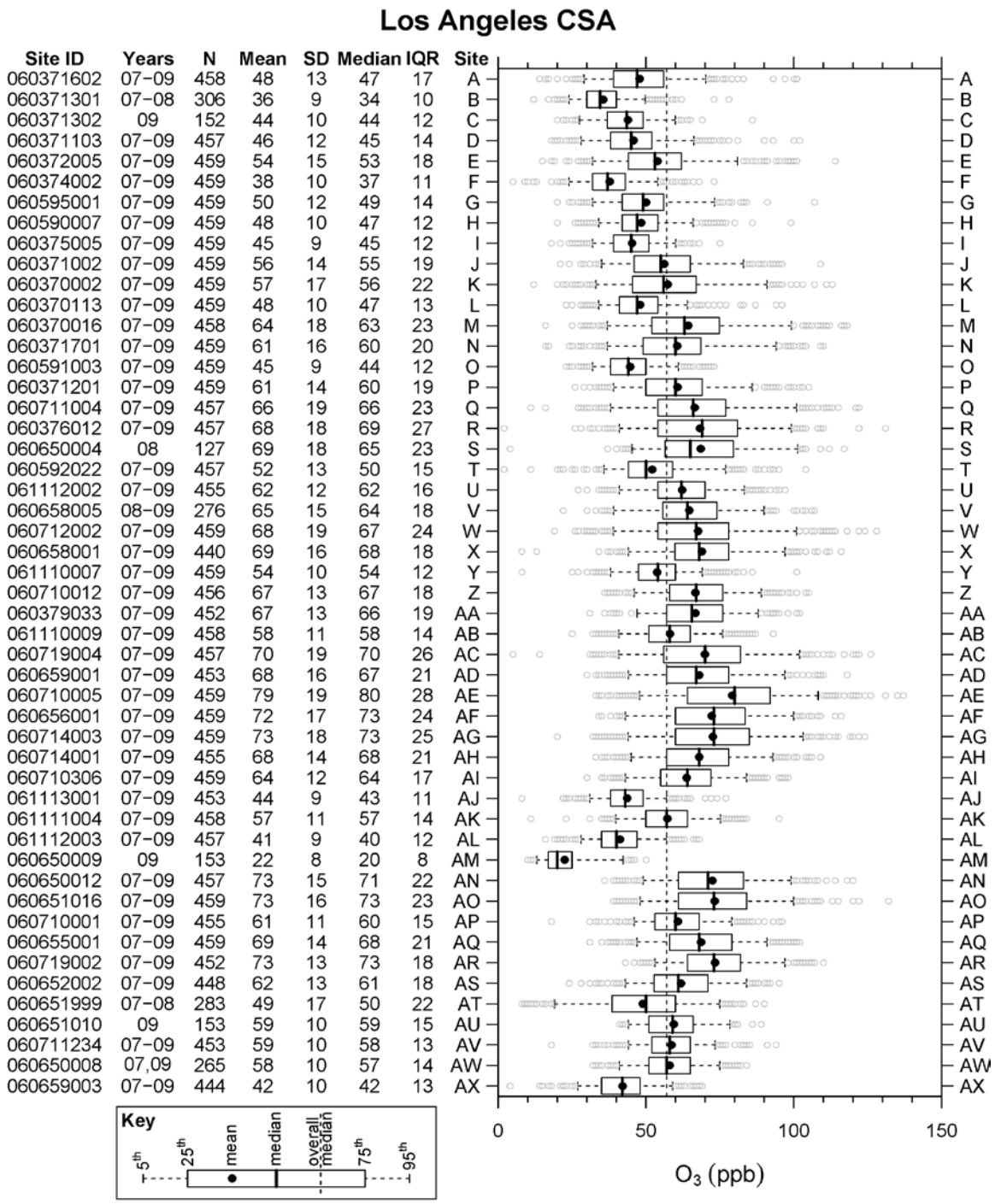



**Figure 3-34 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Los Angeles CSA.**

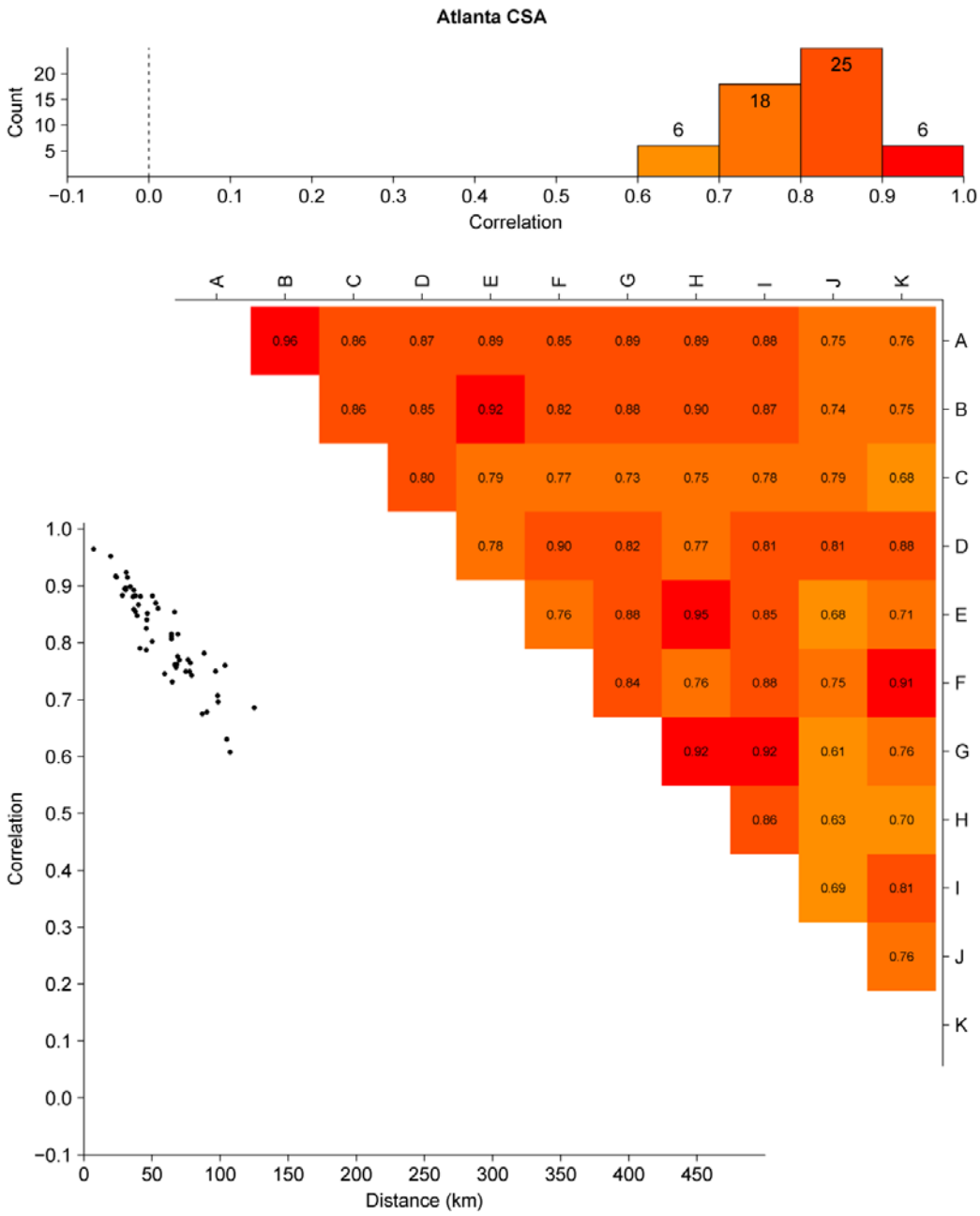
Pair-wise monitor comparisons were used to further evaluate spatial variability between monitors within the 20 focus cities. In the particular case of ground-level $O_3$, central-site monitoring has been justified as a regional measure of exposure mainly on the grounds that correlations between concentrations at neighboring sites measured over time are usually high. In areas with multiple monitoring sites, averages over the monitors have often been used to characterize population exposures. However, substantial differences in concentrations between monitors can exist even though concentrations measured at the monitoring sites are highly correlated, thus leading to the potential for exposure misclassification error. Therefore, both the Pearson correlation coefficient and the coefficient of divergence (COD) were calculated for each monitor pair within the CSA/CBSAs using the 8-h daily max $O_3$ data. The correlation provides an indication of temporal linear dependence across sites while the COD provides an indication of the variability in absolute concentrations across sites. The COD is defined as follows:

$$COD_{jk} = \sqrt{\frac{1}{p}\sum_{i-1}^{p}\left(\frac{X_{ij} - X_{ik}}{X_{ij} + X_{ik}}\right)^2}$$

**Equation 3-1**

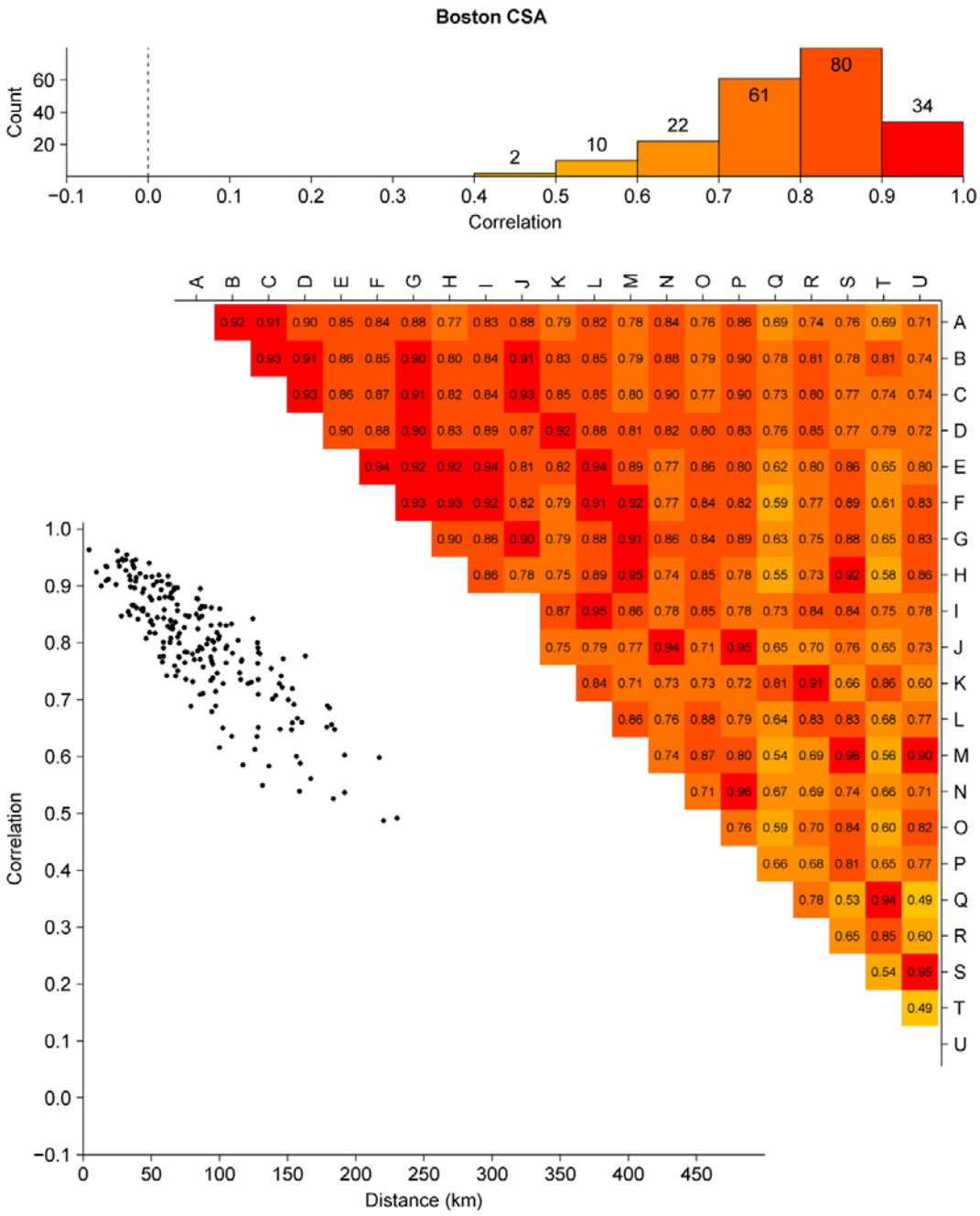
where $X_{ij}$ and $X_{ik}$ represent observed concentrations averaged over some measurement averaging period *i* (hourly, daily, etc.) at sites *j* and *k*, and *p* is the number of paired observations. A COD of 0 indicates there are no differences between concentrations at paired sites (spatial homogeneity), while a COD approaching 1 indicates extreme spatial heterogeneity. These methods for analysis of spatial variability follow those used in previous ISAs for CO, PM, $SO_X$ and $NO_X$ as well as those used in Pinto et al. (2004) for $PM_{2.5}$.

Histograms and contour matrices of the Pearson correlation coefficient between 8-h daily max $O_3$ concentrations from each monitor pair are included as supplemental material in Section 3.9.3, Figure 3-116 through Figure 3-135; examples for Atlanta, Boston and Los Angeles are shown in Figure 3-35 through Figure 3-37. Likewise, histograms, contour matrices, and scatter plots of the coefficient of divergence (COD) between 8-h daily max $O_3$ concentrations from each monitor pair are included as supplemental material in Section 3.9.3, Figure 3-136 through Figure 3-155; examples for Atlanta, Boston and Los Angeles are shown in Figure 3-38 through Figure 3-40. These figures also contain scatter plots of correlation and COD as a function of straight-line distance between monitor pairs.



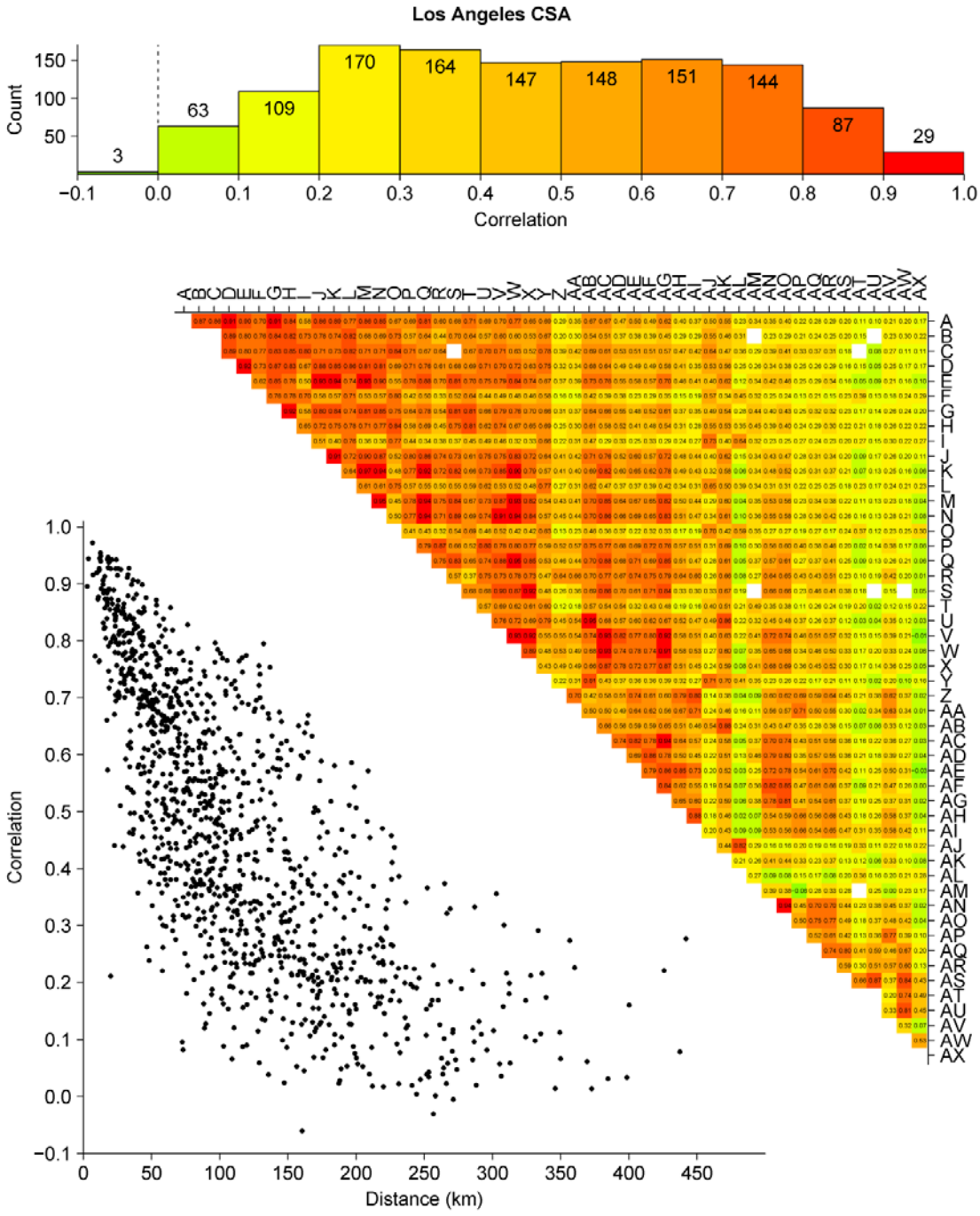

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of the correlations.

**Figure 3-35 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Atlanta CSA.**



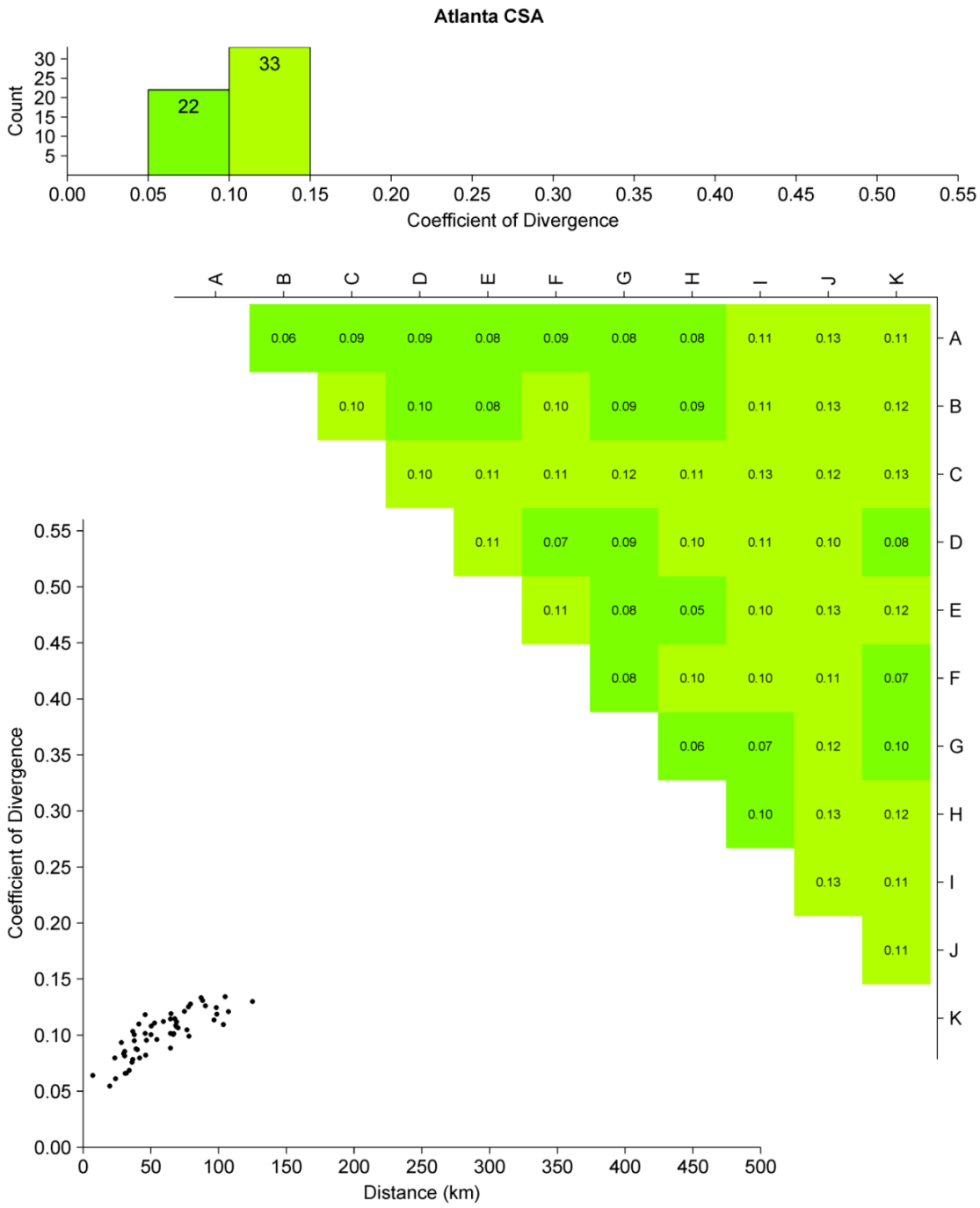

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of the correlations.

**Figure 3-36 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Boston CSA.**



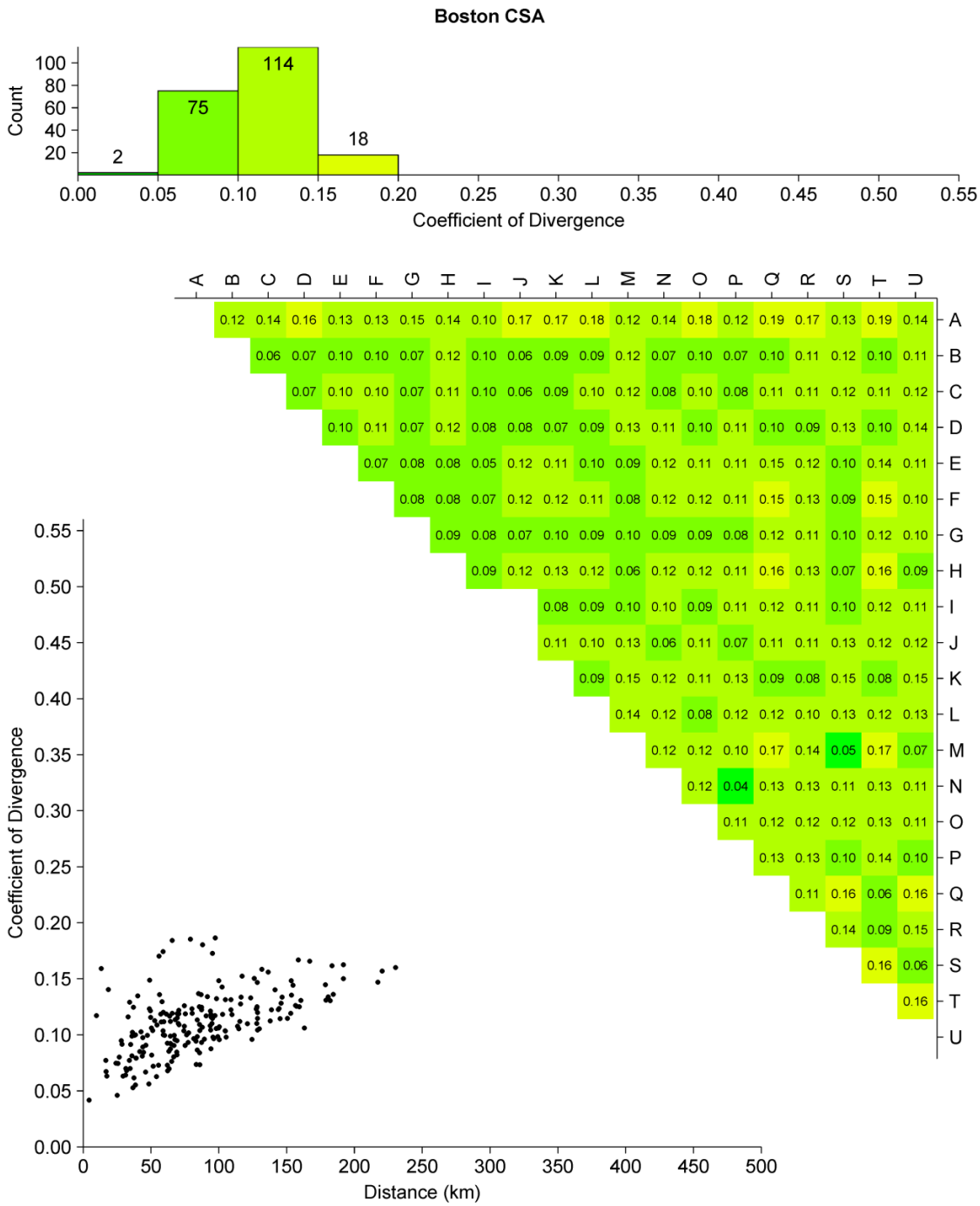

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of the correlations.

**Figure 3-37 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Los Angeles CSA.**



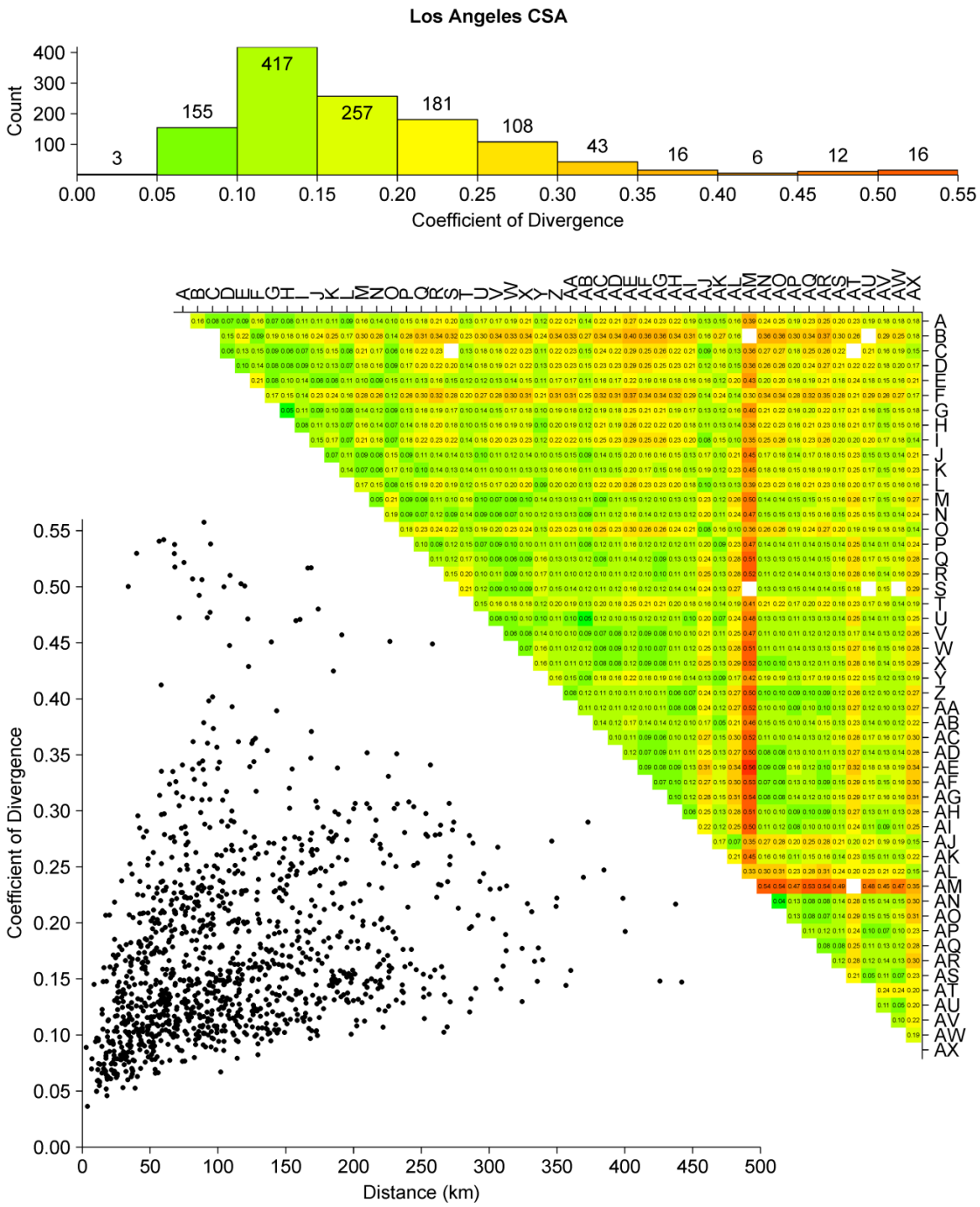

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of the CODs.

**Figure 3-38 Pair-wise monitor coefficient of divergence (COD) expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Atlanta CSA.**




Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of the CODs.

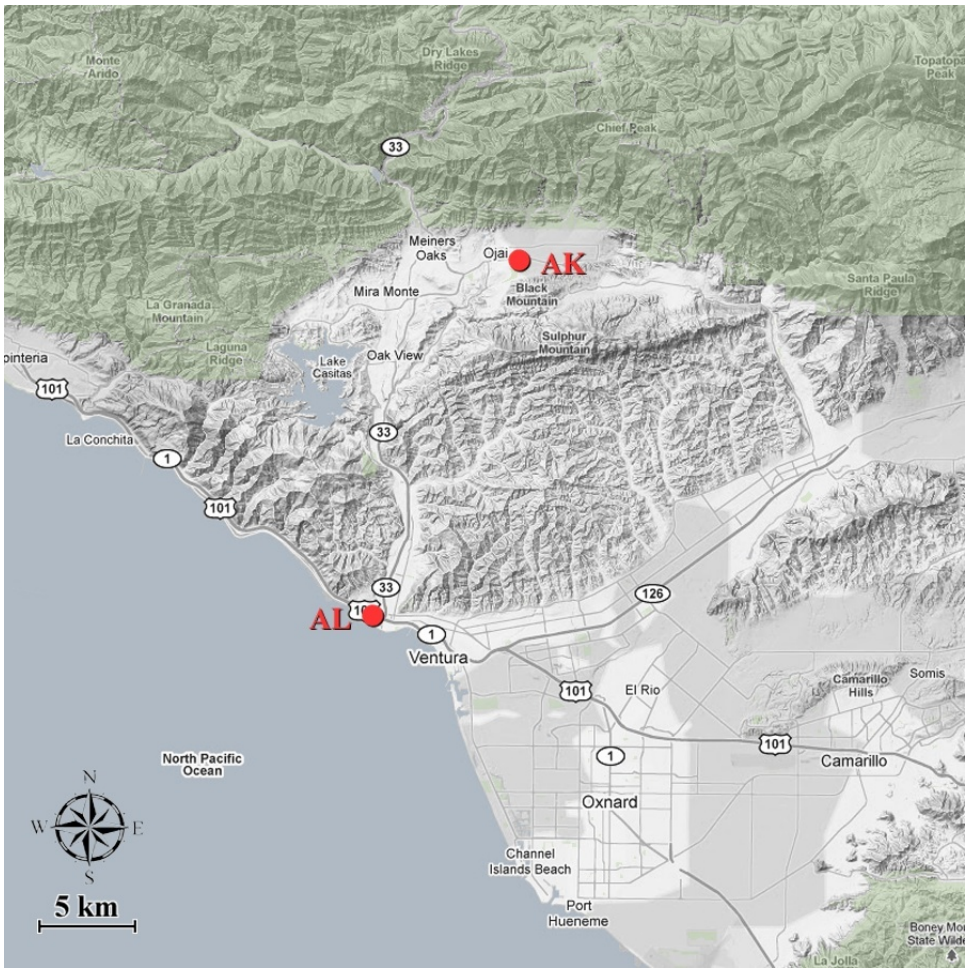
**Figure 3-39 Pair-wise monitor coefficient of divergence (COD) expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Boston CSA.**




Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of the CODs.

**Figure 3-40 Pair-wise monitor coefficient of divergence (COD) expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Los Angeles CSA.**

The monitor pairs within the Atlanta CSA (Figure 3-35) were generally well correlated with correlations between 8-h daily max $O_3$ concentrations ranging from 0.61 to 0.96. The correlations shown in the scatter plot were highest for close monitor pairs and dropped off with distance in a near-linear form. At a monitor separation distance of 50 km or less, the correlations ranged from 0.79 to 0.96. The monitor pairs within the Boston CSA (Figure 3-36) were also generally well correlated with correlations ranging from 0.49 to 0.96. Again, the correlations shown in the scatter plot were highest for close monitor pairs, but there was slightly more scatter in correlation as a function of distance in the Boston CSA compared with the Atlanta CSA. At a monitor separation distance of 50 km or less, the correlations ranged from 0.81 to 0.96. The monitor pairs within the Los Angeles CSA (Figure 3-37) showed a much broader range in correlations, extending from -0.06 to 0.97. At a monitor separation distance of 50 km or less, the correlations shown in the scatter plot ranged from 0.21 to 0.97. The negative and near-zero correlations were between monitors with a relatively large separation distance (>150 km), but even some of the closer monitor pairs were not very highly correlated. For example, Site AL located at Emma Wood State Beach in Ventura and Site AK situated in an agricultural valley surrounded by mountains 20 km inland (see map in Figure 3-41) had a correlation coefficient of only 0.21 over the 2007-2009 warm-season time period. This was slightly lower than the correlation between Site AL and Site AX on the Arizona border, 441 km away (R = 0.28). San Francisco and Seattle (Figure 3-133 and Figure 3-134 in Section 3.9.3) also showed a broad range in pair-wise correlations, likely resulting from their similar geography where background air coming in from the Pacific Ocean rapidly mixes with urban pollutants such as $NO_X$ and VOCs from coastal cities and is transported downwind into diversified terrain to create spatially and temporally varying $O_3$ concentrations.

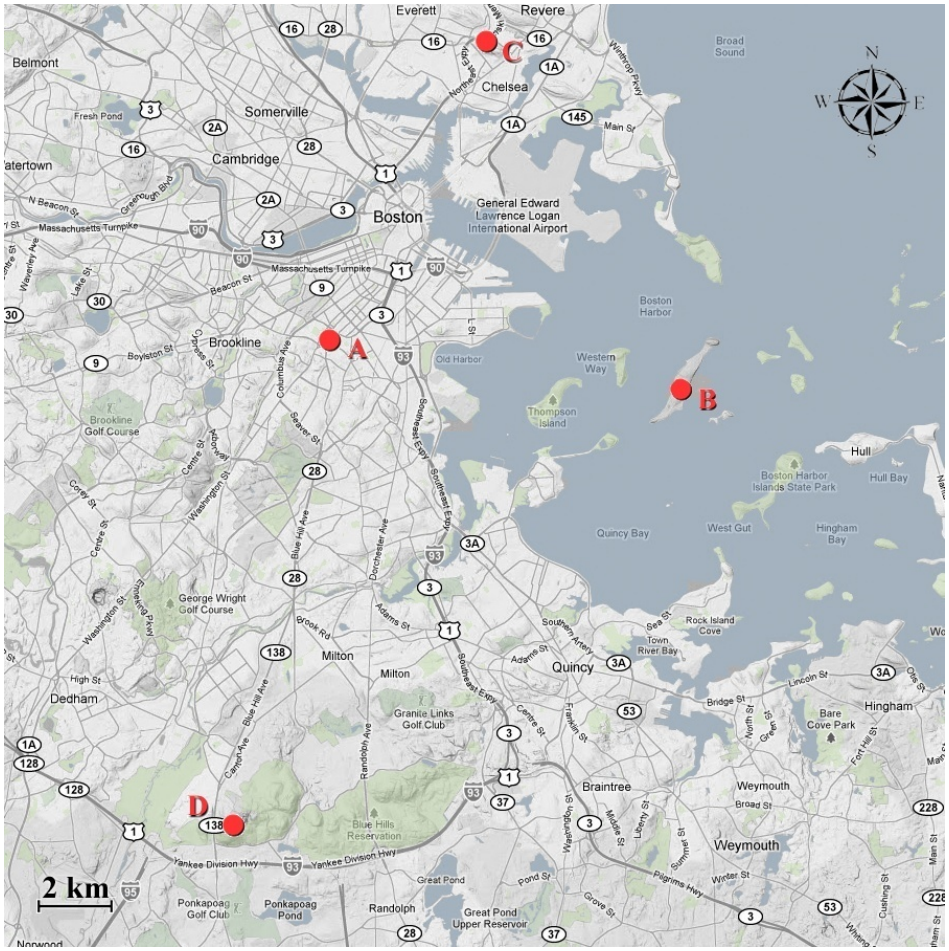



Note: Site AL is near shore, 3 meters above sea level, while Site AK is in an agricultural valley surrounded by mountains, 262 meters above sea level.

**Figure 3-41 Terrain map showing the location of two nearby AQS $O_3$ monitoring sites (red dots) along the western edge of the Los Angeles CSA.**

The COD between 8-h daily max $O_3$ measured at paired monitors in all CSAs/CBSAs (Figure 3-136 through Figure 3-155 in Section 3.9.3) were generally low, with values similar to those shown in Figure 3-38 and Figure 3-39 for Atlanta and Boston. This suggests a generally uniform distribution in the 8-h daily max $O_3$ concentration across monitors within these cities and is consistent with the uniformity observed in the box plots (e.g., Figure 3-32, Figure 3-33, and Figure 3-96 through Figure 3-115 in Section 3.9.2). Los Angeles (Figure 3-34) and San Francisco (Figure 3-153 in Section 3.9.3), however, had several monitor pairs with COD >0.30 indicating greater spatial heterogeneity. This is consistent with the variability observed in the box plots for these two CSAs (Figure 3-34 and Figure 3-113 in Section 3.9.2). In particular, Site AM in the Los Angeles CSA had consistently lower concentrations (median = 20 ppb, IQR = 17-25 ppb) relative to other sites in the CSA

(Figure 3-31), resulting in high CODs with other monitors as shown in Figure 3-40. The $O_3$ monitor at Site AM is located on the Pechanga Tribal Government Building in Temecula, CA, and began collecting data on June 9, 2008. It is located in a suburban setting and is classified as a general background monitor. Another close by site (site ID = 060731201) located in the Pala Reservation, 9.5 km south of this one (just outside the boundary of the Los Angeles CSA) reported similarly low 2009 8-h daily max $O_3$ concentrations (median = 28 ppb, IQR = 23-32 ppb) between May-June, 2009 (the only warm-season months with available data from this site between 2007 and 2009).




Note: Site characteristics range from Site A near downtown at 6 meters above sea level to Site D in a forested area on Blue Hill at 192 meters above sea level.

**Figure 3-42 Terrain map showing the location of four AQS $O_3$ monitoring sites (red dots) located in or near the city limits in the center of the Boston CSA.**

There are instances where sites in an urban area may exhibit substantial differences in median concentrations, but still be moderately well correlated in time. For example, Sites A and D in Boston (see terrain map in Figure 3-42) have an 11 ppb difference in median 8-h daily max $O_3$ concentration (COD = 0.16), but a high correlation (R = 0.90). In this example, Site A is located in the Boston city limits at an elevation of 6 meters while Site D is located 13 km to the south in a forested area on Blue Hill, the highest point in Norfolk County (elevation = 192 meters). The difference in median $O_3$ concentration at these two sites can be attributed to differing degrees of $NO_X$ titration between the neighborhood scale site (Site A) and the regional scale site (Site D) and to the influence of local topography.

Comparison of monitoring data within the selected focus cities has demonstrated considerable variability between cities in the behavior of the $O_3$ concentration fields. Median $O_3$ concentrations vary more within certain urban areas than others. Likewise, pair-wise monitor statistics (R and COD) are dependent on the urban area under investigation. These conclusions are consistent with those drawn in the 2006 $O_3$ AQCD (U.S. EPA, 2006b) where a subset of these focus cities were investigated using similar statistics. As a result, caution should be observed in using data from a sparse network of ambient $O_3$ monitors to approximate community-scale exposures.

### Neighborhood-Scale Variability and the Near-Road Environment

Ozone is a secondary pollutant formed in the atmosphere from precursor emissions and therefore is generally more regionally homogeneous than primary pollutants emitted from stationary or mobile point sources. However, $O_3$ titration from primary NO emissions does result in substantial localized $O_3$ gradients. This is evident in the near-road environment where fresh NO emissions from motor vehicles titrate $O_3$ present in the urban background air, resulting in an $O_3$ gradient down-wind from the roadway. Ozone titration occurring in street canyons where NO emissions are continuously being generated is more efficient because of inhibited transport away from the source of NO.

Several studies have reported $O_3$ concentrations that increase with increasing distance from the roadway, both upwind and downwind of the road. Beckerman et al. (2008) measured $O_3$ profiles in the vicinity of heavily traveled roadways with Annual Average Daily Traffic (AADT) >340,000 vehicles in Toronto, Canada. Ozone was observed to increase with increasing distance from the roadway, both upwind and downwind of the road. This is consistent with scavenging of $O_3$ in the near-road environment by reaction with NO to form $NO_2$. Upwind of the road, concentrations were >75% of the maximum observed value at >100 meters from the road; downwind, concentrations were approximately 60% of the maximum within 200-400 meters of the road. The $O_3$ concentration adjacent to the road on the upwind side was approximately 40% of the maximum value observed at the site. Concentrations measured with Ogawa passive samplers over a 1-week period ranged from 7.3-19.4 ppb with the mean at the two sites ranging from 13.0-14.7 ppb. In a study of patrol cars during trooper work shifts, Riediker et al. (2003) made

simultaneous 9-h $O_3$ measurements inside patrol cars, at the roadside, and at a centrally-located ambient monitoring site. The roadside concentrations were approximately 81% of the ambient values (mean of 22.8 ppb versus 28.3 ppb). Wind direction relative to the roadway was not reported.

Johnson (1995) measured $O_3$, NO, and CO concentrations at upwind and downwind locations near a variety of roadways in Cincinnati, OH. The effects of $O_3$ scavenging by NO were apparent in the $O_3$ reduction in the interval between 9 meters upwind and 82 meters downwind of the road. A similar effect was observed by Rodes and Holland (1981) during an earlier study in which outdoor $O_3$ concentrations were monitored downwind of a freeway in Los Angeles, CA. In this study, $O_3$ concentrations measured near the roadway were approximately 20% of the concentrations measured simultaneously at more distant locations judged to be unaffected by the roadway. Minimal separation distances of the samplers from the roadway to eliminate measurable influence were estimated to be approximately 400-500 meters for NO, $NO_2$, and $O_3$. Similar results have been observed outside the U.S., for example in the city of Daegu, Korea, where the yearly roadside concentrations of CO and $SO_2$ showed a well-defined decreasing trend with distance from the roadway, whereas concentrations of $NO_2$ and $O_3$ exhibited the reverse trend (Jo and Park, 2005). During the peak $O_3$ month of May, $O_3$ concentrations in a residential neighborhood were approximately 40% higher than concentrations at roadside monitors located 1 meter from the edge of multiple-lane freeways.

### 3.6.2.2 Rural-Focused Variability and Ground-level Vertical Gradients

AQS $O_3$ data for monitors located at several rural monitoring sites (e.g., national parks, national forests, state parks, etc.) were used to investigate rural-focused $O_3$ concentration variability in contrast with the urban-focused variability discussed in Section 3.6.2.1. These rural monitoring sites tend to be less directly affected by anthropogenic pollution sources than urban sites. However, they can be regularly affected by transport of $O_3$ or $O_3$ precursors from upwind urban areas, or by local anthropogenic sources within the rural areas such as emissions from motor vehicles, power generation, biomass combustion, or oil and gas operations. As a result, monitoring data from these rural locations are not unaffected by anthropogenic emissions.

Six rural focus areas were selected for their geographic distribution across the U.S. as well as their unique topography and relevance to the ecological assessment in Chapter 9. Table 3-11 lists the rural focus areas and provides some cursory site information along with the number of available AQS monitors reporting year-round and only during the warm-season. Accompanying box plots depicting the distribution of 2007-2009 warm-season 8-h daily max $O_3$ data from each individual monitor in the six rural focus areas are included in Figure 3-43. This analysis was restricted to AQS monitors meeting the same data completeness criteria outlined in Table 3-5 for

a direct comparison with the 20 urban focus areas investigated in Section 3.6.2.1. Given the population-center emphasis of the AQS network, limited monitoring sites (between one and five) were available for each rural focus area. Expanded analyses of $O_3$ concentrations measured using the more rural-focused CASTNET monitoring network are included in Chapter 9.

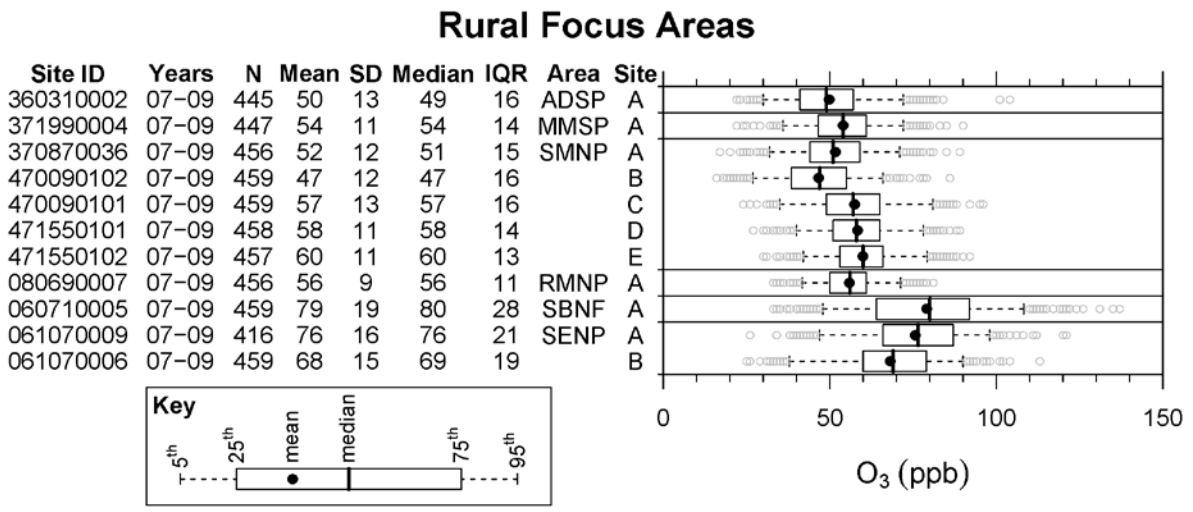
**Table 3-11 Rural focus areas.**

| Focus Area | Short Name | Year-Round $O_3$ Monitoring Sites[a] | Warm-Season $O_3$ Monitoring Sites[b] | Monitor Elevation (meters) | Site Descriptions |
|---|---|---|---|---|---|
| Adirondack State Park, NY | ADSP | 1 | 0 | 1,483 | One site on the summit of Whiteface Mountain in the Adirondack Mountains |
| Mount Mitchell State Park, NC | MMSP | 0 | 1 | 1,982 | One site near the summit of Mount Mitchell (highest point in the eastern U.S.), in the Appalachian Mountains |
| Great Smoky Mountain National Park, NC-TN | SMNP | 2 | 3 | 564 to 2,021 | Five different locations within Great Smoky Mountain National Park in the Appalachia Mountains |
| Rocky Mountain National Park, CO | RMNP | 1 | 0 | 2,743 | One site in a valley at the foot of Longs Peak in the Rocky Mountains |
| San Bernardino National Forest, CA | SBNF[c] | 1 | 0 | 1,384 | One site in Lake Gregory Regional Park (near Crestline, CA) in the San Bernardino Mountains |
| Sequoia National Park, CA | SENP | 2 | 0 | 560 to 1,890 | Two contrasting sites at different elevations within Sequoia NP in the Sierra Nevada Mountains |

[a]Number of AQS monitors meeting the year-round data set inclusion criteria; the year-round data set is limited to these monitors.

[b]Number of AQS monitors meeting the warm-season data set inclusion criteria; the warm-season data set includes May-September data from both the warm-season and year-round monitors.

[c]Same AQS site as Site AE in the Los Angeles CSA shown in Figure 3-31.




Note: Includes: Adirondack State Park, NY (ADSP); Mount Mitchell State Park, NC (MMSP); Great Smoky Mountain National Park, NC-TN (SMNP); Rocky Mountain National Park, Colorado (RMNP); San Bernardino National Forest, CA (SBNF); and Sequoia National Park, CA (SENP).

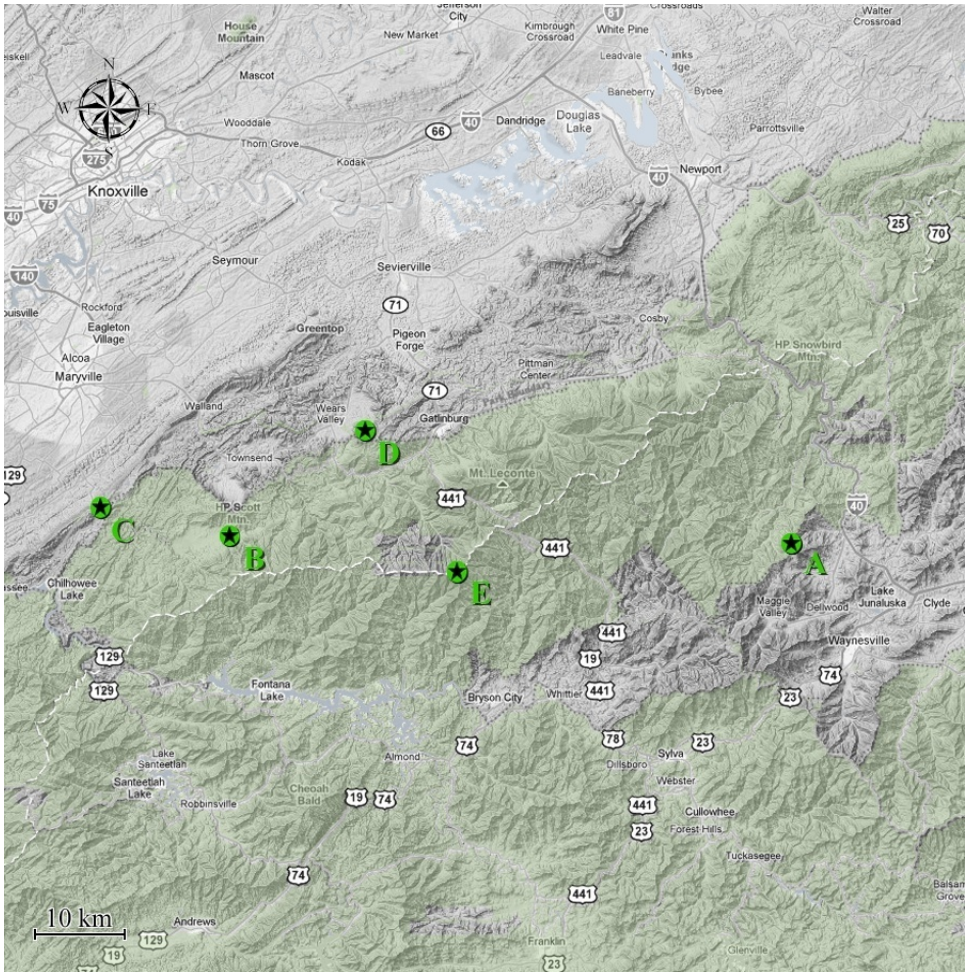
**Figure 3-43 Rural focus area site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the rural focus areas.**

### Eastern Rural Focus Areas

In the East, the distribution in warm-season 8-h daily max $O_3$ concentrations from the Adirondack State Park (ADSP) site on Whiteface Mountain in Upstate NY (median = 49 ppb) (Figure 3-43) was among the lowest of the rural focus monitors investigated, but was still higher than concentration distributions measured in the Boston CSA (medians ranging from 33 to 46 ppb) (Figure 3-33) located 320 km to the southeast. The ADSP AQS site was included in an analysis for the 2006 $O_3$ AQCD (U.S. EPA, 2006b) and had the lowest year-round median hourly $O_3$ concentration of the rural forested sites investigated (including Yellowstone NP, the Great Smoky Mountains NP, and Shenandoah NP). For the Appalachian Mountain monitors in Mount Mitchell State Park, NC (MMSP) and Great Smoky Mountain National Park, NC-TN (SMNP), there was a fair amount of variability in concentration distribution. Within SMNP, the median warm-season 8-h daily max $O_3$ concentration ranged from 47 ppb at the lowest elevation site (elevation = 564 meters; site ID = 470090102) to 60 ppb at the highest elevation site (elevation = 2,021 meters; site ID = 471550102); these sites are shown on the terrain map in Figure 3-44. The warm-season median 8-h daily max $O_3$ concentration gradient between these two sites located 26.2 km apart in SMNP was 0.9 ppb per 100 meters elevation gain.

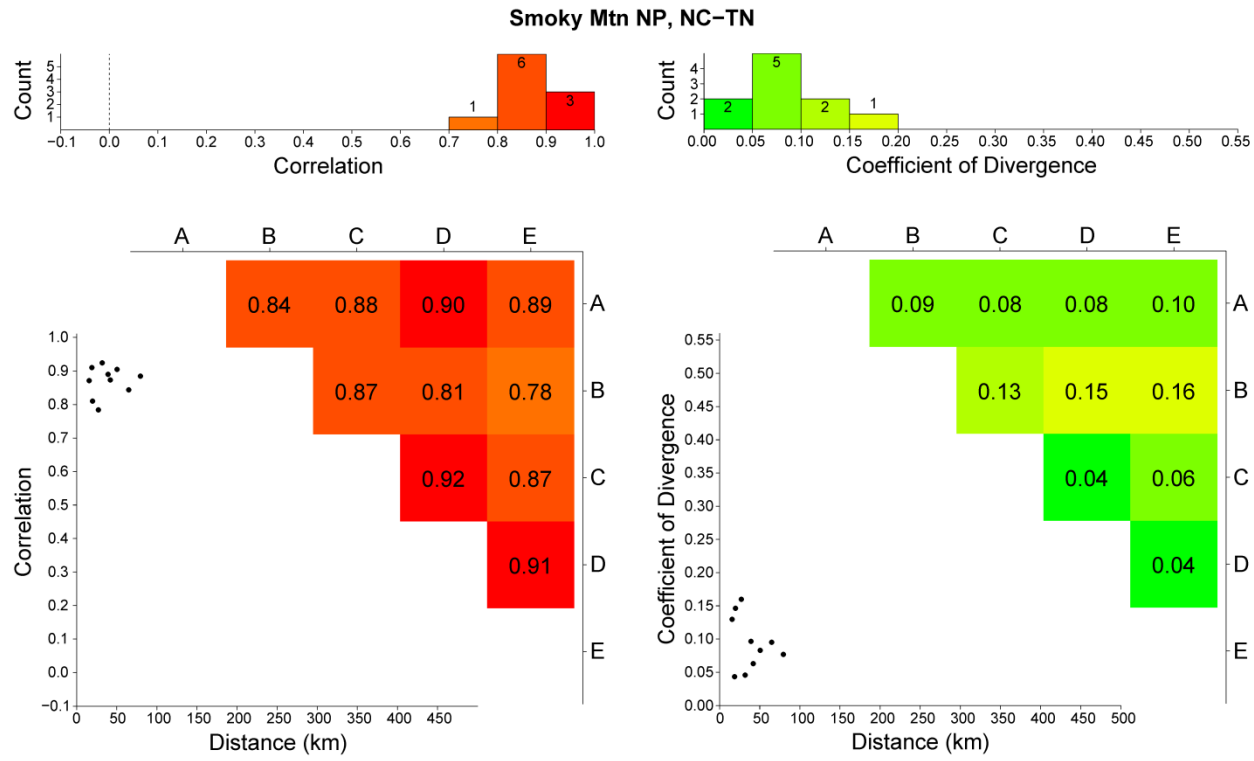
Data from the five sites within SMNP allowed for further investigation of spatial variability within the park; Figure 3-45 contains histograms, contour plots and scatter

plots as a function of distance for the pair-wise correlation and COD (defined in Equation 3-1) for SMNP. The correlations between the five sites ranged from 0.78 to 0.92 and the CODs ranged from 0.04 to 0.16. The plots of correlation and COD as a function of distance between SMNP monitor pairs in Figure 3-45 show a large degree of spatial variability between monitors over relatively short distances. A host of factors may contribute to these variations, including proximity to local $O_3$ precursor emissions, variations in boundary-layer influences, meteorology and stratospheric intrusion as a function of elevation, and differences in wind patterns and transport behavior due to local topography.




Note: The lowest elevation site (Site B) is 564 meters above sea level, while the highest elevation site (Site E) is 2,021 meters above sea level.

**Figure 3-44 Terrain map showing the location of five AQS $O_3$ monitoring sites (green/black stars) in Great Smoky Mountain National Park, NC-TN (SMNP).**



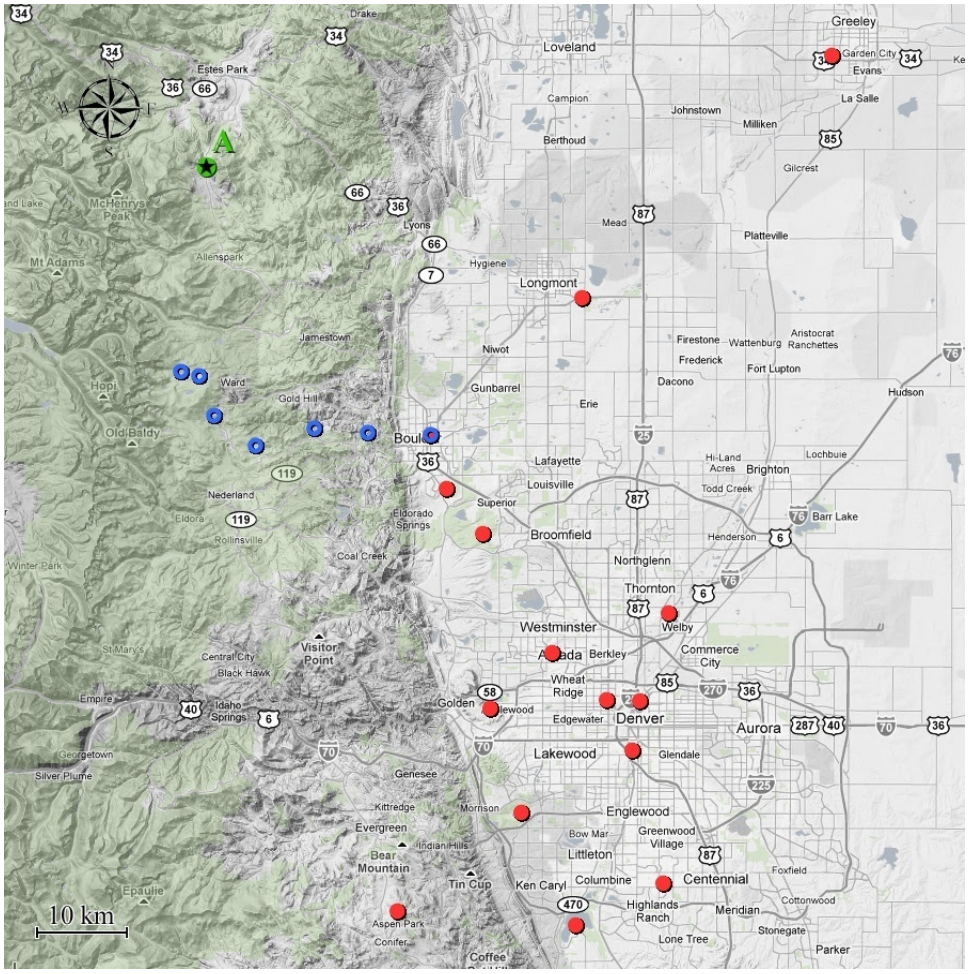

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histograms includes the number of monitor pairs per bin and the contour matrix includes the numeric values of the correlations and CODs.

**Figure 3-45 Pair-wise monitor correlations (left) and coefficients of divergence (CODs) (right) expressed as a histogram (top), contour matrix (middle) and scatter plot vs. distance between monitors (bottom) for 8-h daily max $O_3$ in Great Smoky Mountain National Park, NC-TN (SMNP).**

### Western Rural Focus Areas

The Rocky Mountain National Park (RMNP) site in Colorado at 2,743 meters in elevation had a warm-season 8-h daily max $O_3$ concentration distribution (median = 56 ppb, IQR = 11 ppb) (Figure 3-43) that is comparable to the distributions at sites in the Denver CSA located 75 km southeast at elevations around 1,600 meters (medians ranging from 41 to 59 ppb, IQRs ranging from 10 to 16 ppb; see Figure 3-102 in Section 3.9.1). In nearby Boulder County, Colorado, a 1-year time-series (Sept 2007-Aug 2008) of ambient surface-level $O_3$ measurements was collected by Brodin et al. (2010) along an elevation gradient ranging from 1,608 meters to 3,528 meters. The 7 sites used in this study are shown in Figure 3-46 along with the RMNP site and the 15 Denver CSA sites. In fall, winter, and spring, they observed a clear monotonic increase in $O_3$ concentration with elevation, with a rate of increase in the mean $O_3$ concentration of 1.5 ppb per 100 meters elevation gain during winter. In summer, the $O_3$ gradient was similar in magnitude over the

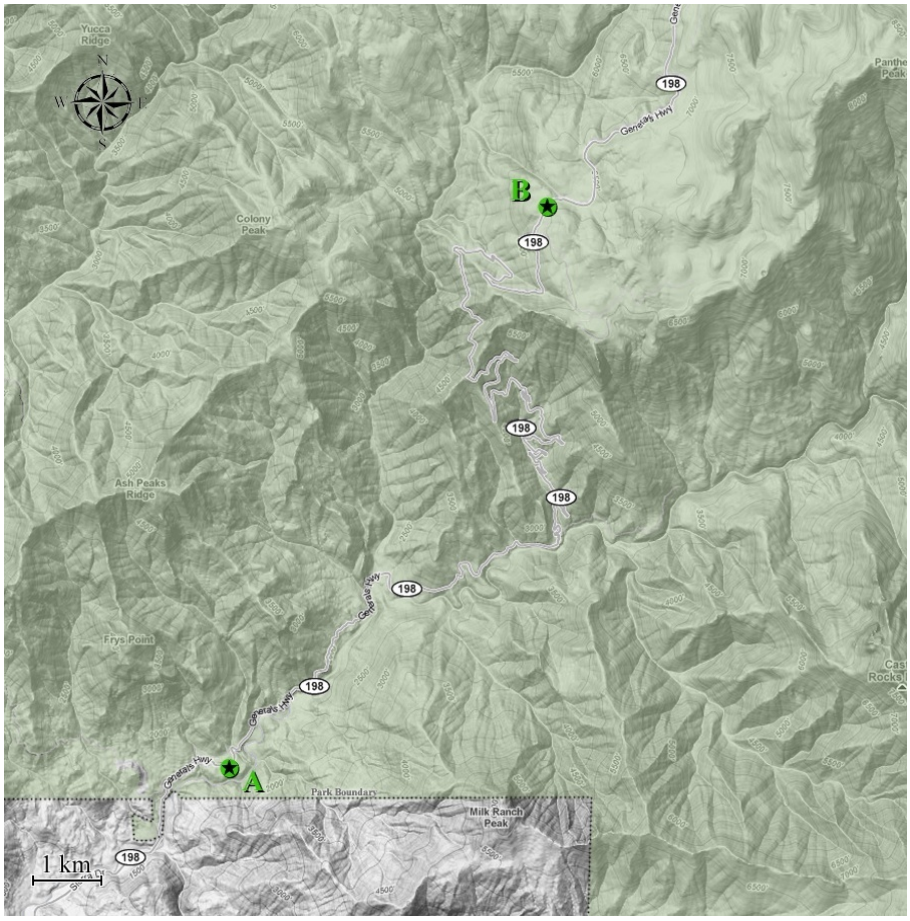
seven-site transect (1.3 ppb per 100 meters), but much less monotonic; the majority of the vertical gradient occurred between the lowest two sites (4.5 ppb per 100 meters) and between the highest two sites (5.5 ppb per 100 meters), with the middle five sites all having approximately equal median $O_3$ concentrations. Ozone concentrations at the lowest site in Boulder were influenced by NO titration as evidenced by traffic-related diel cycles in $O_3$ concentrations, but the remaining six sites were located at elevation in more rural/remote settings and illustrate a positive surface-level $O_3$ elevation gradient similar to that seen in SMNP and typical of areas under less direct influence of boundary layer pollution.




Note: Elevations range from approximately 1,600 meters above sea level in Denver and Boulder, Colorado, to 3,528 meters above sea level at the highest mountainous site. Blue circles indicate monitoring sites used in the Brodin et al. (2010) study.

**Figure 3-46 Terrain map showing the location of the AQS $O_3$ monitoring site in Rocky Mountain National Park, Colorado (black/green star) and the Denver, Colorado, CSA (red dots) along with $O_3$ monitoring sites used in the Brodin et al. (2010) study (blue circles).**

The three sites in California–one in San Bernardino National Forest (SBNF) and two in Sequoia National Park (SENP)–had the highest distribution of 8-h daily max $O_3$ concentrations of the selected rural focus area monitors included in Figure 3-43. The SBNF site had a warm-season 8-h daily max $O_3$ concentration mean of 80 ppb and a maximum of 137 ppb measured on July 1, 2007. This site is located in Crestline, CA, 90 km down-wind of Los Angeles in the San Bernardino Mountains. This site was included in the Los Angeles CSA shown in Figure 3-31 (Site AE) and had the highest median 8-h daily max $O_3$ concentration of any AQS site in the Los Angeles CSA during this time period (Figure 3-34). This site was also included in an analysis performed for the 2006 $O_3$ AQCD (U.S. EPA, 2006b) where similarly high $O_3$ concentrations were observed using 2004 year-round hourly observations.




Note: The lower site (site ID = 061070009) is 560 meters above sea level and the higher site (site ID = 061070006) is 1,890 meters above sea level.

**Figure 3-47 Terrain map showing the location of two AQS $O_3$ monitoring sites (black/green stars) in Sequoia National Park, CA.**

The two sites in SENP are located 9.7 km apart at contrasting elevations as is illustrated in the terrain map in Figure 3-47. The correlation in 8-h daily max $O_3$ between these two sites was 0.86 and the COD was 0.09, which are within the range in correlations and CODs for SMNP (Figure 3-45). The distribution of 8-h daily max $O_3$ concentrations at the lower elevation site (elevation = 560 meters; site ID = 061070009) is shifted slightly higher with a median of 76 ppb compared to the higher elevation site (elevation = 1,890 meters; site ID = 061070006) with a median of 69 ppb. The lower elevation site is located at the entrance to the park and is at a low enough elevation to be influenced by boundary layer pollution coming upwind from Fresno and the San Joaquin Valley. The higher elevation site is in the free troposphere above the planetary boundary layer and is less influenced by such pollution. This gives rise to a negative average surface-level elevation gradient of -0.5 ppb per 100 meters elevation gain in SENP, illustrating the location-specific complexities inherent to high-altitude surface-level $O_3$ concentrations.

Since $O_3$ produced from emissions in urban areas is transported to more rural downwind locations, elevated $O_3$ concentrations can occur at considerable distances from urban centers. In addition, major sources of $O_3$ precursors such as highways, power plants, biomass combustion, and oil and gas operations are commonly found in rural areas, adding to the $O_3$ in these areas. Due to lower chemical scavenging in non-urban areas, $O_3$ tends to persist longer in rural than in urban areas which tends to lead to higher cumulative exposures in rural areas influenced by anthropogenic precursor emissions. The persistently high $O_3$ concentrations observed at many of these rural sites investigated here indicate that cumulative exposures for humans and vegetation in rural areas can be substantial and often higher than cumulative exposures in urban areas.

## 3.6.3 Temporal Variability

### 3.6.3.1 Multiyear Trends

As reported in the 2010 National Air Quality Status and Trends report (U.S. EPA, 2010e), nation-wide surface-level $O_3$ concentrations in the U.S. have declined gradually over the last decade. Figure 3-48 shows the downward trend in the annual 4th-highest 8-h daily max $O_3$ concentration from 870 surface-level monitors across the United States. Figure 3-49 shows a similar trend in the annual 2nd highest 1-h daily max $O_3$ concentration from 875 surface-level monitors. The median annual 4th-highest 8-h daily max dropped from 88 ppb in 1998 to 71 ppb in 2010. Likewise, the median annual 2nd-highest 1-h daily max dropped from 109 ppb in 1998 to 86 ppb in 2010. The large decreases in 2003 and 2004 in both figures coincides with $NO_X$ emissions reductions resulting from implementation of the $NO_X$ State Implementation Plan (SIP) Call rule, which began in 2003 and was fully

implemented in 2004. This rule was designed to reduce $NO_X$ emissions from power plants and other large combustion sources in the eastern United States. Reductions in mobile $NO_X$ emissions nationwide from the implementation of recent vehicle and fuel standards could also be adding to the gradual decline in nationwide surface-level $O_3$ concentrations (Dallmann and Harley, 2010).

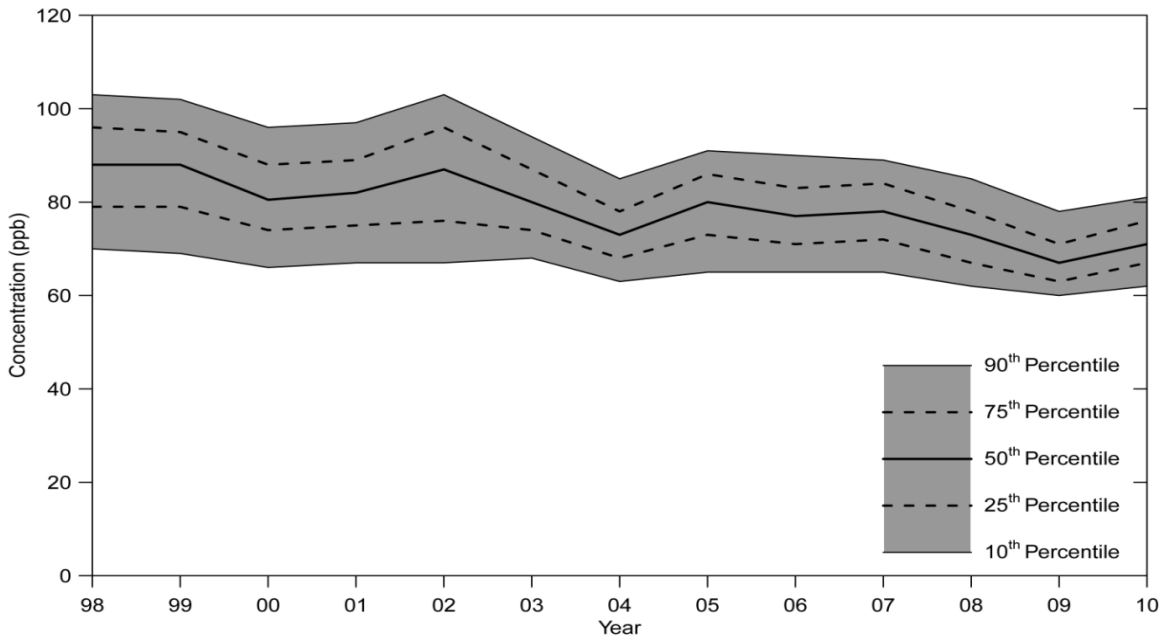



**Figure 3-48 National 8-h daily max $O_3$ trend and distribution across 870 U.S. $O_3$ monitors, 1998-2010 (annual 4th-highest 8-h daily max $O_3$ concentrations in ppm).**

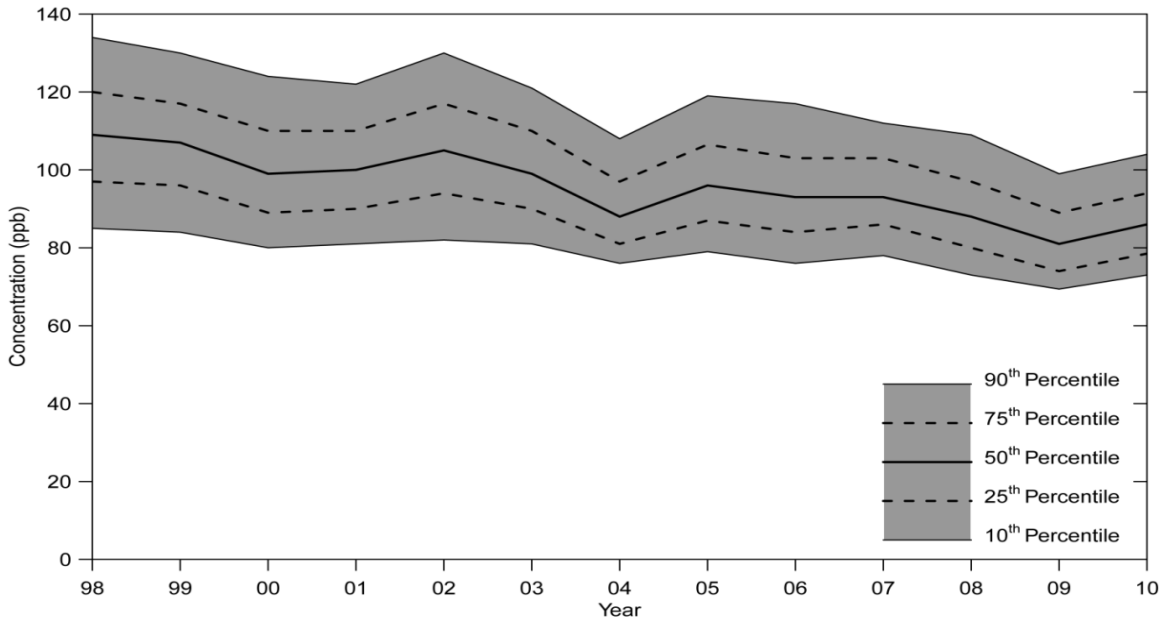



**Figure 3-49 National 1-h daily max $O_3$ trend and distribution across 875 U.S. $O_3$ monitors, 1998-2010 (annual 2nd-highest 1-h daily max $O_3$ concentrations in ppm).**

The distributional percentiles (10th, 25th, 75th, and 90th) displayed in Figure 3-48 and Figure 3-49 reveal a gradual tightening of the $O_3$ concentration distribution observed across monitors. For the annual 4th-highest 8-h daily max $O_3$ concentration, the IQR decreased from 17 ppb in 1998 to 9 ppb in 2010. Likewise, for the annual 2nd highest 1-h daily max $O_3$ concentration, the IQR decreased from 23 ppb in 1998 to 16 ppb in 2010. A similar tightening was observed for the wider percentiles (90th-10th) for both averaging times.

Weather can have a strong influence on the $O_3$ trends shown in Figure 3-48 and Figure 3-49. The number of hot, dry days can substantially alter the number of high $O_3$ days in any given year, even if $O_3$ forming emissions do not change. To better evaluate the progress and effectiveness of emissions reduction programs, EPA uses a statistical model to estimate the influence of atypical weather on $O_3$ formation (U.S. EPA, 2010e). After adjusting for the influence of weather, the downward trend in surface-level national 8-h daily max $O_3$ concentrations between 2001 and 2008 increased slightly from an 8% reduction prior to adjustment for weather to an 11% reduction after adjustment for weather (U.S. EPA, 2010e).

A regional breakdown of the trend in $O_3$ concentrations for the 8-hour and 1-hour metrics is included in Figure 3-50 and Figure 3-51, respectively. In general, the trends are region-specific with a substantial amount of year-to-year variability. The reduction in $NO_X$ and $O_3$ during the 2003-2004 timeframe is particularly evident in the North Central and Northeastern U.S. where the $NO_X$ SIP Call was focused

(U.S. EPA, 2010e). The western region (including Alaska and Hawaii but excluding California) started out with the lowest annual $O_3$ concentration in 1998 and exhibits the least amount of reduction when compared to 2010 concentrations (11% reduction in the average annual 4th-highest 8-h daily max and 13% reduction in the average annual 2nd-highest 1-h daily max). In contrast, California—which has some of the highest concentrations of the identified regions—shows a larger downward trend in $O_3$ concentrations over the same time period (19% reduction in the average annual 4th-highest 8-h daily max and 22% reduction in the average annual 2nd-highest 1-h daily max).

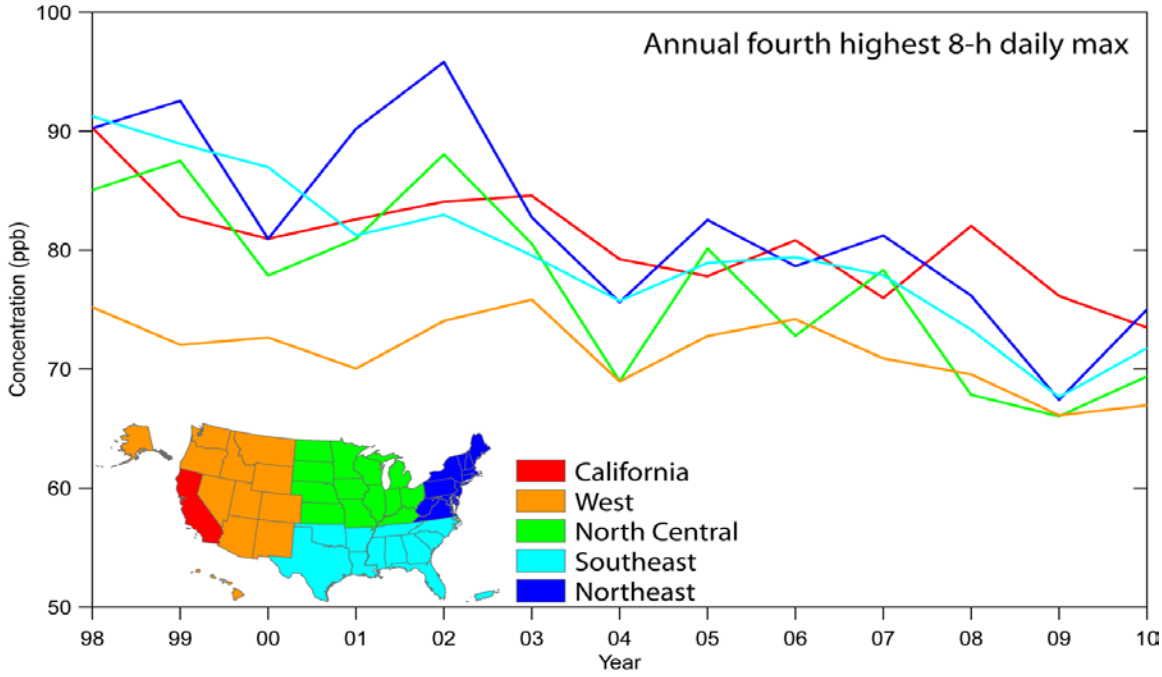



**Figure 3-50 Trend in mean 8-h daily max $O_3$ by region, 1998-2010 (mean of the annual 4th-highest 8-h daily max $O_3$ concentrations in ppm).**

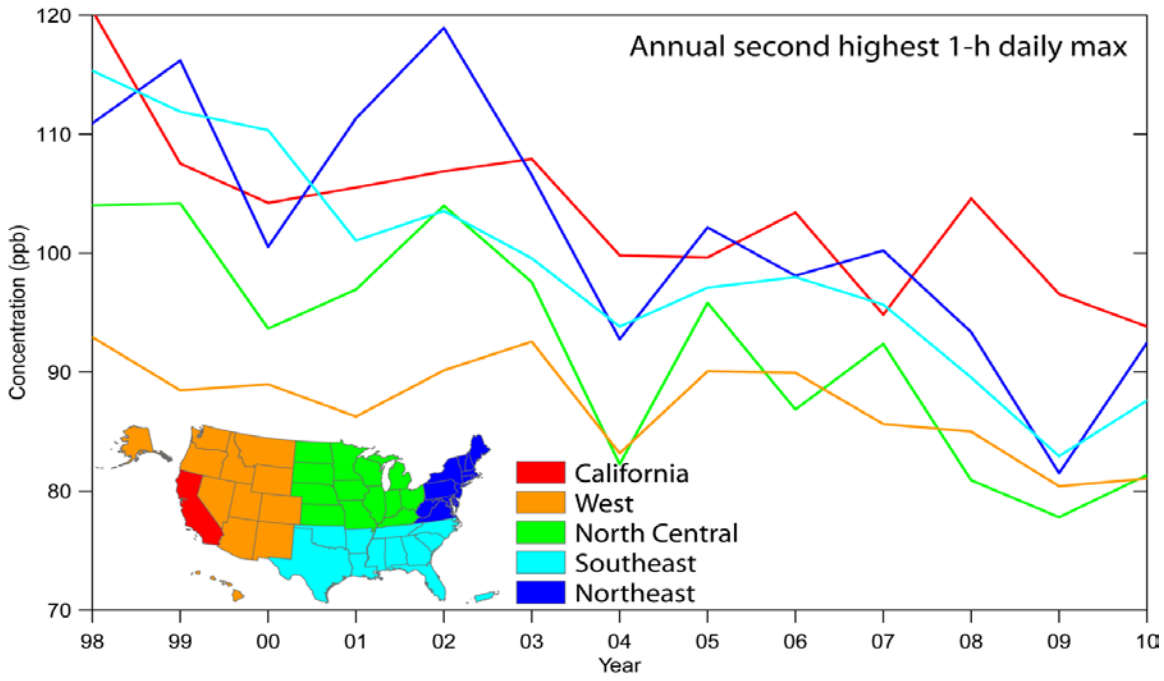



**Figure 3-51 Trend in mean 1-h daily max $O_3$ by region, 1998-2010 (mean of the annual 2nd-highest 1-h daily max $O_3$ concentrations in ppm).**

Narrowing the focus to changes in $O_3$ concentrations at the individual monitor level, Figure 3-52 displays the 8-h $O_3$ design value (4th-highest 8-h daily max $O_3$ concentration occurring within a three-year period) for all available monitors for the 2008-2010 period (Figure 3-52A) as well as the change in this design value between the 2001-2003 period and the 2008-2010 period (Figure 3-52B). Figure 3-53 displays analogous information for a 1-h $O_3$ design value (4th-highest 1-h daily max $O_3$ concentration occurring within a three-year period). As can be seen in both figures, the majority of monitors recorded a decrease in design values when comparing the 2001-2003 period to the 2008-2010 period. Specifically, 699 of 853 sites (82%) included in Figure 3-52B for the 8-h design value and 747 of 869 sites (86%) included in Figure 3-53B for the 1-h design value reported a decrease of at least 6 ppb in the respective design values. The highest density of monitors reporting decreases occurs in the Northeast. Only 8 sites (1%) reported an increase of more than 5 ppb in the 8-h design value and only 16 sites (2%) reported an increase of more than 5 ppb in the 1-h design value. These sites reporting an increase between the 2001-2003 and the 2008-2010 periods were located primarily in the West. More in depth trends analyses have been performed to support the $O_3$ Risk and Exposure Analysis and are available through EPA's Technology Transfer Network website for the $O_3$ review (Wells et al., 2012).

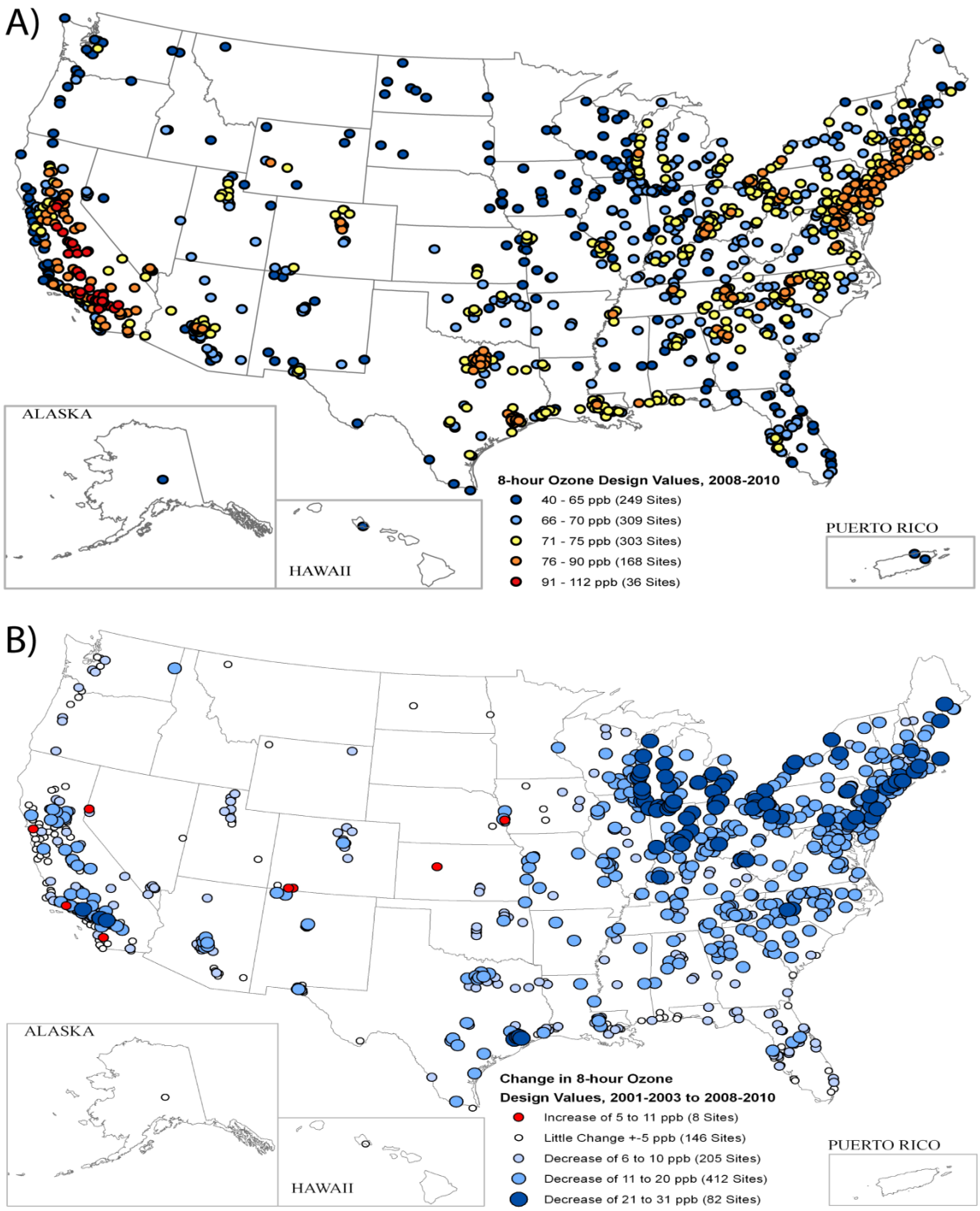



**Figure 3-52 Individual monitor 8-h daily max $O_3$ design values displayed: (A) for the 2008-2010 period, and (B) as the change since the 2001-2003 period.**

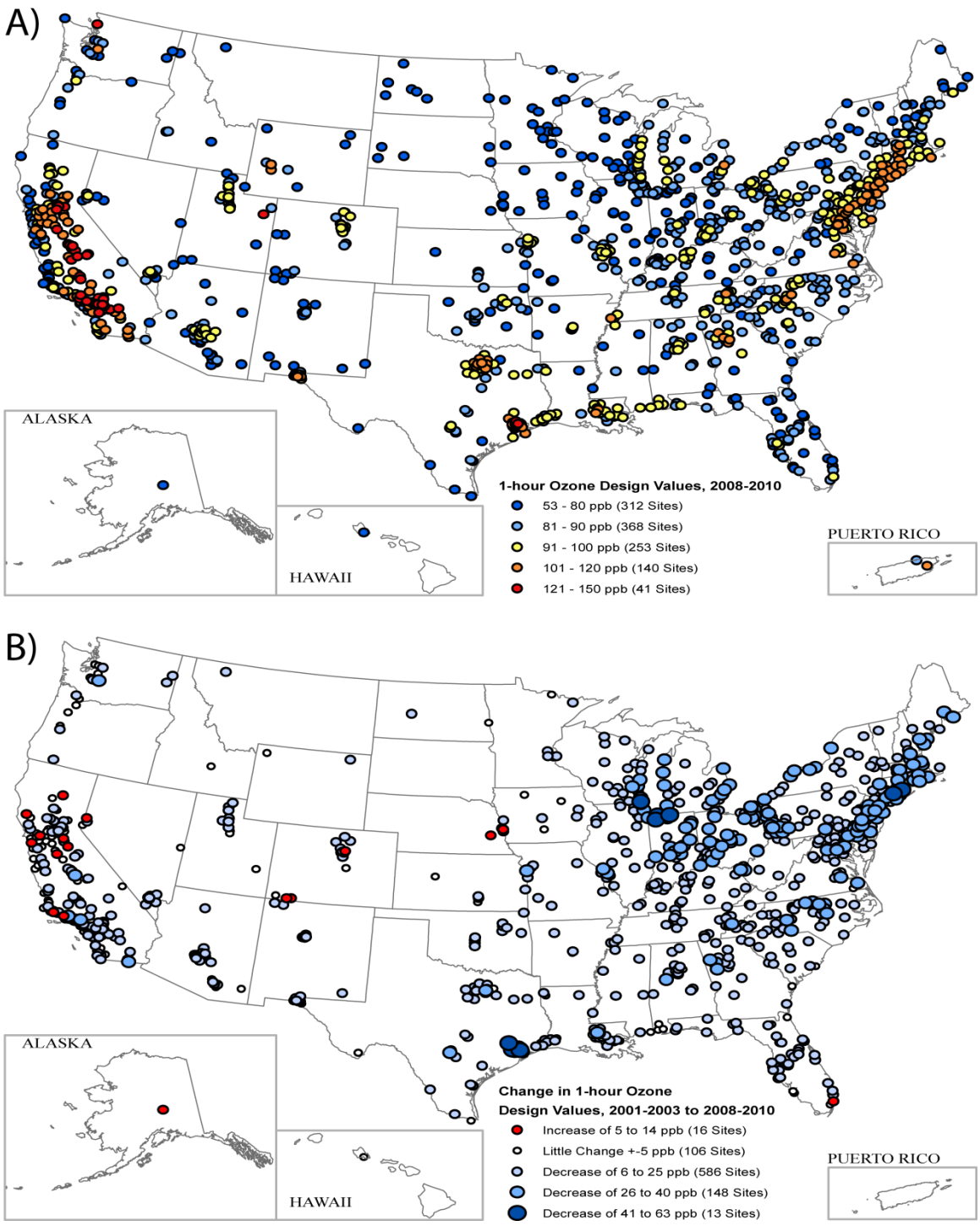



**Figure 3-53 Individual monitor 1-h daily max $O_3$ design values displayed: (A) for the 2008-2010 period, and (B) as the change since the 2001-2003 period.**

Similar findings were reported for regional trends in the 4th-highest 8-h daily max $O_3$ concentration between 2001 and 2008 in the 2010 National Air Quality Status and Trends report (U.S. EPA, 2010e). Individual sites that showed the greatest reduction in $O_3$ between 2001 and 2008 were in or near the following metropolitan areas: Anderson, IN; Chambersburg, PA; Chicago, IL; Cleveland, OH; Houston, TX; Michigan City, IN; Milwaukee, WI; New York, NY; Racine, WI; Watertown, NY; and parts of Los Angeles, CA. Individual sites that showed an increase in $O_3$ over this time period and had measured concentrations above the $O_3$ standard[1] during the 2006-2008 time period were located in or near the following metropolitan areas: Atlanta, GA; Baton Rouge, LA; Birmingham, AL; Denver, Colorado; El Centro, CA; San Diego, CA; Seattle, WA; and parts of Los Angeles, CA.

Pegues et al. (2012) investigated changes in 3-year average 8-h daily max $O_3$ design values between 2003 and 2009 and found reductions at the majority of sites across the U.S.; consistent with the findings in this section and in the 2010 National Air Quality Status and Trends report (U.S. EPA, 2010e). Furthermore, they compared trends in $O_3$ design values between areas that were or were not classified as nonattainment of the 84 ppb $O_3$ standard in the 2004 designations. Monitors designated nonattainment achieved $O_3$ design value reductions of 13.3 ppb on average while monitors designated in attainment achieved reductions of 7.0 ppb on average.

Looking further back in time, Leibensperger et al. (2008) included an analysis of June-August 8-h daily max $O_3$ trends from 1980-2006 using AQS data from over 2000 sites in the contiguous United States. They created an index for "pollution days" representing days when the 8-h daily max $O_3$ concentration was greater than 84 ppb. The observed trend in summertime $O_3$ pollution days over this 27 year period decreased at an average rate of -0.84 days/year. The authors used several methods to deconstruct this trend into a component coming from reductions in $O_3$ precursor emissions (-1.50 days/year) and a component coming from climate change (+0.63 days/year). The climate change impact is a result of decreases in frequency of mid-latitude cyclones which serve to ventilate surface air over the United States. Leibensperger et al. (2008) conclude that the reduction in frequency of mid-latitude cyclones over the 1980-2006 time period has offset almost half of the air quality gains in the Northeastern U.S. that should have been achieved from reductions of anthropogenic emissions alone over that period. This conclusion is based on the assumption of a linear additive relationship between $O_3$ precursor emission changes and cyclone frequency variations on the rate of change of high $O_3$ days, and does not account for nonlinearities inherent in $O_3$ chemistry as discussed in Section 3.2. A more recent analysis by Turner et al. (2012) addressed this issue by utilizing the GFDL CM3 global coupled chemistry climate model to assess relationships between summertime cyclones and $O_3$ pollution episodes. They also found a robust decline in cyclone frequency in modeled scenarios incorporating climate change, but in their models, less than 10% of the variability in high $O_3$ days in the Northeastern U.S. was explained by cyclone activity. As a result, Turner et al. (2012) caution against over-

[1] The 2008 $O_3$ NAAQS include primary and secondary standards of 0.075 ppm (8-h daily max).

interpreting the strong association observed by Leibensperger et al. (2008) between mid-latitude cyclone frequency and the occurrence of high $O_3$ days.

Averaging time can have an impact on perceived trends in surface-level $O_3$ concentrations. Lefohn et al. (2008) investigated the impact of using different exposure indices on trends in surface-level $O_3$ concentrations in the U.S. by comparing the annual 2nd-highest 1-h avg concentration, the annual 4th-highest daily maximum 8-h avg concentration, and the seasonally corrected 24-h W126 cumulative exposure index. Between 1980 and 2005, most of the urban and rural sites across the U.S. included in this study showed decreasing or zero trend for all three of these metrics. However, the magnitude of this trend varied greatly by exposure index. The largest downward trend in the 1-h and 8-h metrics listed above were observed in Southern California (>2%/yr downward trend) but the W126 cumulative exposure metric showed large (>2%/yr) downward trends in many locations across the U.S. including Southern California, the Midwest and Northeast. By contrasting the 1980 – 2005 trends with more recent 1990 – 2005 trends, Lefohn et al. (2008) reported that a large number of sites (44%, 35% and 25% of sites for the 1-h, 8-h and W126 metrics, respectively) shifted from a negative trend to no trend. These shifts in trends were attributed to slow changes in mid-level concentrations (i.e., 60-90 ppb) following a more rapid change in peak concentrations in the early years. A similar conclusion was drawn from nationwide $O_3$ data between 1980 – 2008 (Lefohn et al., 2010b), suggesting a shift in the $O_3$ distribution over this time period.

In contrast to the mostly urban observations included in the Pegues et al. (2012) study above, several studies focusing on rural western monitors have reported positive trends in $O_3$ concentrations over the last few decades. Jaffe and Ray (2007) investigated daytime (10 a.m. – 6 p.m. local time) $O_3$ concentrations at rural sites in the northern and western U.S. between 1987-2004. They found significantly positive trends in seven of the eleven sites selected ranging from 0.19 ppb/yr in Gothic, Colorado to 0.51 ppb/yr in Rocky Mountain NP, Colorado (mean trend of 0.26 ppb/yr at these seven sites). No significant trend was observed for the two sites in Alaska and one site each in Wyoming and Montana. Seasonal analyses were conducted on the sites having the longest records in Rocky Mountain NP, Yellowstone, NP and Lassen NP and positive trends were found for all seasons at all sites. As noted in the 2006 $O_3$ AQCD (U.S. EPA, 2006b), caution should be exercised in using trends calculated at national parks to infer contributions from distant sources either inside or outside of North America because of the influence of regional pollution (see Section 3.4 for a discussion of background $O_3$ concentrations and international transport).

Trends in baseline $O_3$ concentrations, defined as $O_3$ concentrations at a given site in the absence of strong local influences, were estimated by region and season in the U.S. in Chan and Vet (2010). The temperature-adjusted decadal (1997-2006) trends in estimated baseline $O_3$ varied substantially by region and season. In the Pacific coastal regions, the trends increased in all seasons except fall, but none of the trends were statistically significant. In the eastern U.S., negative trends were observed in all

seasons with the exception of (1) insignificant positive trends in northeast Maine in summer, fall and winter; (2) significant positive trends in the Midwest in winter; and 3) significant positive trends at one site in Vermont in the summer. The density of sites in the central and western U.S. were much lower than the coastal and eastern areas, but in general all sites showed trends that tended to be negative in the spring and fall but positive in the summer and winter.

Positive trends in marine boundary layer $O_3$ concentrations at several sites on the Pacific Coast have been reported by other sources in the literature. Parrish et al. (2009) used observations from multiple coastal sites in California and Washington and reported a positive annual mean trend of 0.34 ± 0.09 ppb/yr between the mid-1980s and 2007 (exact dates varied by site depending on available data). A seasonal stratification of the data at these sites showed the largest positive trend in the spring (0.46 ± 0.13 ppb/yr) with a smaller and non-significant positive trend during fall (0.12 ± 0.14 ppb/yr). These results agree with positive trends in springtime $O_3$ mixing ratios reported in an earlier study (Jaffe et al., 2003). Positive trends in $O_3$ measurements in the free troposphere above western North America at altitudes of 3-8 km (above sea level) during April and May of 1995 to 2008 were reported by Cooper et al. (2010) and discussed in Section 3.4.2 as they relate to intercontinental transport. Comparable trends were observed in the median as well as 5th, 33rd, 67th, and 95th percentiles of observations. Note, however, that these results relate to $O_3$ trends above ground level and not to surface $O_3$.

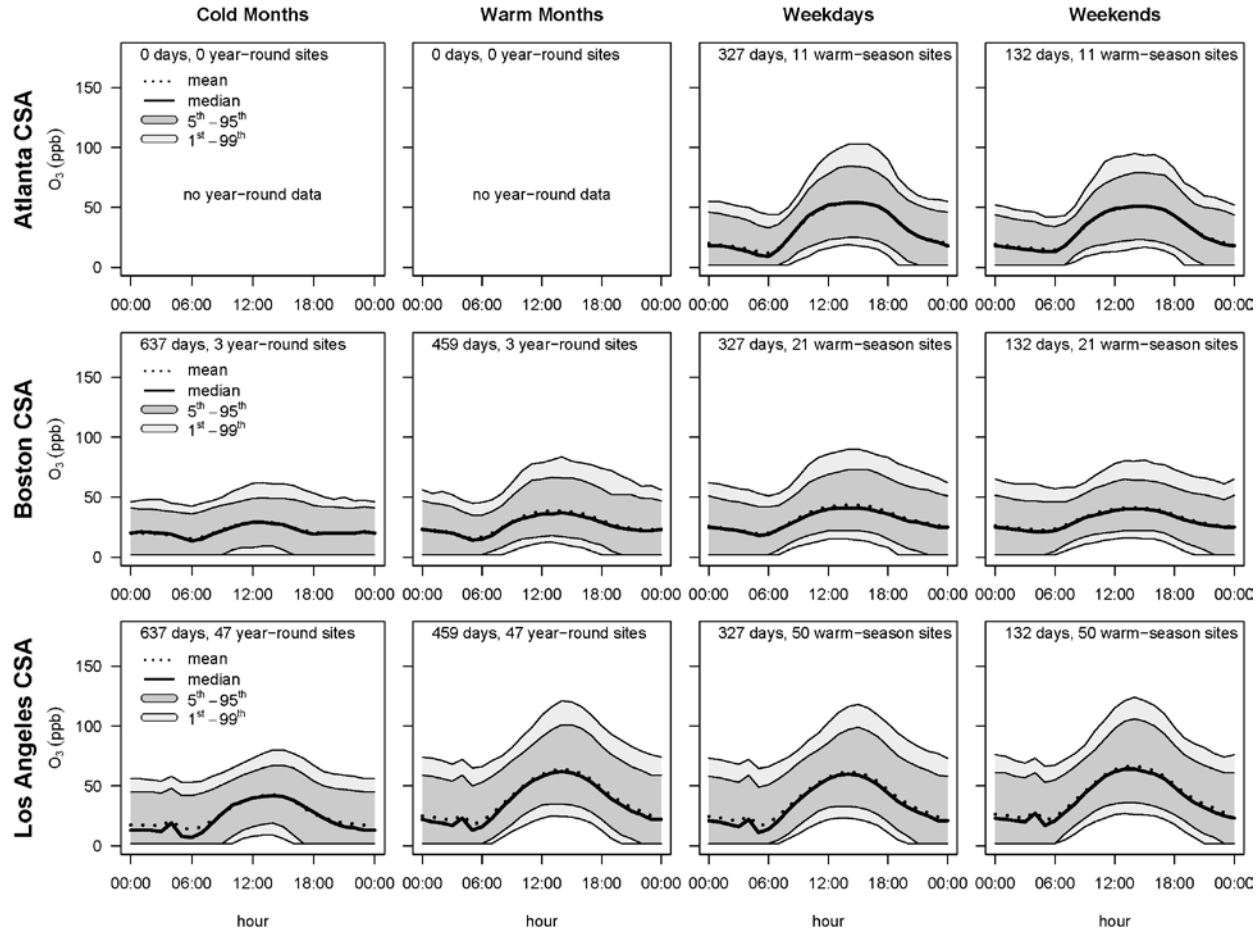
Extending back to the 19th Century, Volz and Kley (1988) report a series of historic $O_3$ measurements from Europe. Comparing these with more contemporary measurements, Parrish et al. (2009) report a 2 to 3 fold increase in boundary layer $O_3$ mixing ratios over the last 130 years with no indication of stabilization in recent years. Other long-term observations of global trends in the burden of tropospheric $O_3$ as they relate to climate change are discussed in Chapter 10, Section 10.3.3.1.

### 3.6.3.2 Hourly Variations

Ozone concentrations frequently possess a strong degree of diel variability resulting from daily patterns in temperature, sunlight, and precursor emissions. Other factors, such as the relative importance of transport versus local photochemical production and loss rates, the timing for entrainment of air from the nocturnal residual boundary layer, and the diurnal variability in mixing layer height also play a role in daily $O_3$ patterns. The 2006 $O_3$ AQCD (U.S. EPA, 2006b) looked at composite urban diel variations from April to October 2000 to 2004 and found 1-h maxima to occur in mid-afternoon and 1-h minima to occur in early morning. On a national basis, however, there was a high degree of spread in these times and caution was raised in extrapolating results from one city to another in determining the time of day for $O_3$ maxima and minima.

Urban diel variability in $O_3$ concentrations was investigated for the 20 focus cities listed in Table 3-9 using 1-h avg $O_3$ data from AQS. The year-round data set

described in Table 3-5 was used to compare diel patterns during cold months (October - April) and warm months (May - September) between 2007 and 2009. The warm-season data set, also described in Table 3-5, was used to compare weekday and weekend diel patterns. Figure 3-156 through Figure 3-160, in the supplemental material in Section 3.9.4, show these patterns for each of the 20 cities; examples for Atlanta, Boston, and Los Angeles are shown in Figure 3-54.




Note: Uses the year-round data set for the cold month/warm month comparison (left half) and the warm-season data set for the weekday/weekend comparison (right half). Atlanta had no year-round monitors available for the cold month/warm month comparison.

**Figure 3-54 Diel patterns in 1-h avg $O_3$ for Atlanta, Boston and Los Angeles between 2007 and 2009.**

In general, all the urban areas showed 1-h daily max concentrations occurring typically in the early afternoon. In all cities, these afternoon peaks were more pronounced in the warm months than in the cold months. However, a small peak was still present during the cold months. During warm months, the difference between the median daily extrema varied considerably by city. For example, in Los Angeles, the median 1-h daily min (10 ppb) at ~5:00 a.m. was 50 ppb less than the median 1-h daily max (60 ppb) at ~2:00 p.m. By contrast, in Boston, the median 1-h daily min (13 ppb) occurred at the same time, but was only 25 ppb less than the median 1-h daily max (38 ppb). Cities with large daily swings (>40 ppb) in median 1-h $O_3$ concentrations included Atlanta, Birmingham, Los Angeles, Phoenix, Pittsburgh, and Salt Lake City (Figure 3-156 through Figure 3-160 in Section 3.9.4). Cities with small daily swings (<25 ppb) in median 1-h $O_3$ concentrations included Boston, Minneapolis, San Francisco and Seattle (Figure 3-156 through Figure 3-160 in Section 3.9.4). These results are very similar to those found in the 2006 $O_3$ AQCD (U.S. EPA, 2006b) where many of these same urban areas were investigated. This supports the conclusions drawn in the previous $O_3$ review that diel patterns in $O_3$ have remained stable over the last 20 years, with times of occurrence of the daily maxima varying by no more than an hour from year to year.

Using the warm-season data, there was little difference in the median diel profiles for weekdays compared with weekends across all urban areas. This result stresses the complexity of $O_3$ formation and the importance of meteorology, entrainment, biogenic precursor emissions, and transport in addition to anthropogenic precursor emissions. There was, however, a subtle deviation between weekdays and weekends in the lower percentiles (1st and 5th) of the distribution. The lower end of the distribution tended to be lower on weekdays relative to weekends. This is consistent with analyses in the 2006 $O_3$ AQCD (U.S. EPA, 2006b) and is a result of lower traffic volumes on weekends relative to weekdays, leading to less NO emissions and $O_3$ titration on the weekends.

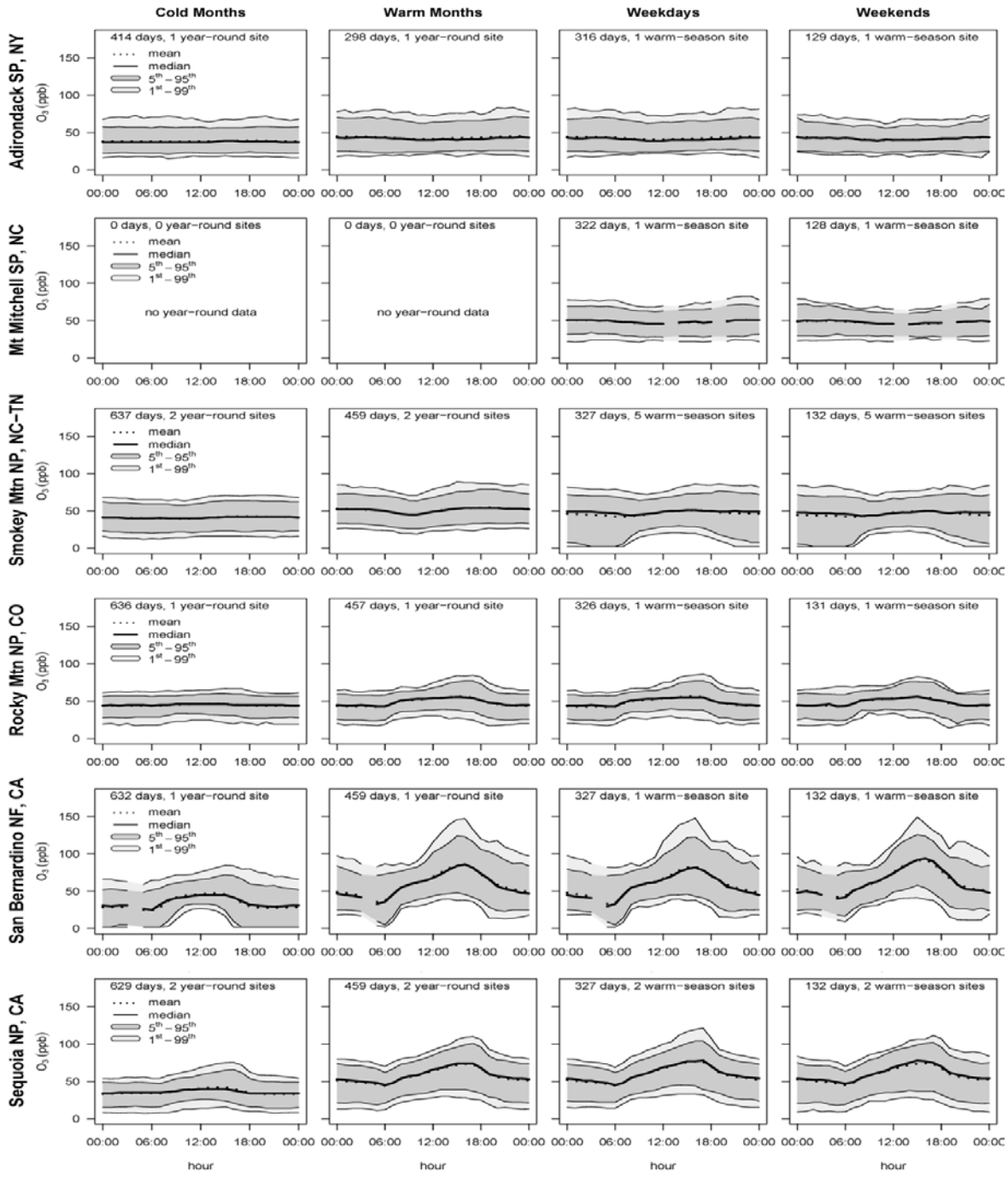
Seasonal and site-to-site variations in diel patterns within a subset of the urban focus areas presented here were investigated in the 2006 $O_3$ AQCD (U.S. EPA, 2006b). In northern cities, there was substantial seasonal variability in the diel patterns with higher extreme values in the $O_3$ distribution during the warm season than during the cold season. In southern cities, the seasonal differences in extreme $O_3$ concentrations were much smaller, and some of the highest $O_3$ concentrations in the Houston CSA were found outside of summer. The general pattern that emerged from investigating site-to-site variability within the urban areas was that peaks in 1-h avg $O_3$ concentrations are higher and tend to occur later in the day at downwind sites relative to sites located in the urban core. Differences between sites were not only related to the distance between them, but also depend on the presence or absence of nearby $O_3$ sources or sinks.

Rural diel variability in $O_3$ concentrations was investigated for the six rural focus areas listed in Table 3-11 using 1-h avg $O_3$ data from AQS. As with the urban analysis, the year-round data set described in Table 3-5 was used to compare diel patterns during cold months (October - April) and warm months (May - September)

between 2007 and 2009. The warm-season data set, also described in Table 3-5, was used to compare weekday and weekend diel patterns. Figure 3-55 shows the diel patterns for each of the rural areas investigated.

There was considerable variability in the diel patterns observed in the six rural focus areas. The selected mountainous eastern sites in ADSP, MMSP, and SMNP exhibited a generally flat profile with little hourly variability in the median concentration and the upper percentiles. In SMNP, there was some diel variability in the lower percentiles, with higher values during the daylight hours in the warm season data. This behavior was not present in the data coming from the two year-round monitors located at lower elevation sites (Sites C and Site D; see map in Figure 3-44), however, possibly resulting from differing impacts from local sources within SMNP. For the western rural areas, there was a clear diel pattern to the hourly $O_3$ data with a peak in concentration in the afternoon similar to those seen in the urban areas in Figure 3-54 and Figure 3-156 through Figure 3-160 in Section 3.9.4. This was especially obvious at the SBNF site which sits 90 km east of Los Angeles in the San Bernardino Mountains at an elevation of 1,384 meters. This site was located here to monitor $O_3$ transported downwind from major urban areas in the South Coast Air Basin. It had the highest 2007-2009 median 8-h daily max $O_3$ concentration of any AQS site in the Los Angeles CSA (see Figure 3-34), and is clearly impacted by the upwind urban plume which has sufficient time and sunlight to form $O_3$ from precursor emissions and concentrate the $O_3$ in the shallow boundary layer present at this elevation.

As with the urban analysis, there was little difference observed in the weekday and weekend diel profiles using the warm-season data, even down at the lower percentiles in the distribution. This is consistent with the regional nature of tropospheric $O_3$. Using the year-round data, there was an upward shift in the distribution going from the cold months to the warm months, and in some instances the general shape of the distribution changed considerably as was seen in several urban sites.



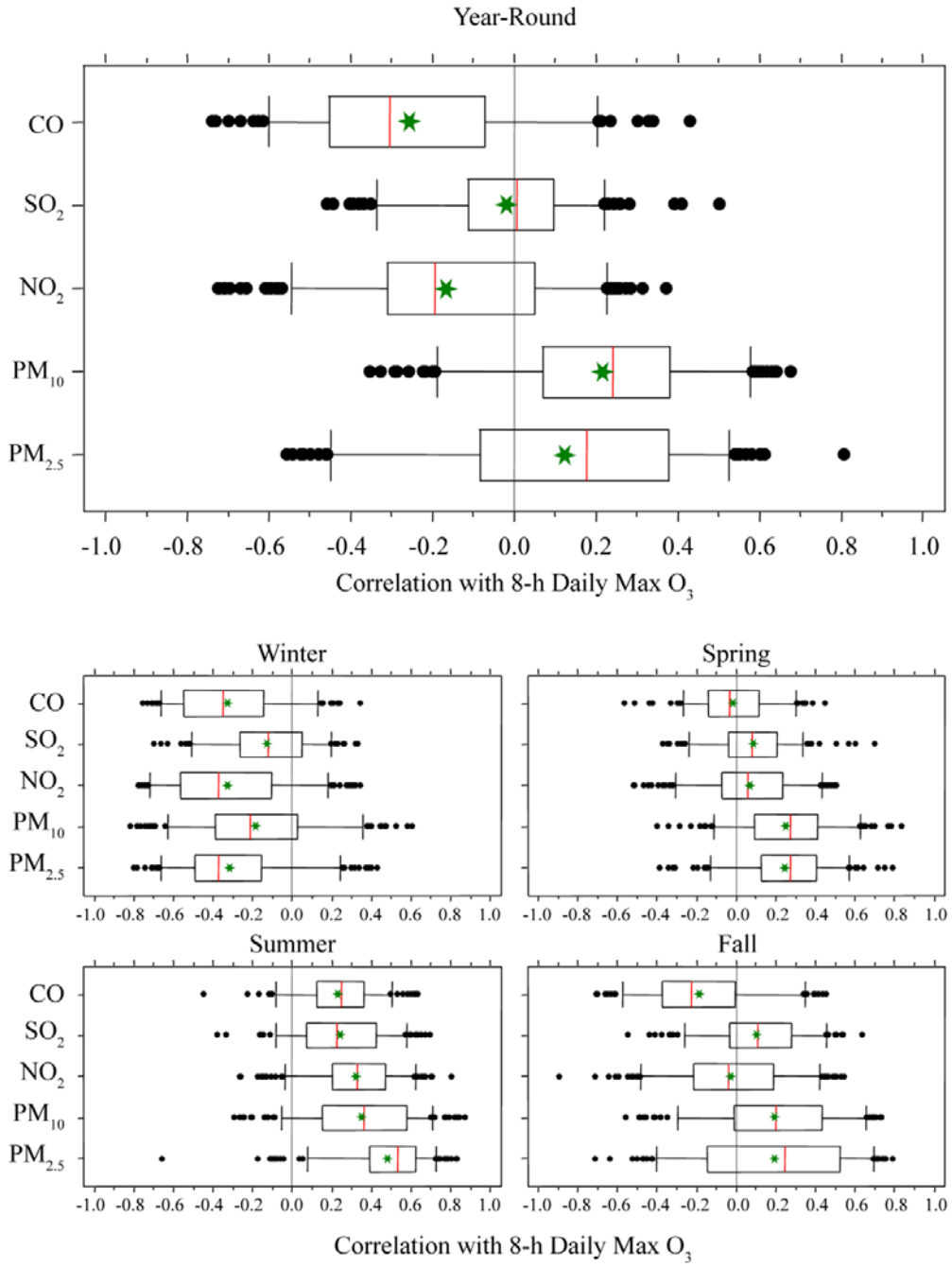

Note: Uses the year-round data set for the cold month/warm month comparison (left half) and the warm-season data set for the weekday/weekend comparison (right half). Mt. Mitchell SP, NC had no year-round monitors available for the cold month/warm month comparison.

**Figure 3-55 Diel patterns in 1-h avg $O_3$ for six rural focus areas between 2007 and 2009.**

### 3.6.4 Associations with Copollutants

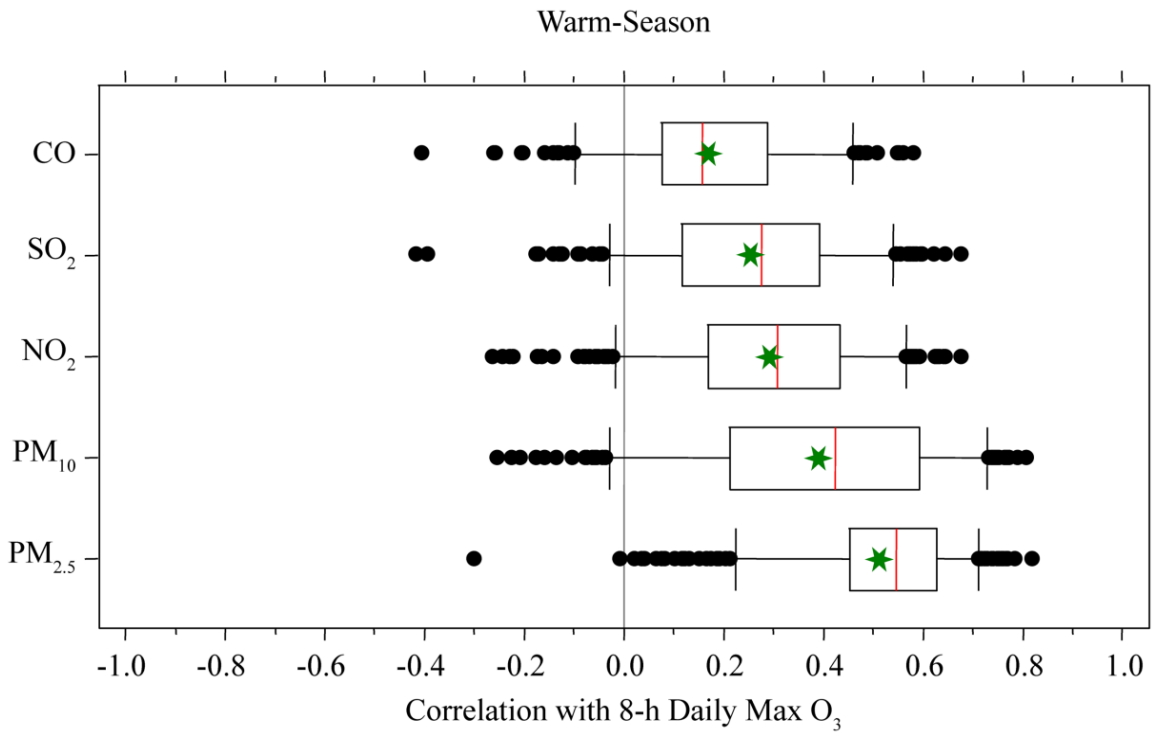
Correlations between $O_3$ and other criteria pollutants are discussed in this section. Since $O_3$ is a secondary pollutant formed in the atmosphere from precursor emissions, its correlation with primary pollutants such as CO and $NO_X$ can vary substantially by location. Furthermore, $O_3$ formation is strongly influenced by meteorology, entrainment, and transport of both $O_3$ and $O_3$ precursors, resulting in a broad range in correlations with other pollutants which can vary substantially with season. This section focuses on correlations between $O_3$ and other criteria pollutants measured at the mostly urban AQS sites: a more detailed discussion of $O_3$ and $O_3$-precursor relationships is included in Section 3.2.4. To investigate correlations with copollutants, 8-h daily max $O_3$ from the year-round and warm-season data sets (Table 3-6 and Table 3-7) were compared with co-located 24-h avg CO, $SO_2$, $NO_2$, $PM_{2.5}$ and $PM_{10}$ obtained from AQS for 2007-2009. Figure 3-56 and Figure 3-57 contain copollutant box plots of the correlation between co-located monitors for the year-round data set and the warm-season data set, respectively.

The year-round 8-h daily max $O_3$ data (Figure 3-56) had a very wide range in correlations with all the 24-h avg copollutants. A clearer pattern emerged when the data were stratified by season (bottom four plots in Figure 3-56) with mostly negative correlations in the winter and mostly positive correlations in the summer for all copollutants. In summer, the IQR in correlations is positive for all copollutants. However, the median seasonal correlations are still modest at best with the highest positive correlation at 0.52 for $PM_{2.5}$ in the summer and the highest negative correlation at -0.38 for $PM_{2.5}$ in the winter. Spring and fall lie in between with spring having a slightly narrower distribution than fall for all copollutants. The warm-season 8-h daily max $O_3$ data (Figure 3-57) shows a very similar distribution to the summer stratification of the year-round data due to their overlap in time periods (May-Sept and June-Aug, respectively).




Note: Year round (Top figure), and with seasonal stratification (Bottom four figures). Shown are the median (red line), mean (green star), inner-quartile range (box), 5th and 95th percentiles (whiskers) and extremes (black circles).

**Figure 3-56 Distribution of Pearson correlation coefficients for comparison of 8-h daily max $O_3$ from the year-round data set with co-located 24-h avg CO, $SO_2$, $NO_2$, $PM_{10}$ and $PM_{2.5}$ from AQS, 2007-2009.**




Note: Shown are the median (red line), mean (green star), inner-quartile range (box), 5th and 95th percentiles (whiskers), and extremes (black circles).

**Figure 3-57 Distribution of Pearson correlation coefficients for comparison of 8-h daily max $O_3$ from the warm-season (May-Sept) data set with co-located 24-h avg CO, $SO_2$, $NO_2$, $PM_{10}$ and $PM_{2.5}$ from AQS, 2007-2009.**

The seasonal fluctuations in correlations present in Figure 3-56 result in part from the mixture of primary and secondary sources for the copollutants. For example, $O_3$ is a secondary pollutant whereas $PM_{2.5}$ has both primary and secondary origins and these two pollutants show the largest summertime/wintertime swing in correlation distributions. This situation arises because the secondary component to $PM_{2.5}$ is larger during the summer and is formed in conditions conducive to secondary $O_3$ formation. This results in positive correlations between $O_3$ and $PM_{2.5}$ during the summer. During the winter, photochemical production of $O_3$ is much smaller than during summer and $O_3$ comes mainly from aloft, i.e., the free troposphere (see Section 3.4.1.1 for further details). In addition, concentrations of $PM_{2.5}$ are much lower aloft. On relatively clean days, this can lead to high concentrations of $O_3$ and lower concentrations of primary pollutants such as $PM_{2.5}$ or NO. On relatively dirty days with elevated NO and $PM_{2.5}$, the intruding $O_3$ is readily titrated by NO in the boundary layer. These processes result in negative correlations between $O_3$ and $PM_{2.5}$ during the winter.

## 3.7 Chapter Summary

This section contains a summary of the major topics included in this chapter on the atmospheric chemistry and ambient concentrations of tropospheric $O_3$ and other related photochemical oxidants. This chapter has built upon information previously reported in the 2006 $O_3$ AQCD (U.S. EPA, 2006b) and includes updated material on: (1) physical and chemical processes of $O_3$ formation and removal; (2) atmospheric modeling; (3) background $O_3$ concentrations; (4) monitoring techniques and networks; and (5) ambient concentrations.

### 3.7.1 Physical and Chemical Processes

Ozone in the troposphere is a secondary pollutant; it is formed by photochemical reactions of precursor gases and is not directly emitted from specific sources. Ozone precursor gases originate from both anthropogenic and natural source categories. Ozone attributed to anthropogenic sources is formed in the atmosphere by photochemical reactions involving sunlight and precursor pollutants including VOCs, $NO_X$, and CO. Ozone attributed to natural sources is formed through similar photochemical reactions involving natural emissions of precursor pollutants from vegetation, microbes, animals, biomass burning, lightning, and geogenic sources. The distinction between natural and anthropogenic sources of $O_3$ precursors is often difficult to make in practice, as human activities affect directly or indirectly emissions from what would have been considered natural sources during the pre-industrial era. The formation of $O_3$, other oxidants, and oxidation products from these precursors is a complex, nonlinear function of many factors including: (1) the intensity and spectral distribution of sunlight reaching the lower troposphere; (2) atmospheric mixing; (3) concentrations of precursors in the ambient air and the rates of chemical reactions of these precursors; and (4) processing on cloud and aerosol particles.

Ozone is present not only in polluted urban atmospheres but throughout the troposphere, even in remote areas of the globe. The same basic processes involving sunlight-driven reactions of $NO_X$, VOCs, and CO contribute to $O_3$ formation throughout the troposphere. These processes also lead to the formation of other photochemical products, such as PAN, nitric acid, and sulfuric acid, and to other compounds, such as formaldehyde and other carbonyl compounds. In urban areas, $NO_X$, VOCs, and CO are all important for $O_3$ formation. In non-urban vegetated areas, biogenic VOCs emitted from vegetation tend to be the most important precursor to $O_3$ formation. In the remote troposphere, methane (structurally the simplest VOC) and CO are the main carbon-containing precursors to $O_3$ formation. Ozone is subsequently removed from the troposphere through a number of gas phase reactions and deposition to surfaces.

Convective processes and turbulence transport $O_3$ and other pollutants both upward and downward throughout the planetary boundary layer and the free troposphere.

In many areas of the U.S., $O_3$ and its precursors can be transported over long distances, aided by vertical mixing. The transport of pollutants downwind of major urban centers is characterized by the development of urban plumes. Meteorological conditions, small-scale circulation patterns, localized chemistry, and mountain barriers can influence mixing on a smaller scale, resulting in frequent heterogeneous $O_3$ concentrations across individual urban areas.

### 3.7.2 Atmospheric Modeling

CTMs have been widely used to compute the interactions among atmospheric pollutants and their transformation products, and the transport and deposition of pollutants. They have also been widely used to improve basic understanding of atmospheric chemical processes and to develop control strategies. The domains of CTMs extend from a few hundred kilometers on a side to the entire globe.

Most major regional (i.e., sub-continental) scale air-related modeling efforts at EPA rely on the CMAQ modeling system. The horizontal domain for CMAQ typically extends over North America with efforts underway to extend it over the entire Northern Hemisphere. The upper boundary for CMAQ is typically set at 100 hPa, which is located on average at an altitude of ~16 km. CMAQ is most often driven by the MM5 mesoscale meteorological model, though it may be driven by other meteorological models including the WRF model and the RAMS. Other major air quality systems used for regional scale applications include CAMx and WRF/Chem.

Fine scale resolution is necessary to resolve features which can affect pollutant concentrations such as urban heat island circulation; sea breezes; mountain and valley breezes; and the nocturnal low-level jet. Horizontal domains are typically modeled by nesting a finer grid model within a larger domain model of coarser resolution. Caution must be exercised in using nested models because certain parameterizations like those for convection might be valid on a relatively coarse grid scale but may not be valid on finer scales and because incompatibilities can occur at the model boundaries. The use of finer resolution in CTMs will require advanced parameterizations of meteorological processes such as boundary layer fluxes, deep convection, and clouds, and necessitate finer-scale inventories of land use, source locations, and emission inventories.

Because of the large number of chemical species and reactions that are involved in the oxidation of realistic mixtures of anthropogenic and biogenic hydrocarbons, condensed mechanisms must be used to simplify atmospheric models. These mechanisms can be tested by comparison with smog chamber data. However, the existing chemical mechanisms often neglect many important processes such as the formation and subsequent reactions of long-lived carbonyl compounds, the incorporation of the most recent information on reactions of halogenated species, and heterogeneous reactions involving cloud droplets and aerosol particles. As a result, models such as CMAQ have had difficulties with capturing the regional nature of $O_3$

episodes, in part because of uncertainty in the chemical pathways converting $NO_X$ to isoprene nitrates and recycling of $NO_X$.

Errors in photochemical modeling arise from meteorological, chemical, and emissions inputs to the model. Algorithms must be used for simulating meteorological processes that occur on spatial scales smaller than the model's grid spacing and for calculating the dependence of emissions on meteorology and time. Large uncertainties exist in the mechanism for oxidizing compounds of importance for atmospheric chemistry such as isoprene. Appreciable errors in emissions can occur if inappropriate assumptions are used in these parameterizations.

The performance of CTMs must be evaluated by comparison with field data as part of a cycle of model evaluations and subsequent improvements. Discrepancies between model predictions and observations can be used to point out gaps in current understanding of atmospheric chemistry and to spur improvements in parameterizations of atmospheric chemical and physical processes.

### 3.7.3 Background Concentrations

Because the mean tropospheric lifetime of $O_3$ is on the order of a few weeks, $O_3$ can be transported from continent to continent. The degree of influence from intercontinental transport varies greatly by location and time. For instance, high elevation sites are most susceptible to the intercontinental transport of pollution, particularly during spring. However, because the atmospheric chemistry of $O_3$ is quite complex and can be highly non-linear in environments close to sources of precursors, isolating the influence of intercontinental transport of $O_3$ and $O_3$ precursors on urban air quality is particularly problematic.

A number of recent studies indicate that natural sources such as wildfires and stratospheric intrusions and the intercontinental transport of pollution can significantly affect $O_3$ air quality in the United States. Two major modeling/field studies that focused on discerning the contributions of Asian emissions to air quality in California were the IONS-2010 and the CalNex studies conducted in May through June of 2010. Modeling and observational components of these studies found evidence for substantive contributions from stratospheric intrusions and Eurasian pollution to boundary layer $O_3$. In particular, one modeling study found evidence of Asian contributions of 8 -15 ppb in surface air during strong transport events in southern California. These contributions are in addition to contributions from dominant local pollution sources. Their results suggest that the influence of background sources on high $O_3$ concentrations at the surface is not always confined to high elevation sites. It is not clear to what extent the contributions inferred by these studies are likely to be found in other years, during other seasons, or in other locations. To gain a broader perspective and to isolate the influence of natural or transported $O_3$, estimates from CTMs must be used. This is because observations within the U.S. (even at relatively remote monitoring sites) are impacted by transport from anthropogenic source regions within the U.S. borders.

In the context of a review of the NAAQS, it is useful to define background $O_3$ concentrations in a way that distinguishes between concentrations that result from precursor emissions that are relatively less controllable from those that are relatively more controllable through U.S. policies. For this assessment, three definitions of background $O_3$ concentrations are considered, including (1) NA background (simulated $O_3$ concentrations that would exist in the absence of anthropogenic emissions from the U.S., Canada and Mexico), (2) U.S. background (simulated $O_3$ concentrations that would exist in the absence of anthropogenic emissions from the U.S.), and (3) natural background (simulated $O_3$ concentrations in the absence of all anthropogenic emissions globally). Each definition of background $O_3$ includes contributions resulting from emissions from natural sources (e.g., stratospheric intrusion, wildfires, biogenic methane and more short-lived VOC emissions) throughout the globe. There is no chemical difference between background $O_3$ and $O_3$ attributable to U.S. or North American anthropogenic sources. However, to inform policy considerations regarding the current or potential alternative standards, it is useful to understand how total $O_3$ concentrations can be attributed to different sources.

Since background $O_3$ concentrations as defined above are a construct that cannot be directly measured, the range of background $O_3$ concentrations is estimated using CTMs. For the current assessment, recently published results from Zhang et al. (2011) using the GEOS-Chem model at 0.5° × 0.667° (~50 km × 50 km) horizontal resolution and Emery et al. (2012) using a GEOS-Chem/CAMx model (hereafter referred to as CAMx) at finer horizontal resolution (12 km × 12 km) were used. Results from these models represent the latest estimates for background $O_3$ concentrations documented in the peer-reviewed literature.

The main results from these modeling efforts can be summarized as follows. Simulated regional and seasonal means of base-case $O_3$ using both models generally agree to within a few pbb with observations for most of the United States. However, neither model is currently capable of simulating day specific base-case $O_3$ concentrations within reasonable bounds. Both models show background concentrations vary spatially and temporally. NA background concentrations are generally higher in spring than in summer across the United States. Simulated mean NA background concentrations are highest in the Intermountain West (i.e., at high altitude) in spring and in the Southwest in summer. Lowest estimates of NA background occur in the East in the spring and the Northeast in summer. NA background concentrations tend to increase with total (i.e., base case) $O_3$ concentrations at high elevation; but that tendency is not as clear at low elevations. Comparison of NA background and natural background indicate that methane is a major contributor to NA background $O_3$, accounting for slightly less than half of the increase in background since the pre-industrial era; and whose relative contribution is projected to grow in the future. U.S. background concentrations are on average 2.6 ppb higher than NA background concentrations during spring and 2.7 ppb during summer across the U.S. with highest increases above NA background over the Northern Tier of New York State (19.1 ppb higher than NA background) in summer. High values for U.S. background are also found in other areas bordering Canada and

Mexico. Contributions to background $O_3$ can be episodic or non-episodic; high background concentrations are driven primarily by the episodic events such as stratospheric intrusions and wildfires. The most pronounced differences between these model results and observations are at the upper end of the concentration distribution, particularly at high elevations. In general, these model simulations provide a consistent representation of average background concentrations over seasons and broad spatial areas, but are not able to capture background concentrations at finer spatial (i.e., urban) and temporal (i.e., specific day) scales.

Note that the calculations of background concentrations presented in this chapter were formulated to answer the question, "what would $O_3$ concentrations be if there were no anthropogenic sources." This is different from asking, "how much of the $O_3$ measured or simulated in a given area is due to background contributions." Because of potentially strong non-linearities—particularly in many urban areas—these estimates by themselves should not be used to answer the second question posed above. The extent of these non-linearities will generally depend on location and time, the strength of concentrated sources, and the nature of the chemical regime. Further work is needed on how these estimates of background concentrations can be used to help determine the contributions of background sources of $O_3$ to urban concentrations.

### 3.7.4 Monitoring

The FRM for $O_3$ measurement is the CLM and is based on the detection of chemiluminescence resulting from the reaction of $O_3$ with ethylene gas. Almost all of the SLAMS that reported data to AQS from 2005 to 2009 used UV absorption photometer FEMs and greater than 96% of $O_3$ monitors met precision and bias goals during this period.

State and local monitoring agencies operate $O_3$ monitors at various locations depending on the area size and typical peak concentrations (expressed in percentages below, or near the $O_3$ NAAQS). SLAMS make up the ambient air quality monitoring sites that are primarily needed for NAAQS comparisons and include PAMS, NCore, and all other State or locally-operated stations except for the monitors designated as SPMs.

In 2010, there were 1250 SLAMS $O_3$ monitors reporting values to the EPA AQS database. Since $O_3$ levels decrease appreciably in the colder parts of the year in many areas, $O_3$ is required to be monitored at SLAMS monitoring sites only during the "ozone season" which varies by state. PAMS provides more comprehensive data on $O_3$ in areas classified as serious, severe, or extreme nonattainment for $O_3$. There were a total of 119 PAMS reporting values to the EPA AQS database in 2009. NCore is a new multipollutant monitoring network currently being implemented to meet multiple monitoring objectives. Each state is required to operate at least one NCore site and the network will consist of about 60 urban and 20 rural sites nationwide.

CASTNET is a regional monitoring network established to assess trends in acidic deposition and also provides concentration measurements of $O_3$. CASTNET $O_3$ monitors operate year round and are primarily located in rural areas. At the beginning of 2010, there were 80 CASTNET sites located in, or near, rural areas. The NPS also operates a POMS network. The POMS couples the small, low-power $O_3$ monitor with a data logger, meteorological measurements, and solar power in a self contained system for monitoring in remote locations. Twenty NPS POMS reported $O_3$ data to AQS in 2010. A map of the current and proposed rural NCore sites, along with the CASTNET, and the NPS POMS sites was shown in Figure 3-22.

Satellite observations for $O_3$ are growing as a resource for many purposes, including model evaluation, assessing emissions reductions, pollutant transport, and air quality management. Satellite retrievals are conducted using the solar backscatter or thermal infrared emission spectra and a variety of algorithms. Most satellite measurement systems have been developed for measurement of the total $O_3$ column. Mathematical techniques have been developed and must be applied to derive information from these systems about tropospheric $O_3$. Satellite observations of $O_3$ precursors such as CO, $NO_2$, and HCHO are also available and useful for constraining model predictions of emissions of precursors and formation of $O_3$ during intercontinental transport.

### 3.7.5 Ambient Concentrations

Ozone is the only photochemical oxidant other than $NO_2$ that is routinely monitored and for which a comprehensive database exists. Other photochemical oxidants are typically only measured during special field studies. Therefore, the concentration analyses contained in this chapter have been limited to widely available $O_3$ data obtained directly from AQS for the period from 2007 to 2009.

The median 24-h avg, 8-h daily max, and 1-h daily max $O_3$ concentrations across all U.S. sites reporting data to AQS between 2007 and 2009 were 29, 40, and 44 ppb, respectively. Representing the upper end of the distribution, the 99th percentiles of these same metrics across all sites were 60, 80, and 94 ppb, respectively.

To investigate urban-scale $O_3$ variability, 20 focus cities were selected for closer analysis; these cities were selected based on their importance in $O_3$ epidemiologic studies and on their geographic distribution across the United States. Several of these cities had relatively little spatial variability in 8-h daily max $O_3$ concentrations (e.g., inter-monitor correlations ranging from 0.61 to 0.96 in Atlanta) while other cities exhibited considerably more variability in $O_3$ concentrations (e.g., inter-monitor correlations ranging from -0.06 to 0.97 for Los Angeles). The negative and near-zero correlations in Los Angeles were between monitors with a relatively large separation distance (>150 km), but even some of the closer monitor pairs were not very highly correlated. Similar to the correlation, the coefficient of divergence was found to be highly dependent on the urban area under investigation. As a result,

caution should be observed in using data from a sparse network of ambient $O_3$ monitors to approximate community-scale exposures.

To investigate rural-focused $O_3$ variability using AQS data, all monitors located within six rural monitoring areas were examined. These rural monitoring sites are impacted by transport of $O_3$ or $O_3$ precursors from upwind urban areas, and by local anthropogenic emissions within the rural areas such as emissions from motor vehicles, power generation, biomass combustion, or oil and gas operations. As a result, monitoring data from these rural locations are not unaffected by anthropogenic emissions. The rural area investigated with the largest number of available AQS monitors was Great Smoky Mountain National Park in NC and TN where the median warm-season 8-h daily max $O_3$ concentration ranged from 47 ppb at the lowest elevation site (elevation = 564 meters; site ID = 470090102) to 60 ppb at the highest elevation site (elevation = 2,021 meters; site ID = 471550102), with correlations between the 5 sites ranging from 0.78 to 0.92 and CODs ranging from 0.04 to 0.16. A host of factors may contribute to variations observed at these rural sites, including proximity to local $O_3$ precursor emissions, variations in boundary-layer influences, meteorology and stratospheric intrusion as a function of elevation, and differences in wind patterns and transport behavior due to local topography.

Since $O_3$ produced from emissions in urban areas is transported to more rural downwind locations, elevated $O_3$ concentrations can occur at considerable distances from urban centers. In addition, major sources of $O_3$ precursors such as highways, power plants, biomass combustion, and oil and gas operations are commonly found in rural areas, adding to the $O_3$ in these areas. Due to lower chemical scavenging in non-urban areas, $O_3$ tends to persist longer in rural than in urban areas which tends to lead to higher cumulative exposures in rural areas influenced by anthropogenic precursor emissions. The persistently high $O_3$ concentrations observed at many of these rural sites investigated here indicate that cumulative exposures for humans and vegetation in rural areas can be substantial and often higher than cumulative exposures to $O_3$ in urban areas.

Nation-wide surface-level $O_3$ concentrations in the U.S. have declined gradually over the last decade. A noticeable decrease in $O_3$ concentrations between 2003 and 2004, particularly in the eastern U.S., coincided with $NO_X$ emissions reductions resulting from implementation of the $NO_X$ SIP Call rule, which began in 2003 and was fully implemented in 2004. This rule was designed to reduce $NO_X$ emissions from power plants and other large combustion sources in the eastern United States. Downward trends in $O_3$ concentrations in the western U.S. have not been as substantial and several individual monitors have reported increases in $O_3$ concentrations when 2001-2003 design values are compared with 2008-2010 design values. Over a longer time scale, several observational studies investigating $O_3$ concentrations in the marine layer off the Pacific Coast of the U.S. have reported a steady rise in $O_3$ concentrations over the last few decades. And global scale observations have indicated a general rise in $O_3$ by a factor of 2 or more since pre-industrial times, as discussed in Chapter 10, Section 10.3.3.1.

Urban $O_3$ concentrations show a strong degree of diel variability resulting from daily patterns in temperature, sunlight, and precursor emissions. Other factors, such as the relative importance of transport versus local photochemical production and loss rates, the timing for entrainment of air from the nocturnal residual boundary layer, and the diurnal variability in mixing layer height also play a role in daily $O_3$ patterns. Urban diel variations investigated in this assessment show no substantial change in patterns since the 2006 $O_3$ AQCD (U.S. EPA, 2006b). The 1-h max concentrations tend to occur in mid-afternoon and 1-h min concentrations tend to occur in early morning, with more pronounced peaks in the warm months relative to the cold months. There is city-to-city variability in these times, however, and caution is raised in extrapolating results from one city to another in determining the time of day for $O_3$ maxima and minima.

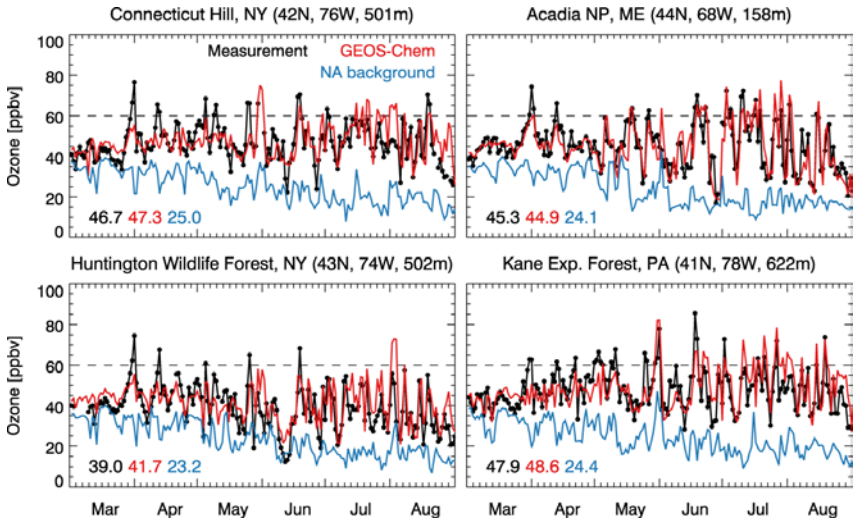
Rural $O_3$ concentrations show a varying degree of diel variability depending on their location relative to larger urban areas. Three rural areas investigated in the east showed relatively little diel variability, reflecting the regional nature of $O_3$ in the east. In contrast, three rural areas investigated in the west did display diel variability resulting from their proximity to fresh urban emissions. These six areas investigated were selected as illustrative examples and do not represent all rural areas in the U.S.

Since $O_3$ is a secondary pollutant formed in the atmosphere from precursor emissions, its correlation with primary pollutants such as CO and $NO_X$ can vary substantially by location. Furthermore, $O_3$ formation is strongly influenced by meteorology, entrainment, and transport of both $O_3$ and $O_3$ precursors, resulting in a broad range in correlations with other pollutants which can vary substantially with season. In the copollutant analyses shown in Figure 3-56, the year-round 8-h daily max $O_3$ data exhibited a very wide range in correlations with all the criteria pollutants. A clearer pattern emerged when the data are stratified by season with mostly negative correlations in the winter and mostly positive correlations in the summer for all copollutants. The median seasonal correlations are modest at best with the highest positive correlation at 0.52 for $PM_{2.5}$ in the summer and the highest negative correlation at -0.38 for $PM_{2.5}$ in the winter. Therefore, statistical analyses that may be sensitive to correlations between copollutants need to take seasonality into consideration, particularly when $O_3$ is being investigated.

## 3.8 Supplemental Information on $O_3$ Model Predictions

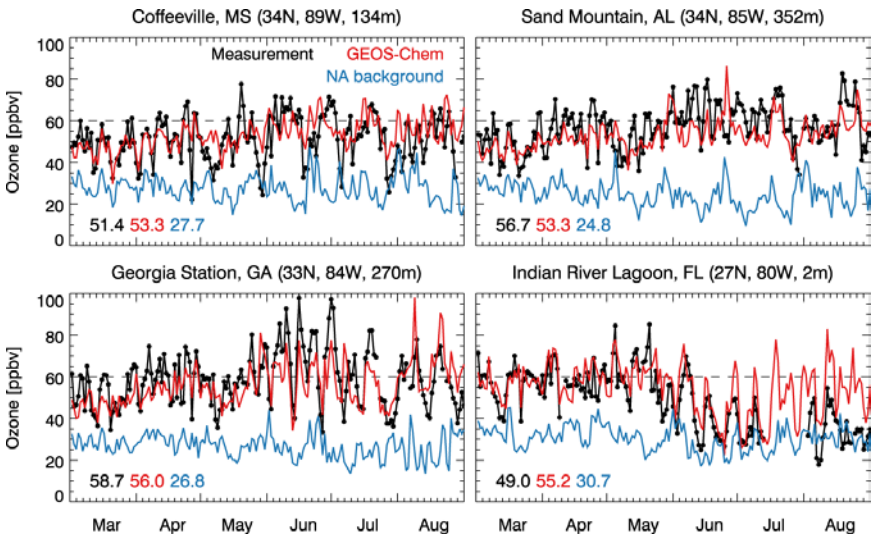
This section contains supplemental comparisons between GEOS-Chem simulations of MDA8 $O_3$ concentrations with observations for 2006 from Zhang et al. (2011) and Emery et al. (2012). Further details on these simulations can be found in Section 3.4.3. Figure 3-58 through Figure 3-64 show GEOS-Chem predictions for the base model (i.e., model including all anthropogenic and natural sources; labeled as GEOS-Chem in the figure) and the NA background model (i.e., model including natural sources everywhere in the world and anthropogenic sources outside the U.S., Canada, and Mexico; labeled as NA background in the figure) along with

measurements obtained from selected CASTNET sites (labeled as Measurement in the figure). Figure 3-65 shows a comparison of GEOS-Chem output with measurements at Mt. Bachelor, OR, and Trinidad Head, CA, from March-August, 2006. Figure 3-66 shows a comparison of vertical profiles (mean ± 1 standard deviation) calculated by GEOS-Chem with ozonesondes launched at Trinidad Head, California and Boulder, Colorado. Figure 3-67 and Figure 3-68 show a comparison of AM3 simulations of individual stratospheric intrusions during May-June 2010. Figure 3-69 through Figure 3-74 show box plots for measurements at CASTNET sites, GEOS-Chem predictions from Zhang et al. (2011) and CAMx predictions from Emery et al. (2012) for both the base case and NA background. Figure 3-75 shows time series of AM3 simulations at approximately 2° × 2° at Gothic, Colorado, for 2006.



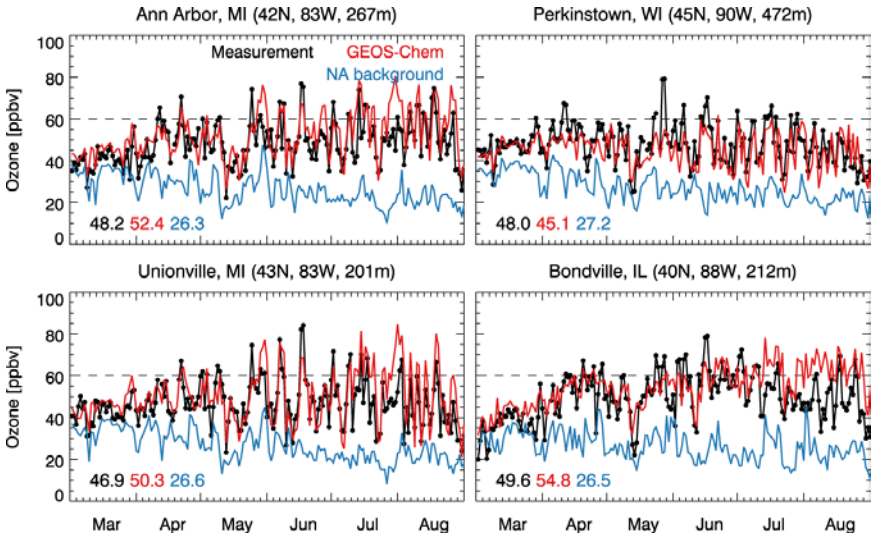

Source: Zhang et al. (2011).

**Figure 3-58 Comparison of time series of measurements of MDA8 $O_3$ concentrations at four CASTNET sites in the Northeast with GEOS-Chem predictions for the base case and for the North American background case during March-August, 2006.**



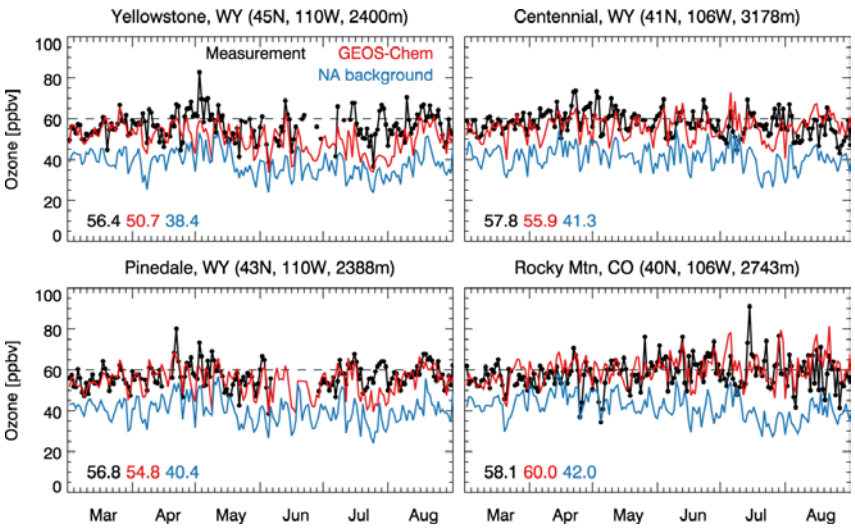

Source: Zhang et al. (2011).

**Figure 3-59 Comparison of time series of measurements of MDA8 $O_3$ concentrations at four CASTNET sites in the Southeast with GEOS-Chem predictions for the base case and for the North American background case during March-August, 2006.**



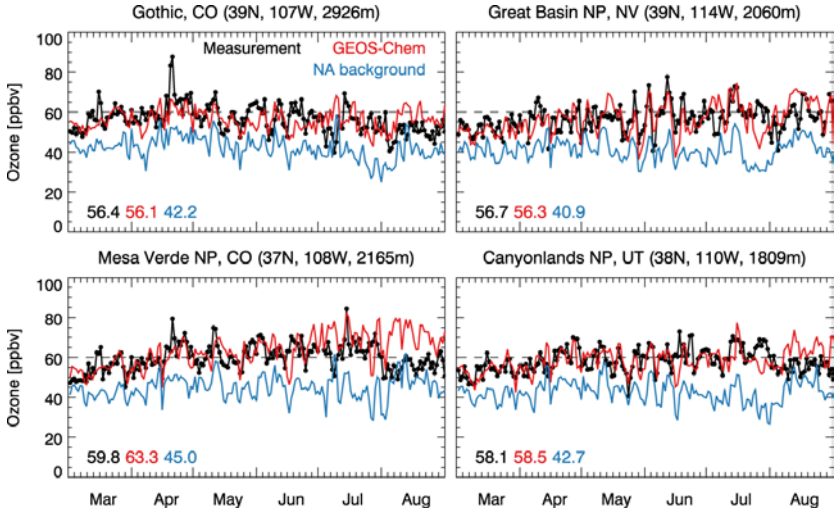

Source: Zhang et al. (2011).

**Figure 3-60 Comparison of time series of measurements of MDA8 $O_3$ concentrations at four CASTNET sites in the Upper Midwest with GEOS-Chem predictions for the base case and for the North American background case during March-August, 2006.**



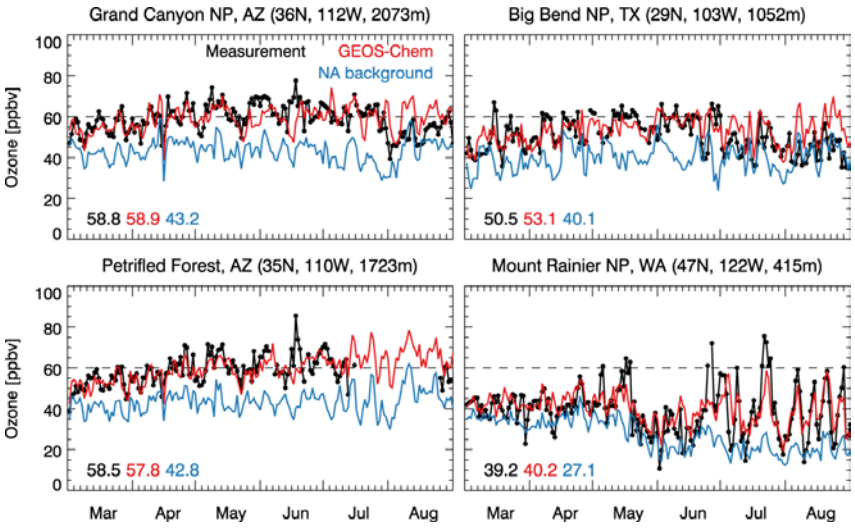

Source: Zhang et al. (2011).

**Figure 3-61 Comparison of time series of measurements of MDA8 $O_3$ concentrations at four CASTNET sites in the Intermountain West with GEOS-Chem predictions for the base case and the North American background case during March-August, 2006.**



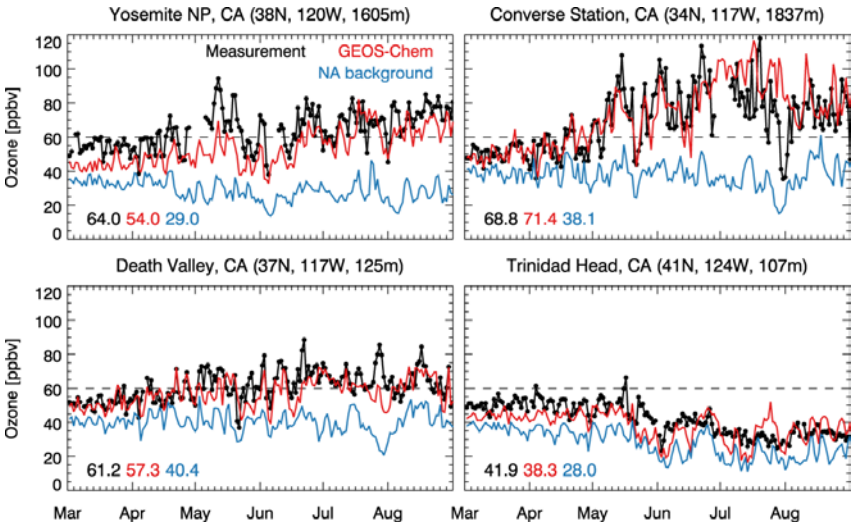

Source: Zhang et al. (2011).

**Figure 3-62 Comparison of time series of measurements of MDA8 $O_3$ concentrations at four CASTNET sites in the Intermountain West with GEOS-Chem predictions for the base case and the North American background case during March-August, 2006.**



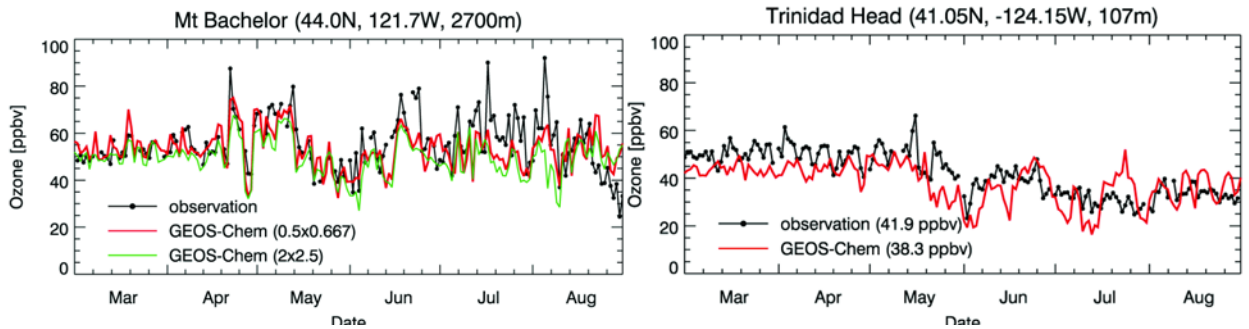

Source: Zhang et al. (2011).

**Figure 3-63 Comparison of time series of measurements of MDA8 $O_3$ concentrations at four CASTNET sites in the West with GEOS-Chem predictions for the base case and the North American background case during March-August, 2006.**



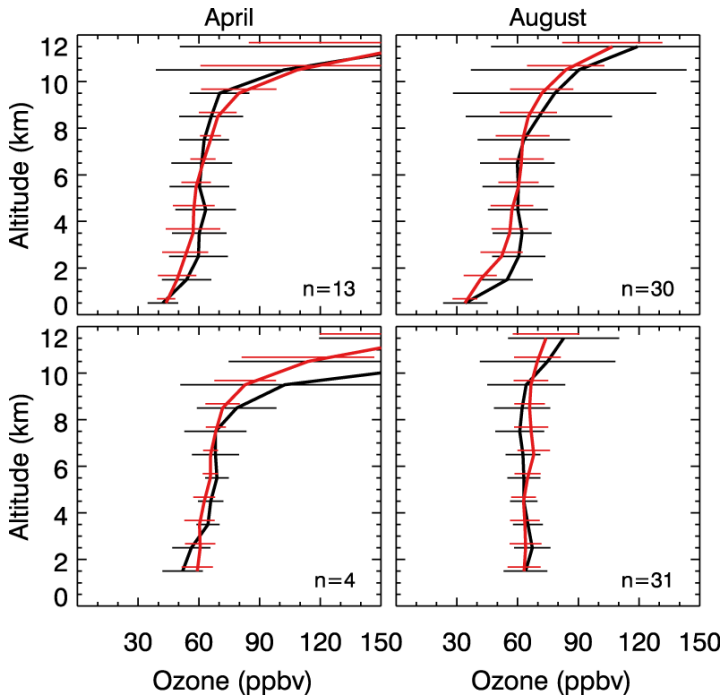

Source: Zhang et al. (2011).

**Figure 3-64 Comparison of time series of measurements of MDA8 $O_3$ concentrations at three CASTNET sites and the Trinidad Head site in California with GEOS-Chem predictions for the base case and the North American background case during March-August, 2006.**




Source: Zhang et al. (2011).

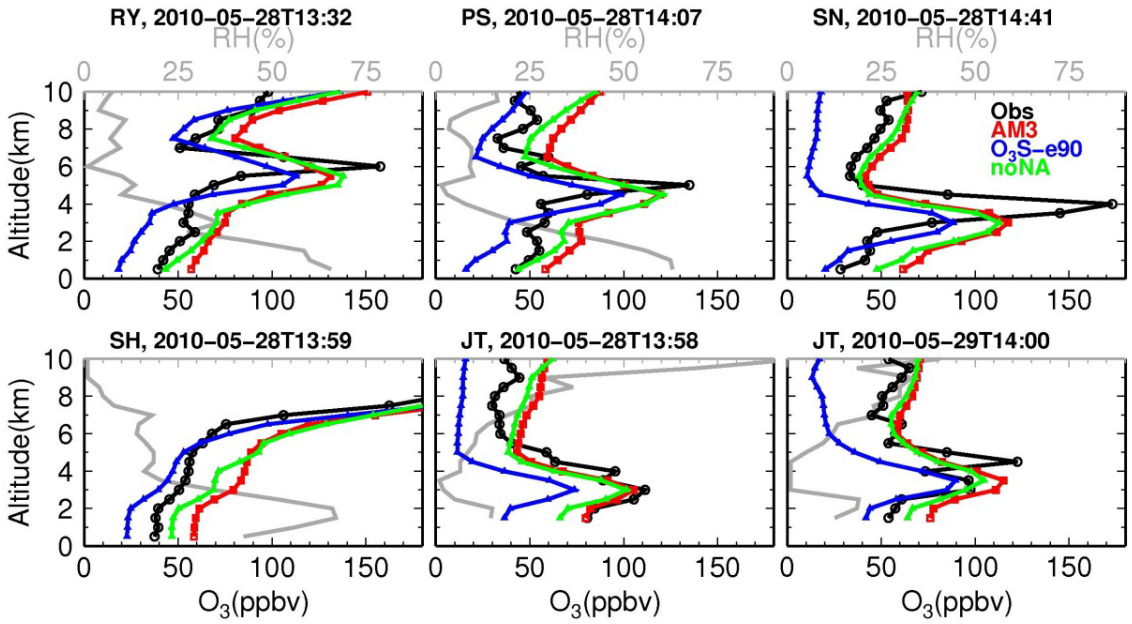
**Figure 3-65 Comparison of MDA8 $O_3$ predicted using GEOS-Chem at 0.5° × 0.667° (and 2° × 2.5° resolution; left figure only) with measurements at Mount Bachelor, Oregon (left); and at Trinidad Head, California (right) from March to August 2006.**




Note: The letter 'n' refers to the number of ozonesonde profiles, and the model was sampled on the same days as the ozonesonde launches. As can be seen from the figure, variability in both model and measurements increases with altitude, but variability in the model results is much smaller at high altitudes than seen in the observations at both sites.

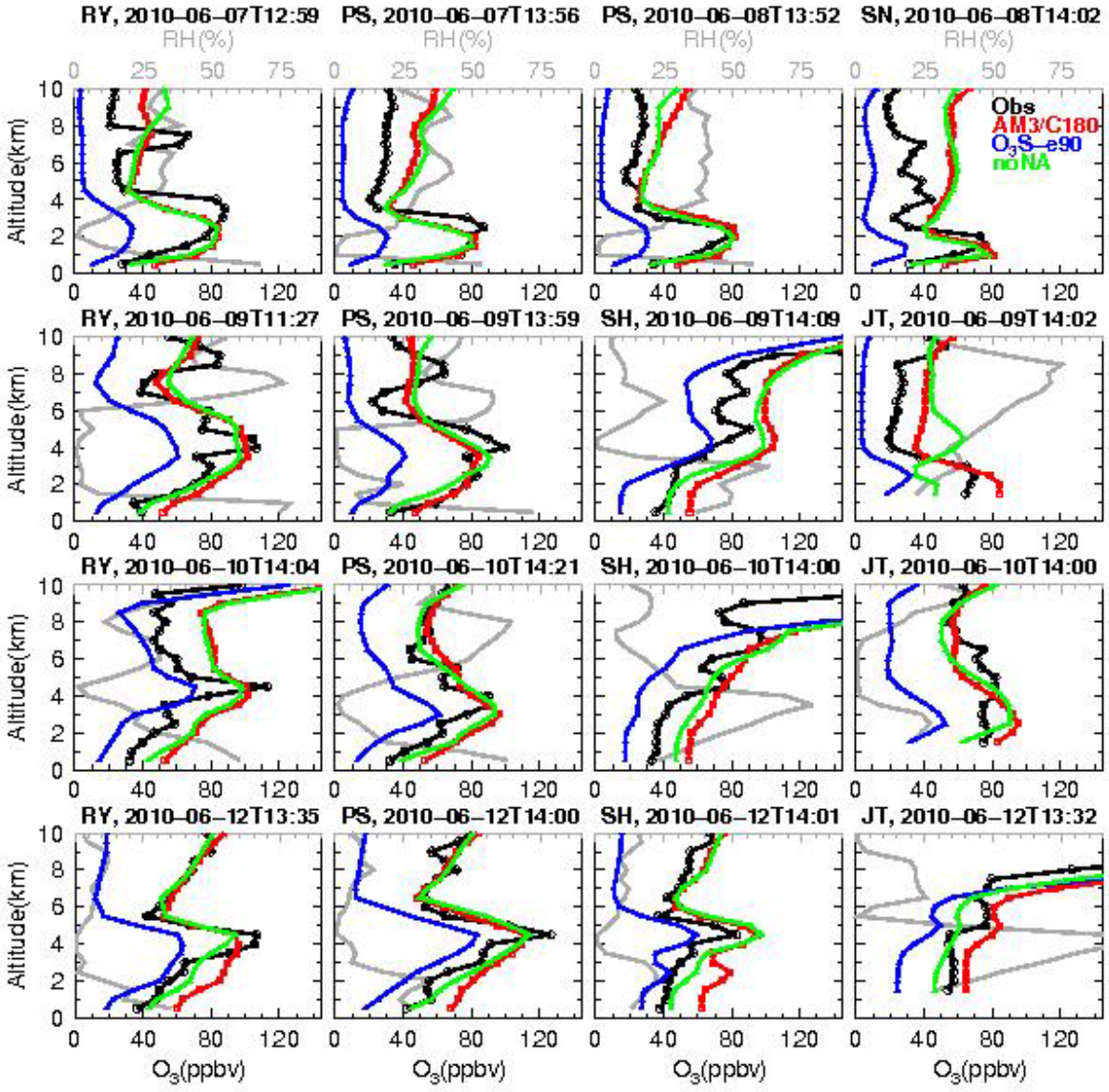
Source: Zhang et al. (2011).

**Figure 3-66 Comparison of monthly mean (± 1 standard deviation) $O_3$ calculated GEOS-Chem (in red) with ozonesondes (in black) at Trinidad Head, CA (top) and Boulder, Colorado (bottom) during April and August 2006.**



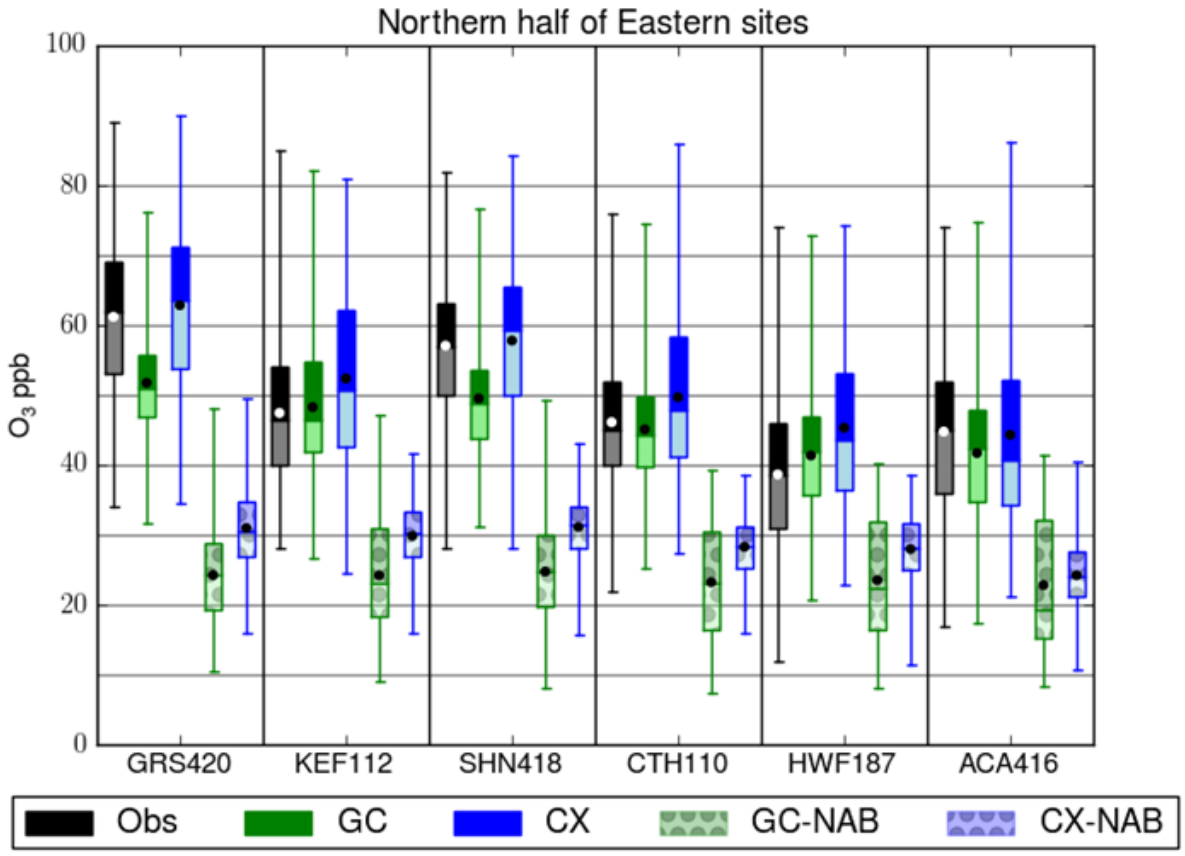

Note: Shows $O_3$ profiles at multiple sites as observed (black) by ozonesondes and simulated (red) by the GFDL AM3 model at ~50 × 50 km resolution. Also shown are observed relative humidity (gray) and AM3 estimates of $O_3$ concentrations in the absence of North American anthropogenic emissions (green) and the stratospheric contribution (blue). Model results have been interpolated to sonde pressure and averaged over 0.5-km altitude bins.

**Figure 3-67 A deep stratospheric $O_3$ intrusion over California on May 28 to May 29, 2010.**




Note: Shows $O_3$ profiles at multiple sites as observed (black) by ozonesondes and simulated (red) by the GFDL AM3 model at ~50 × 50 km resolution. Also shown are observed relative humidity (gray) and AM3 estimates of $O_3$ concentrations in the absence of North American anthropogenic emissions (green) and the stratospheric contribution (blue). Model results have been interpolated to sonde pressure and averaged over 0.5-km altitude bins.

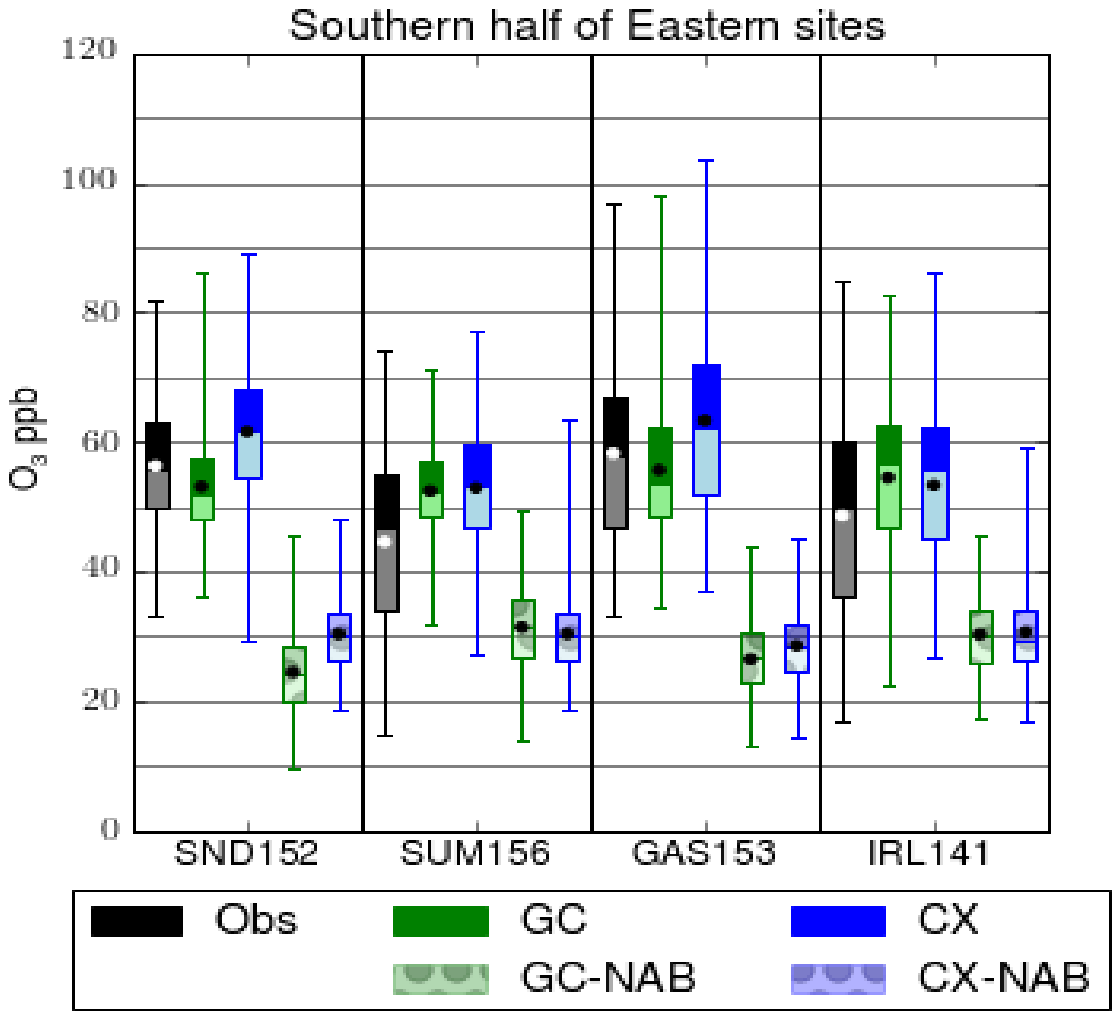
**Figure 3-68 A deep stratospheric $O_3$ intrusion over California on June 7 to June 12, 2010.**




Note: Stippled boxes indicate North American background. GRS = Great Smoky NP (North Carolina and Tennessee); KEF = Kane Exp. Forest (Pennsylvania); SHN = Shenandoah NP (Virginia); CTH = Connecticut Hill Management Wildlife Area (New York); HWF = Huntington Wildlife Forest (New York); ACA = Acadia NP (Maine).

Source: Adapted from Emery et al. (2012) and Zhang et al. (2011).

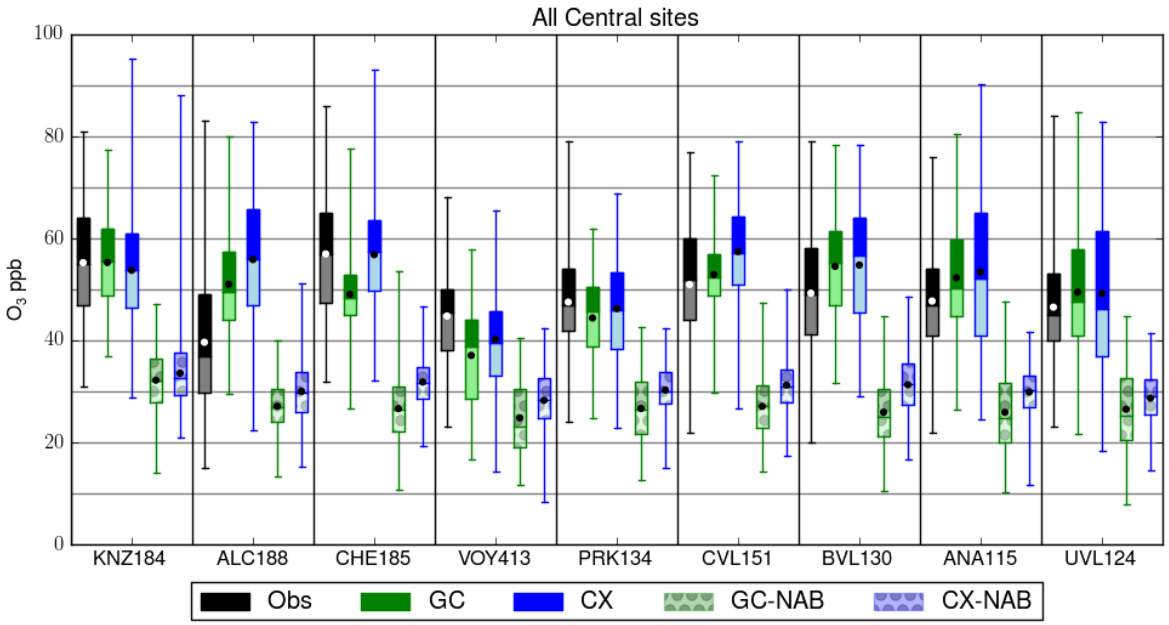
**Figure 3-69 Box plots showing maximum, interquartile range and minimum $O_3$ concentrations measured at CASTNET sites (black) in the Northeast and predictions from GEOS-Chem at ~50 × 50 km resolution (green) and CAMx at 12 × 12 km resolution (blue) for May-August 2006.**




Note: Stippled boxes indicate North American background. SND = Sand Mountain (Alabama); SUM = Sumatra (Florida); GAS = Georgia Station (Georgia); IRL = Indian River Lagoon (Florida).

Source: Adapted from Emery et al. (2012) and Zhang et al. (2011).

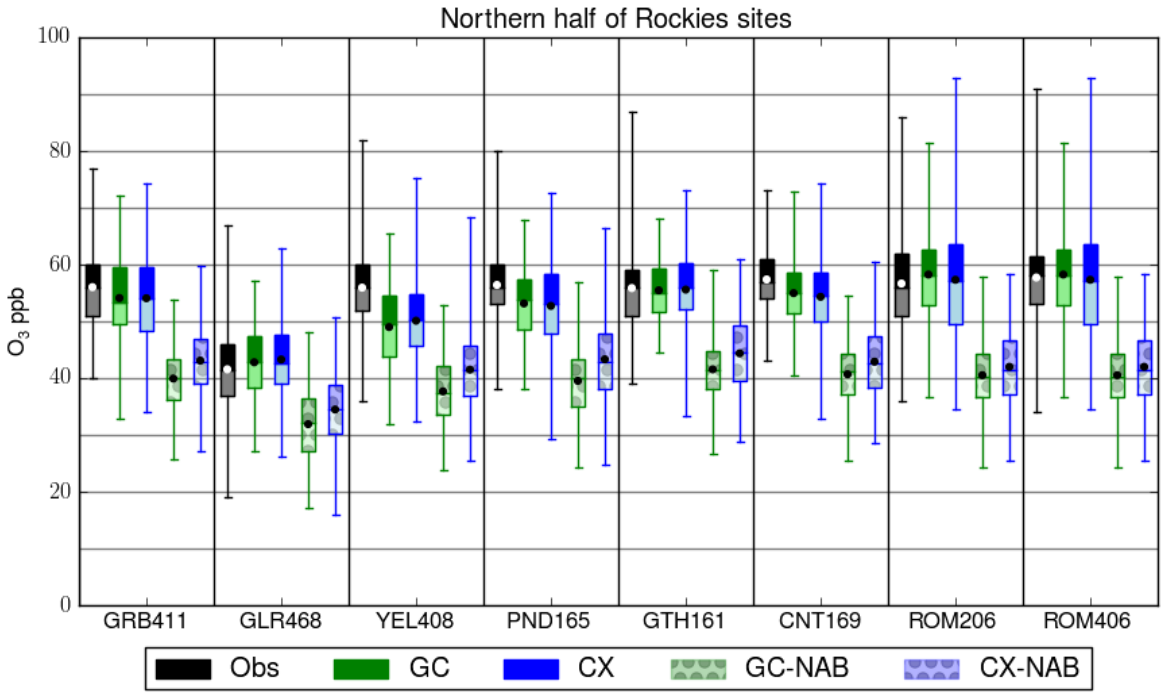
**Figure 3-70 Box plots showing maximum, interquartile range and minimum $O_3$ concentrations measured at CASTNET sites (black) in the Southeast and predictions from GEOS-Chem at ~50 × 50 km resolution (green) and CAMx at 12 × 12 km resolution (blue) for May-August 2006.**




Note: Stippled boxes indicate North American background. KNZ = Konza Prairie (Kansas); ALC = Alabama-Coushatta (Texas); CHE = Cherokee Nation (Oklahoma); VOY = Voyageurs NP (Minnesota); PRK = Perkinstown (Wisconsin); CVL = Coffeeville (Mississippi); BVL = Bondsville (Illinois); ANA = Ann Arbor (Michigan); UVL = Unionville (Michigan).

Source: Adapted from Emery et al. (2012) and Zhang et al. (2011).

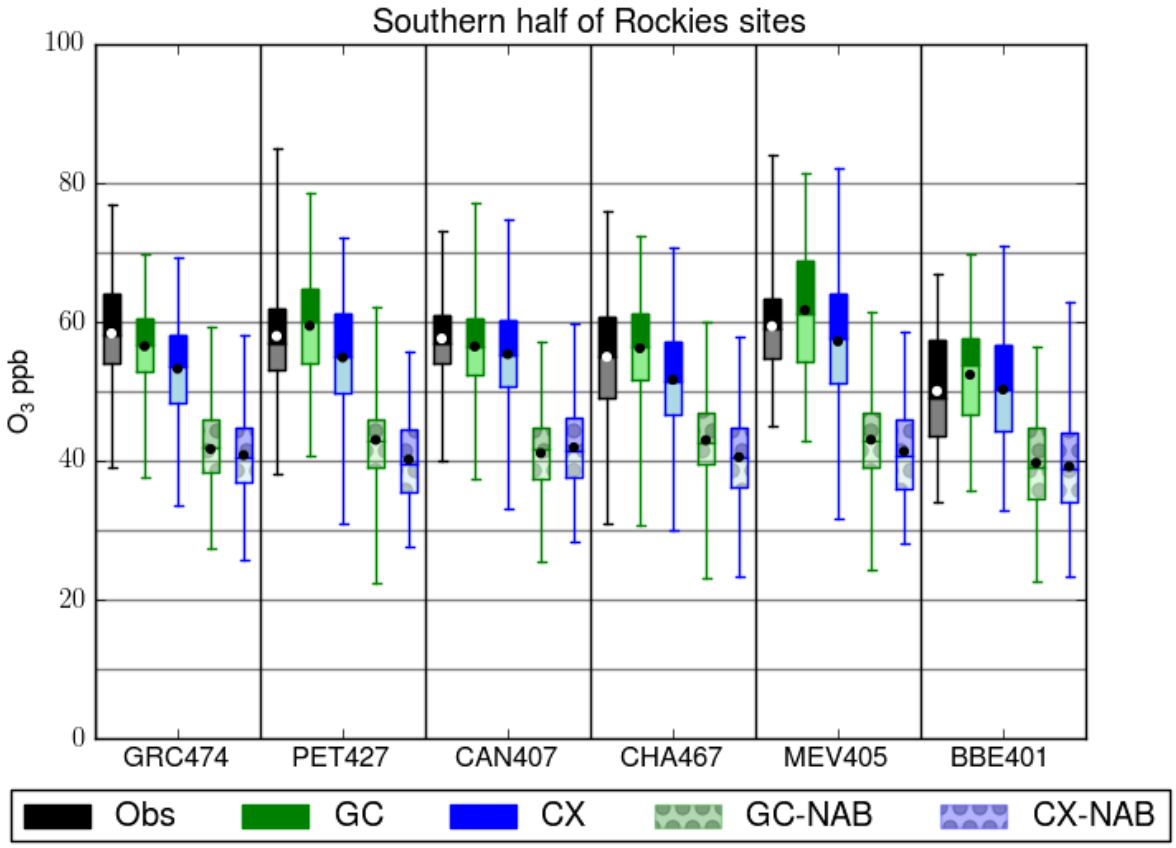
**Figure 3-71 Box plots showing maximum, interquartile range and minimum $O_3$ concentrations measured at CASTNET sites (black) in the Central U.S. and predictions from GEOS-Chem at ~50 × 50 km resolution (green) and CAMx at 12 × 12 km resolution (blue) for May-August 2006.**




Note: Stippled boxes indicate North American background. GRB = Great Basin NP (Nevada); GLR = Glacier NP (Montana); YEL = Yellowstone NP (Wyoming); PND = Pinedale (Wyoming); GTH = Gothic (Colorado); CNT = Centennial (Wyoming); ROM = Rocky Mountain NP (Colorado, co-located sites).

Source: Adapted from Emery et al. (2012) and Zhang et al. (2011).

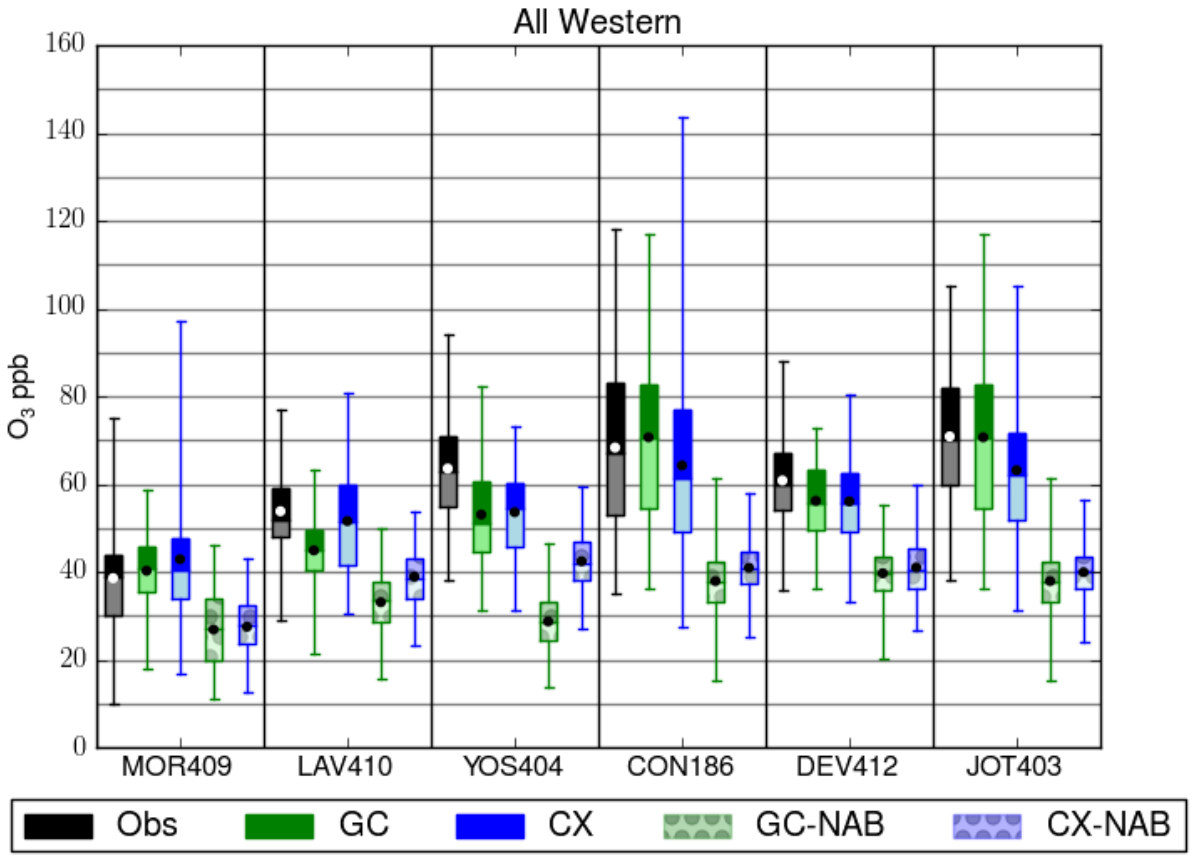
**Figure 3-72 Box plots showing maximum, interquartile range and minimum $O_3$ concentrations measured at CASTNET sites (black) in the Northern Rockies and predictions from GEOS-Chem at ~50 × 50 km resolution (green) and CAMx at 12 × 12 km resolution (blue) for May-August 2006.**




Note: Stippled boxes indicate North American background. GRC = Grand Canyon NP (Arizona); PET = Petrified Forest (Arizona); CAN = Canyonlands NP (Utah); CHA = Chiracahua NM (Arizona); MEV = Mesa Verde NP (Colorado); BBE = Big Bend NP (Texas).

Source: Adapted from Emery et al. (2012) and Zhang et al. (2011).

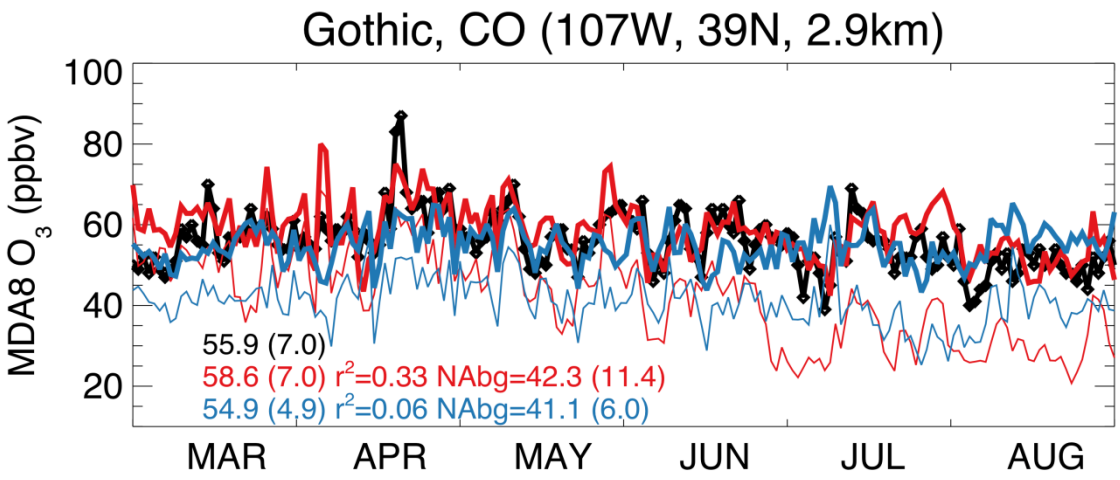
**Figure 3-73 Box plots showing maximum, interquartile range and minimum $O_3$ concentrations measured at CASTNET sites (black) in the Southern Rockies and predictions from GEOS-Chem at ~50 × 50 km resolution (green) and CAMx at 12 × 12 km resolution (blue) for May-August 2006.**




Note: Stippled boxes indicate North American background. MOR = Mount Ranier NP (Washington); LAV = Lassen Volcanic NP (California); YOS = Yosemite NP (California); CON = Converse Station (California); DEV = Death Valley NM (California); JOT = Joshua Tree NM (California).

Source: Adapted from Emery et al. (2012) and Zhang et al. (2011).

**Figure 3-74 Box plots showing maximum, interquartile range and minimum $O_3$ concentrations measured at CASTNET sites (black) in the West and predictions from GEOS-Chem at ~50 × 50 km resolution (green) and CAMx at 12 × 12 km resolution (blue) for May-August 2006.**




Note: Observed (black) and simulated by the GEOS-Chem (blue; horizontal resolution is 0.5° × 0.667°) and AM3 (red; horizontal resolution is approximately 2° × 2°) global models. Also shown are the model estimates for North American background (thin lines); the spike in mid-April likely corresponds to a stratospheric intrusion. The model correlations with observations, average (over the entire March through August period) total $O_3$ and North American background (NAbg) $O_3$ estimates, and their standard deviations (shown in parentheses) are presented in the lower left.

**Figure 3-75 MDA8 $O_3$ in surface air at Gothic, Colorado for March through August 2006.**

## 3.9 Supplemental Figures of Observed Ambient $O_3$ Concentrations

### 3.9.1 Ozone Monitor Maps for the Urban Focus Cities

This section contains supplemental maps showing the location of $O_3$ monitors reporting to AQS for each of the 20 urban focus cities introduced in Section 3.6.2.1. The monitors are delineated in the maps as year-round or warm-season based on their inclusion in the year-round data set and the warm-season data set discussed in Section 3.6.2.1. The maps also include the CSA/CBSA boundary selected for monitor inclusion, the location of urban areas and water bodies, the major roadway network, as well as the population gravity center based on the entire CSA/CBSA and the individual focus city boundaries. Population gravity center is calculated from the average longitude and latitude values for the input census tract centroids and represents the mean center of the population in a given area. Census tract centroids are weighted by their population during this calculation.

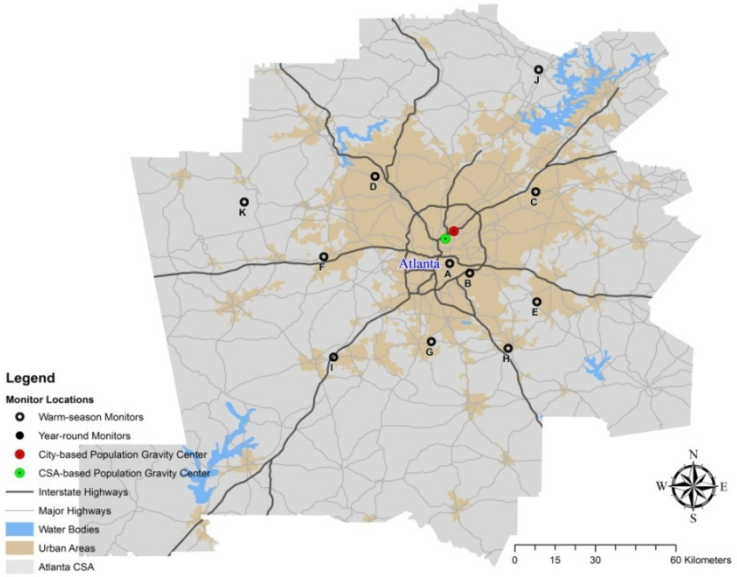



**Figure 3-76 Map of the Atlanta, Georgia, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**

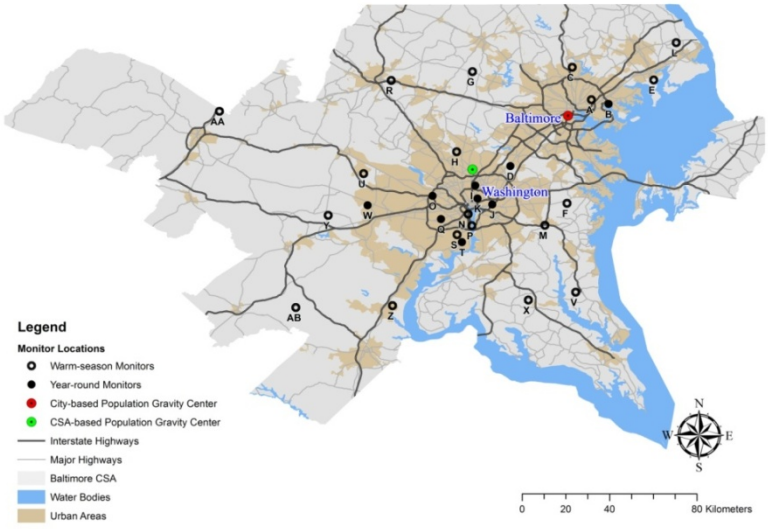



**Figure 3-77 Map of the Baltimore, Maryland, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**

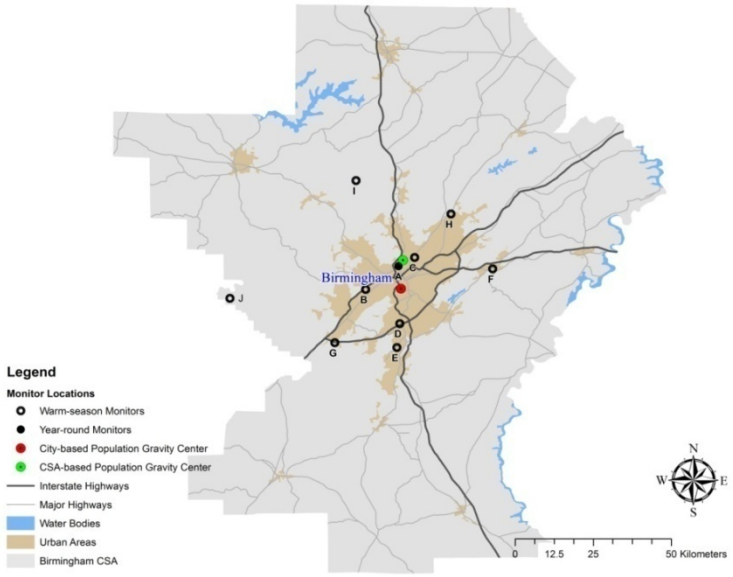



**Figure 3-78 Map of the Birmingham, Alabama, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**




**Figure 3-79 Map of the Boston, Massachusetts, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**

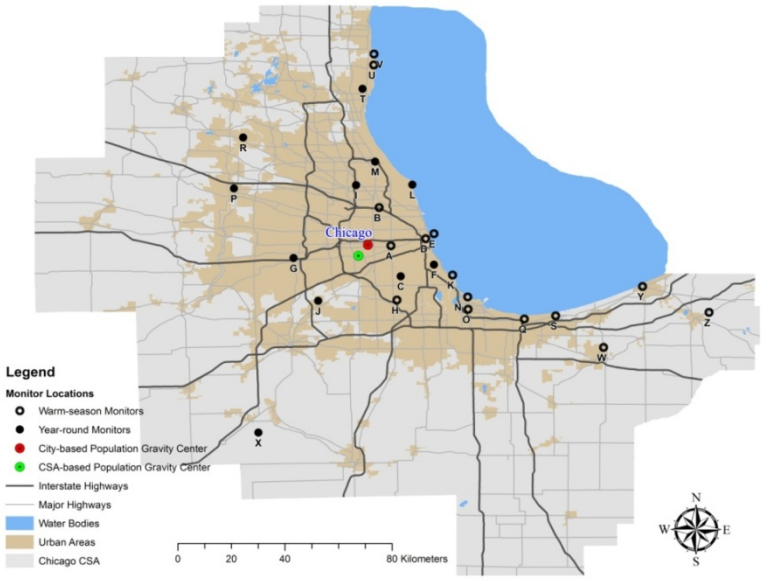



**Figure 3-80 Map of the Chicago, Illinois, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**

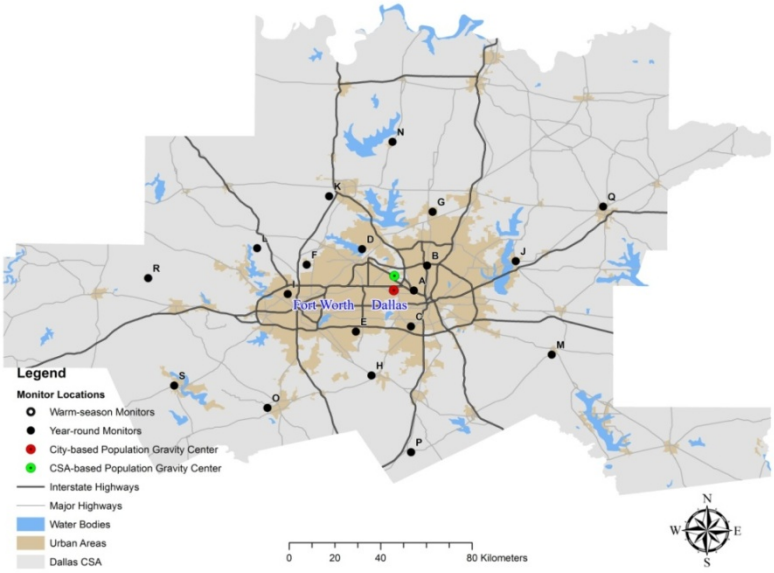



**Figure 3-81 Map of the Dallas, Texas, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**

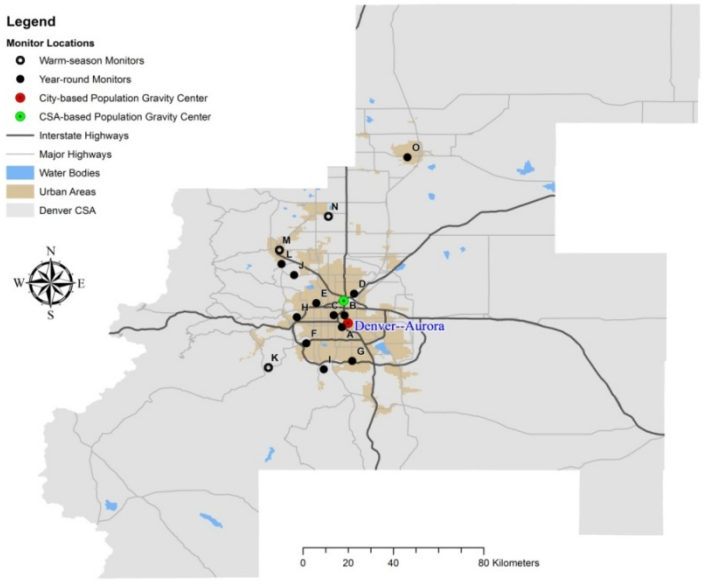



**Figure 3-82 Map of the Denver, Colorado, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**

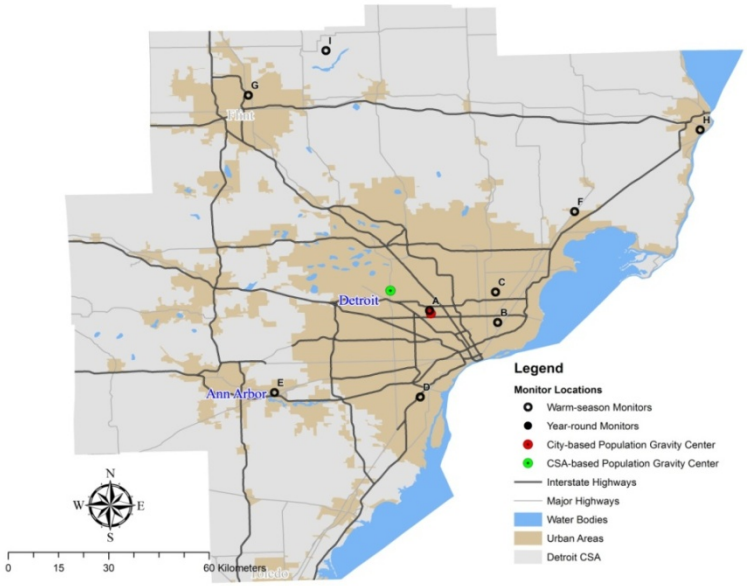



**Figure 3-83 Map of the Detroit, Michigan, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**

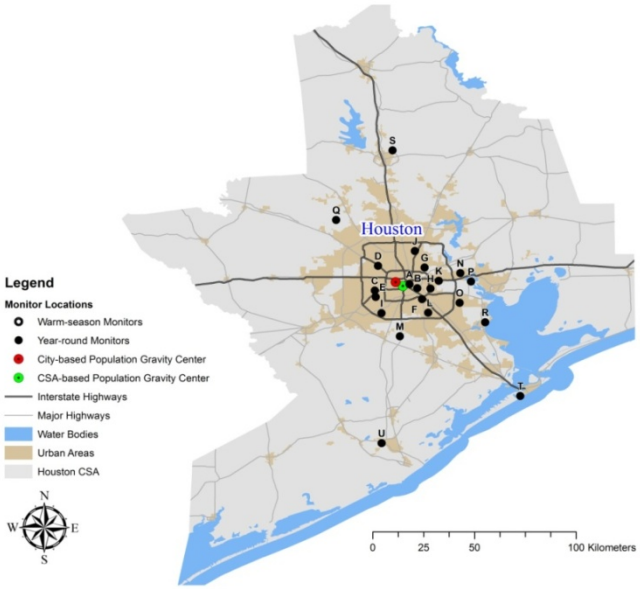



**Figure 3-84 Map of the Houston, Texas, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**




**Figure 3-85 Map of the Los Angeles, California, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**

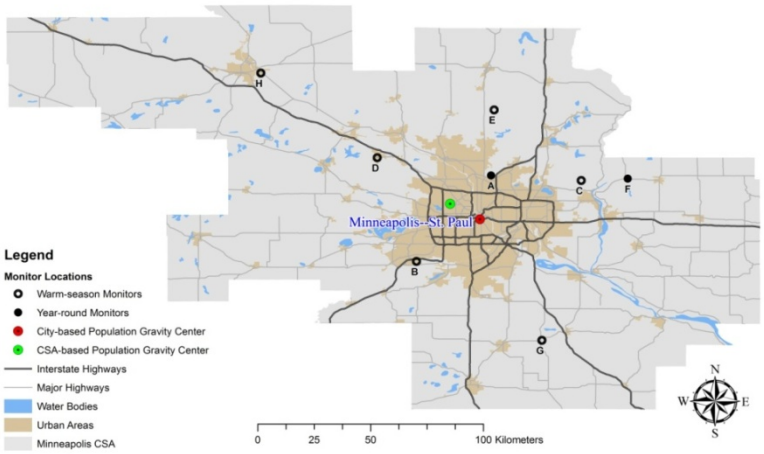



**Figure 3-86 Map of the Minneapolis, Minnesota, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**

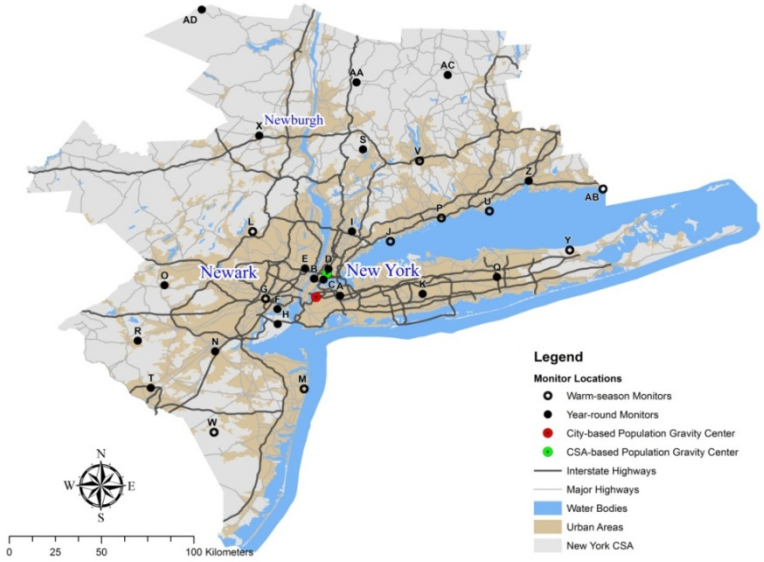



**Figure 3-87 Map of the New York City, New York, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**

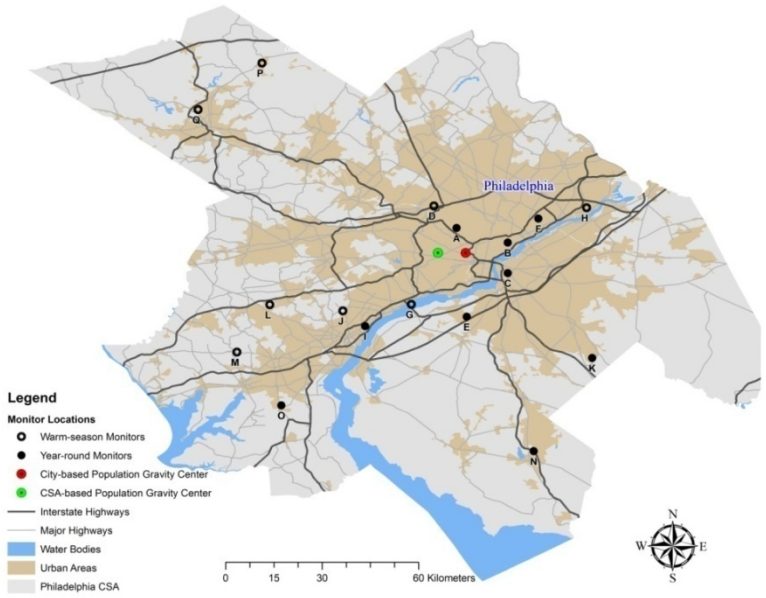



**Figure 3-88 Map of the Philadelphia, Pennsylvania, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**

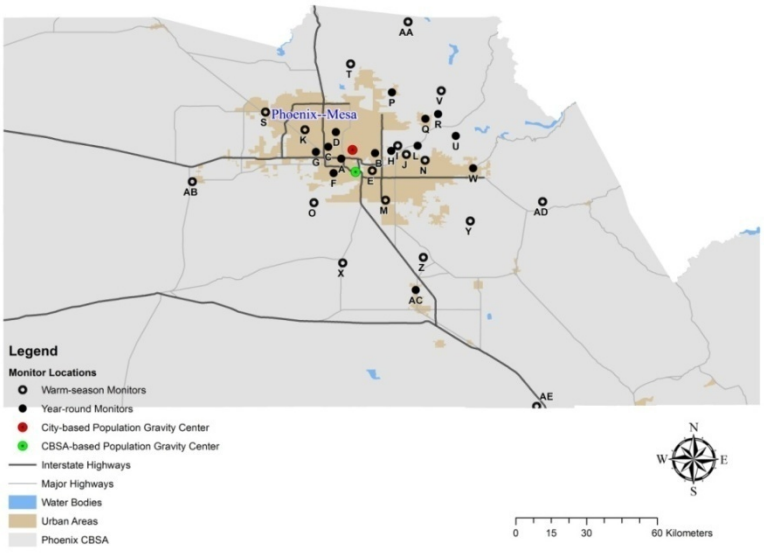



**Figure 3-89 Map of the Phoenix, Arizona, CBSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**

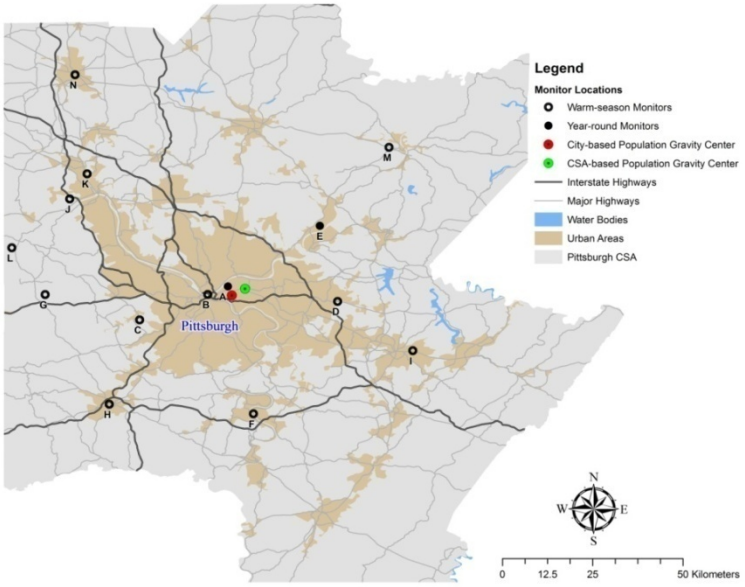



**Figure 3-90 Map of the Pittsburgh, Pennsylvania, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**

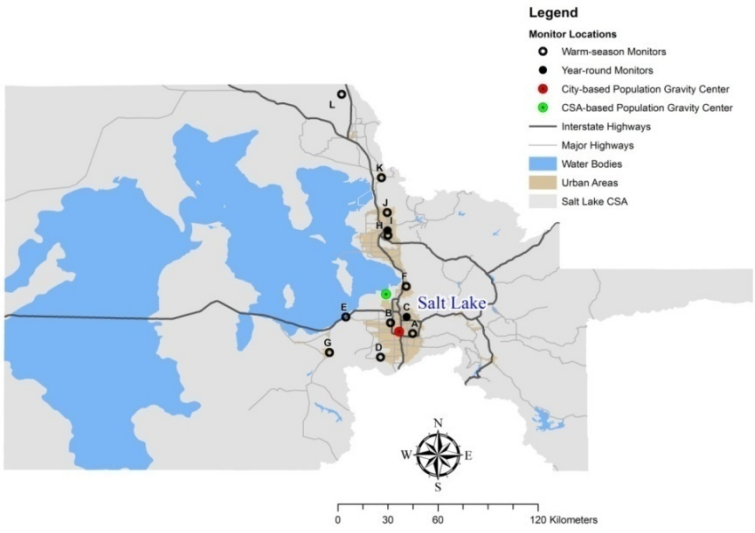



**Figure 3-91 Map of the Salt Lake City, Utah, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**

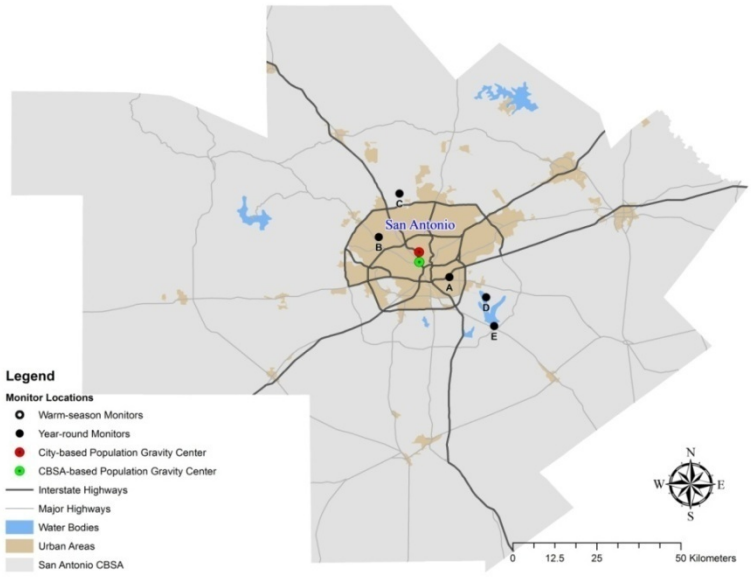



**Figure 3-92 Map of the San Antonio, Texas, CBSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**

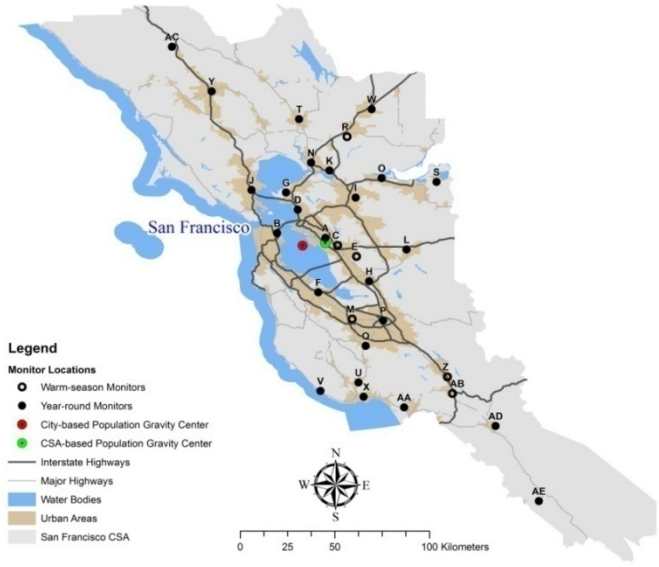



**Figure 3-93 Map of the San Francisco, California, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**

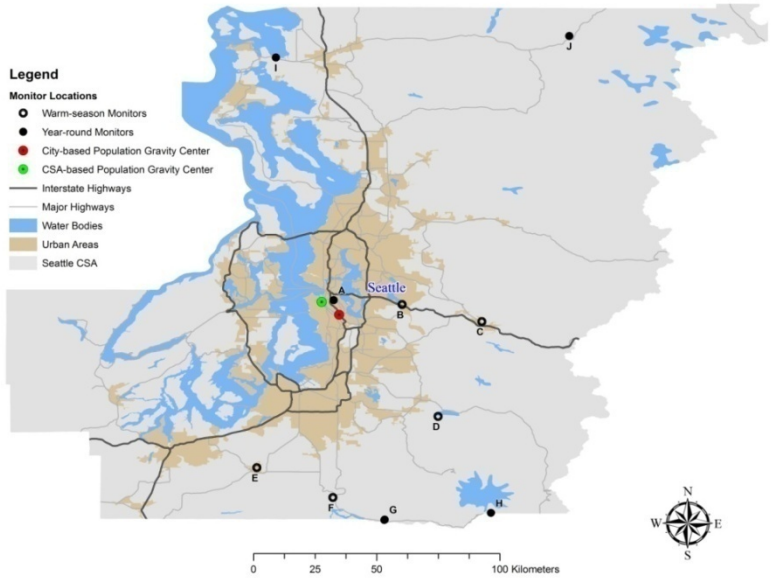



**Figure 3-94 Map of the Seattle, Washington, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**

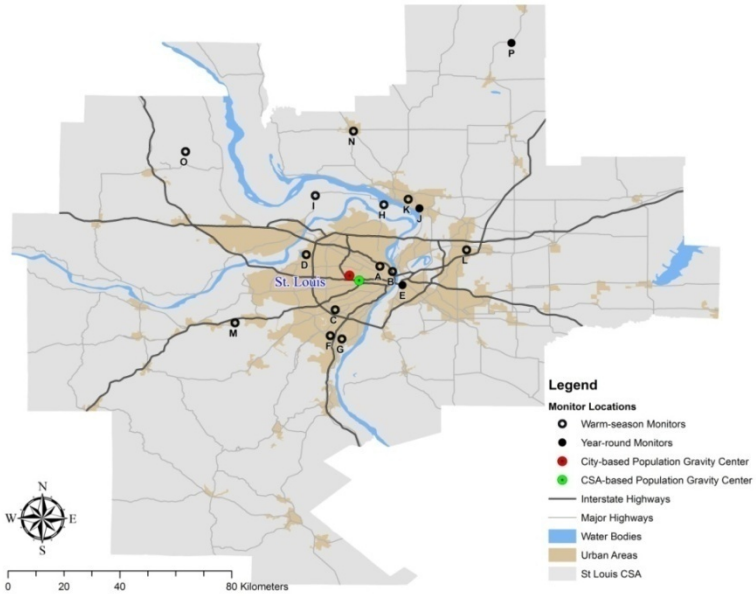



**Figure 3-95 Map of the St. Louis, Missouri, CSA including $O_3$ monitor locations, population gravity centers, urban areas, and major roadways.**

### 3.9.2 Ozone Concentration Box Plots for the Urban Focus Cities

This section contains box plots depicting the distribution of 2007-2009 warm-season 8-h daily max $O_3$ data from each individual monitor in the 20 urban focus cities introduced in Section 3.6.2.1. Monitor information including the AQS site id, the years containing qualifying data between 2007 and 2009, and the number of 8-h daily max $O_3$ observations included in the data set are listed next to the box plot. Statistics including the mean, standard deviation (SD), median and inner quartile range (IQR) are also shown for each monitor with the site letter corresponding to the sites listed in the figures above.

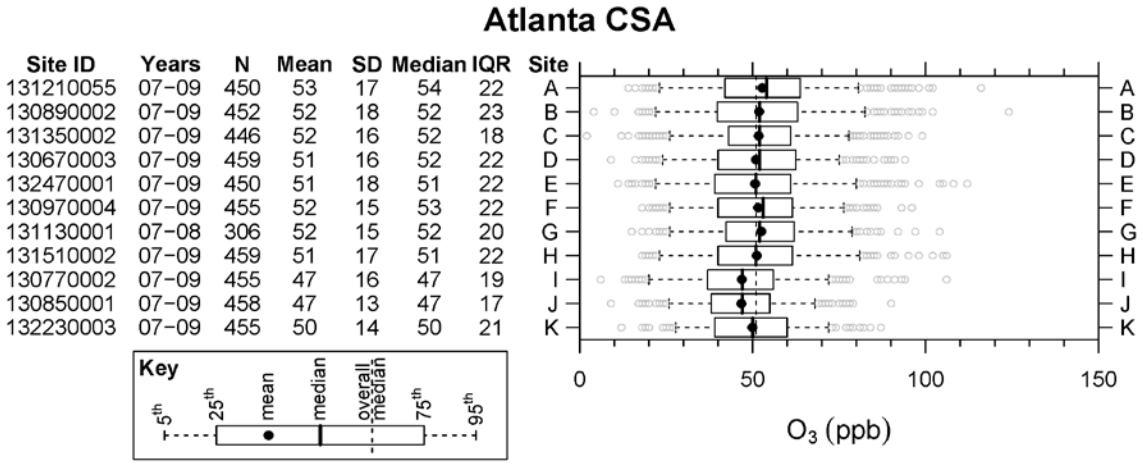



**Figure 3-96 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Atlanta, Georgia, CSA.**

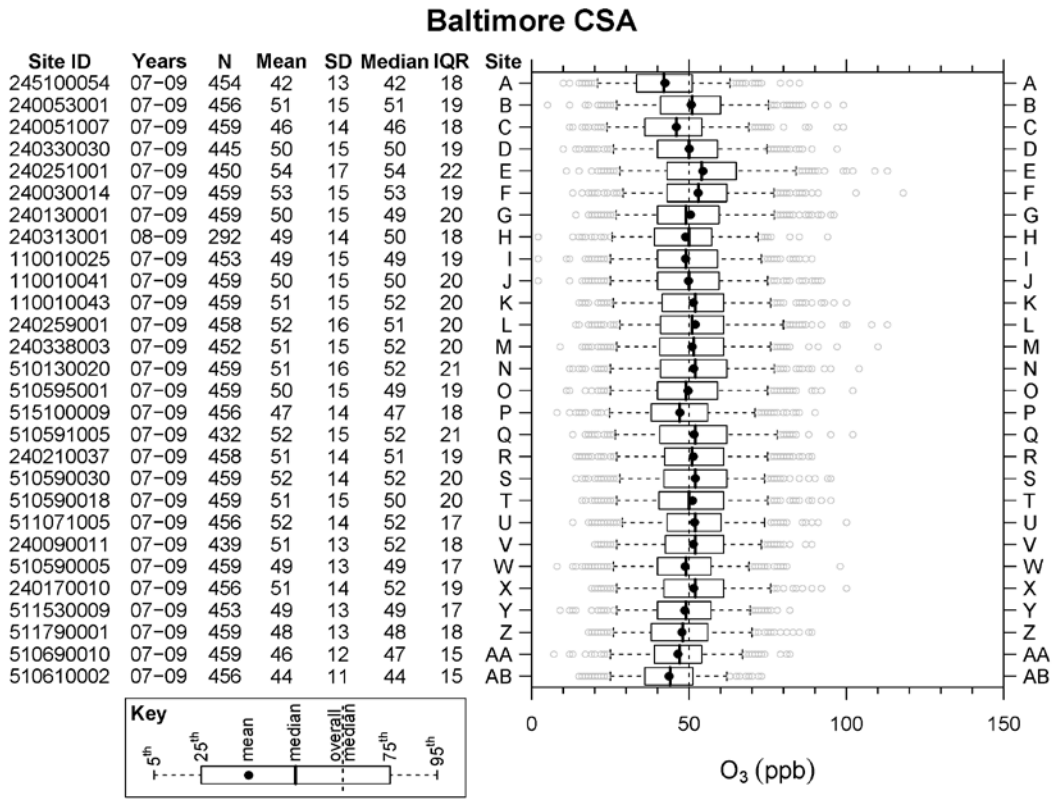



**Figure 3-97 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Baltimore, Maryland, CSA.**

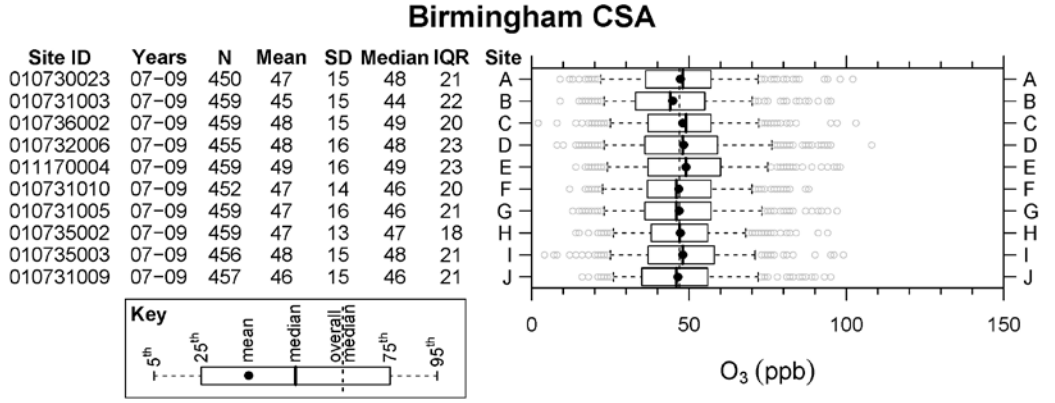



**Figure 3-98 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Birmingham, Alabama, CSA.**

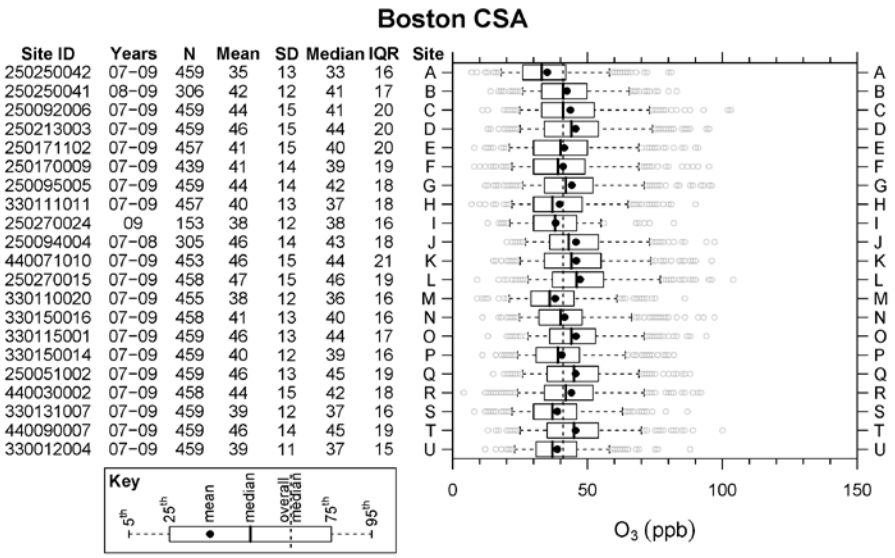



**Figure 3-99 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Boston, Massachusetts, CSA.**

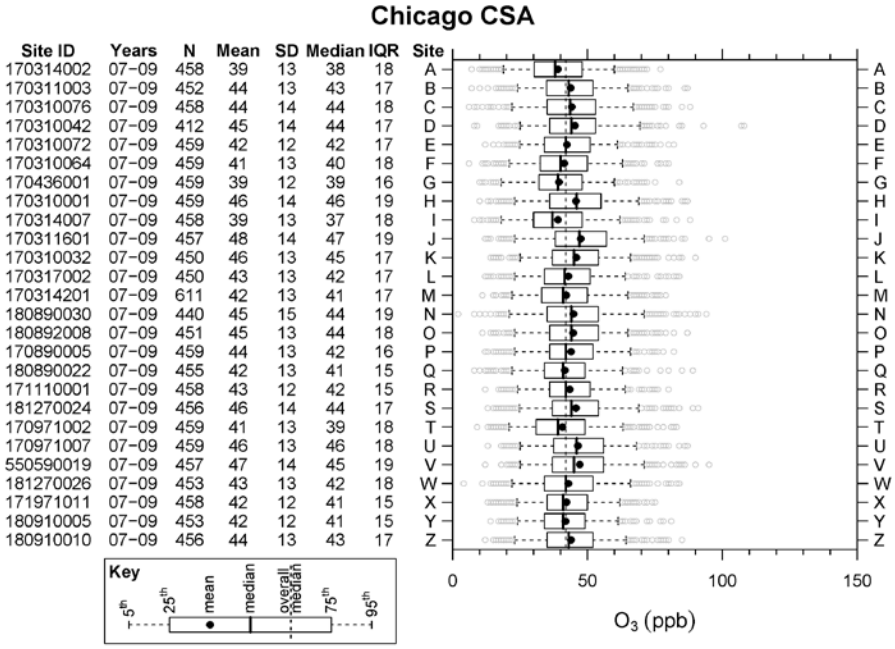



**Figure 3-100 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Chicago, Illinois, CSA.**

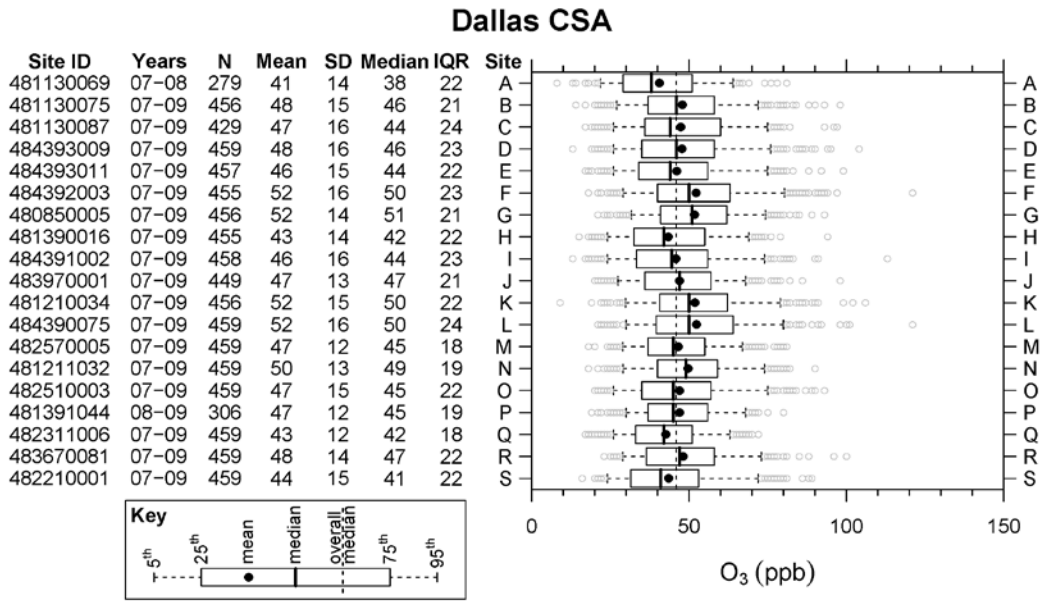



**Figure 3-101 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Dallas, Texas, CSA.**

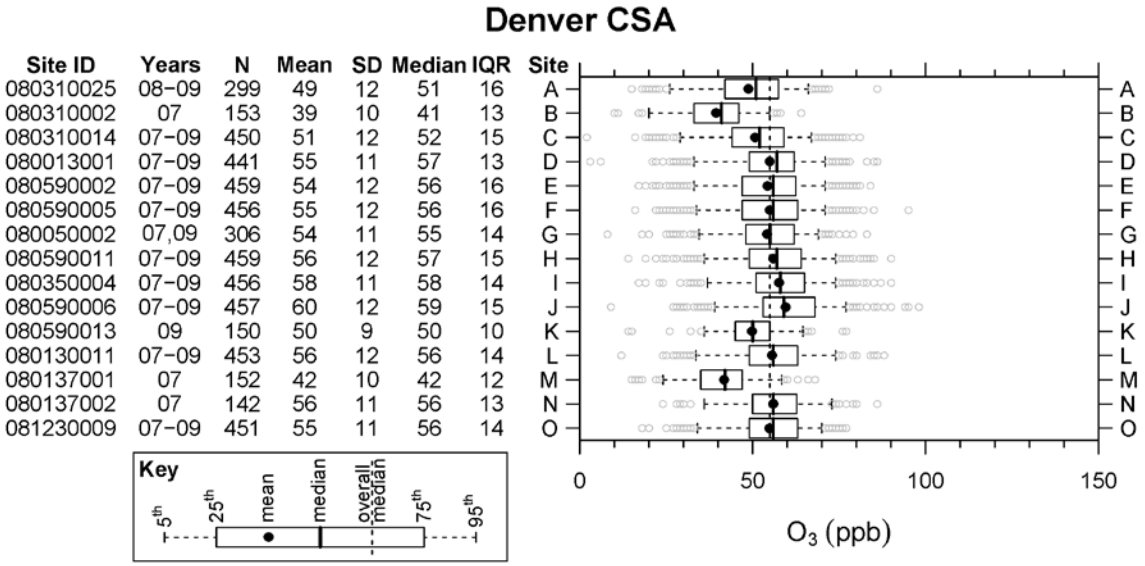



**Figure 3-102 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Denver, Colorado, CSA.**

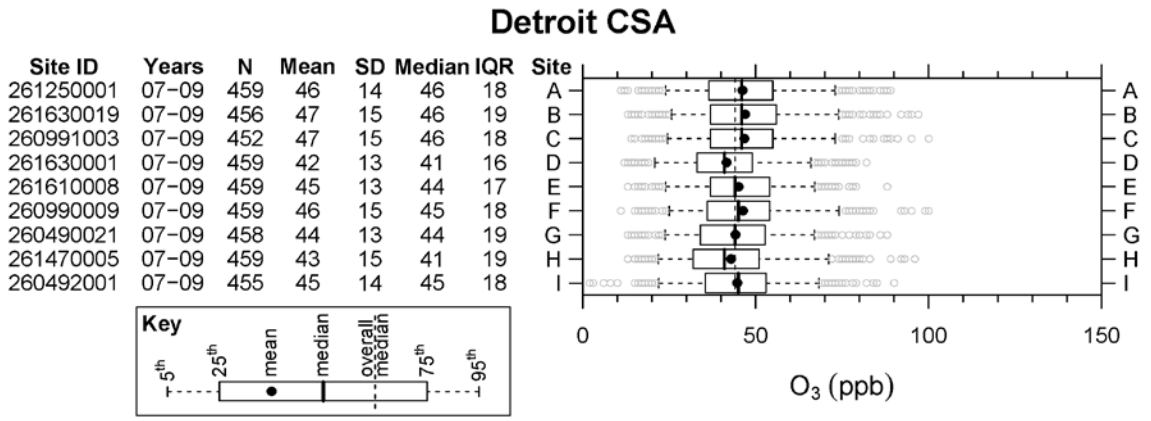



**Figure 3-103 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Detroit, Michigan, CSA.**

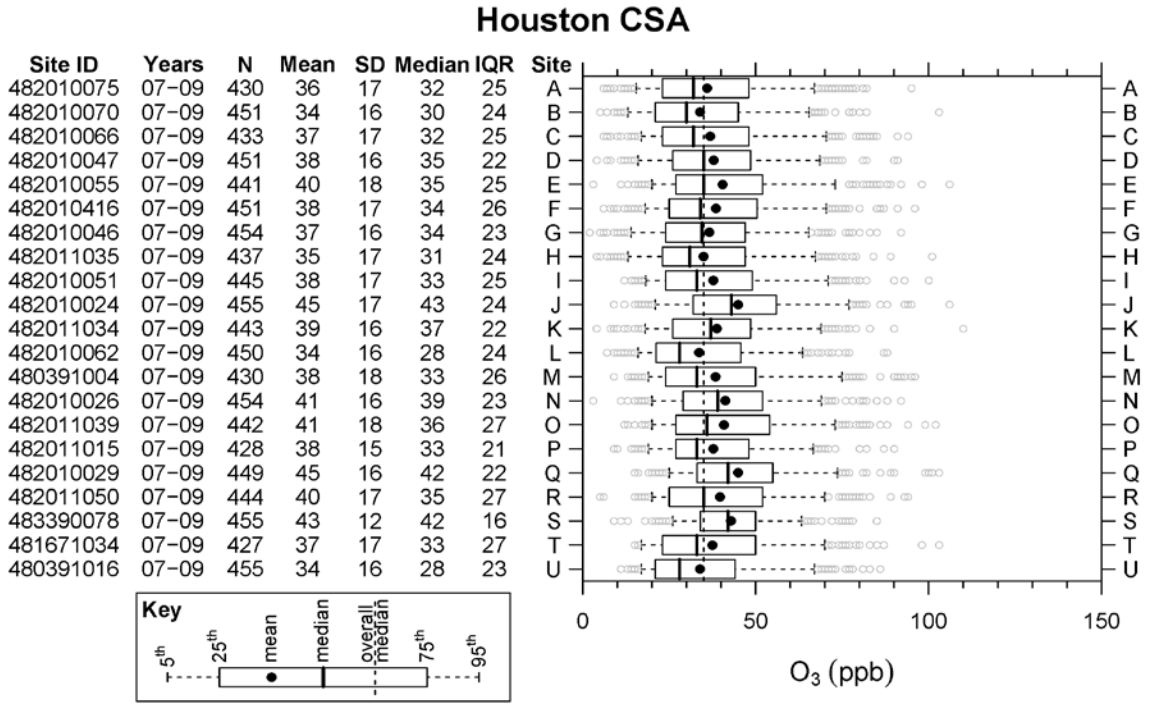



**Figure 3-104 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Houston, Texas, CSA.**

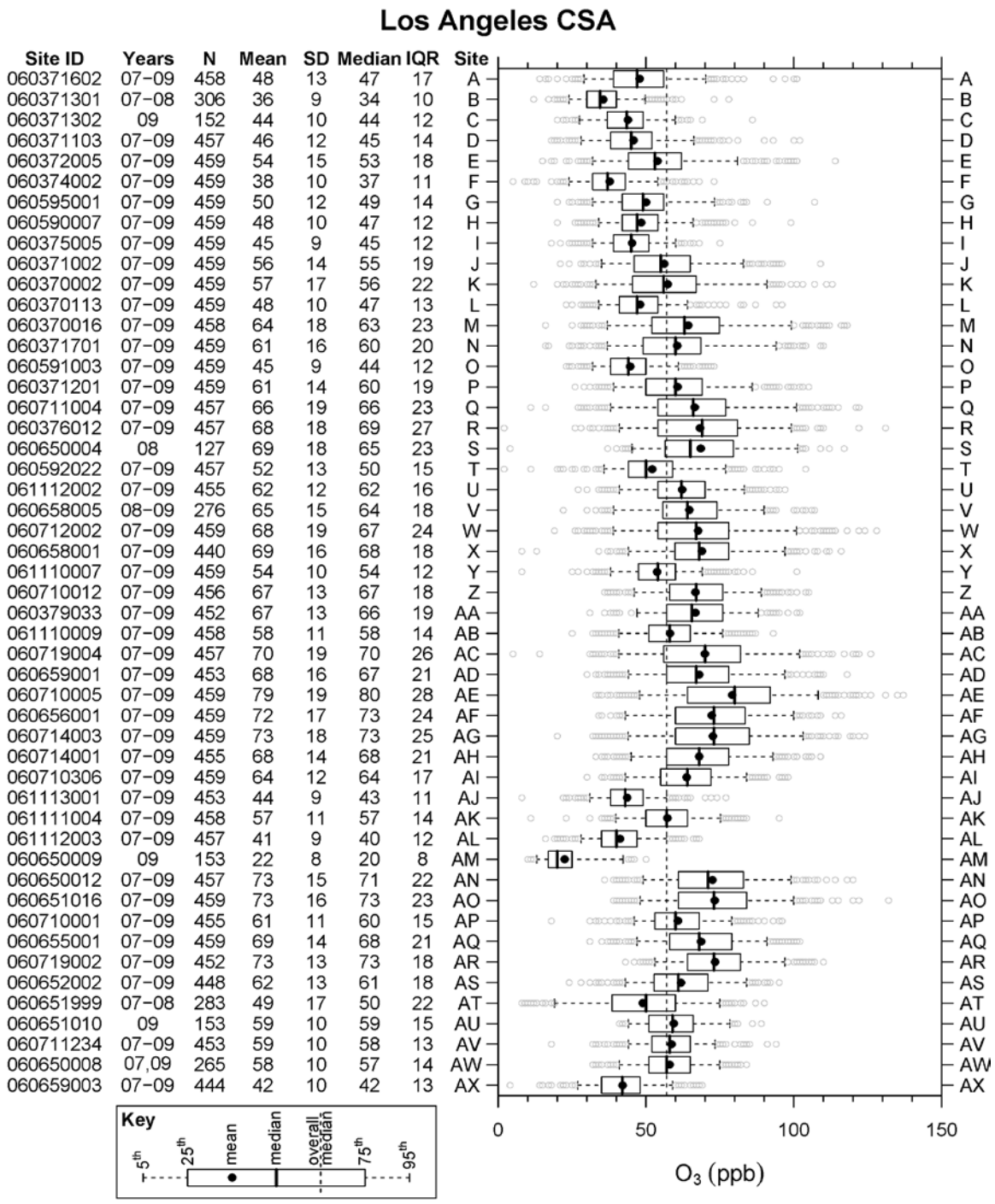



**Figure 3-105 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Los Angeles, California, CSA.**

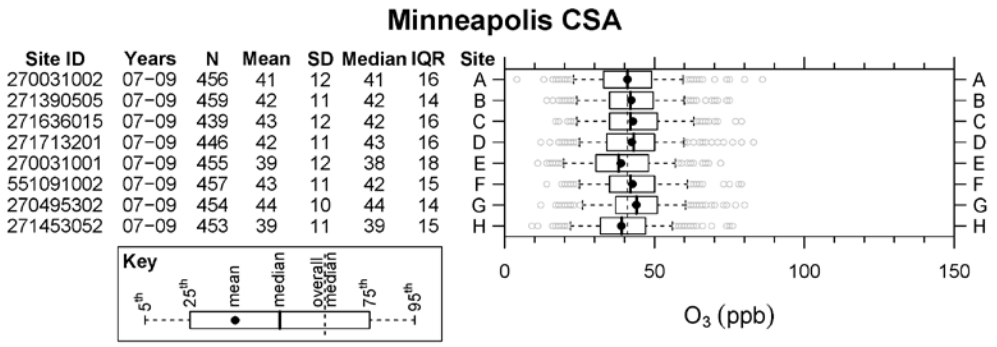



**Figure 3-106 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Minneapolis, Minnesota, CSA.**

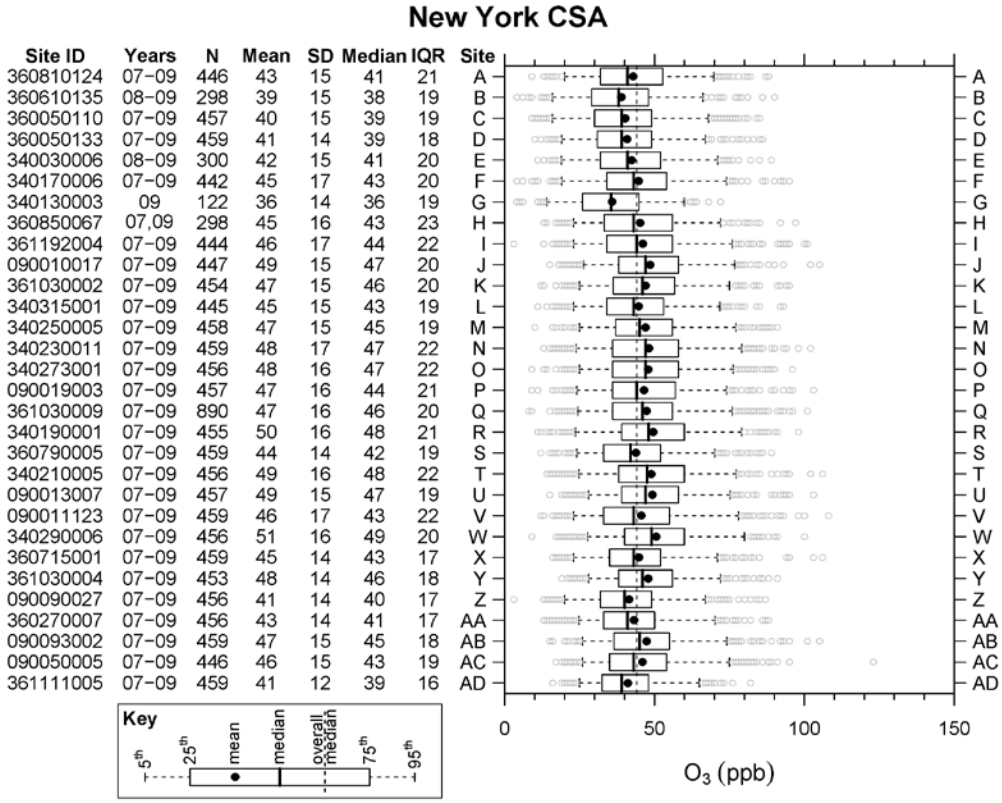



**Figure 3-107 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the New York City, New York, CSA.**

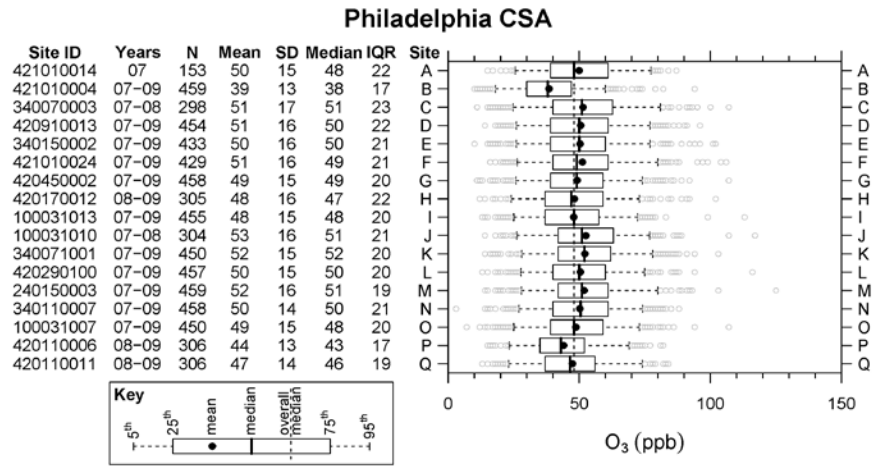



**Figure 3-108 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Philadelphia, Pennsylvania, CSA.**

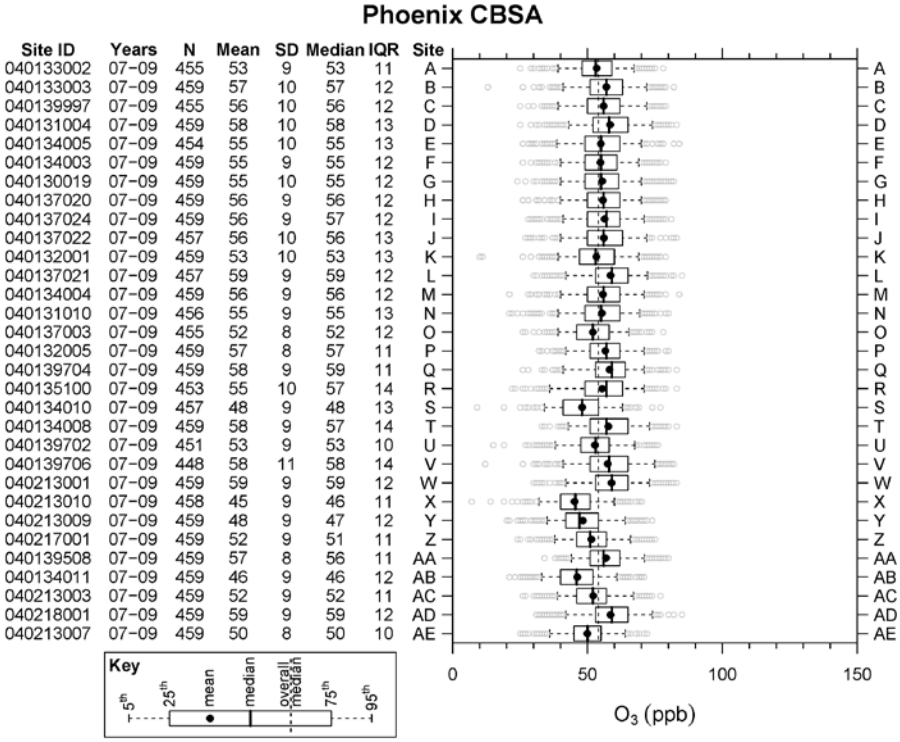



**Figure 3-109 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Phoenix, Arizona, CBSA.**

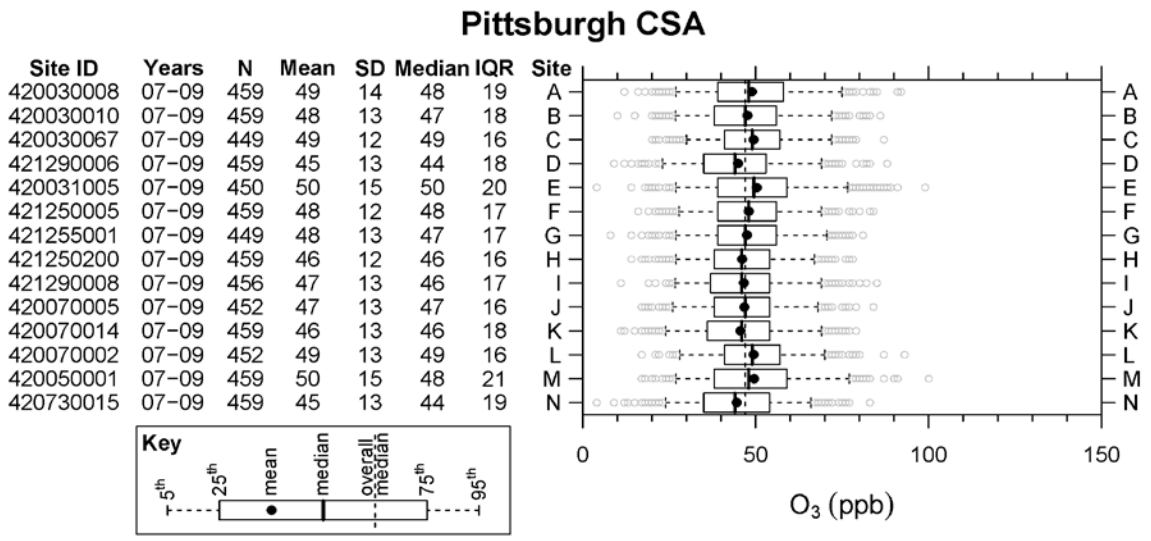



**Figure 3-110 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Pittsburgh, Pennsylvania, CSA.**

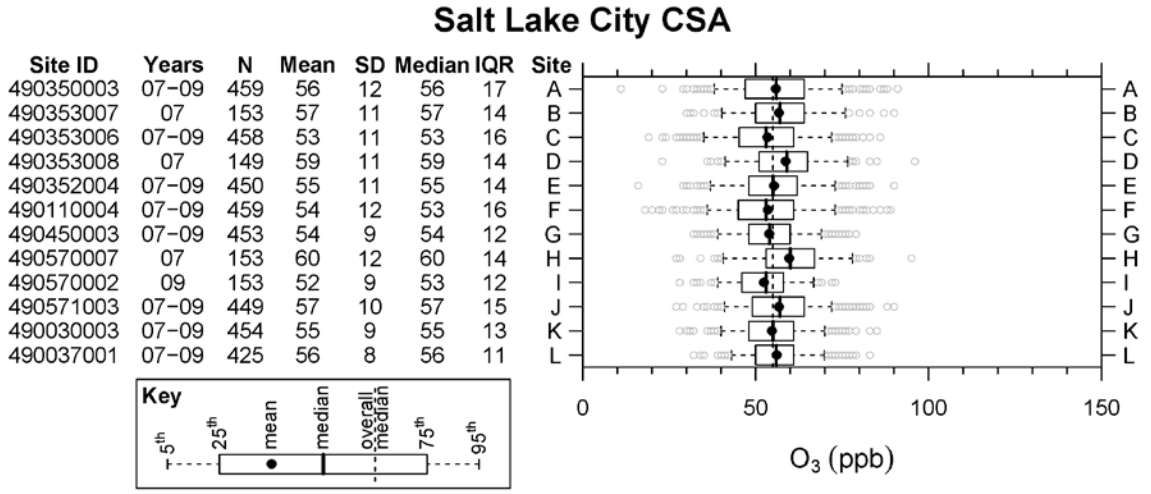



**Figure 3-111 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Salt Lake City, Utah, CSA.**

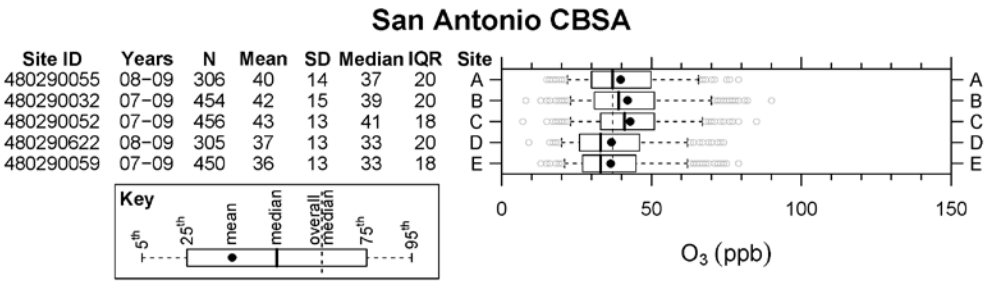



**Figure 3-112 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the San Antonio, Texas, CBSA.**

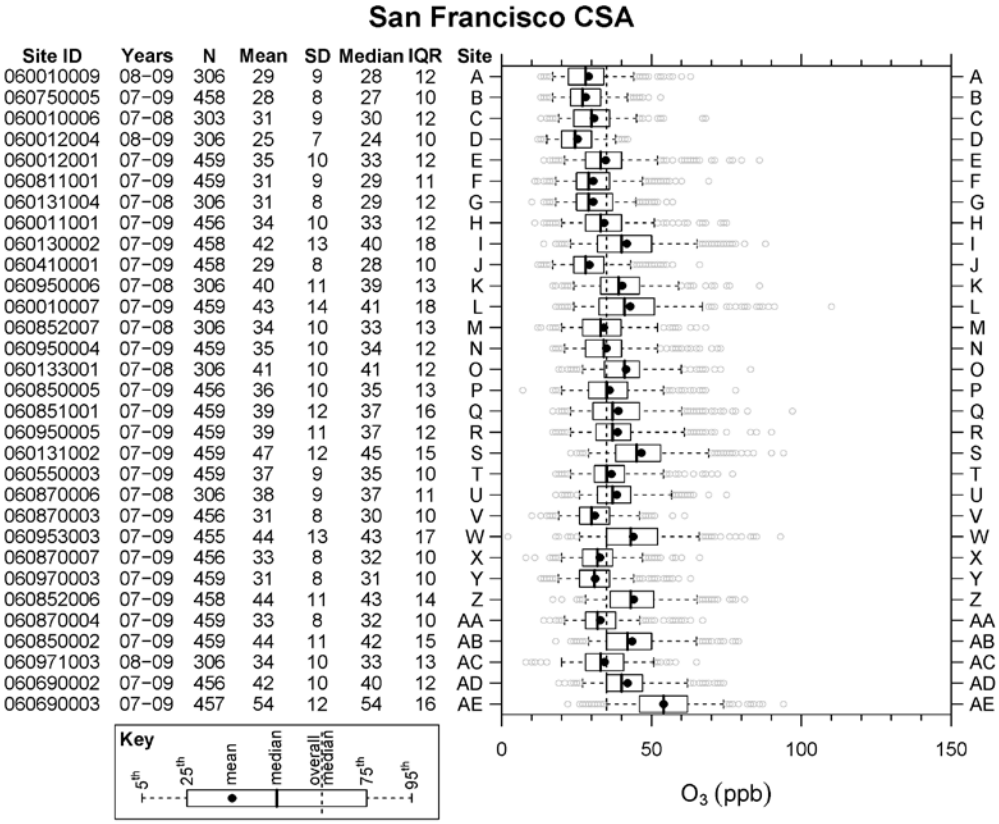



**Figure 3-113 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the San Francisco, California, CSA.**

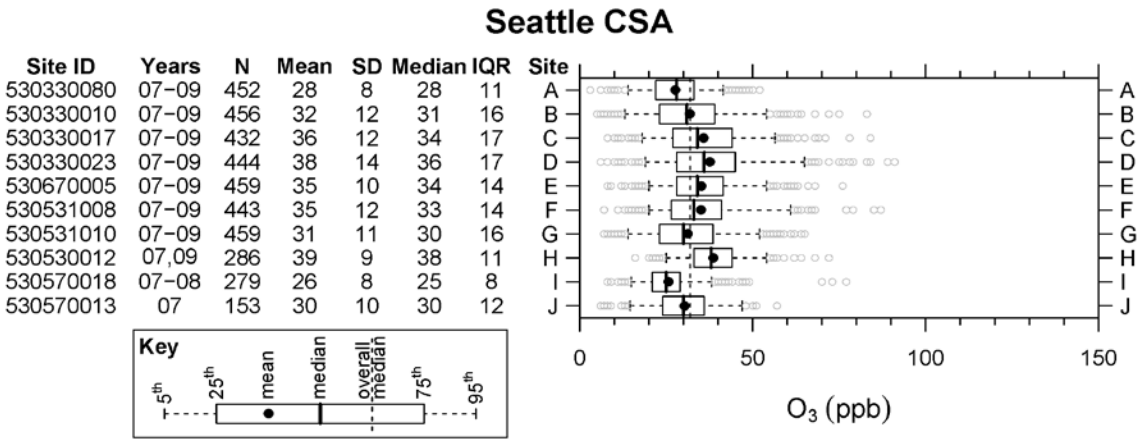



**Figure 3-114 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the Seattle, Washington, CSA.**

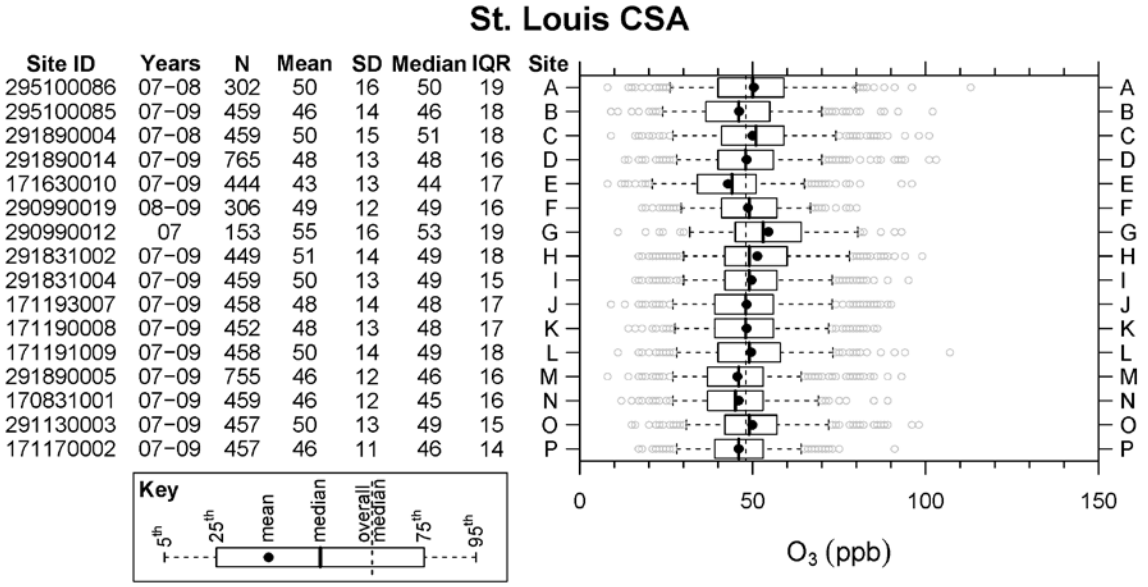
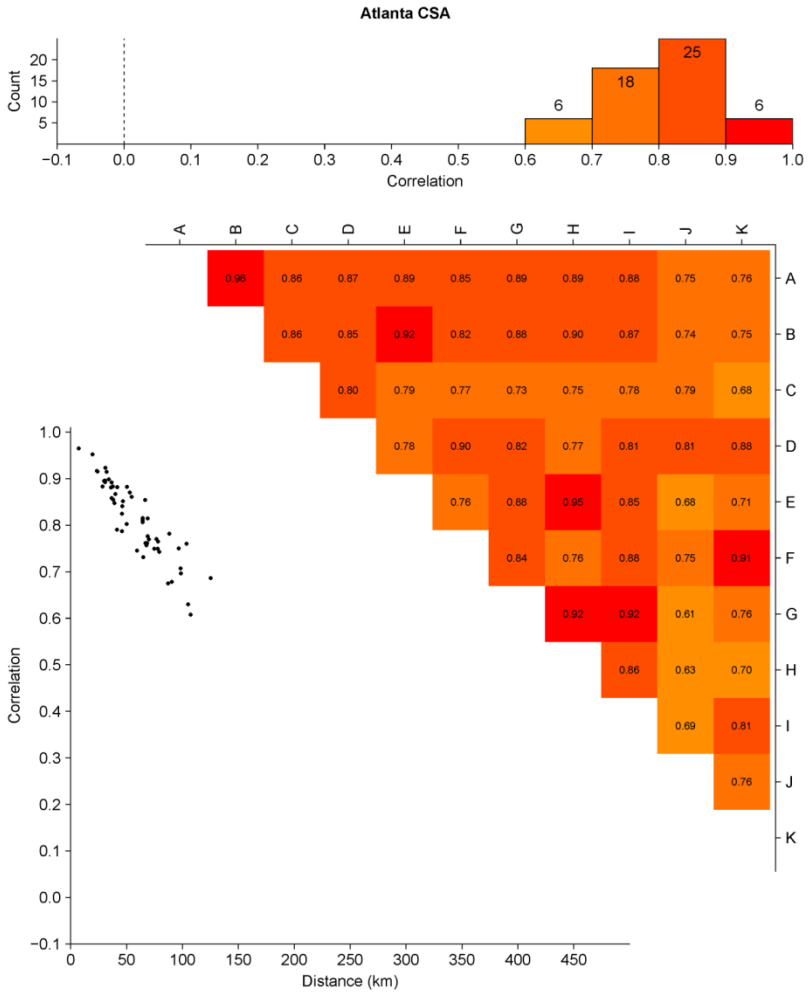



**Figure 3-115 Site information, statistics and box plots for 8-h daily max $O_3$ from AQS monitors meeting the warm-season data set inclusion criteria within the St. Louis, Missouri, CSA.**

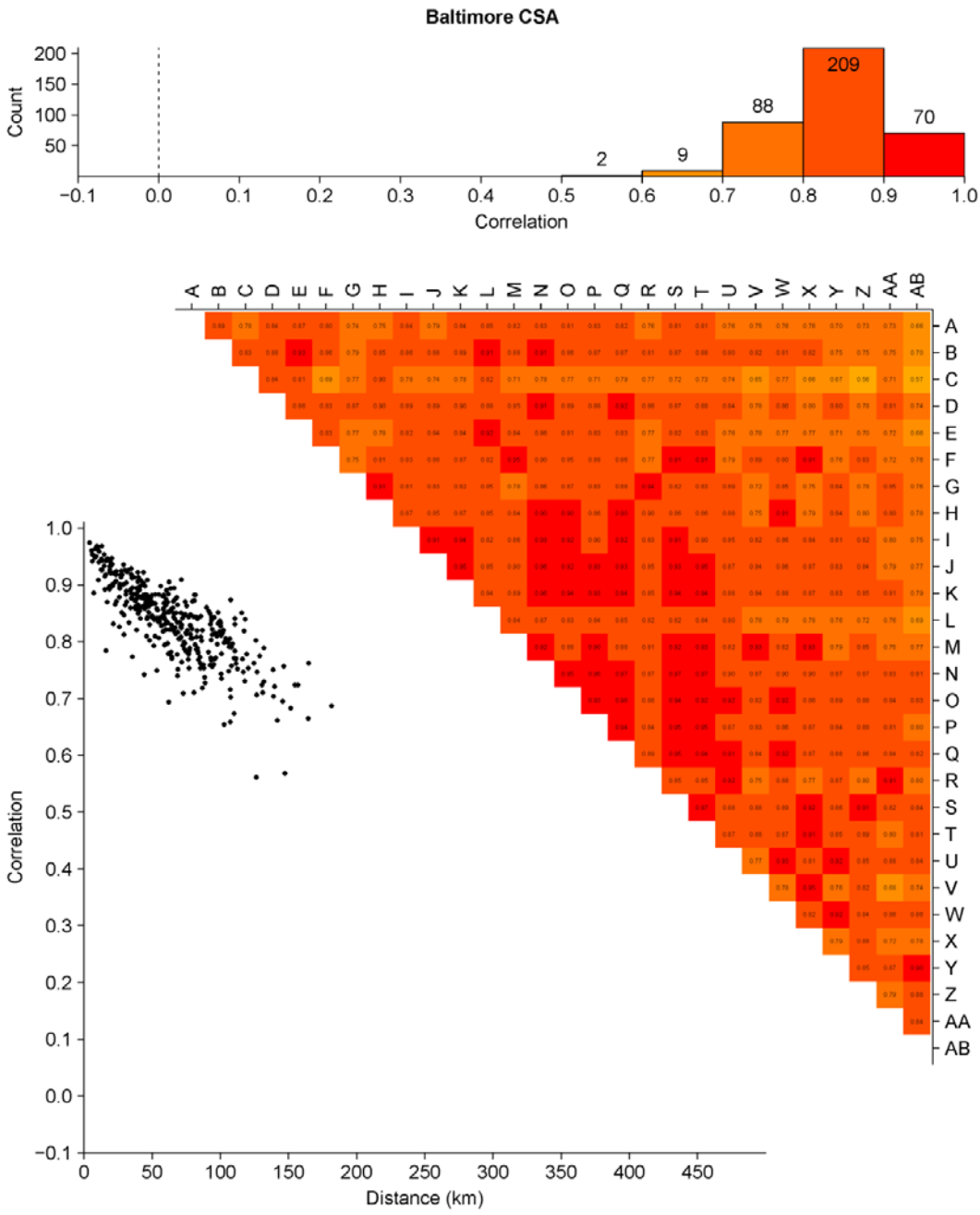
### 3.9.3 Ozone Concentration Relationships for the Urban Focus Cities

This section contains histograms and contour matrices of the Pearson correlation coefficient (R) and the coefficient of divergence (COD) between 8-h daily max $O_3$ concentrations from each monitor pair within the 20 urban focus cities discussed in Section 3.6.2.1. These figures also contain scatter plots of R and COD as a function of straight-line distance between monitor pairs.



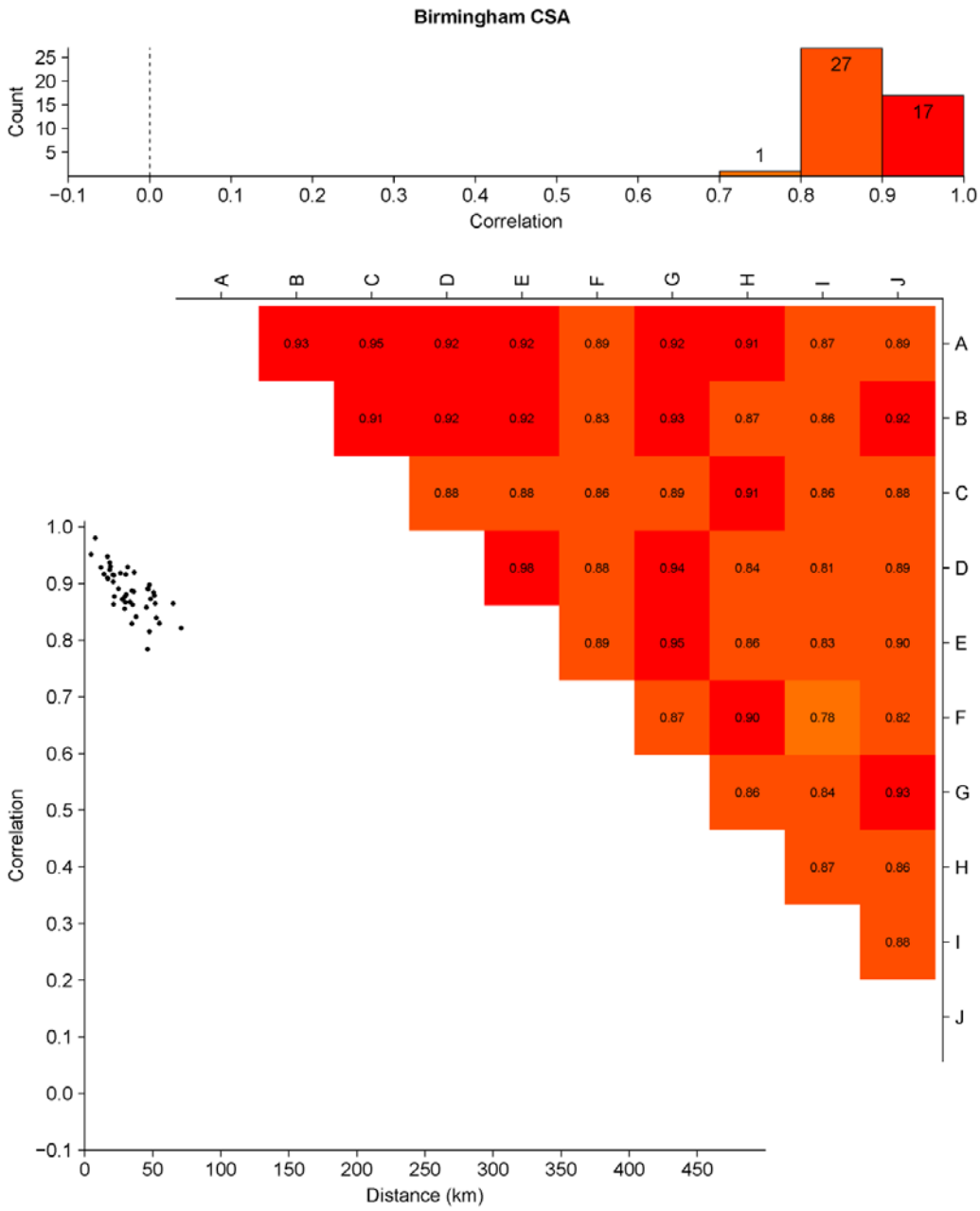

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of R.

**Figure 3-116 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Atlanta, Georgia, CSA.**



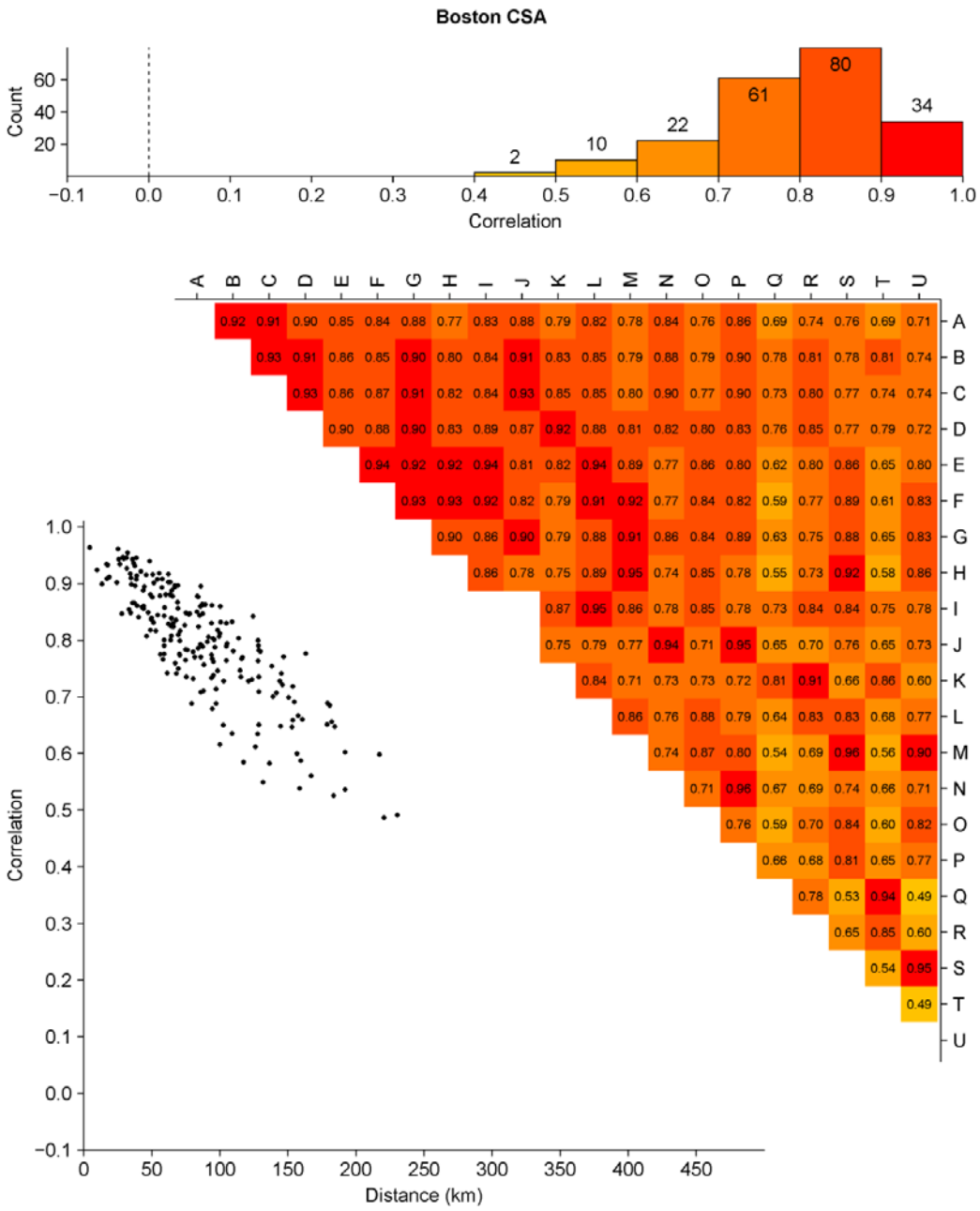

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of R.

**Figure 3-117 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Baltimore, Maryland, CSA.**



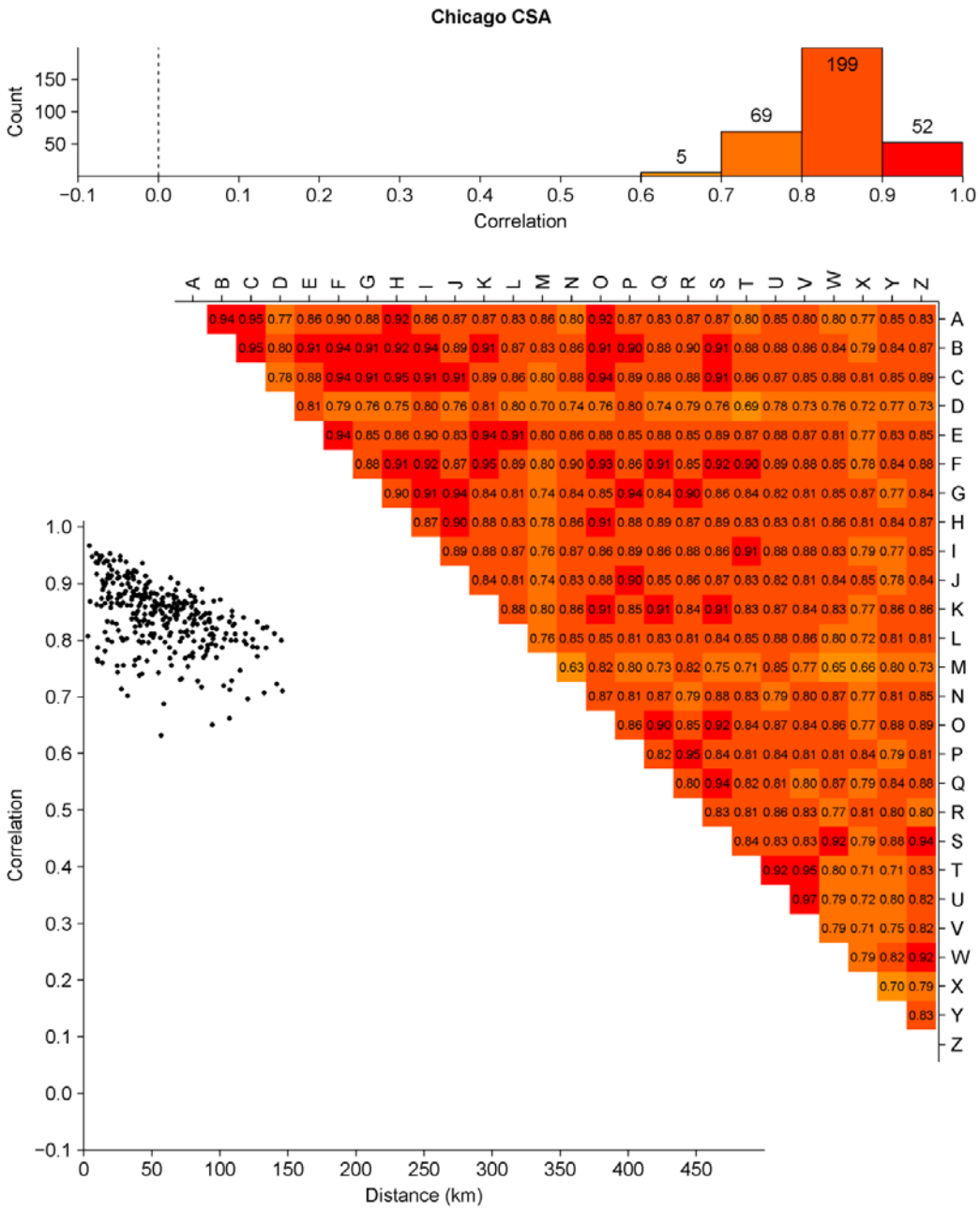

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of R.

**Figure 3-118 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Birmingham, Alabama, CSA.**



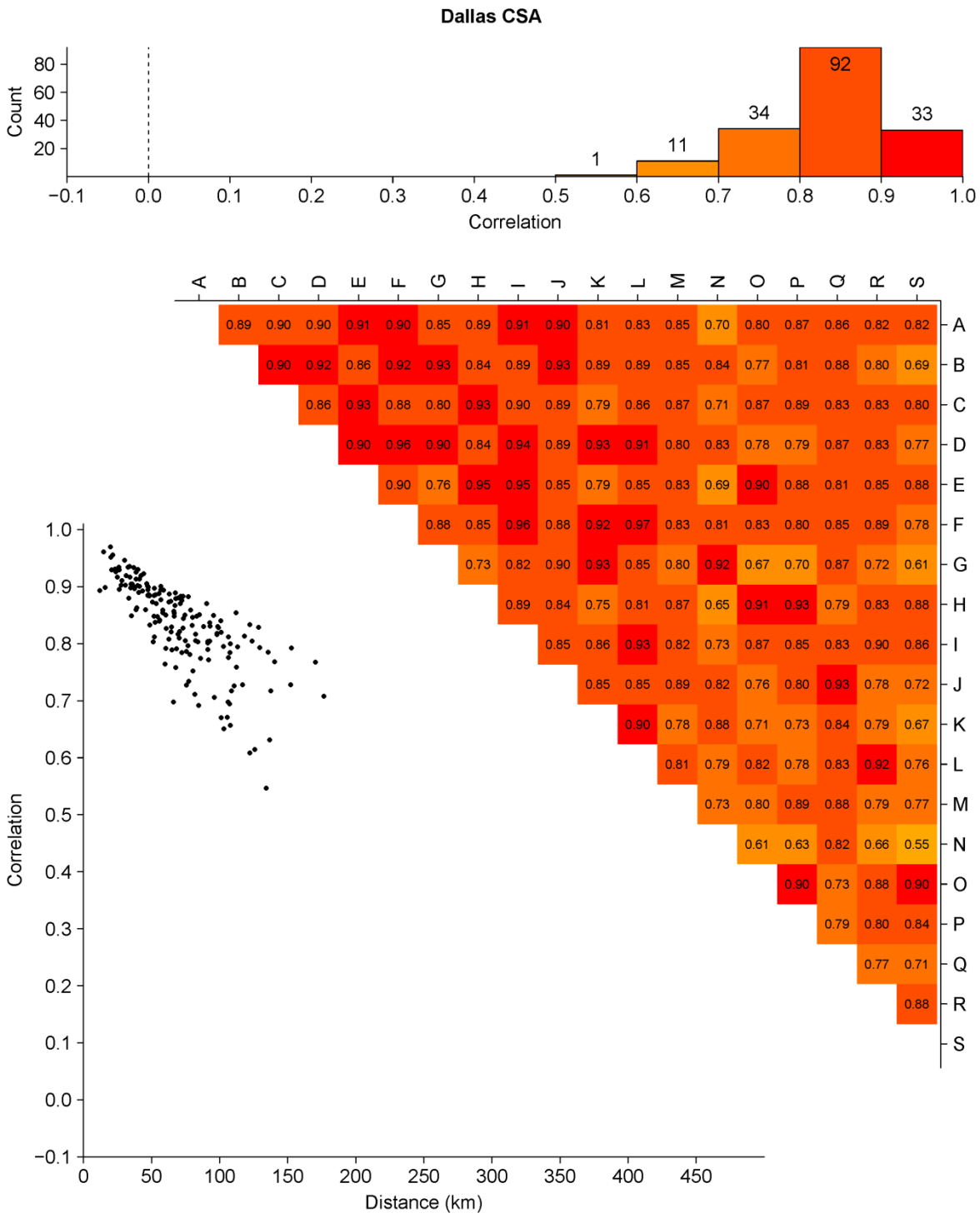

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of R.

**Figure 3-119 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Boston, Massachusetts, CSA.**



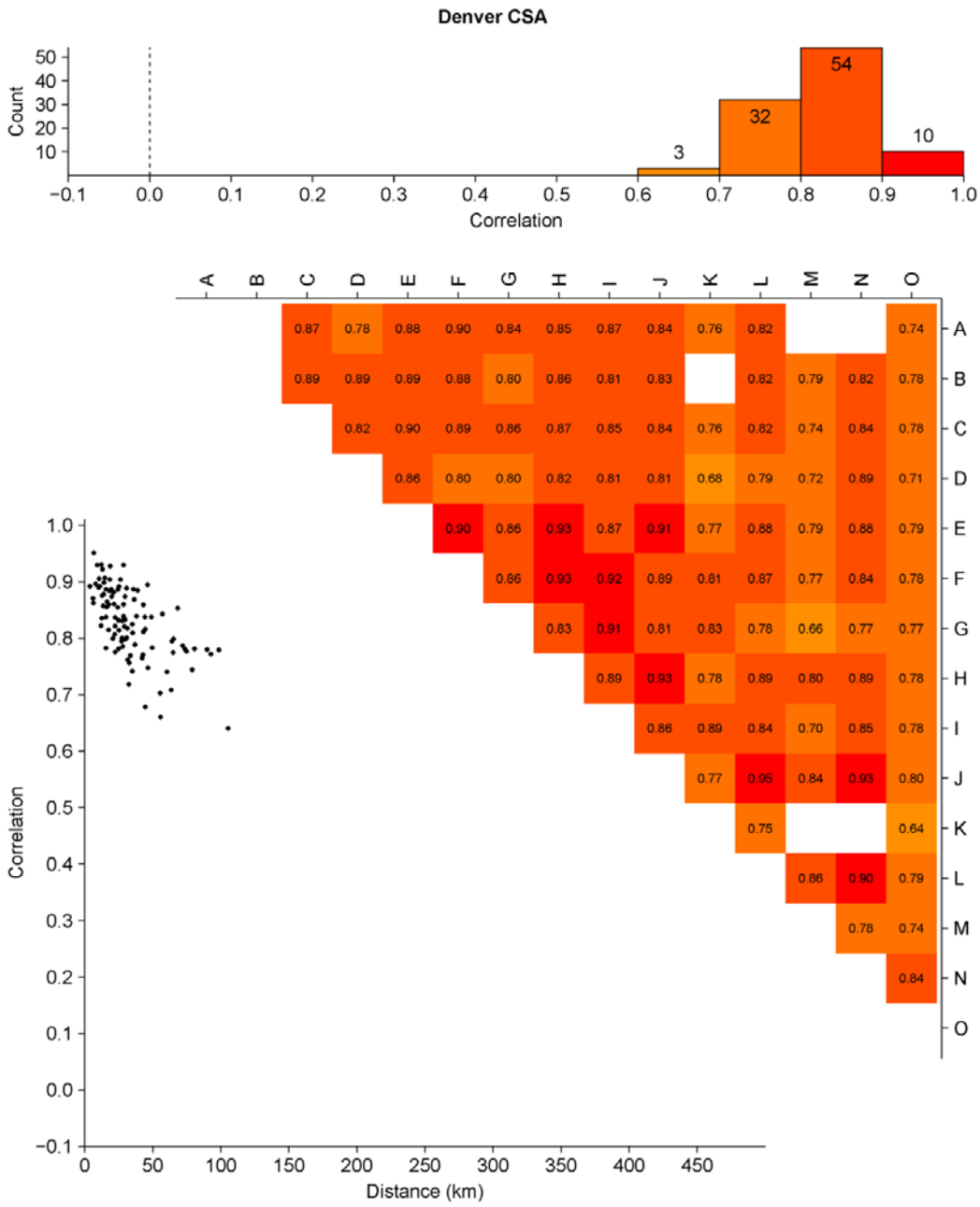

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of R.

**Figure 3-120 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Chicago, Illinois, CSA.**



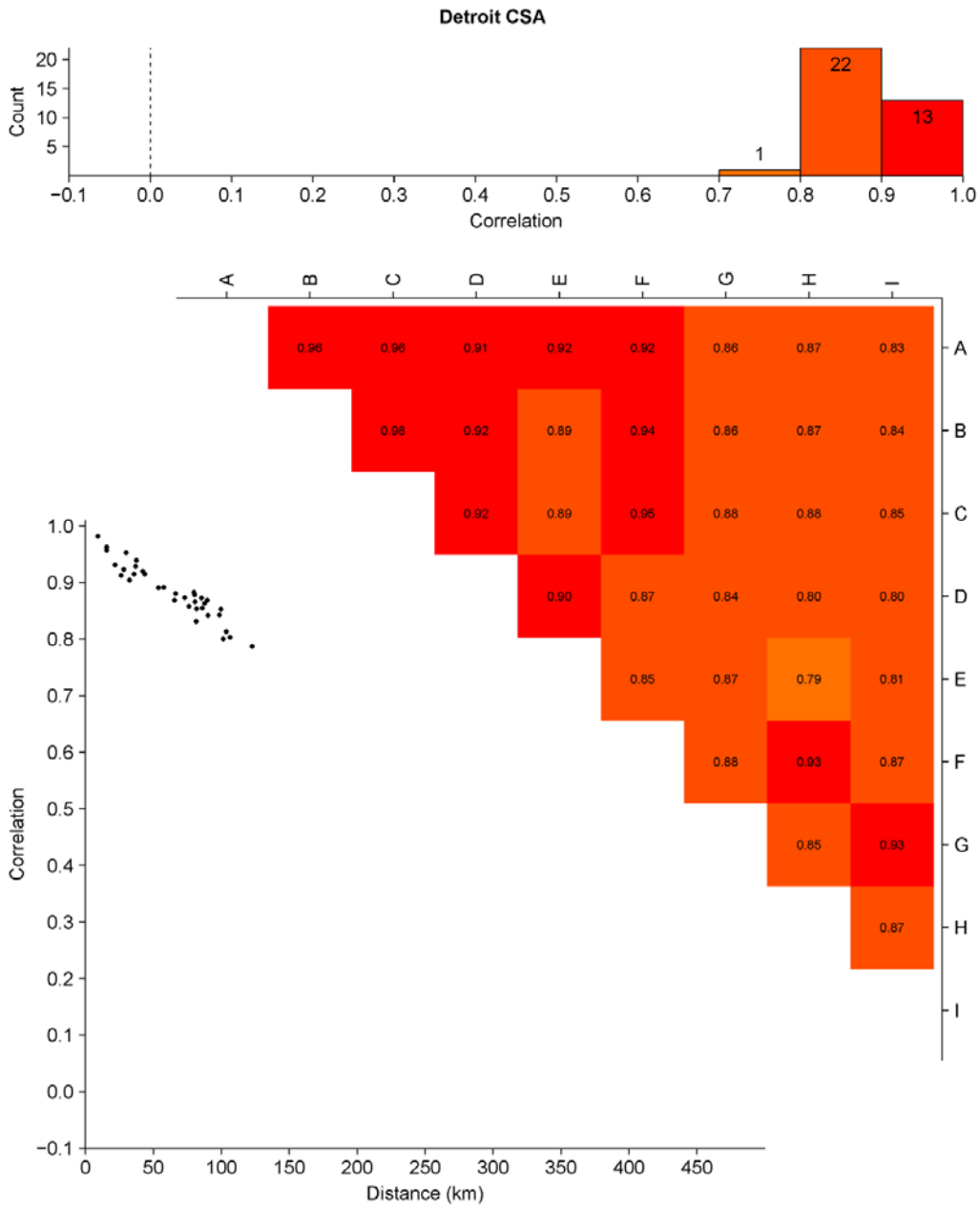

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of R.

**Figure 3-121 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Dallas, Texas, CSA.**



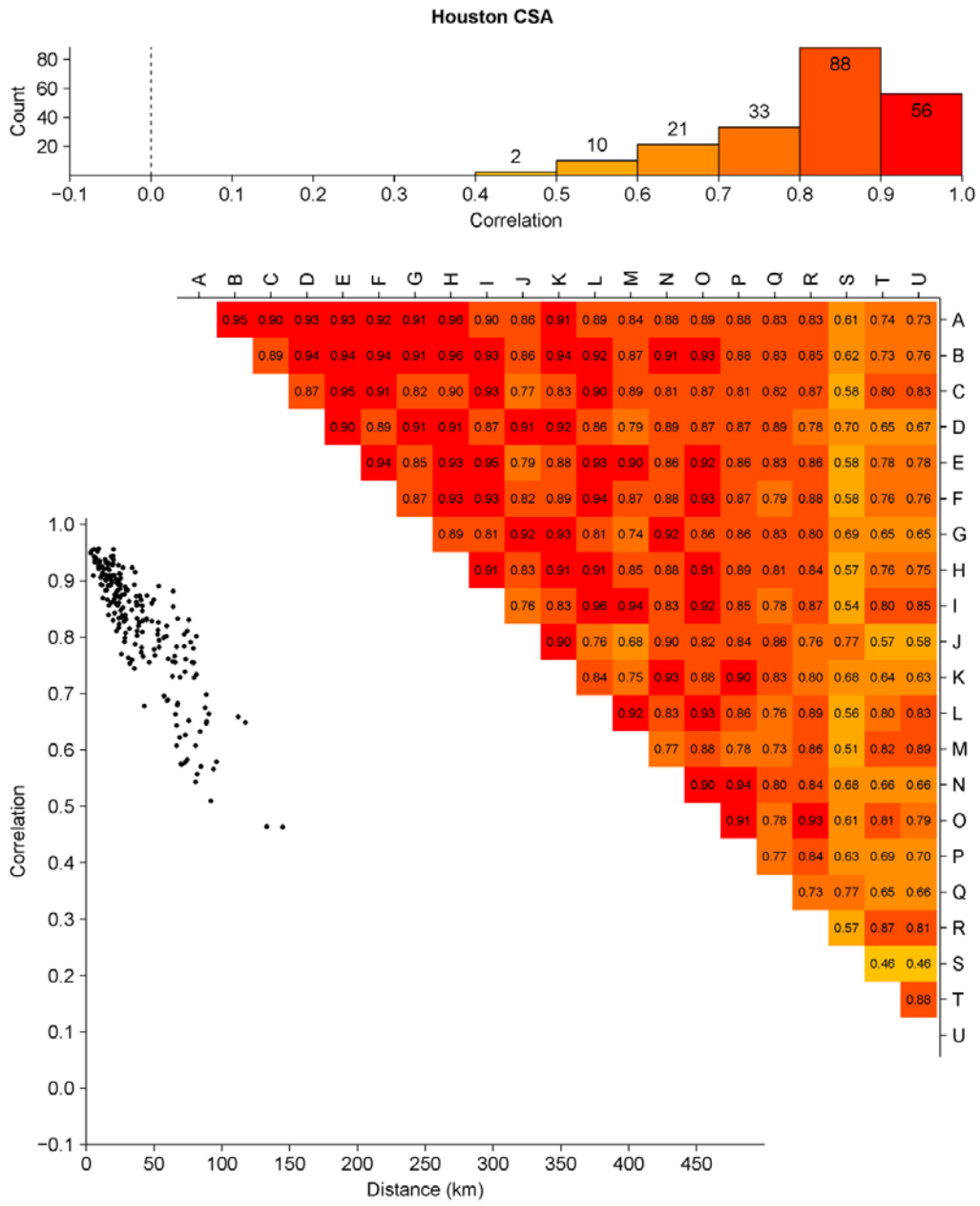

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of R.

**Figure 3-122 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Denver, Colorado, CSA.**



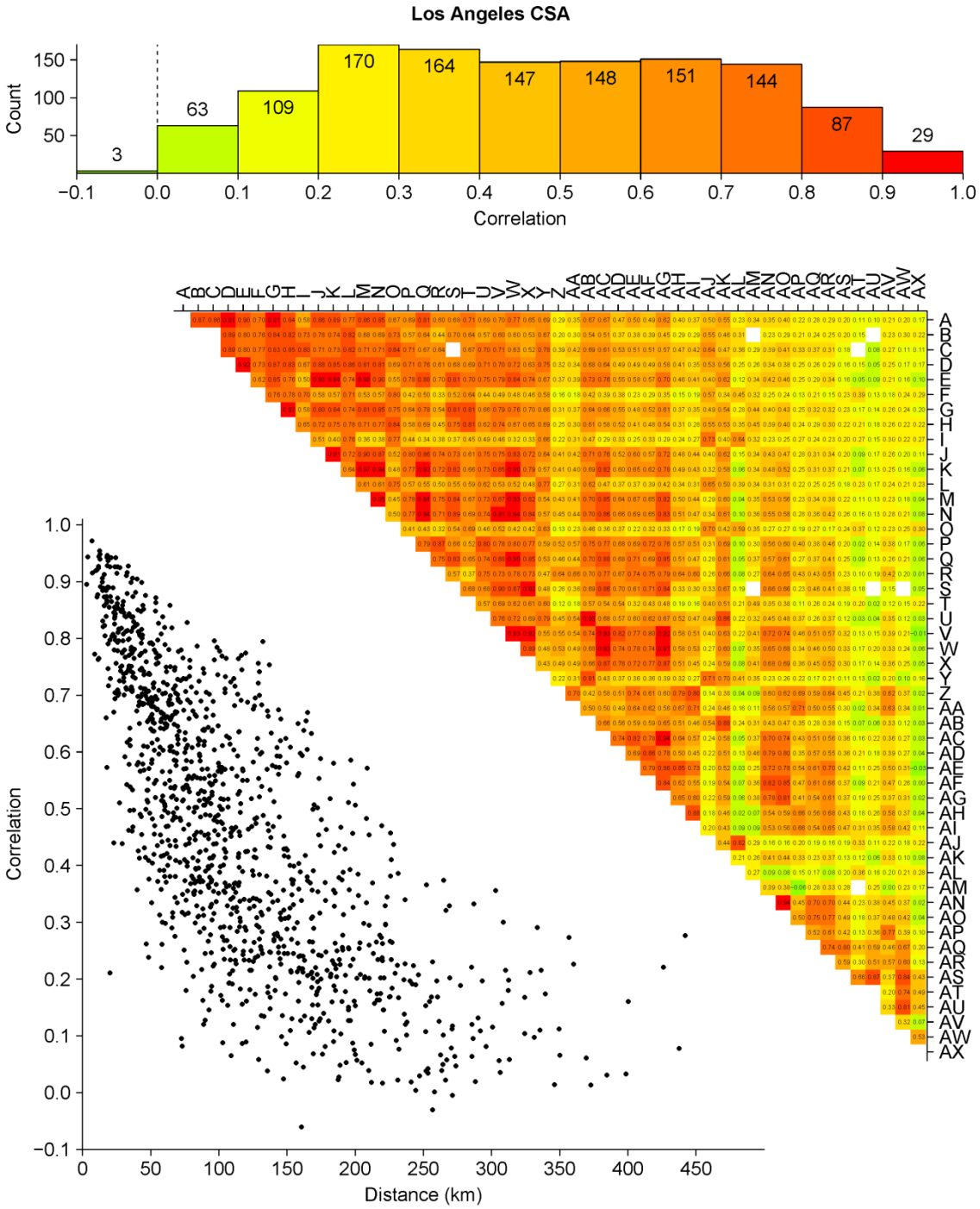

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of R.

**Figure 3-123 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Detroit, Michigan, CSA.**



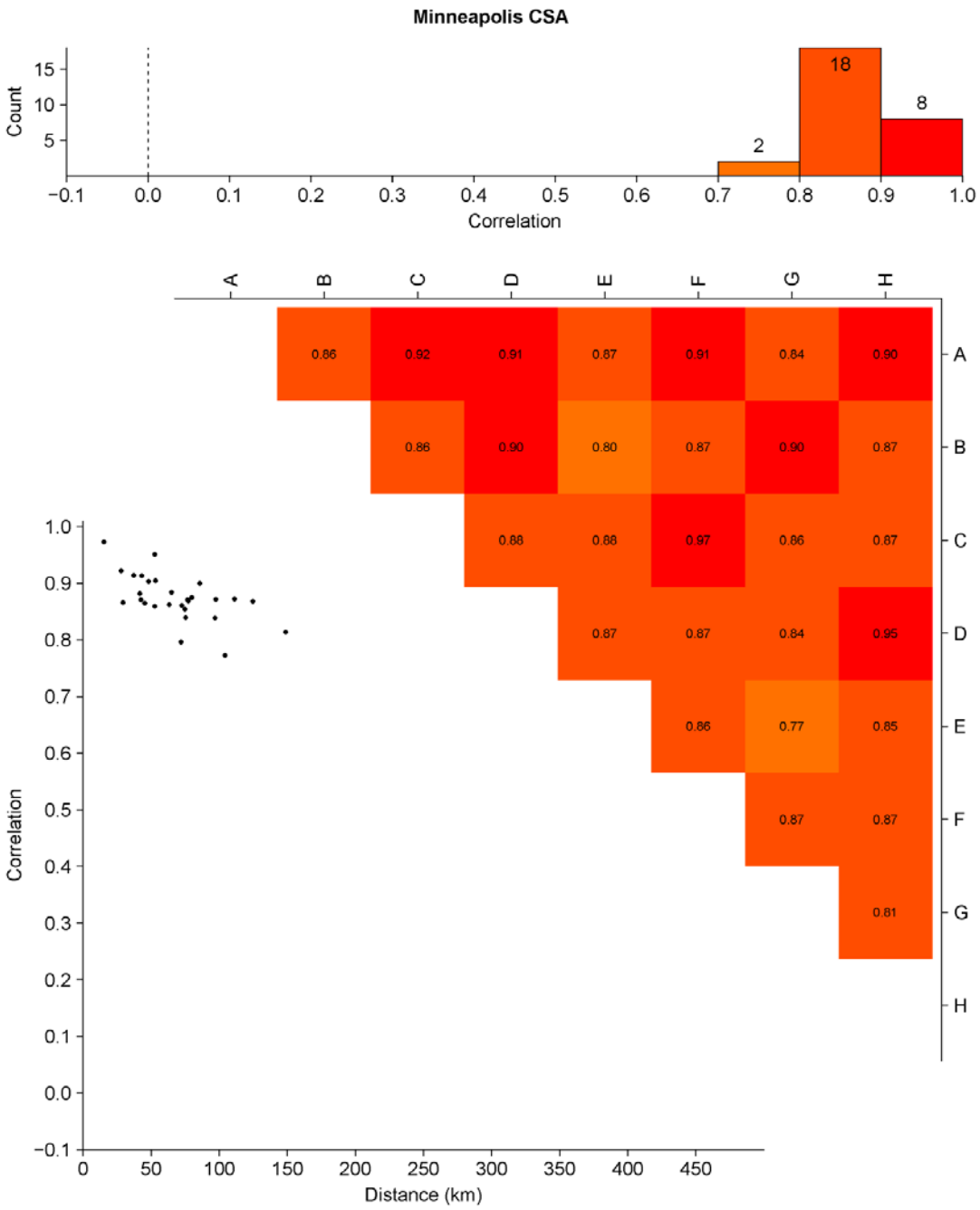

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of R.

**Figure 3-124 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Houston, Texas, CSA.**



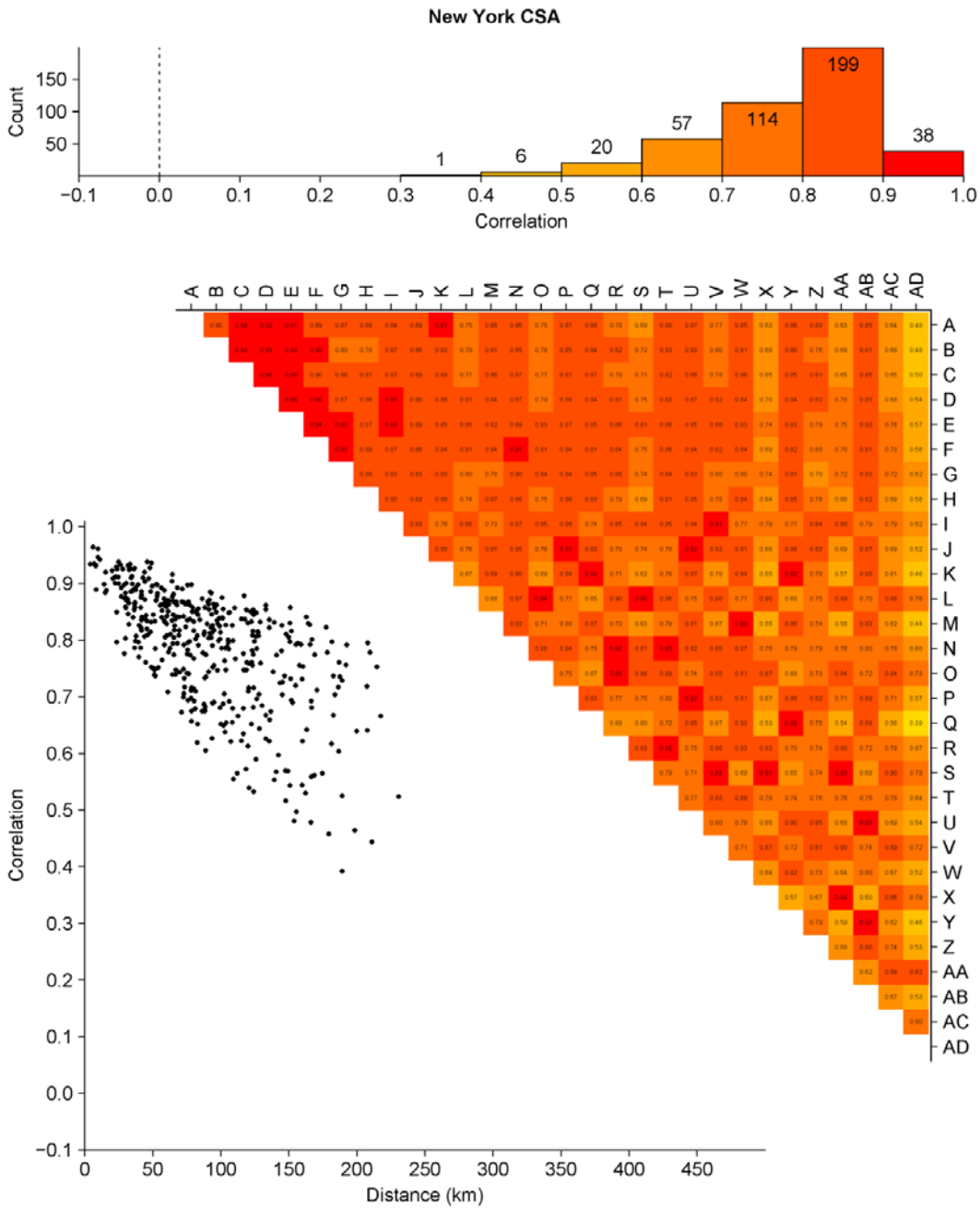

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of R.

**Figure 3-125 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Los Angeles, California, CSA.**



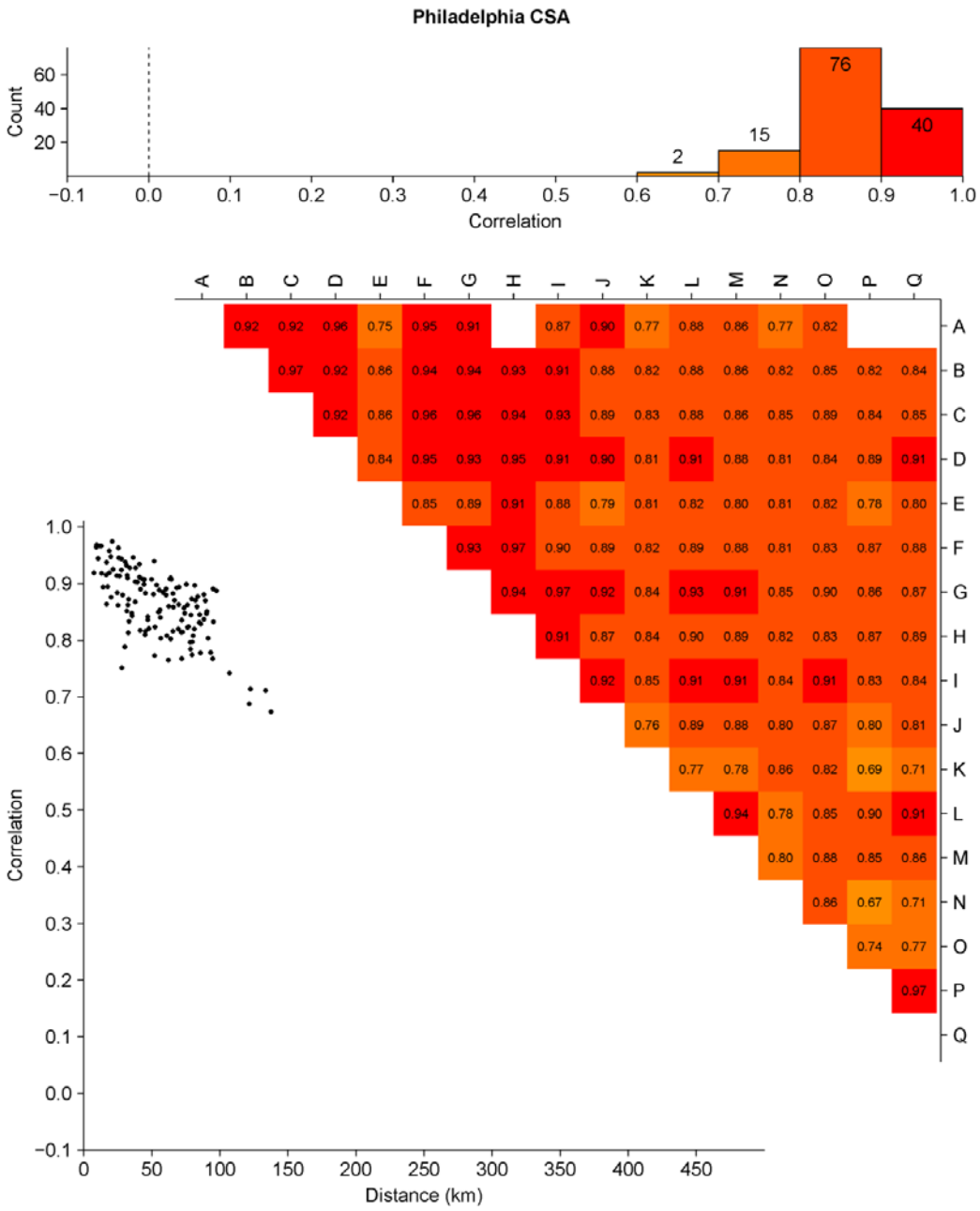

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of R.

**Figure 3-126 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Minneapolis, Minnesota, CSA.**



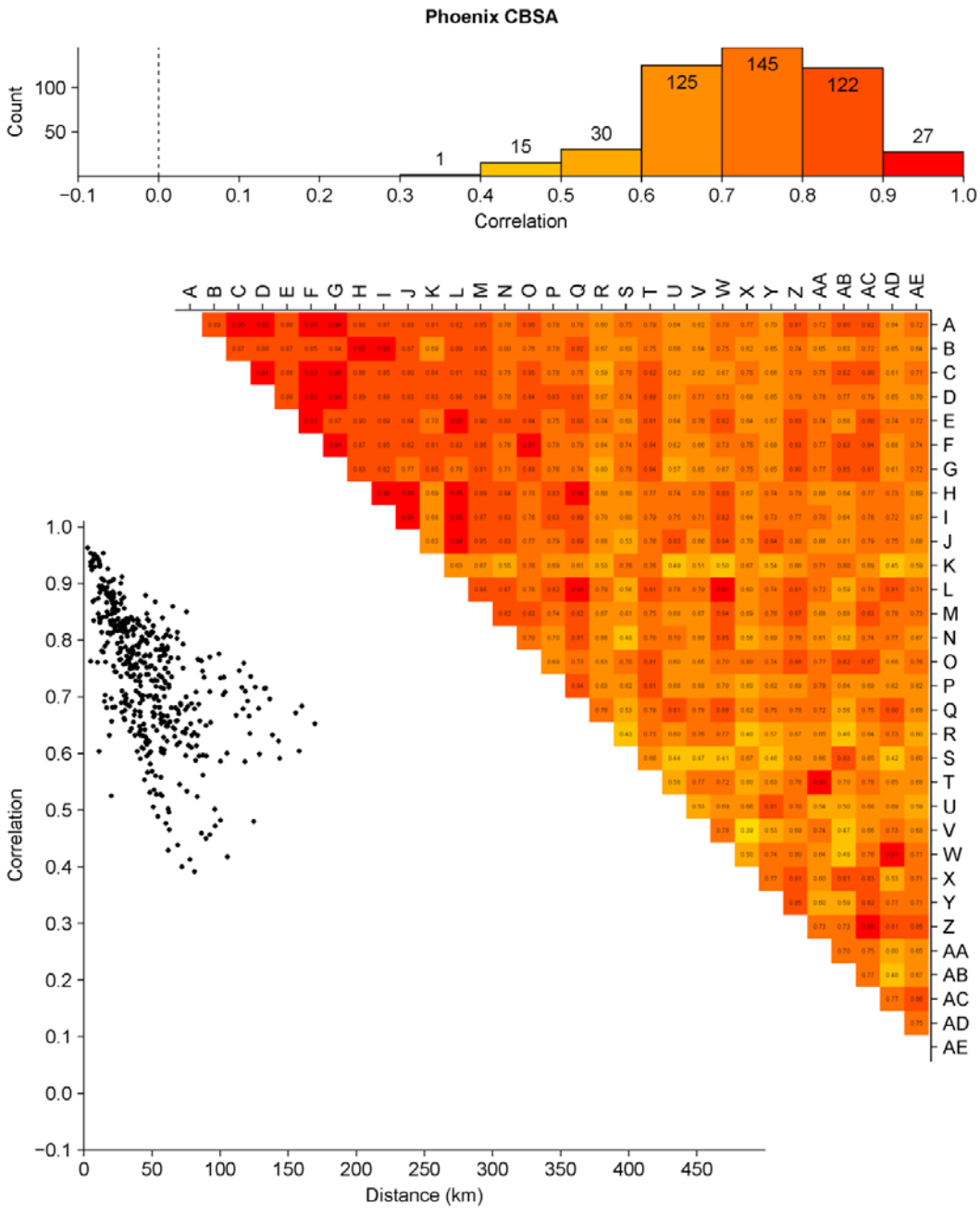

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of R.

**Figure 3-127 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the New York City, New York, CSA.**



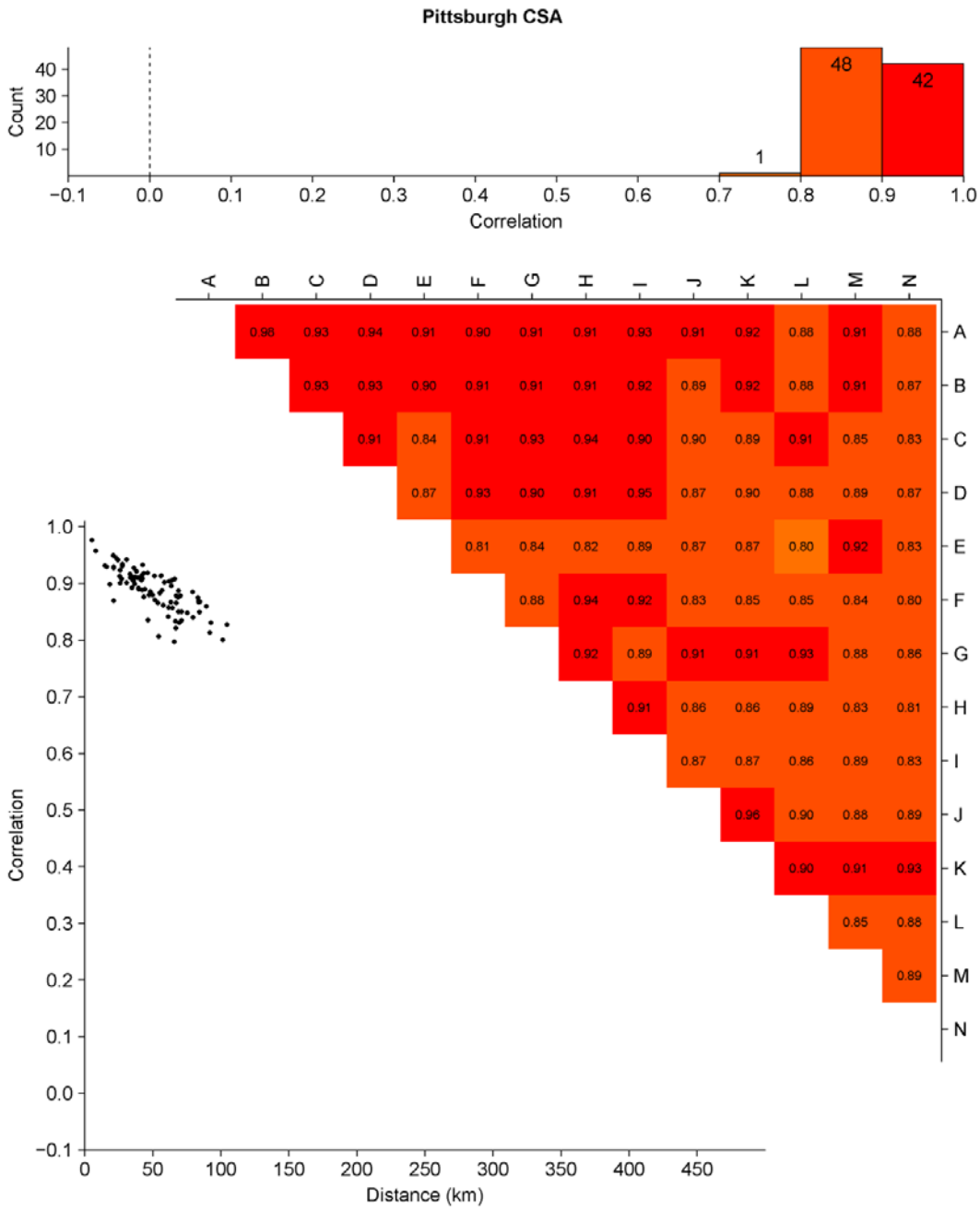

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of R.

**Figure 3-128 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Philadelphia, Pennsylvania, CSA.**



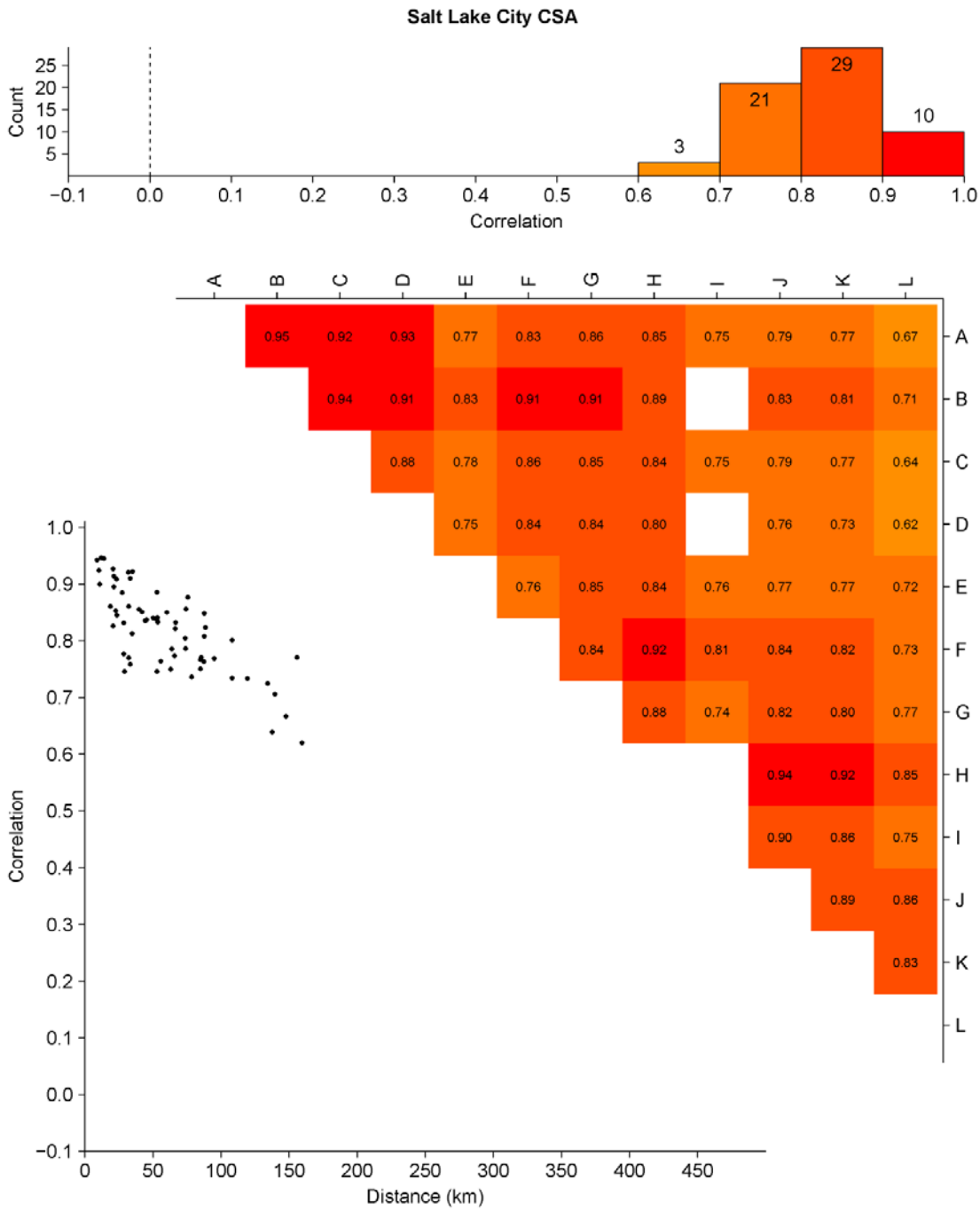

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of R.

**Figure 3-129 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Phoenix, Arizona, CBSA.**



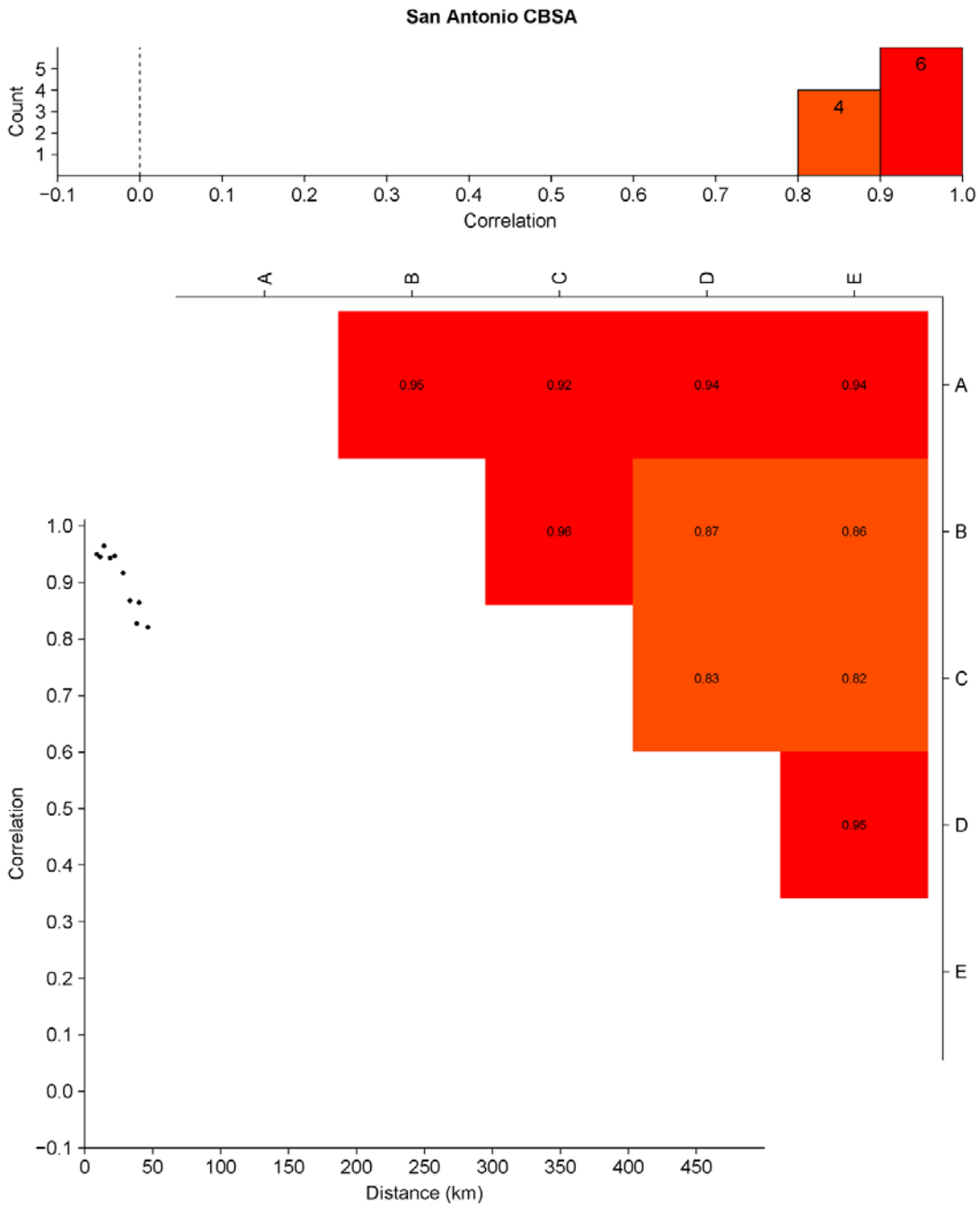

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of R.

**Figure 3-130 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Pittsburgh, Pennsylvania, CSA.**



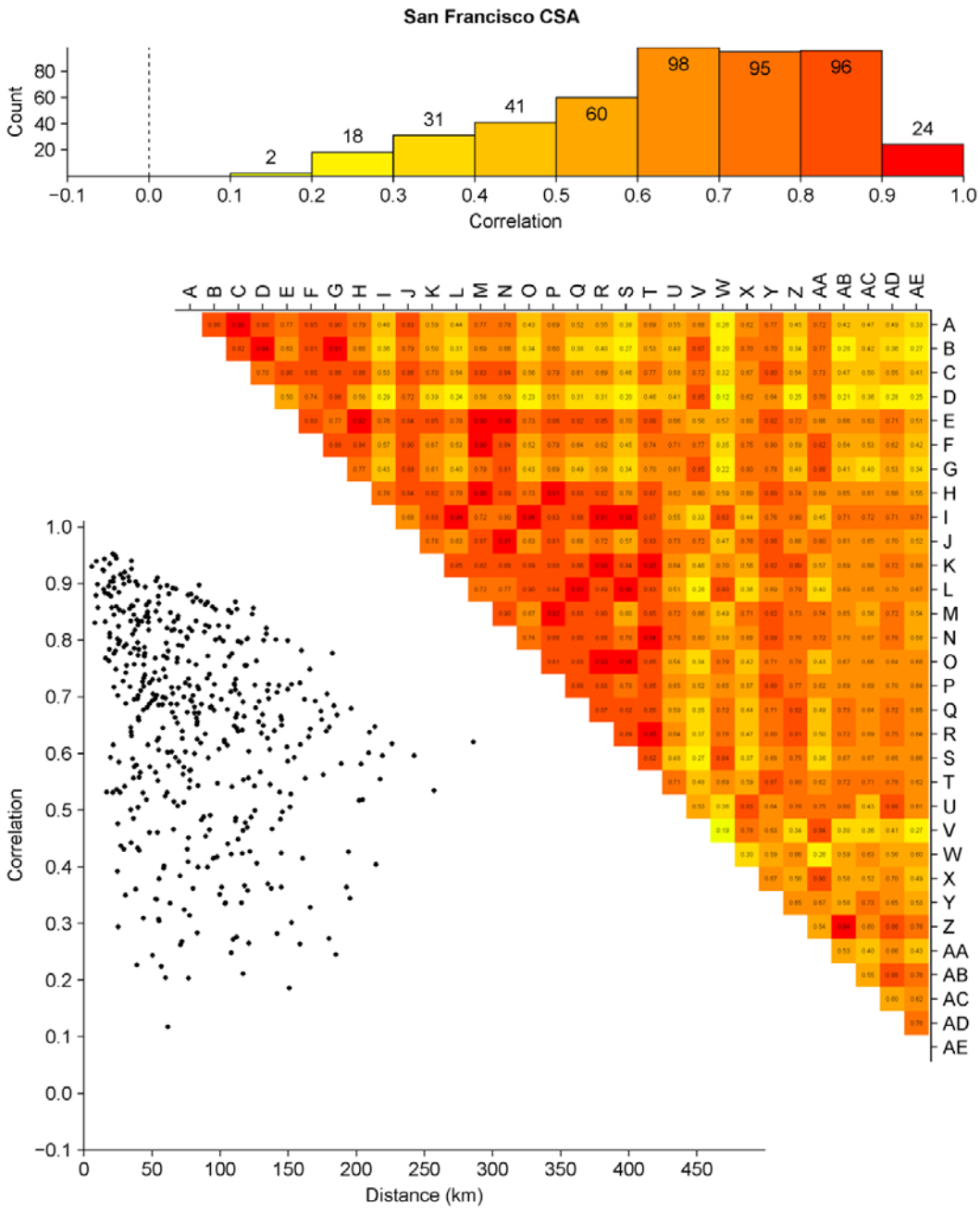

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of R.

**Figure 3-131 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Salt Lake City, Utah, CSA.**



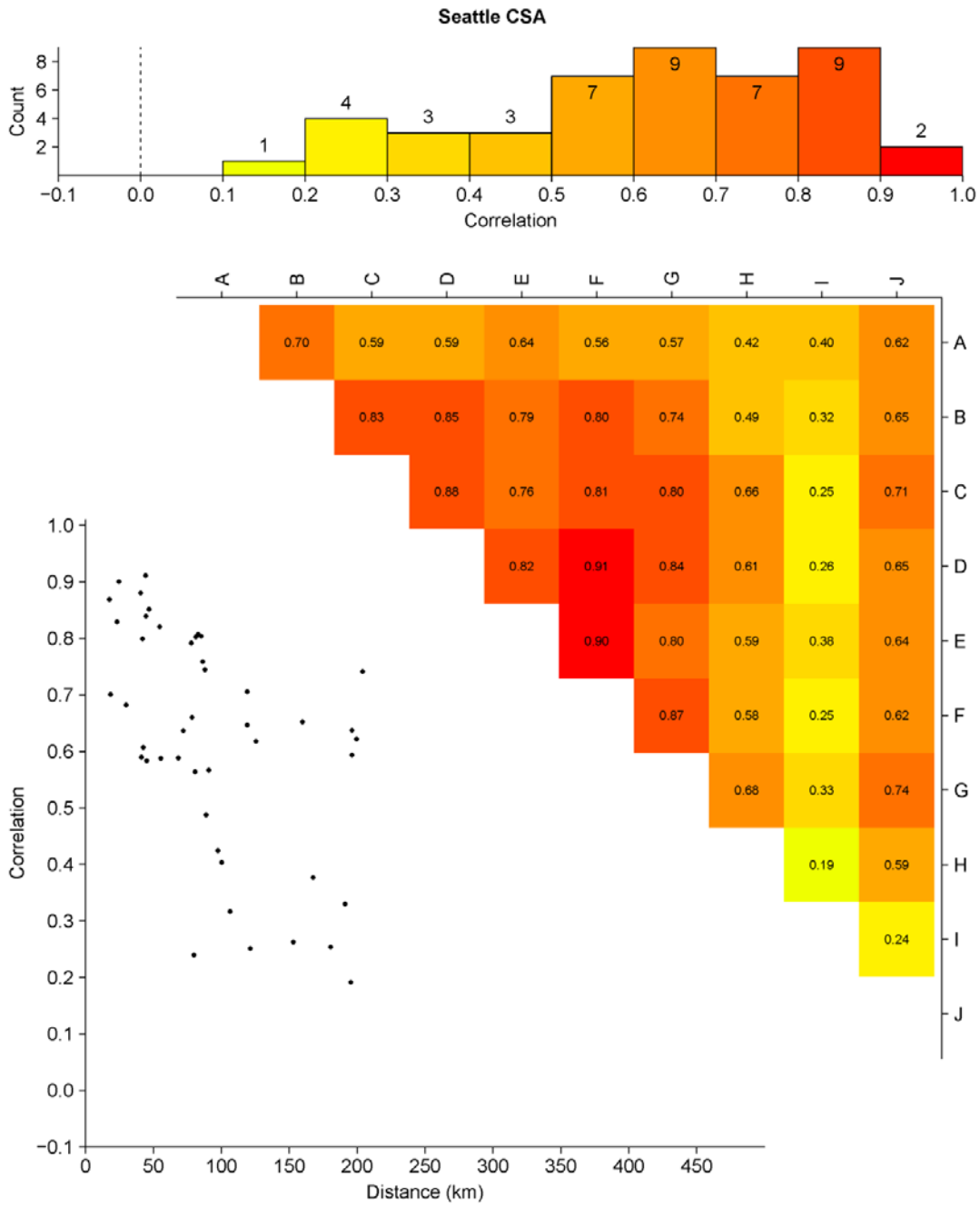

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of R.

**Figure 3-132 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the San Antonio, Texas, CBSA.**



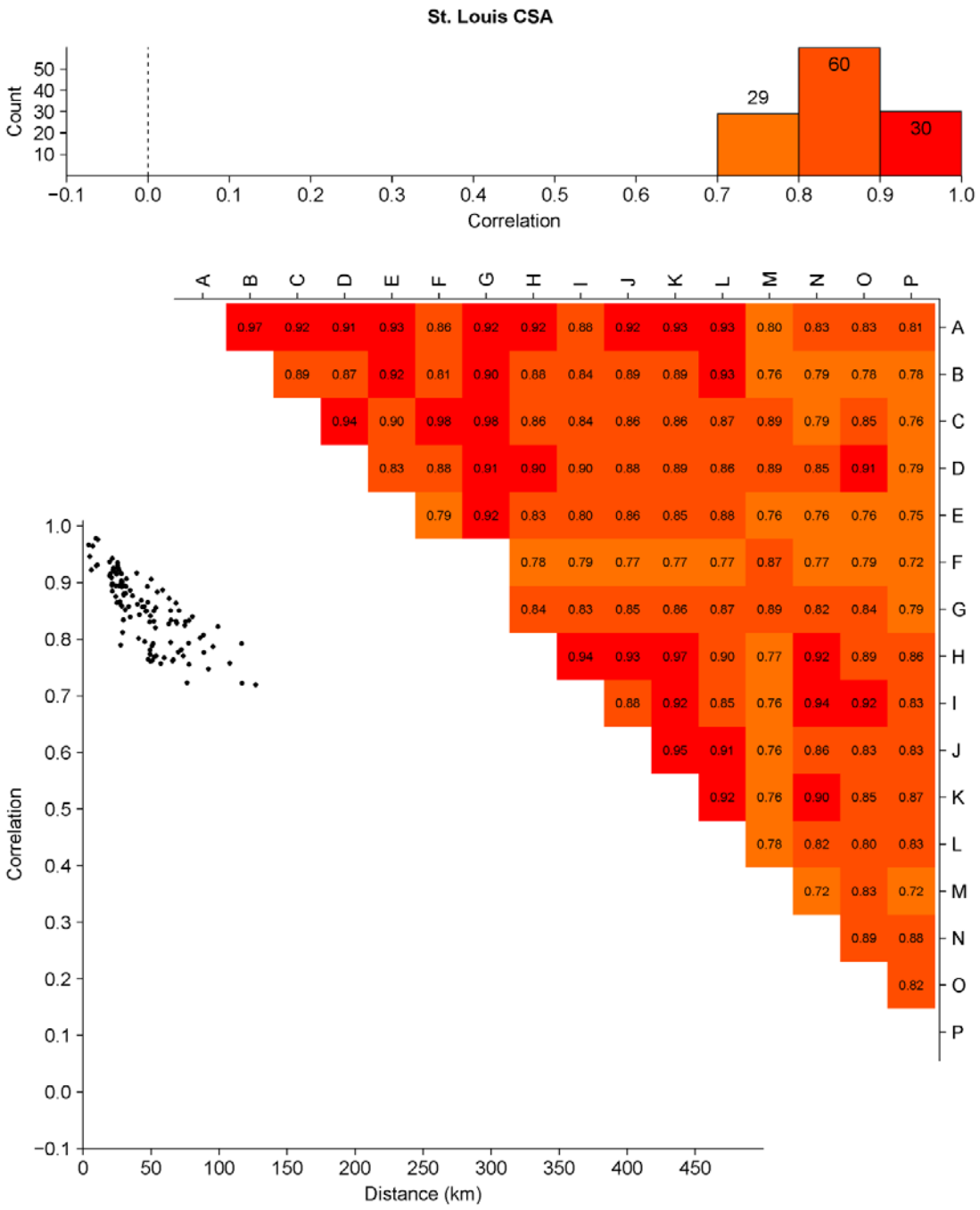

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of R.

**Figure 3-133 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the San Francisco, California, CSA.**



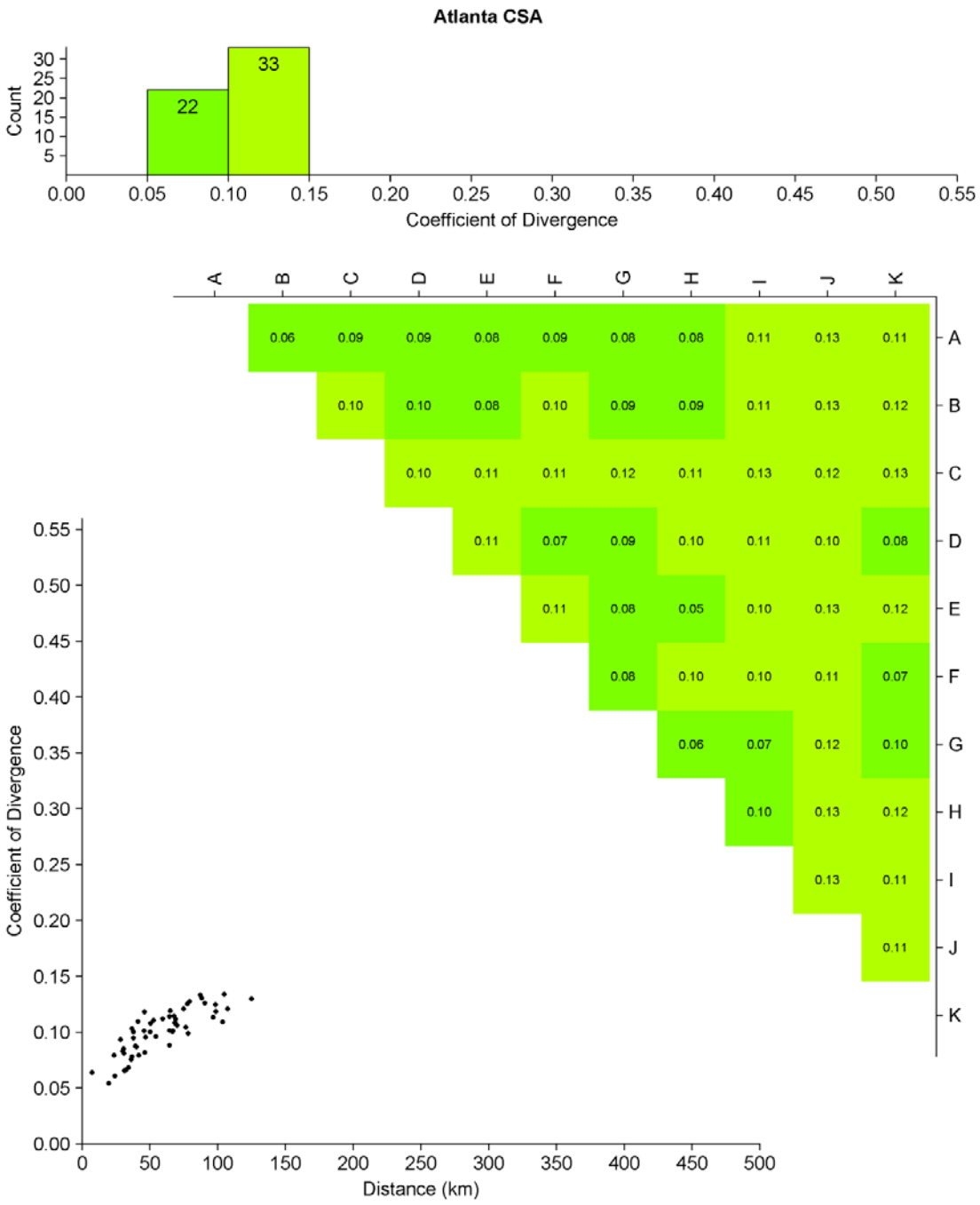

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of R.

**Figure 3-134 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Seattle, Washington, CSA.**



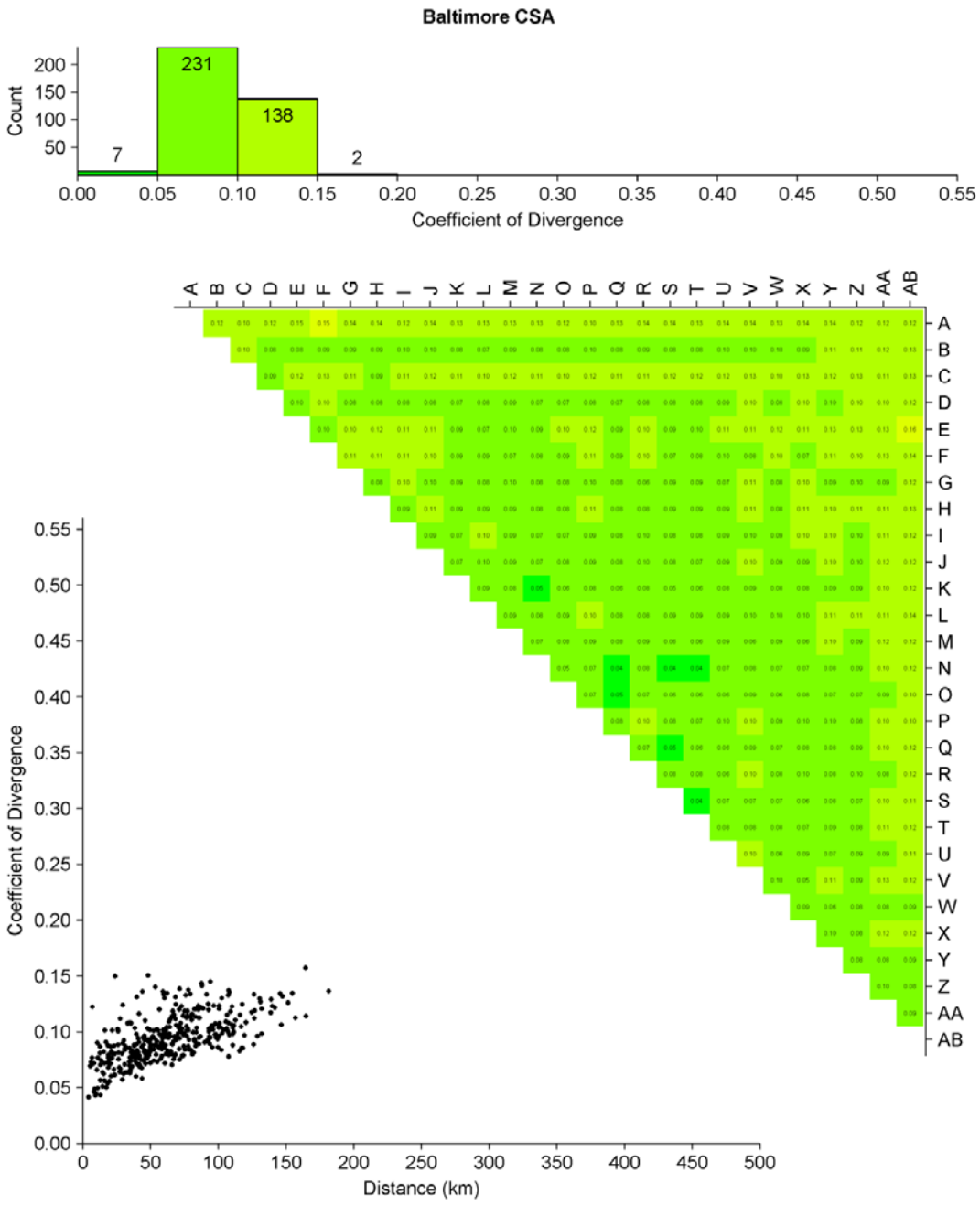

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of R.

**Figure 3-135 Pair-wise monitor correlations expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the St. Louis, Missouri, CSA.**



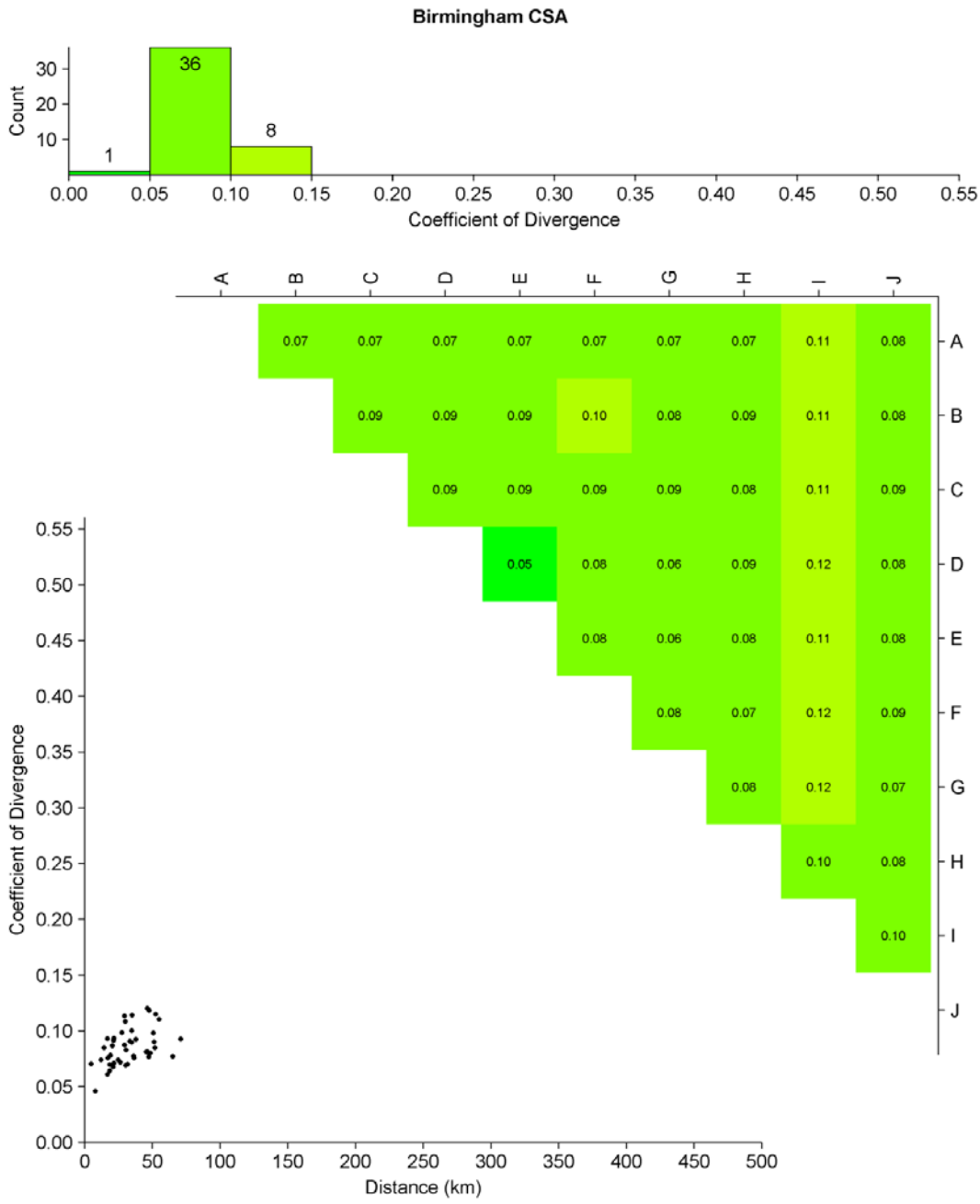

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of COD.

**Figure 3-136 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Atlanta, Georgia, CSA.**



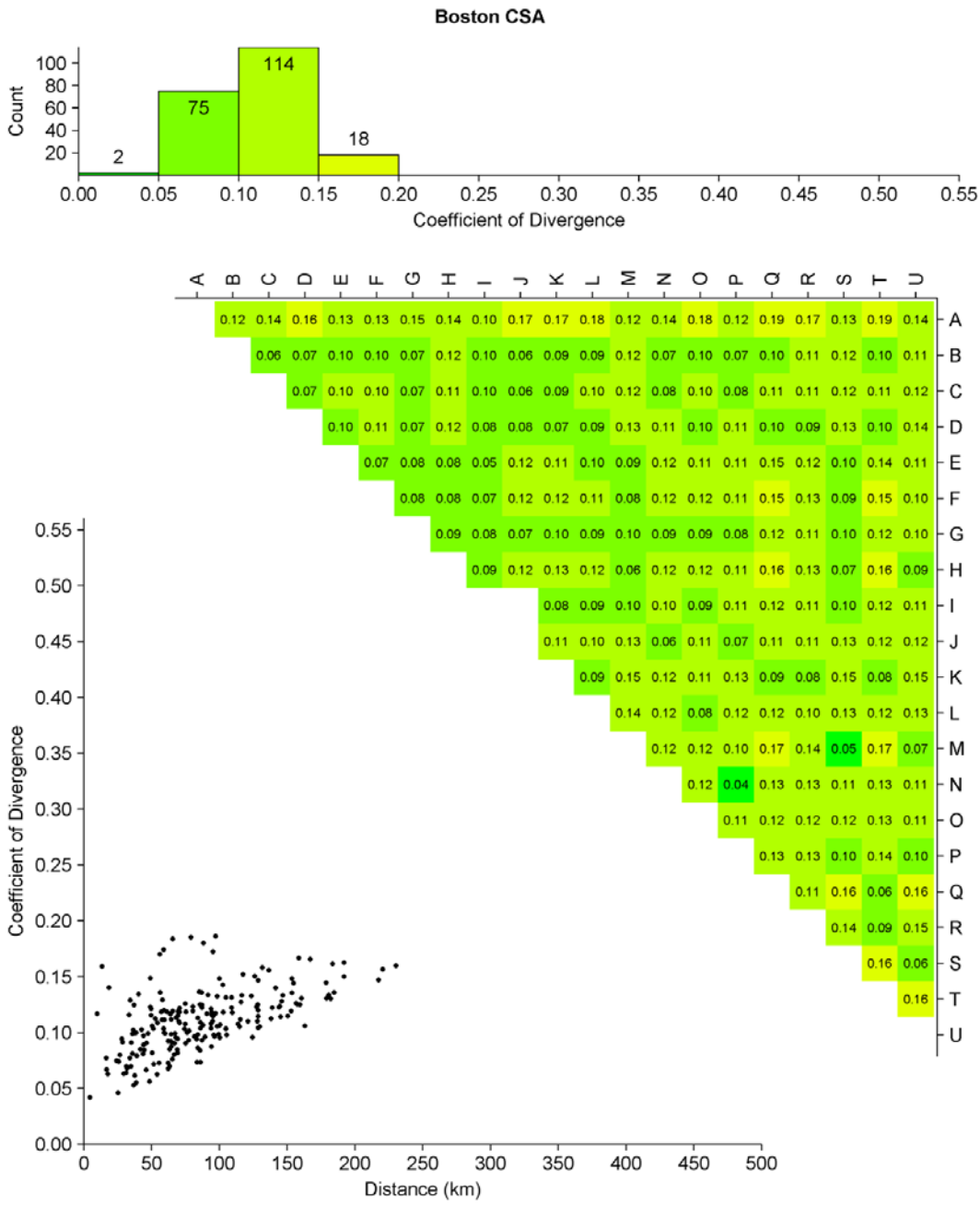

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of COD.

**Figure 3-137 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Baltimore, Maryland, CSA.**



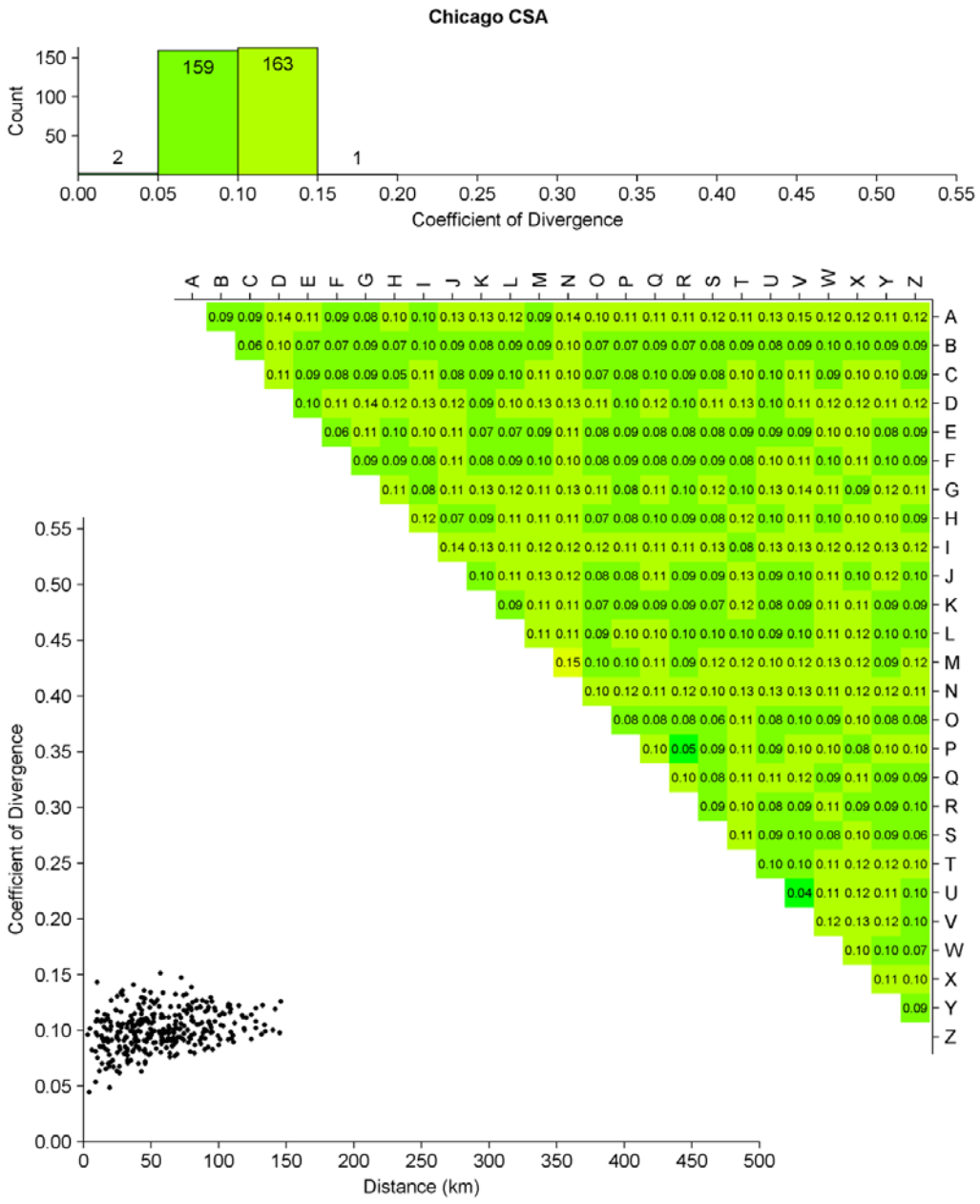

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of COD.

**Figure 3-138 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Birmingham, Alabama, CSA.**



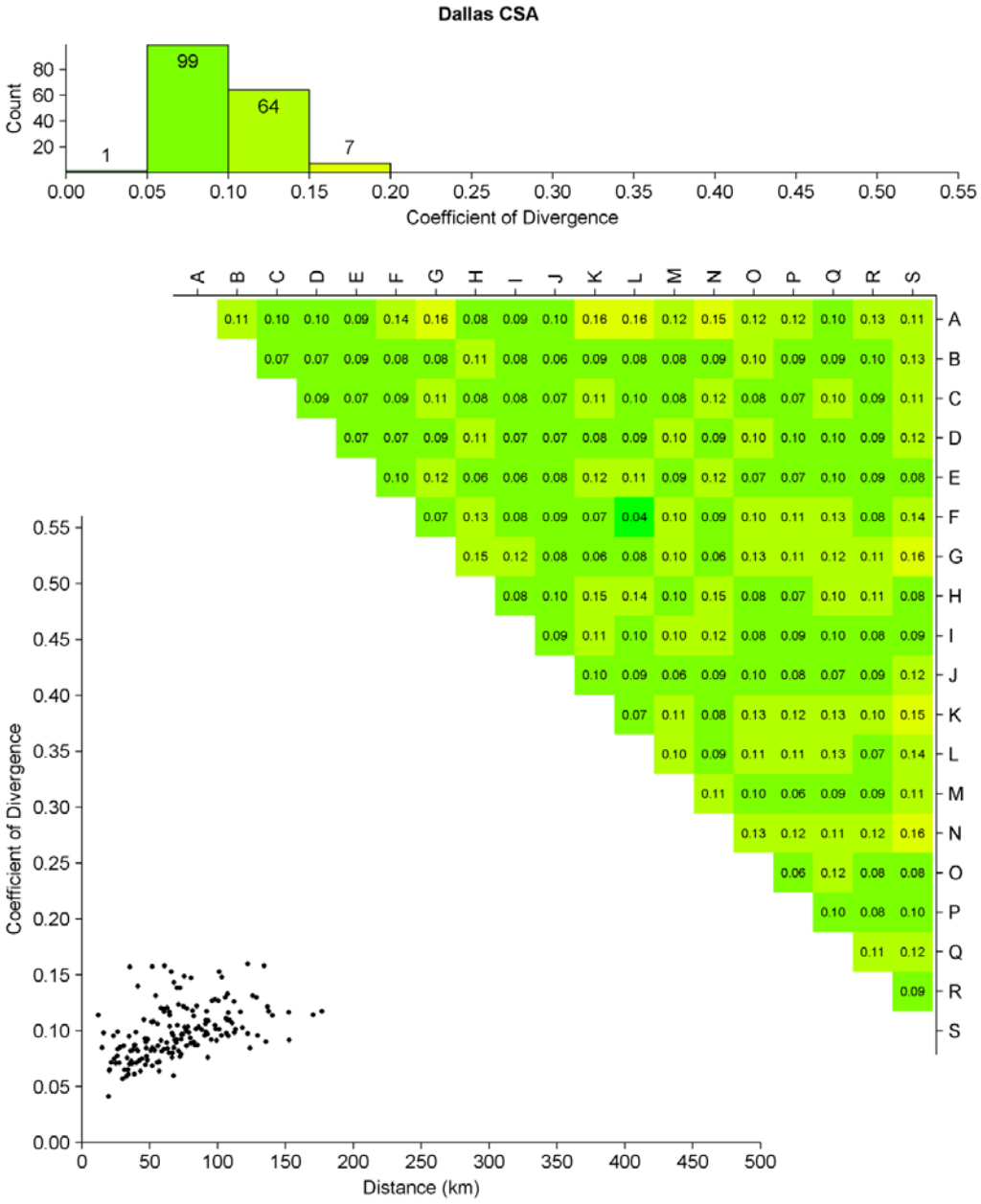

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of COD.

**Figure 3-139 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Boston, Massachusetts, CSA.**



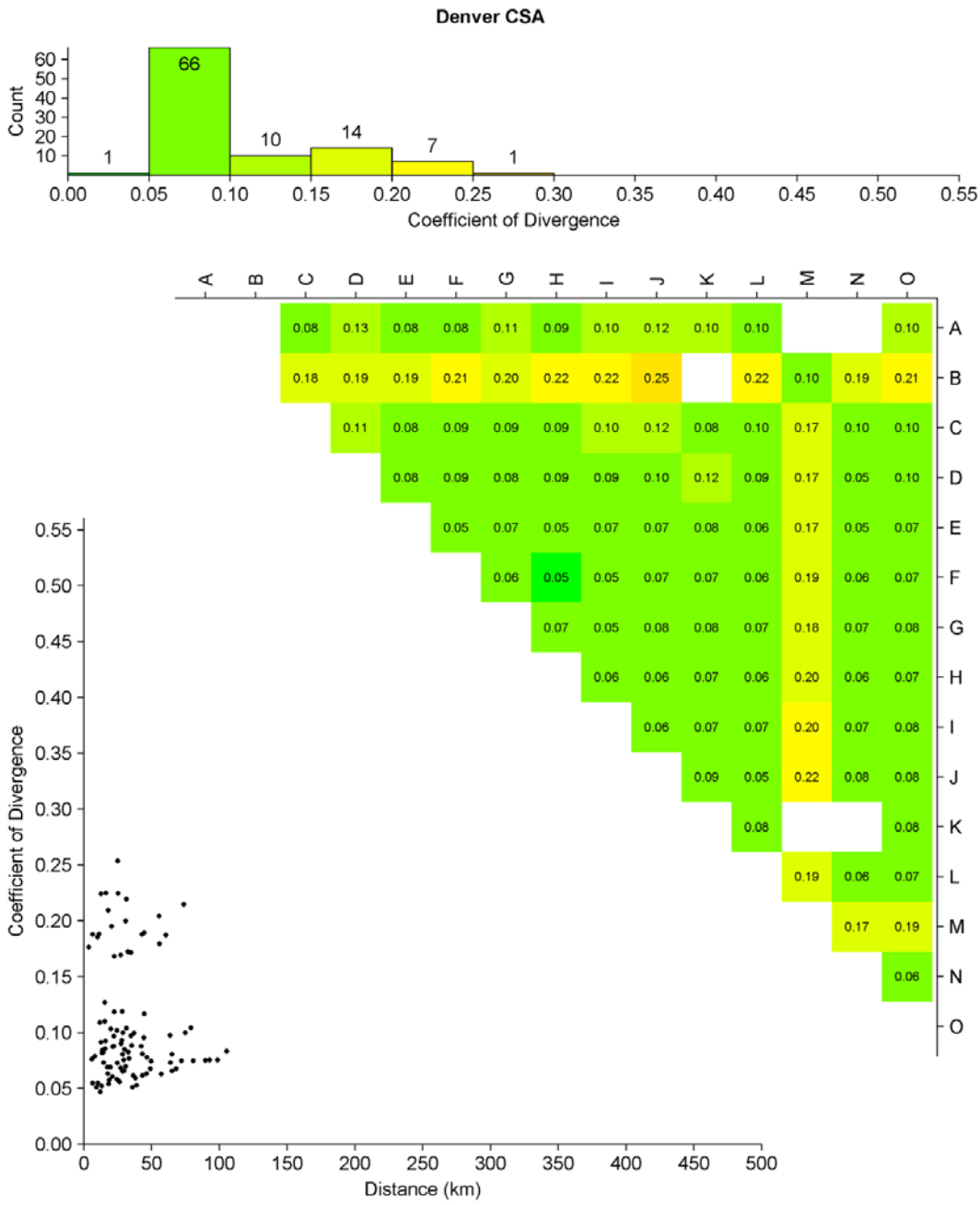

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of COD.

**Figure 3-140 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Chicago, Illinois, CSA.**



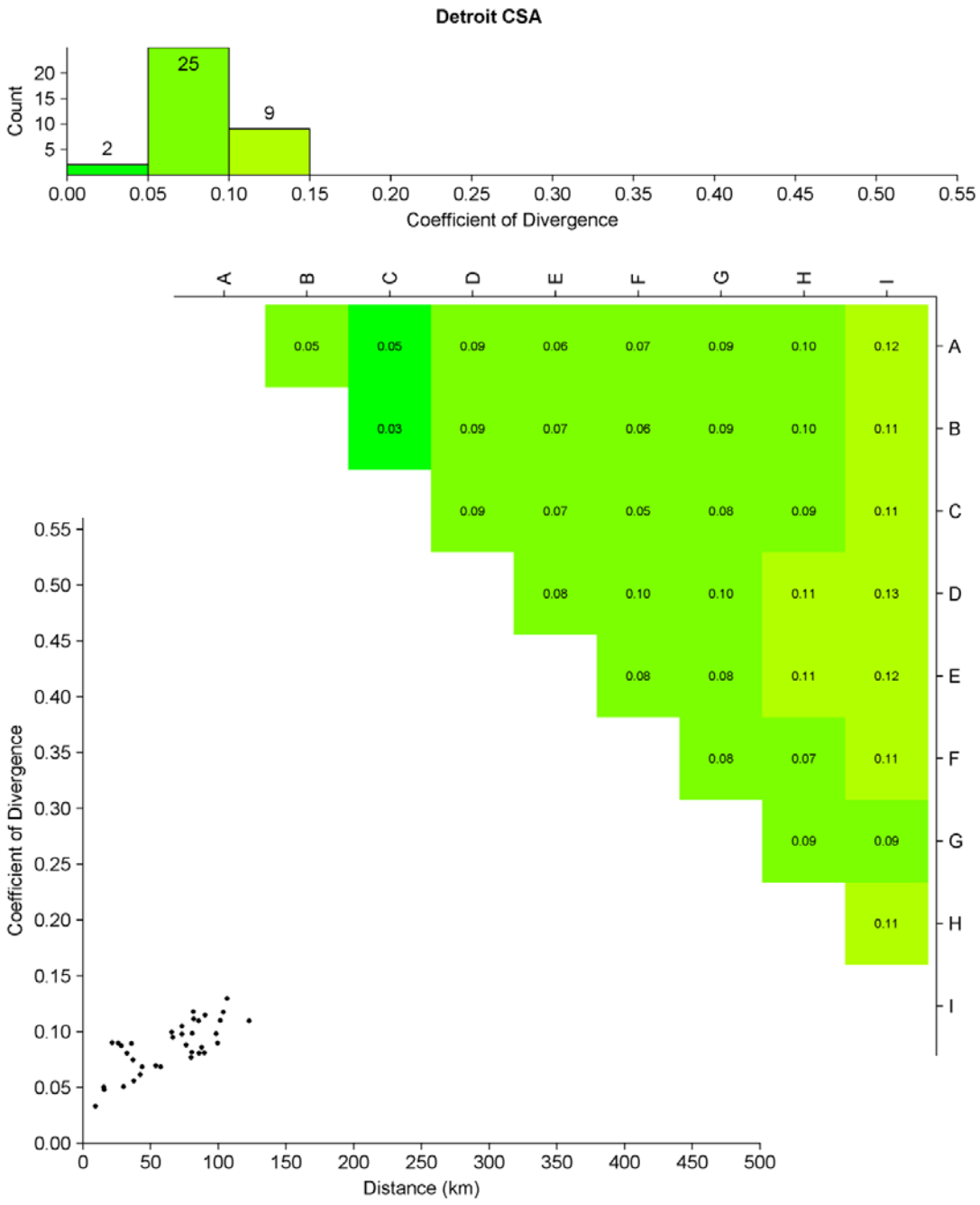

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of COD.

**Figure 3-141 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Dallas, Texas, CSA.**



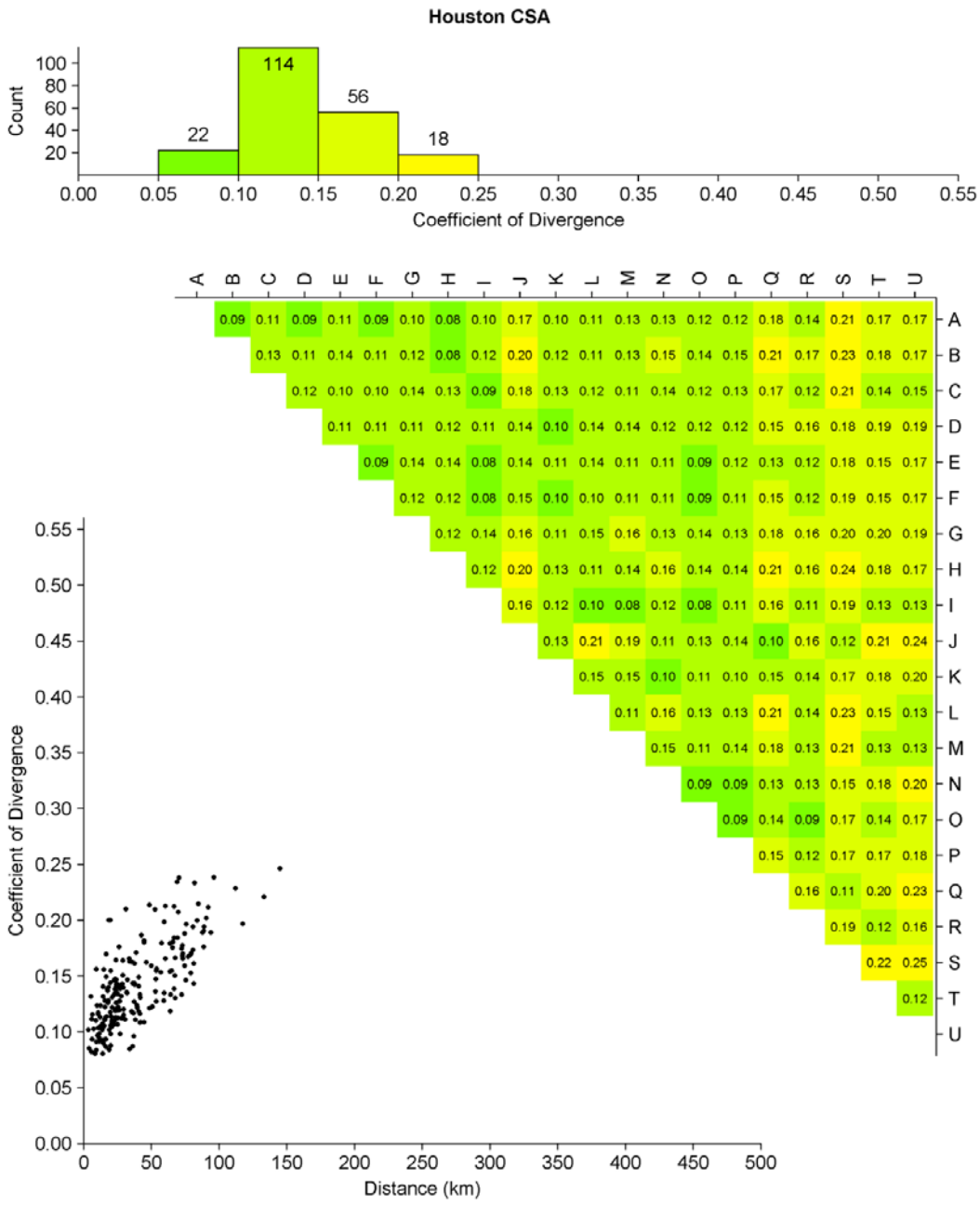

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of COD.

**Figure 3-142 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Denver, Colorado, CSA.**



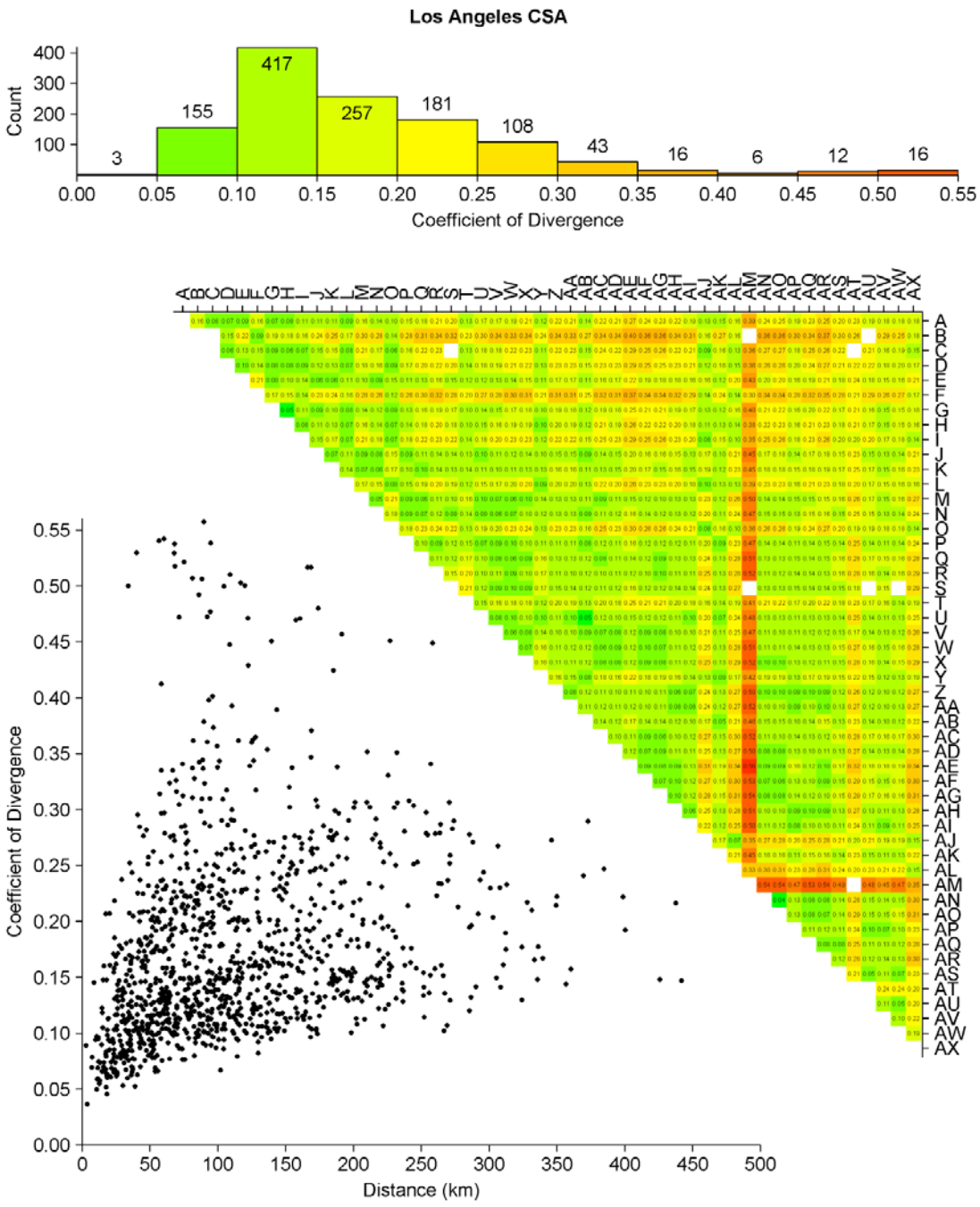

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of COD.

**Figure 3-143 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Detroit, Michigan, CSA.**



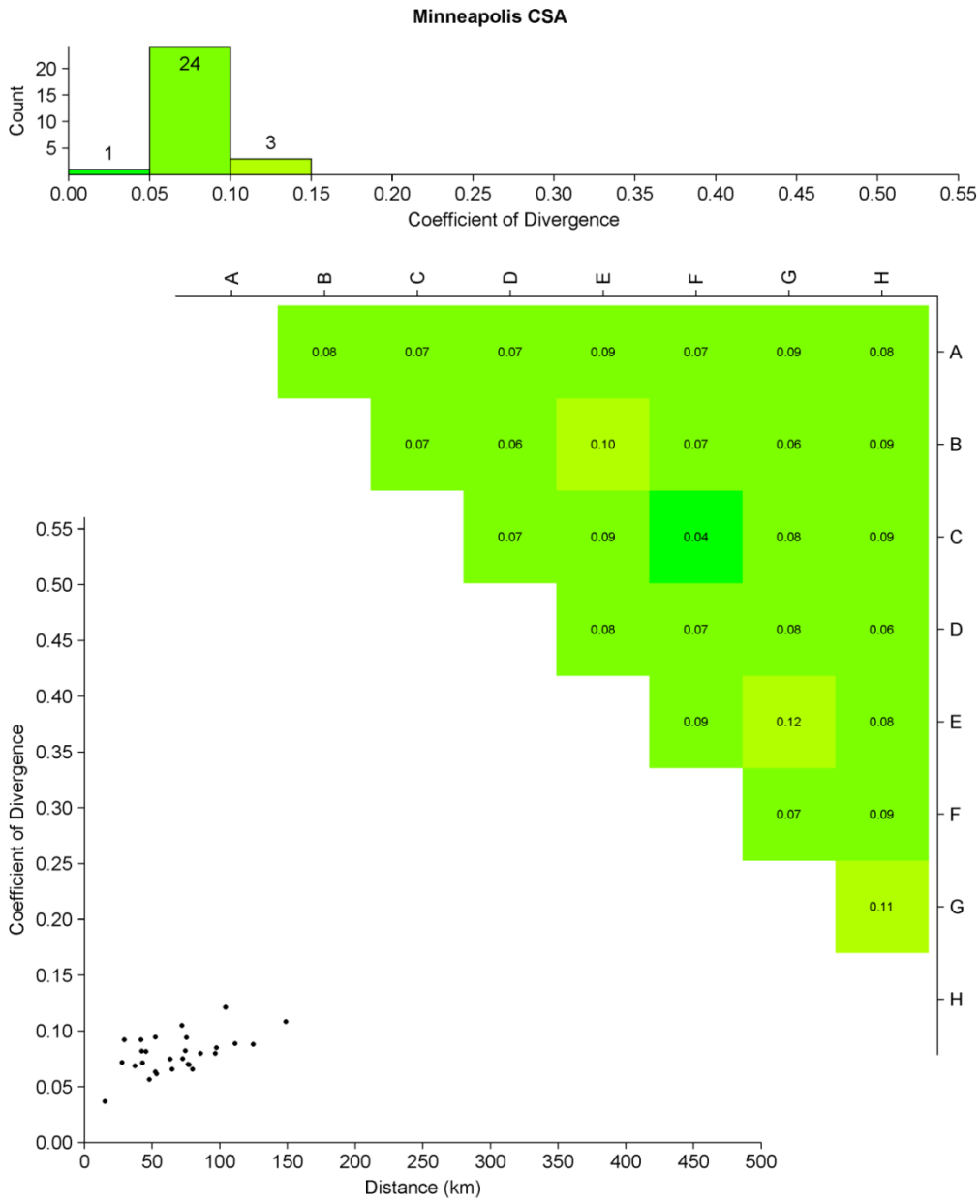

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of COD.

**Figure 3-144 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Houston, Texas, CSA.**



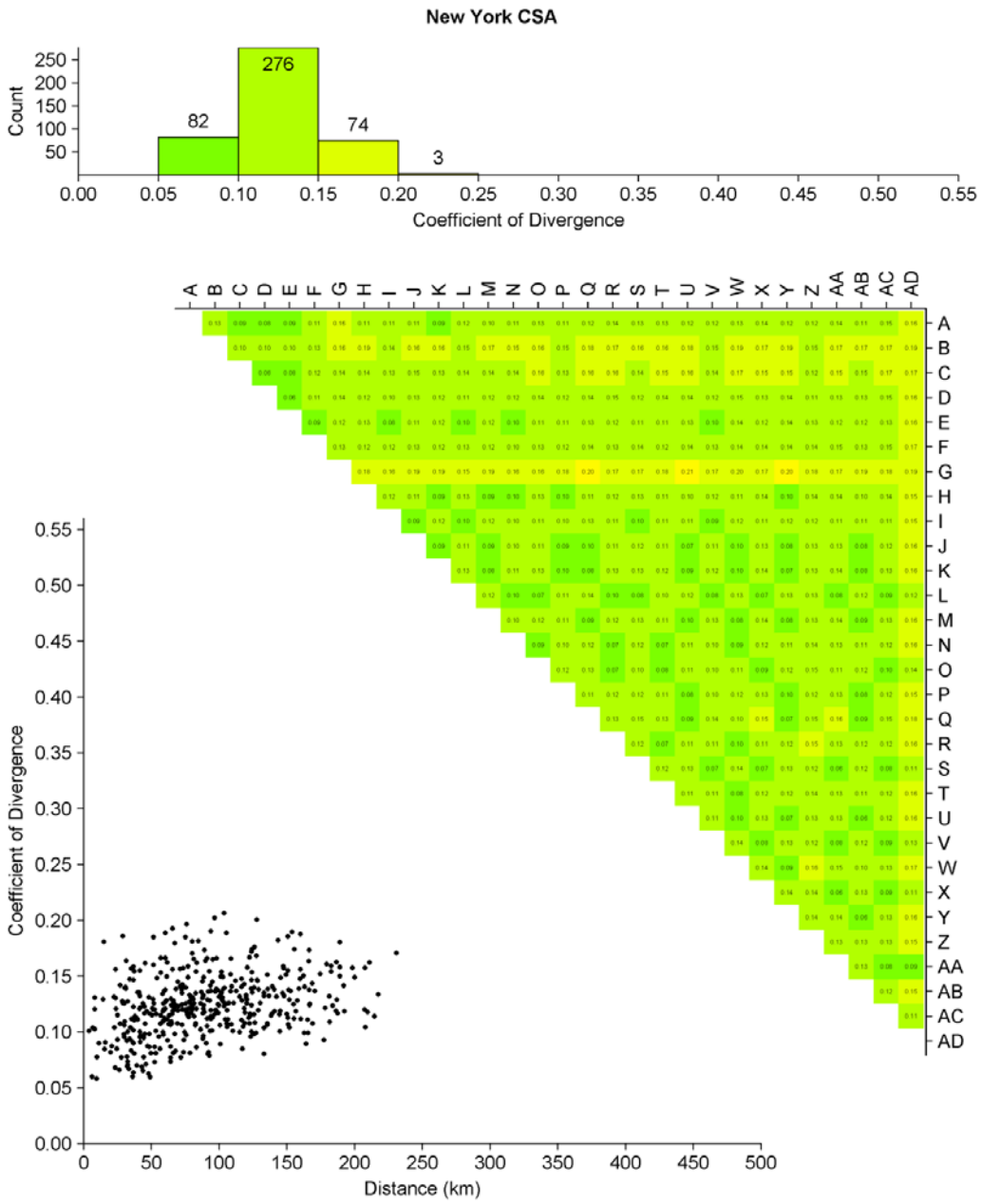

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of COD.

**Figure 3-145 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Los Angeles, California, CSA.**



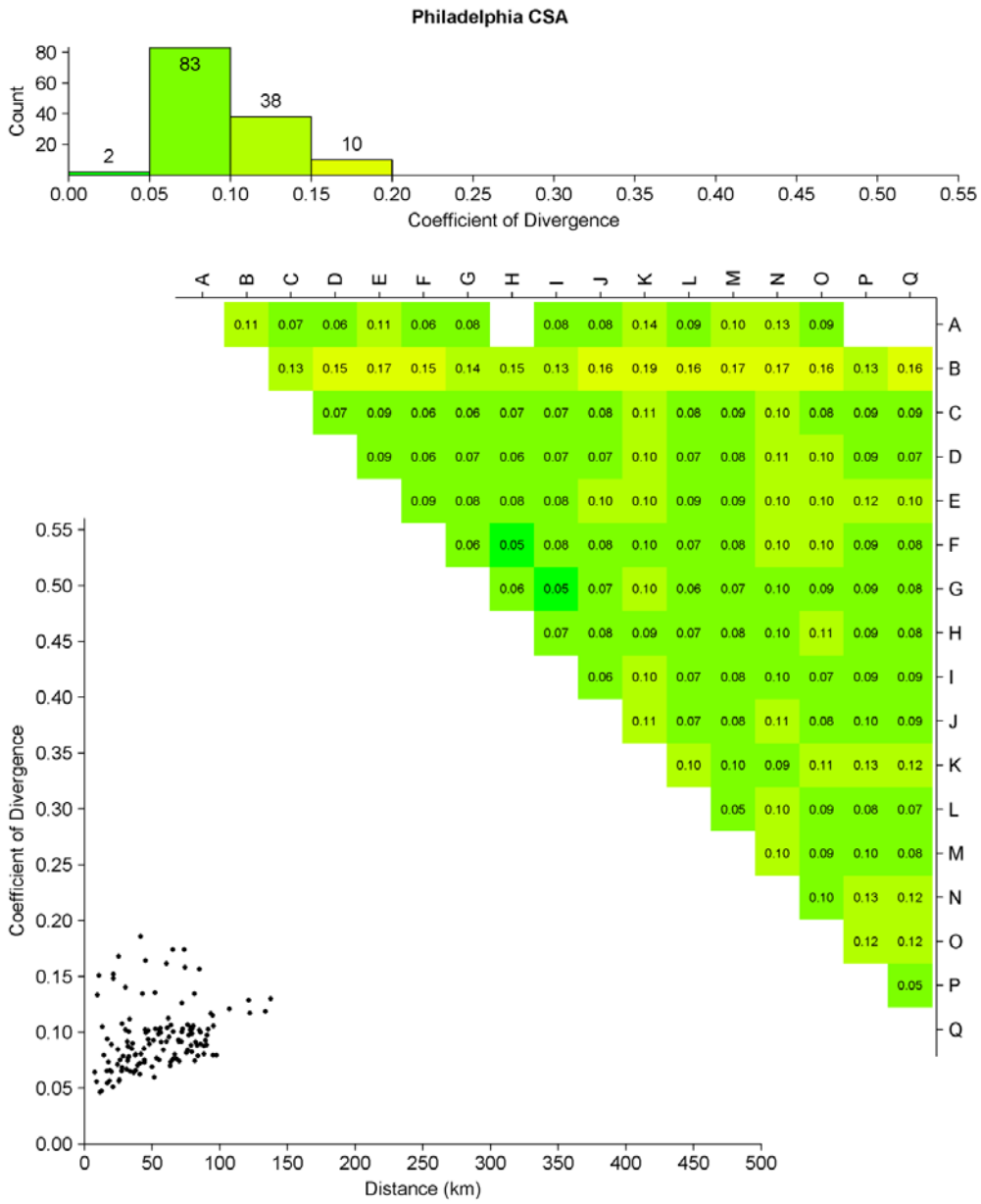

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of COD.

**Figure 3-146 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Minneapolis, Minnesota, CSA.**



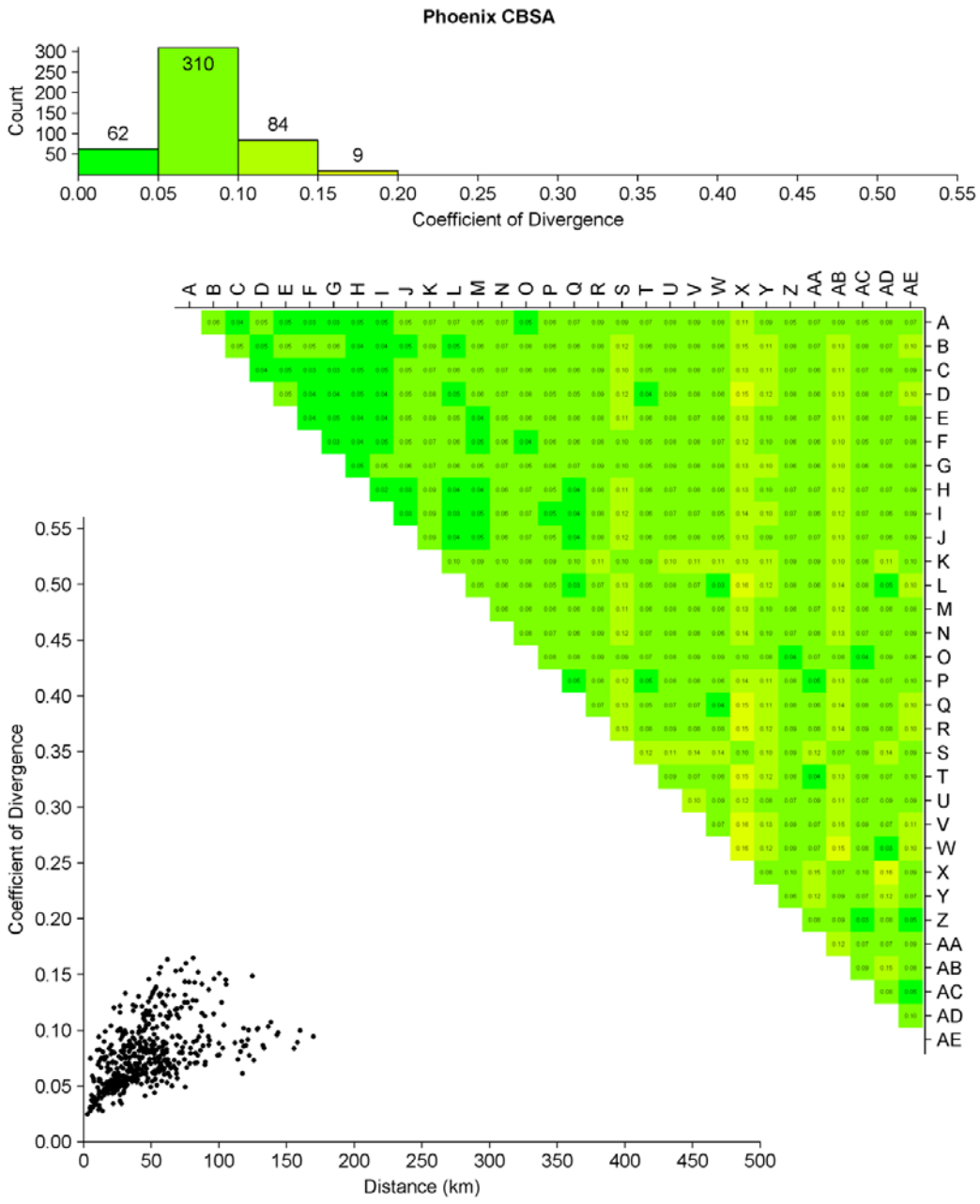

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of COD.

**Figure 3-147 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the New York City, New York, CSA.**



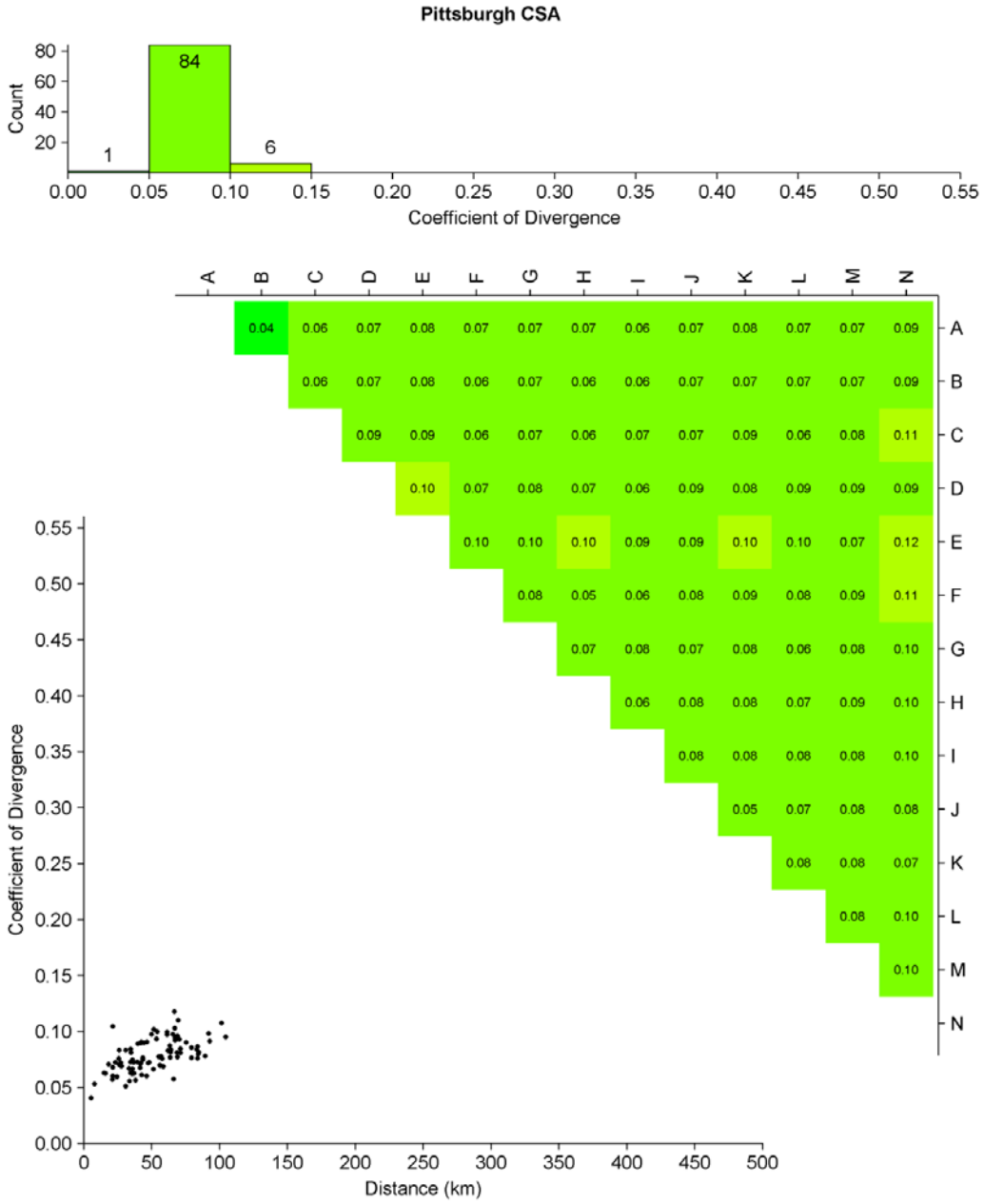

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of COD.

**Figure 3-148 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Philadelphia, Pennsylvania, CSA.**



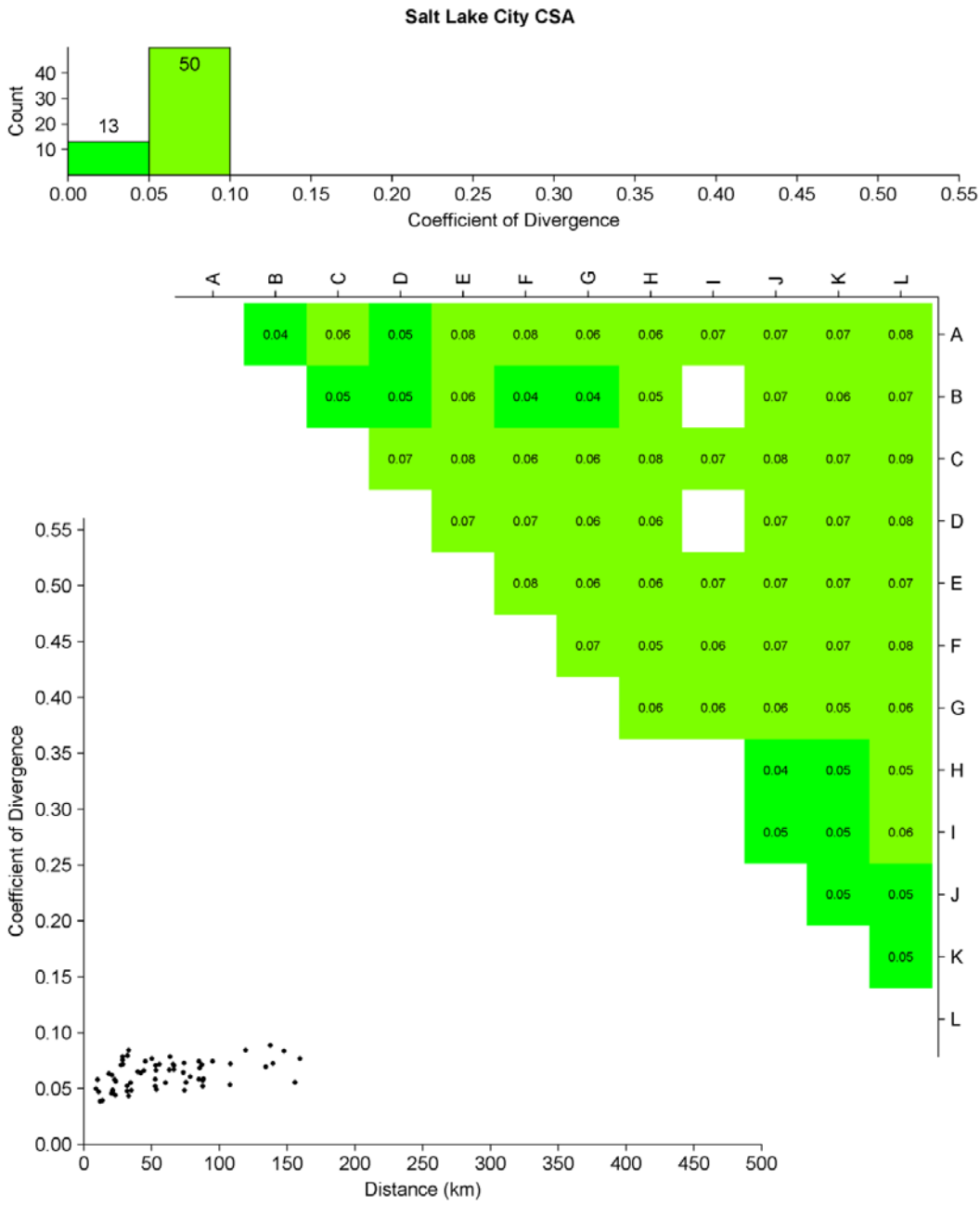

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of COD.

**Figure 3-149 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Phoenix. Arizona, CBSA.**



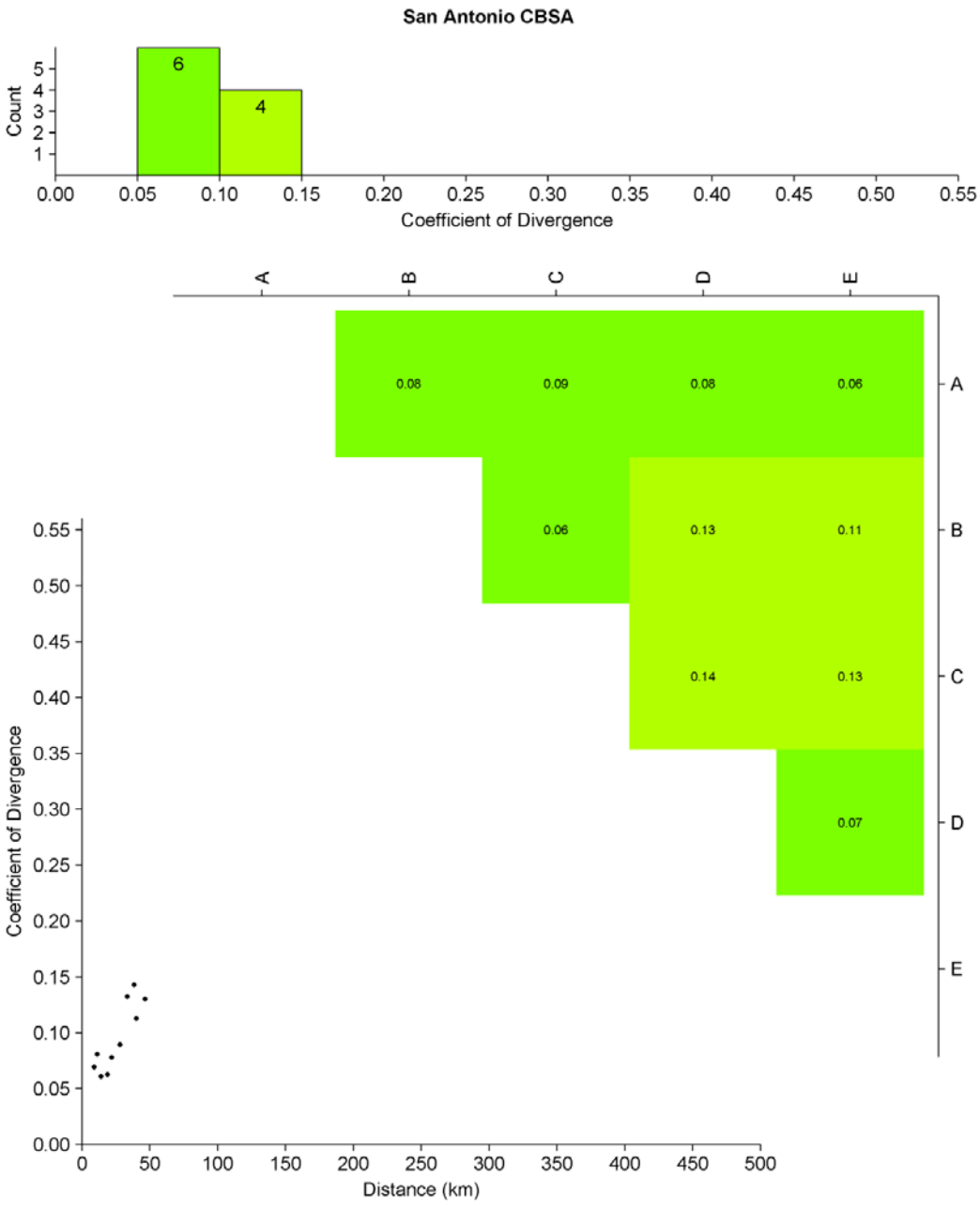

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of COD.

**Figure 3-150 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Pittsburgh, Pennsylvania, CSA.**



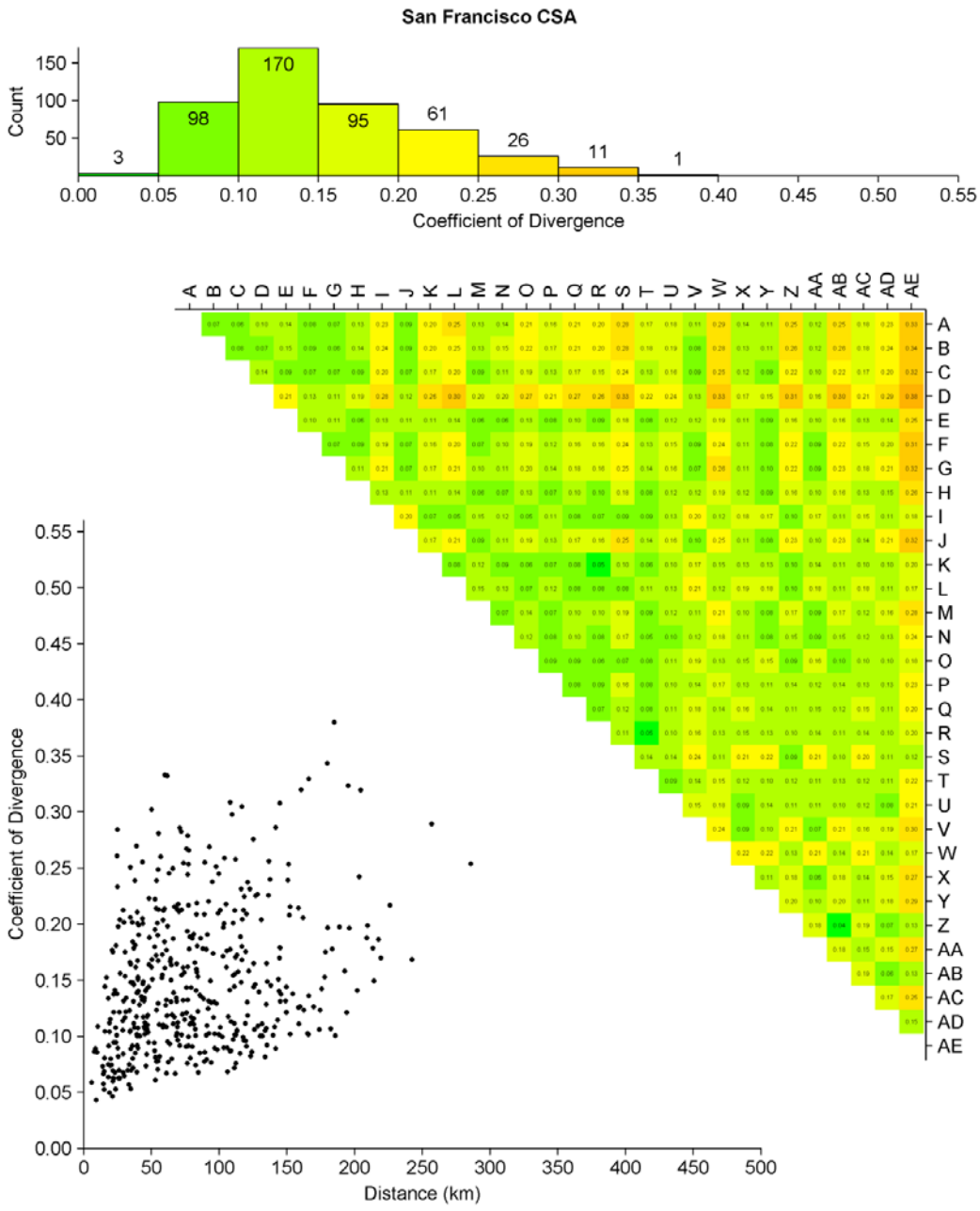

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of COD.

**Figure 3-151 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Salt Lake City, Utah, CSA.**



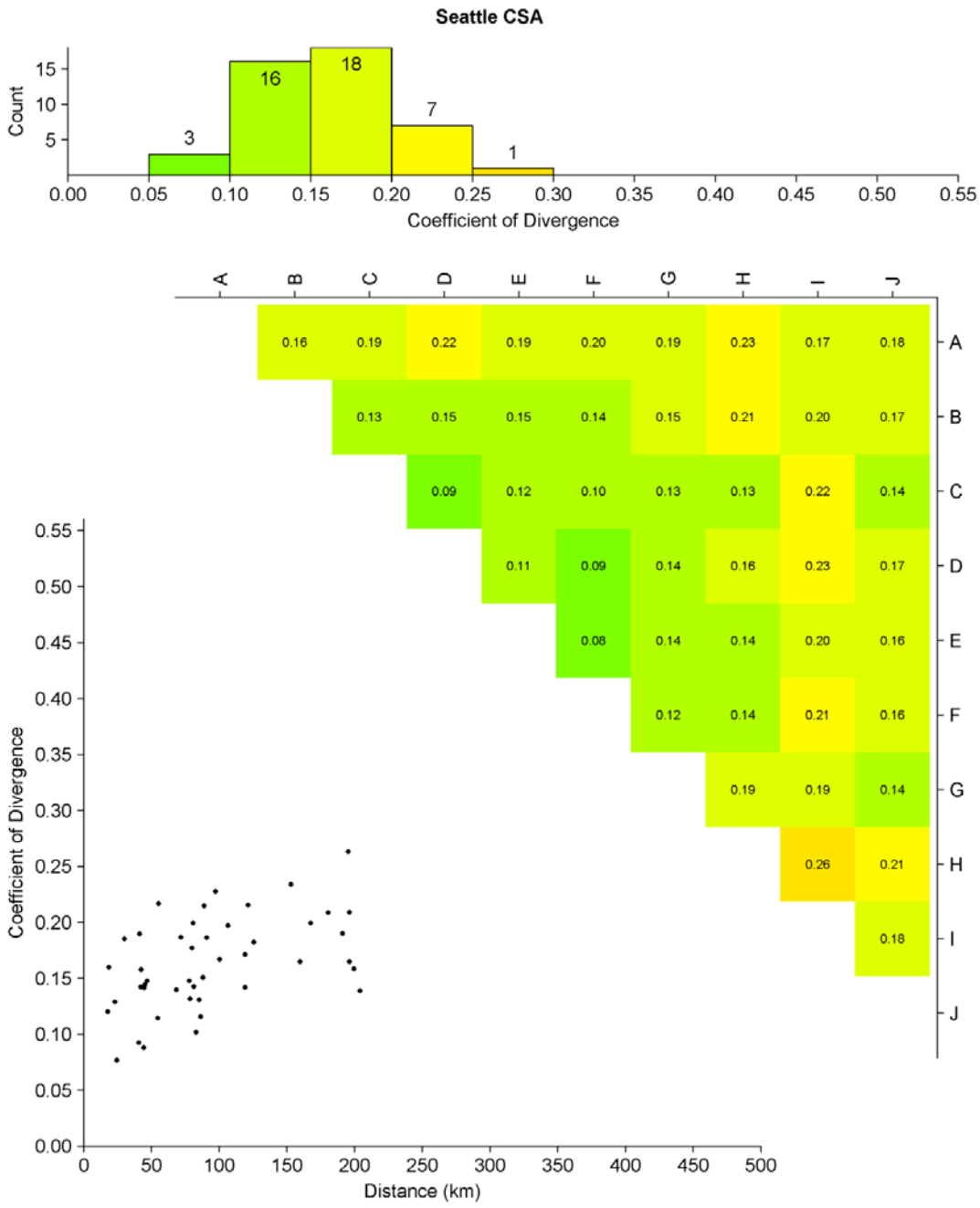

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of COD.

**Figure 3-152 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the San Antonio, Texas, CBSA.**



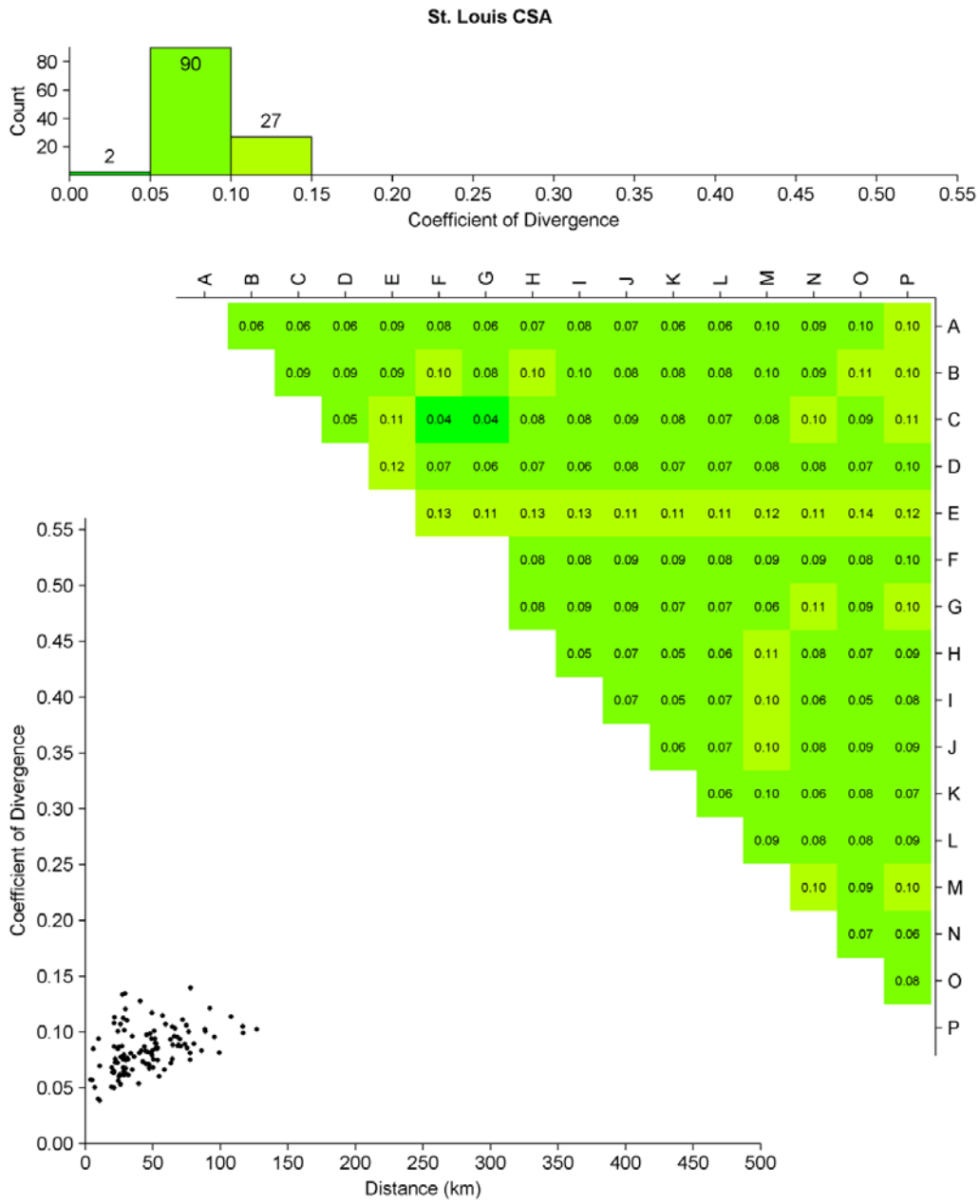

Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of COD.

**Figure 3-153 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the San Francisco, California, CSA.**




Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of COD.

**Figure 3-154 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the Seattle, Washington, CSA.**

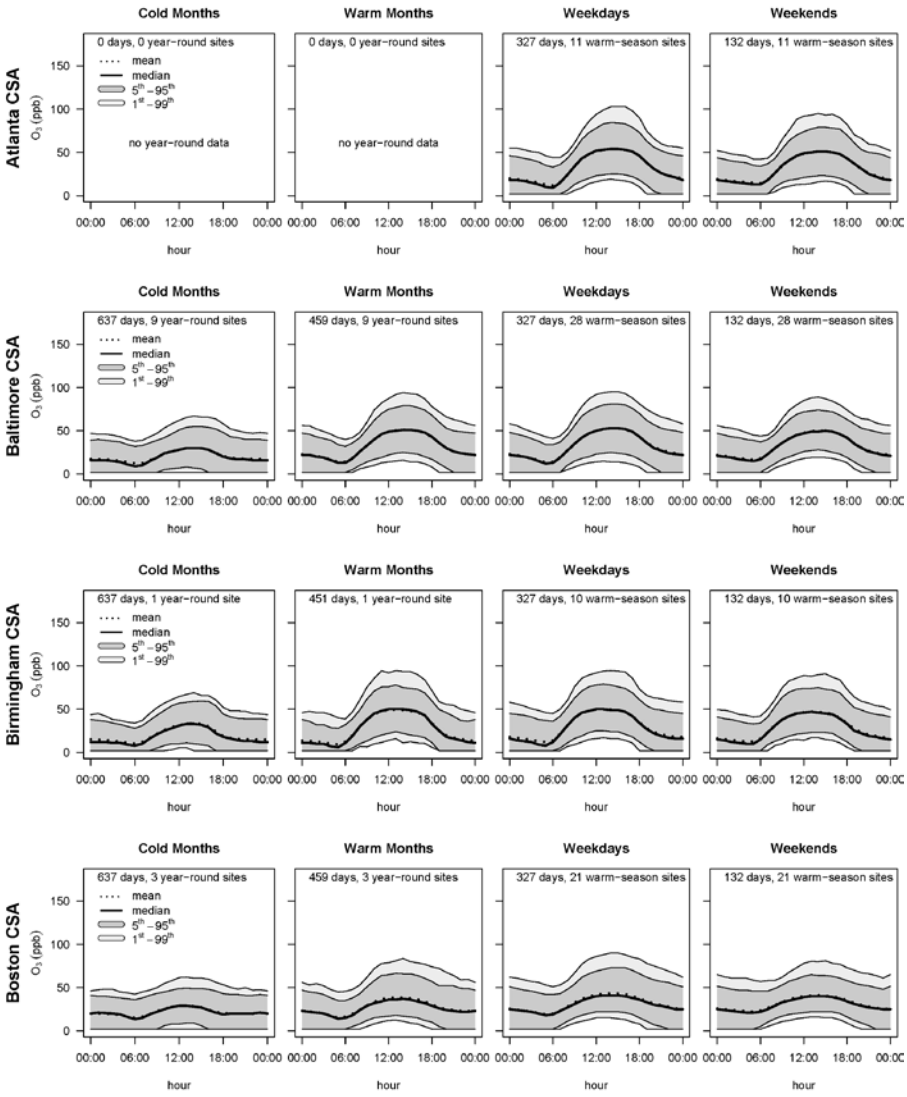



Note: The colors in the histogram bins correspond to the levels of the contour matrix. The histogram includes the number of monitor pairs per bin and the contour matrix includes the numeric values of COD.

**Figure 3-155 Pair-wise monitor coefficient of divergence expressed as a histogram (top), contour matrix (middle) and scatter plot versus distance between monitors (bottom) for 8-h daily max $O_3$ in the St. Louis, Missouri, CSA.**

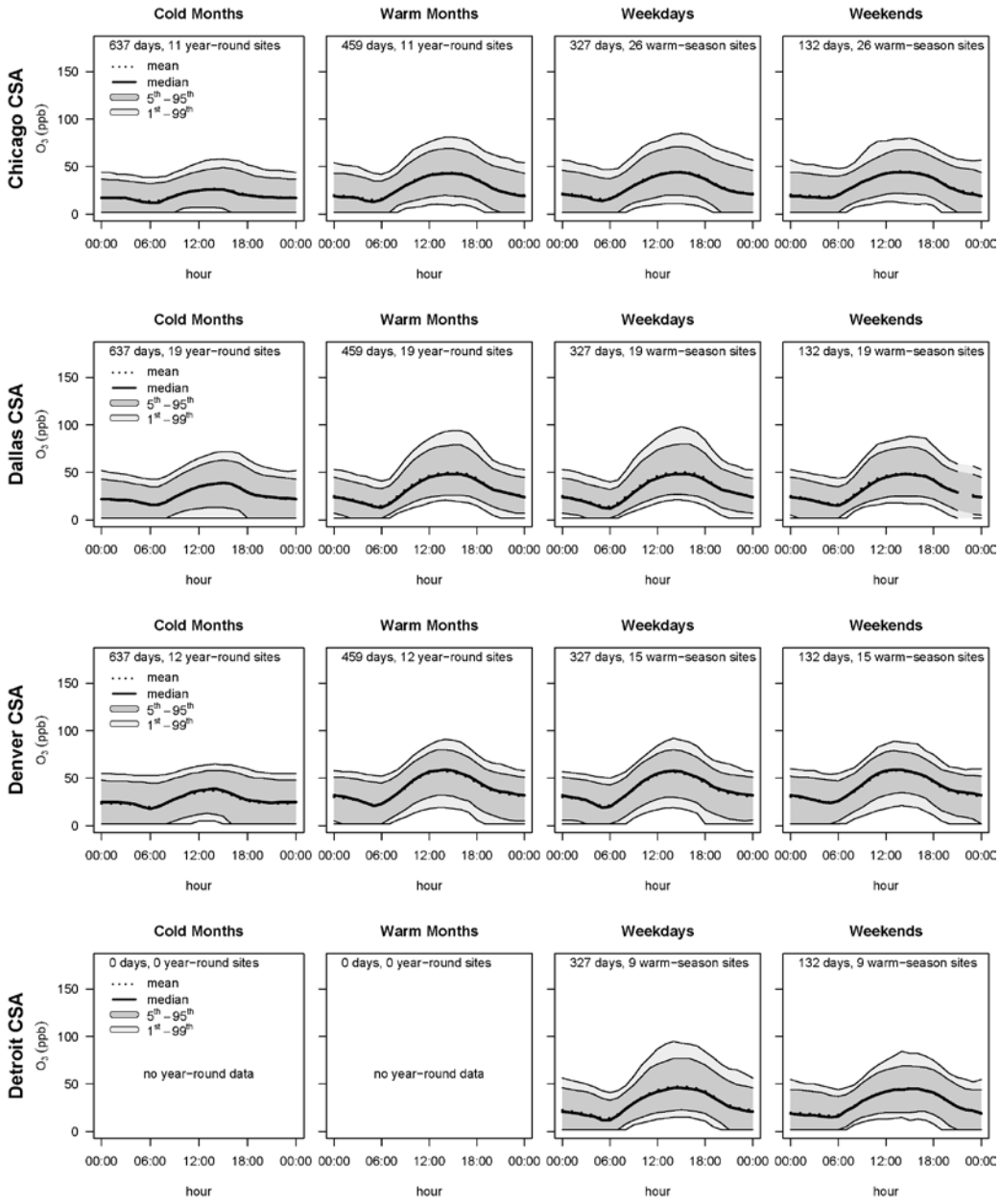
### 3.9.4 Hourly Variations in $O_3$ for the Urban Focus Cities

This section contains diel plots of 1-h avg $O_3$ data to supplement the discussion on hourly variations in $O_3$ concentrations from Section 3.6.3.2 using data from the 20 urban focus cities first introduced in Section 3.6.2.1. Comparisons are made between cold months (October-April) and warm months (May-September), using the year-round data set, and between weekdays (Mon-Fri) and weekends (Sat-Sun) using the warm-season data set.




Note: No year-round monitors were available for the cold month/warm month comparison in the Atlanta CSA.

**Figure 3-156 Diel patterns in 1-h avg $O_3$ for select CSAs between 2007 and 2009 using the year-round data set for the cold month/warm month comparison (left half) and the warm-season data set for the weekday/weekend comparison (right half).**




Note: No year-round monitors were available for the cold month/warm month comparison in the Detroit CSA.

**Figure 3-157 Diel patterns in 1-h avg $O_3$ for select CSAs between 2007 and 2009 using the year-round data set for the cold month/warm month comparison (left half) and the warm-season data set for the weekday/weekend comparison (right half).**

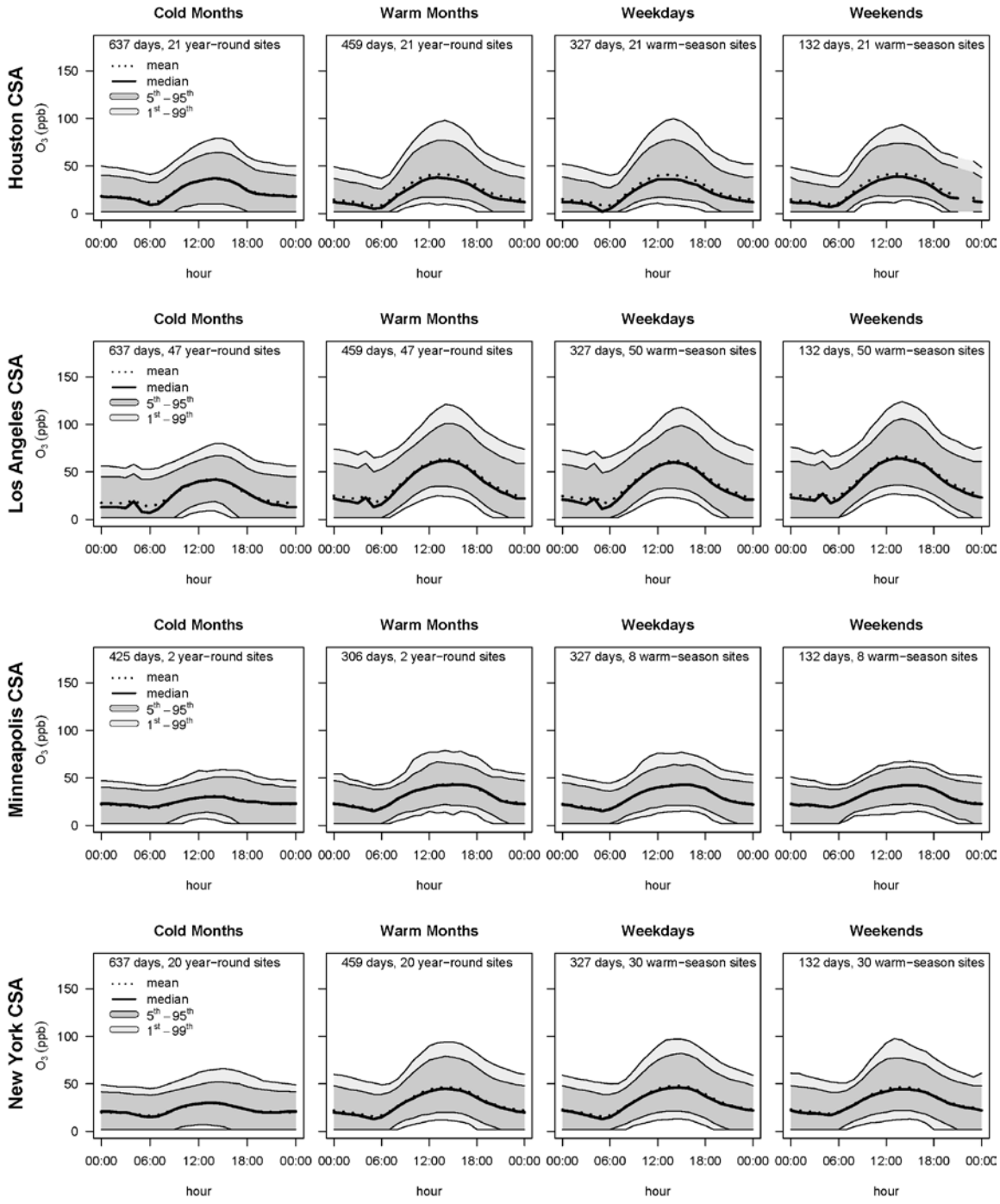



**Figure 3-158 Diel patterns in 1-h avg $O_3$ for select CSAs between 2007 and 2009 using the year-round data set for the cold month/warm month comparison (left half) and the warm-season data set for the weekday/weekend comparison (right half).**

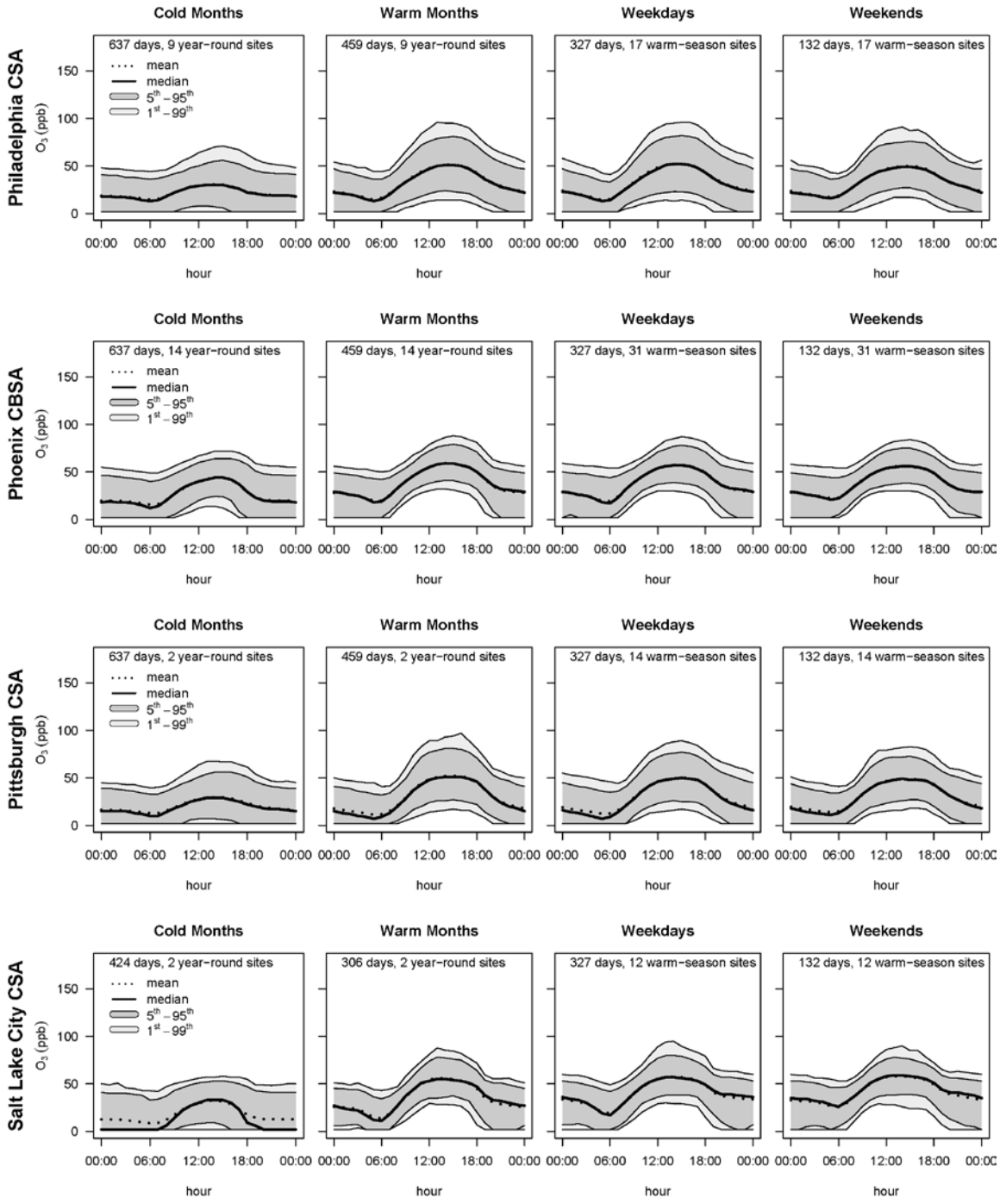



**Figure 3-159 Diel patterns in 1-h avg $O_3$ for select CSAs/CBSAs between 2007 and 2009 using the year-round data set for the cold month/warm month comparison (left half) and the warm-season data set for the weekday/weekend comparison (right half).**

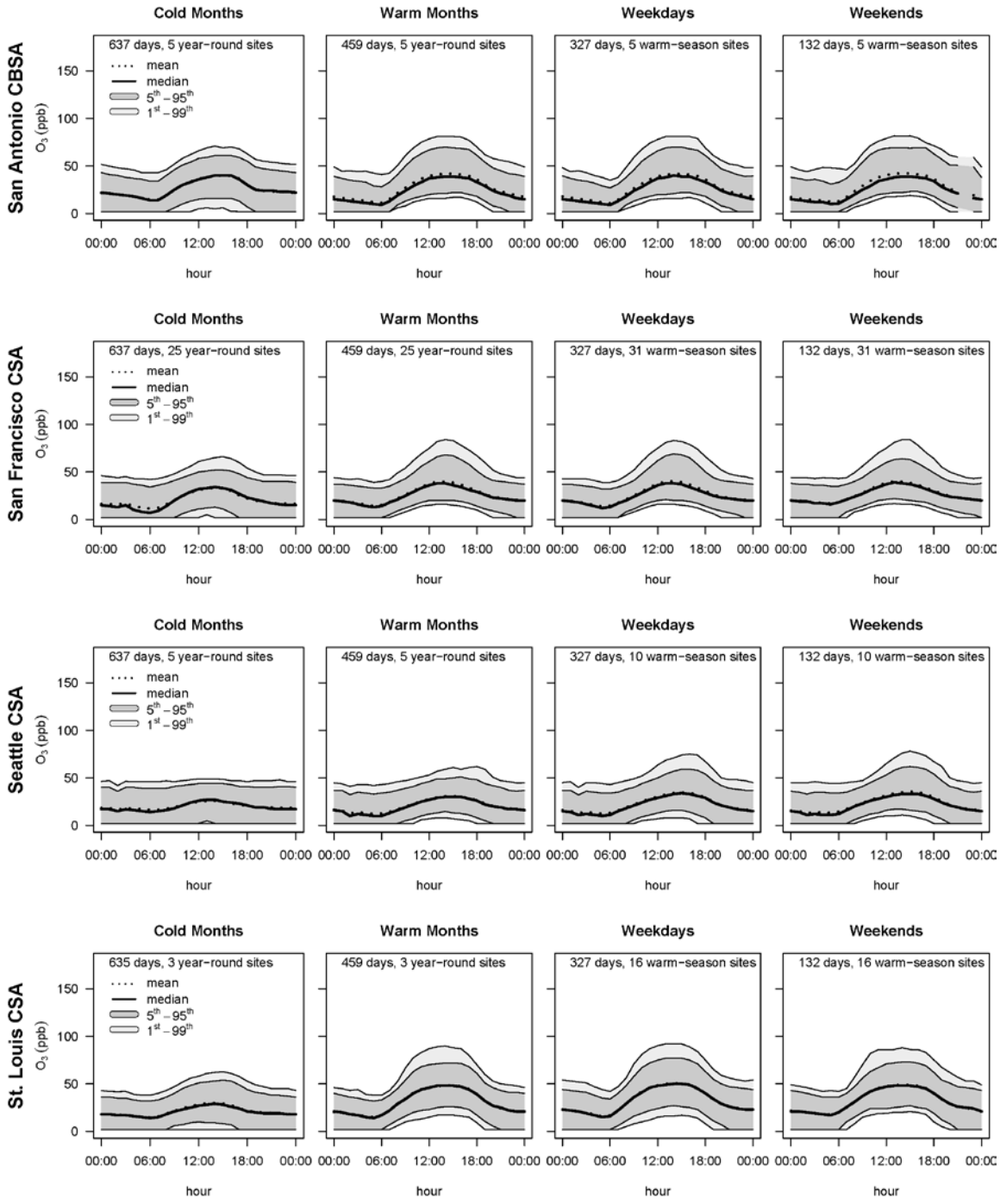



**Figure 3-160 Diel patterns in 1-h avg $O_3$ for select CSAs/CBSAs between 2007 and 2009 using the year-round data set for the cold month/warm month comparison (left half) and the warm-season data set for the weekday/weekend comparison (right half).**

## References


Acker, K; Febo, A; Trick, S; Perrino, C; Bruno, P; Wiesen, P; Möller; Wieprecht, W; Auel, R; Giusto, M; Geyer, A; Platt, U; Allegrini, I. (2006). Nitrous acid in the urban area of Rome. Atmos Environ 40: 3123-3133. http://dx.doi.org/10.1016/j.atmosenv.2006.01.028

Andreae, MO. (1991). Biomass burning: its history, use, and distribution and its impact on environmental quality and global climate. In JS Levine (Ed.), Global Biomass Burning: Atmospheric, Climatic, and Biospheric Implications (pp. 1-21). Cambridge, MA: MIT Press.

Andreae, MO; Merlet, P. (2001). Emission of trace gases and aerosols from biomass burning. Global Biogeochem Cycles 15: 955. http://dx.doi.org/10.1029/2000GB001382

Antón, M; López, M; Vilaplana, JM; Kroon, M; McPeters, R; Bañón, M; Serrano, A. (2009). Validation of OMI-TOMS and OMI-DOAS total ozone column using five Brewer spectroradiometers at the Iberian peninsula. J Geophys Res 114: D14307. http://dx.doi.org/10.1029/2009JD012003

Appel, KW; Gilliland, A; Eder, B. (2005). An operational evaluation of the 2005 release of Models-3 CMAQ version 45. Presentation presented at 4th Annual CMAS Models-3 Users' Conference, September 26-28, 2005, Chapel Hill, NC.

Archibald, AT; Cooke, MC; Utembe, SR; Shallcross, DE; Derwent, RG; Jenkin, ME. (2010). Impacts of mechanistic changes on HOx formation and recycling in the oxidation of isoprene. Atmos Chem Phys 10: 8097-8118. http://dx.doi.org/10.5194/acp-10-8097-2010

Archibald, AT; Levine, JG; Abraham, NL; Cooke, MC; Edwards, PM; Heard, DE; Jenkin, ME; Karunaharan, A; Pike, RC; Monks, PS; Shallcross, DE; Telford, PJ; Whalley, LK; Pyle, JA. (2011). Impacts of HOx regeneration and recycling in the oxidation of isoprene: Consequences for the composition of past, present and future atmospheres. Geophys Res Lett 38: L05804. http://dx.doi.org/10.1029/2010GL046520

Arnold, JR; Dennis, RL; Tonnesen, GS. (2003). Diagnostic evaluation of numerical air quality models with specialized ambient observations: testing the Community Multiscale Air Quality modeling system (CMAQ) at selected SOS 95 ground sites. Atmos Environ 37: 1185-1198.

Arshinov, MY; Belan, BD; Krasnov, OA; Kovalevskii, VK; Pirogov, VA; Plotnikov, AP; Tolmachev, GN; Fofonov, AV. (2002). Comparison of ultraviolet and chemiluminescent ozonometers. Atmos Ocean 15: 656-658.

Arunachalam, S. (2009). Peer review of source apportionment tools in CAMx and CMAQ. (Contract No EP-D-07-102). University of North Carolina Institute for the Environment, prepared for US Environmental Protection Agency. http://www.epa.gov/scram001/reports/SourceApportionmentPeerReview.pdf

ATMET (Atmospheric, Meteorological, and Environmental Technologies). (2011). Atmospheric, meteorological, and environmental technologies. Available online at http://atmet.com/ (accessed January 28, 2011).

Barrie, LA; Bottenheim, JW; Schnell, RC; Crutzen, PJ; Rasmussen, RA. (1988). Ozone destruction and photochemical reactions at polar sunrise in the lower Arctic atmosphere. Nature 334: 138-141.

Beckerman, B; Jerrett, M; Brook, JR; Verma, DK; Arain, MA; Finkelstein, MM. (2008). Correlation of nitrogen dioxide with other traffic pollutants near a major expressway. Atmos Environ 42: 275-290. http://dx.doi.org/10.1016/j.atmosenv.2007.09.042

Beer, R. (2006). TES on the aura mission: Scientific objectives, measurements, and analysis overview. IEEE Trans Geosci Remote Sens 44: 1102-1105. http://dx.doi.org/10.1109/TGRS.2005.863716

Berkowitz, CM; Fast, JD; Sprinston, SR; Larsen, RJ; Spicer, CW; Doskey, PV; Hubbe, JM; Plastridge, R. (1998). Formation mechanisms and chemical characteristics of elevated photochemical layers over the northeast United States. J Geophys Res 103: 10,631-610,647.

Berkowitz, CM; Shaw, WJ. (1997). Airborne measurements of boundary layer chemistry during the Southern Oxidant Study: A case study. J Geophys Res 102: 12,795-712,804. http://dx.doi.org/10.1029/97JD00417

Binkowski, F; Roselle, S. (2003). Models-3 Community Multiscale Air Quality(CMAQ) model aerosol component 1. Model description. J Geophys Res 108: 4183. http://dx.doi.org/10.1029/2001JD001409

Binkowski, FS; Arunachalam, S; Adelman, Z; Pinto, JP. (2007). Examining photolysis rates with a prototype online photolysis module in CMAQ. J Appl Meteor Climatol 46: 1252-1256.

Bishop, GA; Stedman, DH. (2008). A decade of on-road emissions measurements. Environ Sci Technol 42: 1651-1656. http://dx.doi.org/10.1021/es702413b

Bloomer, BJ; Stehr, JW; Piety, CA; Salawitch, RJ; Dickerson, RR. (2009). Observed relationships of ozone air pollution with temperature and emissions. Geophys Res Lett 36: L09803. http://dx.doi.org/10.1029/2009GL037308

Blumenthal, DL; Lurmann, FW; Kumar, N; Dye, TS; Ray, SE; Korc, ME; Londergan, R; Moore, G. (1997). Transport and mixing phenomena related to ozone exceedances in the northeast US (analysis based on NARSTO-northeast data). Santa Rosa, CA: Sonoma Technology. http://capita.wustl.edu/otag/reports/otagrept/otagrept.html

Boersma, KF; Eskes, HJ; Brinksma, EJ. (2004). Error analysis for tropospheric NO2 retrieval from space. J Geophys Res 109: D04311. http://dx.doi.org/10.1029/2003JD003962

Bonn, B; Von Kuhlmann, R; Lawrence, MG. (2004). High contribution of biogenic hydroperoxides to secondary organic aerosol formation. Geophys Res Lett 31: L10108. http://dx.doi.org/10.1029/2003GL019172

Brodin, M; Helmig, D; Oltmans, S. (2010). Seasonal ozone behavior along an elevation gradient in the Colorado Front Range Mountains. Atmos Environ 44: 5305-5315. http://dx.doi.org/10.1016/j.atmosenv.2010.06.033

Burgard, DA; Bishop, GA; Stedman, DH; Gessner, VH; Daeschlein, C. (2006). Remote sensing of in-use heavy-duty diesel trucks. Environ Sci Technol 40: 6938-6942. http://dx.doi.org/10.1021/es060989a

Burley, JD; Ray, JD. (2007). Surface ozone in Yosemite National Park. Atmos Environ 41: 6048-6062.

Buzica, D; Gerboles, M; Plaisance, H. (2008). The equivalence of diffusive samplers to reference methods for monitoring O3, benzene and NO2 in ambient air. J Environ Monit 10: 1052-1059. http://dx.doi.org/10.1039/b802260g

Byun, D; Schere, KL. (2006). Review of the governing equations, computational algorithms, and other components of the models-3 community multiscale air quality (CMAQ) modeling system [Review]. Appl Mech Rev 59: 51-77. http://dx.doi.org/10.1115/1.2128636

Byun, DW; Ching, JKS. (1999). Science algorithms of the EPA models-3 community multiscale air quality (CMAQ) modeling system. (EPA/600-R-99-030). Washington, DC: U.S. Environmental Protection Agency. http://www.epa.gov/asmdnerl/CMAQ/CMAQscienceDoc.html

Carter, WPL. (1995). Computer modeling of environmental chamber studies of maximum incremental reactivities of volatile organic compounds. Atmos Environ 29: 2513-2527.

Carter, WPL; Seinfeld, JH. (2012). Winter ozone formation and VOC incremental reactivities in the Upper Green River Basin of Wyoming. Atmos Environ 50: 255-266. http://dx.doi.org/10.1016/j.atmosenv.2011.12.025

Chan, E; Vet, RJ. (2010). Baseline levels and trends of ground level ozone in Canada and the United States. Atmos Chem Phys 10: 8629-8647. http://dx.doi.org/10.5194/acp-10-8629-2010

Chen, X; Hopke, PK; Carter, WP. (2011). Secondary organic aerosol from ozonolysis of biogenic volatile organic compounds: Chamber studies of particle and reactive oxygen species formation. Environ Sci Technol 45: 276-282. http://dx.doi.org/10.1021/es102166c

Ching, J; Herwehe, J; Swall, J. (2006). On joint deterministic grid modeling and sub-grid variability conceptual framework for model evaluation. Atmos Environ 40: 4935-4945.

Civerolo, KL; Mao, HT; Rao, ST. (2003). The airshed for ozone and fine particulate pollution in the eastern United States. Pure Appl Geophys 160: 81-105.

Conrad, R; Seiler, W. (1985). Influence of temperature, moisture, and organic carbon on the flux of H2 and CO between soil and atmosphere: Field studies in subtropical regions. J Geophys Res 90: 5699-5709.

Cooper, OR; Oltmans, SJ; Johnson, BJ; Brioude, J; Angevine, W; Trainer, M; Parrish, DD; Ryerson, TR; Pollack, I; Cullis, PD; Ives, MA; Tarasick, DW; Al-Saadi, J; Stajner, I. (2011). Measurement of western U.S. baseline ozone from the surface to the tropopause and assessment of downwind impact regions. J Geophys Res 116: 1-22.

Cooper, OR; Parrish, DD; Stohl, A; Trainer, M; Nedelec, P; Thouret, V; Cammas, JP; Oltmans, SJ; Johnson, BJ; Tarasick, D; Leblanc, T; Mcdermid, IS; Jaffe, D; Gao, R; Stith, J; Ryerson, T; Aikin, K; Campos, T; Weinheimer, A; Avery, MA. (2010). Increasing springtime ozone mixing ratios in the free troposphere over western North America. Nature 463: 344-348. http://dx.doi.org/10.1038/nature08708

Corsmeier, U; Kalthhoff, N; Kolle, O; Motzian, M; Fiedler, F. (1997). Ozone concentration jump in the stable nocturnal boundary layer during a LLJ-event. Atmos Environ 31: 1977-1989.

Crounse, JD; Paulot, F; Kjaergaard, HG; Wennberg, PO. (2011). Peroxy radical isomerization in the oxidation of isoprene. Phys Chem Chem Phys 13: 13607-13613. http://dx.doi.org/10.1039/c1cp21330j

D'Anna, B; Jammoul, A; George, C; Stemmler, K; Fahrni, S; Ammann, M; Wisthaler, A. (2009). Lightinduced ozone depletion by humic acid films and submicron aerosol particles. J Geophys Res 114: D12301. http://dx.doi.org/10.1029/2008JD011237

Dallmann, TR; Harley, RA. (2010). Evaluation of mobile source emission trends in the United States. J Geophys Res 115: D14305. http://dx.doi.org/10.1029/2010JD013862

de Gouw, JA; Brock, CA; Atlas, EL; Bates, TS; Fehsenfeld, FC; Goldan, PD; JS, H; Kuster, WC; Lerner, BM; Matthew, BM; Middlebrook, AM; Onasch, TB; Peltier, RE; Quinn, PK; Senff, CJ; Stohl, A; Sullivan, AP; Trainer, M; Warneke, C; Weber, RJ; Williams, EJ. (2008). Sources of particulate matter in the northeastern United States in summer: 1. Direct emissions and secondary formation of organic matter in urban plumes. J Geophys Res 113: D08301.

Dickerson, RR; Rhoads, KP; Carsey, TP; Oltmans, SJ; Burrows, JP; Crutzen, PJ. (1999). Ozone in the remote marine boundary layer: A possible role for halogens. J Geophys Res 104: 21,385-321,395.

Docherty, KS; Wu, W; Lim, YB; Ziemann, PJ. (2005). Contributions of organic peroxides to secondary aerosol formed from reactions of monoterpenes with O3. Environ Sci Technol 39: 4049-4059. http://dx.doi.org/10.1021/es050228s

Doyle, M; Sexton, KG; Jeffries, H; Bridge, K; Jaspers, I. (2004). Effects of 1,3-butadiene, isoprene, and their photochemical degradation products on human lung cells. Environ Health Perspect 112: 1488-1495. http://dx.doi.org/10.1289/ehp.7022

Doyle, M; Sexton, KG; Jeffries, H; Jaspers, I. (2007). Atmospheric photochemical transformations enhance 1,3-butadiene-induced inflammatory responses in human epithelial cells: The role of ozone and other photochemical degradation products. Chem Biol Interact 166: 163-169. http://dx.doi.org/10.1016/j.cbi.2006.05.016

Duncan, BN; Yoshida, Y; Olson, JR; Sillman, S; Martin, RV; Lamsal, L; Hu, Y; Pickering, KE; Retscher, C; Allen, DJ. (2010). Application of OMI observations to a space-based indicator of NOx and VOC controls on surface ozone formation. Atmos Environ 44: 2213-2223. http://dx.doi.org/10.1016/j.atmosenv.2010.03.010

Dunker, AM; Yarwood, G; Ortmann, JP; Wilson, GM. (2002). Comparison of Source Apportionment and Source Sensitivity of Ozone in a Three-Dimensional Air Quality Model. Environ Sci Technol 36: 2953-2964. http://dx.doi.org/10.1021/es011418f

Dunlea, EJ; Herndon, SC; Nelson, DD; Volkamer, RM; Lamb, BK; Allwine, EJ; Grutter, M; Ramos Villegas, CR; Marquez, C; Blanco, S; Cardenas, B; Kolb, CE; Molina, LT; Molina, MJ. (2006). Technical note: Evaluation of standard ultraviolet absorption ozone monitors in a polluted urban environment. Atmos Chem Phys Discuss 6: 2241-2279.

Ebeling, D; Patel, V; Findlay, M; Stetter, J. (2009). Electrochemical ozone sensor and instrument with characterization of the electrode and gas flow effects. Sens Actuators B 137: 129-133. http://dx.doi.org/10.1016/j.snb.2008.10.038

Eder, B; Yu, S. (2005). A performance evaluation of the 2004 release of Models-3 CMAQ. Atmos Environ 40: 4811-4824. http://dx.doi.org/10.1016/j.atmosenv.2005.08.045

Eisele, FL; Mount, GH; Tanner, D; Jefferson, A; Shetter, R; Harder, JW; Williams, EJ. (1997). Understanding the production and interconversion of the hydroxyl radical during the tropospheric OH photochemistry experiment. J Geophys Res 102: 6457-6465. http://dx.doi.org/10.1029/96JD02207

Emery, C; Jung, J; Downey, N; Johnson, J; Jimenez, M; Yarvvood, G; Morris, R. (2012). Regional and global modeling estimates of policy relevant background ozone over the United States. Atmos Environ 47: 206-217. http://dx.doi.org/10.1016/j.atmosenv.2011.11.012

Emmerson, KM; Evans, MJ. (2009). Comparison of tropospheric gas-phase chemistry schemes for use within global models. Atmos Chem Phys 9: 1831-1845. http://dx.doi.org/10.5194/acpd-8-19957-2008

ENVIRON (ENVIRON Holdings Inc.). (2005). CAMx. Available online at http://www.camx.com/over/ (accessed January 28, 2011).

Fang, Y; Fiore, AM; Horowitz, LW; Levy II, H; Hu, Y; Russell, AG. (2010). Sensitivity of the NOy budget over the United States to anthropogenic and lightning NOx in summer. J Geophys Res 115: D18312. http://dx.doi.org/10.1029/2010JD014079

Fehsenfeld, FC; Ancellet, G; Bates, TS; Goldstein, AH; Hardesty, RM; Honrath, R; Law, KS; Lewis, AC; Leaitch, R; McKeen, S; Meagher, J; Parrish, DD; Pszenny, AAP; Russell, PB; Schlager, H; Seinfeld, J; Talbot, R; Zbinden, R. (2006). International consortium for atmospheric research on transport and transformation (ICARTT): North America to Europe: Overview of the 2004 summer field study. J Geophys Res 111: D23S01.21-D23S01.36. http://dx.doi.org/10.1029/2006JD007829

Fehsenfeld, FC; Trainer, M; Parrish, DD; Volz-Thomas, A; Penkett, S. (1996). North Atlantic Regional Experiment (NARE) 1993 summer intensive: Foreword. J Geophys Res 101: 28869-28875.

Finlayson-Pitts, BJ; Pitts, JN, Jr. (1986). Atmospheric chemistry: Fundamentals and experimental techniques. New York, NY: John Wiley & Sons.

Fiore, A; Dentener, F; Wild, O; Cuvelier, C; Schultz, M; Hess, P; Textor, C; Schulz, M; Doherty, R; Horowitz, L; MacKenzie, I; Sanderson, M; Shindell, D; Stevenson, D; Szopa, S; Van Dingenen, R; Zeng, G; Atherton, C; Bergmann, D; Bey, I; Carmichael, G; Collins, W; Duncan, B; Faluvegi, G; Folberth, G; Gauss, M; Gong, S; Hauglustaine, D; Holloway, T; Isaksen, I; Jacob, D; Jonson, J; Kaminski, J; Keating, T; Lupu, A; Marmer, E; Montanaro, V; Park, R; Pitari, G; Pringle, K; Pyle, J; Schroeder, S; Vivanco, M; Wind, P; Wojcik, G; Wu, S; Zuber, A. (2009). Multimodel estimates of intercontinental source-receptor relationships for ozone pollution. J Geophys Res 114: D04301. http://dx.doi.org/10.1029/2008JD010816

Fiore, A; Jacob, DJ; Liu, H; Yantosca, RM; Fairlie, TD; Li, Q. (2003). Variability in surface ozone background over the United States: Implications for air quality policy. J Geophys Res 108: 4787. http://dx.doi.org/10.1029/2003JD003855

Fiore, AM; Horowitz, LW; Purves, DW; Levy, H, II; Evans, MJ; Wang, Y; Li, Q; Yantosca, RM. (2005). Evaluating the contribution of changes in isoprene emissions to surface ozone trends over the eastern United States. J Geophys Res 110: D12303. http://dx.doi.org/10.1029/2004JD005485

Fischer, EV. (2004). Summertime ozone at Mount Washington: Meteorological controls at the highest peak in the northeast. J Geophys Res 109: D24303. http://dx.doi.org/10.1029/2004JD004841

Fuentes, JD; Wang, D; Bowling, DR; Potosnak, M; Monson, RK; Goliff, WS; Stockwell, WR. (2007). Biogenic hydrocarbon chemistry within and above a mixed deciduous forest. J Atmos Chem 56: 165-185. http://dx.doi.org/10.1007/s10874-006-9048-4

Fuentes, M; Raftery, AE. (2005). Model evaluation and spatial interpolation by Bayesian combination of observations with outputs from numerical models. Biometrics 61: 36-45.

Fusco, AC; Logan, JA. (2003). Analysis of 1970-1995 trends in tropospheric ozone at Northern Hemisphere midlatitudes with the GEOS-CHEM model. J Geophys Res 108: 4449. http://dx.doi.org/10.1029/2002JD002742

Gaydos, TM; Pinder, R; Koo, B; Fahey, KM; Yarwood, G; Pandis, SN. (2007). Development and application of a three-dimensional aerosol chemical transport model, PMCAMx. Atmos Environ 41: 2594-2611. http://dx.doi.org/10.1016/j.atmosenv.2006.11.034

Generoso, S; Bey, I; Attié, JL; Bréon, FM. (2007). A satellite- and model-based assessment of the 2003 Russian fires: Impact on the arctic region. J Geophys Res 112: 5302.

Gilliland, AB; Hogrefe, C; Pinder, RW; Godowitch, JM; Foley, KL; Rao, ST. (2008). Dynamic evaluation of regional air quality models: Assessing changes in O3 stemming from changes in emissions and meteorology. Atmos Environ 42: 5110-5123. http://dx.doi.org/10.1016/j.atmosenv.2008.02.018

Godowitch, JM; Gilliland, AB; Draxler, RR; Rao, ST. (2008). Modeling assessment of point source NOx emission reductions on ozone air quality in the eastern United States. Atmos Environ 42: 87-100. http://dx.doi.org/10.1016/j.atmosenv.2007.09.032

Goldstein, A; Galbally, I. (2007). Known and unexplored organic constituents in the earths atmosphere. Environ Sci Technol 41: 15141521. http://dx.doi.org/10.1021/es072476p

Goldstein, AH; Millet, DB; McKay, M; Jaegle, L; Horowitz, L; Cooper, O; Hudman, R; Jacob, DJ; Oltmans, S; Clarke, A. (2004). Impact of Asian emissions on observations at Trinidad Head, California, during ITCT 2K2. J Geophys Res 109: D23S17. http://dx.doi.org/10.1029/2003JD004406

Gottardini, E; Cristofori, A; Cristofolini, F; Ferretti, M. (2010). Variability of ozone concentration in a montane environment, northern Italy. Atmos Environ 44: 147-152. http://dx.doi.org/10.1016/j.atmosenv.2009.10.017

Greenberg, JP; Guenther, AB; Turnipseed, A. (2009). Tethered balloon-based soundings of ozone, aerosols, and solar radiation near Mexico City during MIRAGE-MEX. Atmos Environ 43: 2672-2677. http://dx.doi.org/10.1016/j.atmosenv.2009.02.019

Grell, GA; Emeis, S; Stockwell, WR; Schoenemeyer, T; Forkel, R; Michalakes, J; Knoche, R; Seidl, W. (2000). Application of a multiscale, coupled MM5/chemistry model to the complex terrain of the VOTALP valley campaign. Atmos Environ 34: 1435-1453.

Guenther, A; Geron, C; Pierce, T; Lamb, B; Harley, P; Fall, R. (2000). Natural emissions of non-methane volatile organic compounds, carbon monoxide, and oxides of nitrogen from North America. Atmos Environ 34: 2205-2230. http://dx.doi.org/10.1016/S1352-2310(99)00465-3

Guenther, A; Karl, T; Harley, P; Wiedinmyer, C; Palmer, PI; Geron, C. (2006). Estimates of global terrestrial isoprene emissions using MEGAN (Model of Emissions of Gases and Aerosols from Nature). Atmos Chem Phys 6: 3181-3210. http://dx.doi.org/10.5194/acp-6-3181-2006

Hains, JC; Taubman, BF; Thompson, AM; Stehr, JW; Marufu, LT; Doddridge, BG; Dickerson, RR. (2008). Origins of chemical pollution derived from Mid-Atlantic aircraft profiles using a clustering technique. Atmos Environ 42: 1727-1741. http://dx.doi.org/10.1016/j.atmosenv.2007.11.052

Hameed, S; Pinto, JP; Stewart, RW. (1979). Sensitivity of the predicted CO-OH-CH4 perturbation to tropospheric NOx concentrations. J Geophys Res 84: 763-768.

Harley, RA; Marr, LC; Lehner, JK; Giddings, SN. (2005). Changes in motor vehicle emissions on diurnal to decadal time scales and effects on atmospheric composition. Environ Sci Technol 39: 5356-5362.

Harvard University. (2010a). GEOSChem Model [Computer Program]. Cambridge, MA. Retrieved from http://acmg.seas.harvard.edu/geos/

Harvard University. (2010b). GEOSChem Overview. Available online at http://acmg.seas.harvard.edu/geos/geos_overview.html (accessed January 28, 2011).

Henderson, BH; Pinder, RW; Crooks, J; Cohen, RC; Hutzell, WT; Sarwar, G; Goliff, WS; Stockwell, WR; Fahr, A; Mathur, R; Carlton, AG; Vizuete, W. (2011). Evaluation of simulated photochemical partitioning of oxidized nitrogen in the upper troposphere. Atmos Chem Phys 11: 275-291. http://dx.doi.org/10.5194/acp-11-275-2011

Hess, GD; Carnovale, F; Cope, ME; Johnson, GM. (1992). The evaluation of some photochemical smog reaction mechanismsI. Temperature and initial composition effects. Atmos Environ 26: 625-641. http://dx.doi.org/10.1016/0960-1686(92)90174-J

Hocking, WK; Carey-Smith, T; Tarasick, DW; Argall, PS; Strong, K; Rochon, Y; Zawadzki, I; Taylor, PA. (2007). Detection of stratospheric ozone intrusions by windprofiler radars. Nature 450: 281-284. http://dx.doi.org/10.1038/nature06312

Hofzumahaus, A; Rohrer, F; Keding, L; Bohn, B; Brauers, T; Chih-Chung, C; Fuchs, H; Holland, F; Kita, K; Kondo, Y; Xin, L; Shengrong, L; Min, S; Limin, Z; Wahner, A; Yuanhang, Z. (2009). Amplified trace gas removal in the troposphere. Science 324: 1702-1704. http://dx.doi.org/10.1126/science.1164566

Horowitz, LW; Fiore, AM; Milly, GP; Cohen, RC; Perring, A; Wooldridge, PJ; Hess, PG; Emmons, LK; Lamarque, JF. (2007). Observational constraints on the chemistry of isoprene nitrates over the eastern United States. J Geophys Res 112: D12S08. http://dx.doi.org/10.1029/2006JD007747

Hoskins, BJ. (1972). Non-boussinesq effects and further development in a model of upper tropospheric frontogenesis. Q J Roy Meteorol Soc 98: 532-541. http://dx.doi.org/10.1002/qj.49709841705

Hsu, J; Prather, MJ. (2009). Stratospheric variability and tropospheric ozone. J Geophys Res 114: D06102. http://dx.doi.org/10.1029/2008JD010942

Hudman, RC; Jacob, DJ; Turquety, S; Leibensperger, EM; Murray, LT; Wu, S; Gilliland, AB; Avery, M; Bertram, TH; Brune, W; Cohen, RC; Dibb, JE; Flocke, FM; Fried, A; Holloway, J; Neuman, JA; Orville, R; Perring, A; Ren, X; Sachse, GW; Singh, HB; Swanson, A; Wooldridge, PJ. (2007). Surface and lightning sources of nitrogen oxides over the United States: Magnitudes, chemical evolution, and outflow. J Geophys Res 112: D12S05. http://dx.doi.org/10.1029/2006JD007912

Hudman, RC; Murray, LT; Jacob, DJ; Millet, DB; Turquety, S; Wu, S; Blake, DR; Goldstein, AH; Holloway, J; Sachse, GW. (2008). Biogenic versus anthropogenic sources of CO in the United States. Geophys Res Lett 35: L04801. http://dx.doi.org/10.1029/2007gl032393

Husar, RB; Renard, WP. (1998). Ozone as a function of local wind speed and direction: Evidence of local and regional transport. 91st annual meeting and exhibition of the Air & Waste Management Association, San Diego, CA.

Inman, RE; Ingersoll, RB; Levy, EA. (1971). Soil: A natural sink for carbon monoxide. Science 172: 1229-1231. http://dx.doi.org/10.1126/science.172.3989.1229

Ito, A; Sillman, S; Penner, JE. (2009). Global chemical transport model study of ozone response to changes in chemical kinetics and biogenic volatile organic compounds emissions due to increasing temperatures: sensitivities to isoprene nitrate chemistry and grid resolution. J Geophys Res 114: D09301. http://dx.doi.org/10.1029/2008jd011254

Jacob, DJ. (1999). Introduction to atmospheric chemistry. New Jersey: Princeton University Press.

Jacob, DJ; Horowitz, LW; Munger, JW; Heikes, BG; Dickerson, RR; Artz, RS; Keene, WC. (1995). Seasonal transition from NOx- to hydrocarbon-limited conditions for ozone production over the eastern United States in September. J Geophys Res 100: 9315-9324.

Jacobson, MZ. (2002). Atmospheric pollution: History, science, and regulation. New York: Cambridge University Press.

Jacobson, MZ. (2005). Fundamentals of atmospheric modeling (2 ed.). New York: Cambridge University Press.

Jaegle, L; Jacob, DJ; Brune, WH; Wennberg, PO. (2001). Chemistry of HOx radicals in the upper troposphere. Atmos Environ 35: 469-489. http://dx.doi.org/10.1016/S1352-2310(00)00376-9

Jaffe, D; Chand, D; Hafner, W; Westerling, A; Spracklen, D. (2008). Influence of fires on O-3 concentrations in the western US. Environ Sci Technol 42: 5885-5891. http://dx.doi.org/10.1021/es800084k

Jaffe, D; Price, H; Parrish, D; Goldstein, A; Harris, J. (2003). Increasing background ozone during spring on the west coast of North America. Geophys Res Lett 30: 1613. http://dx.doi.org/10.1029/2003GL017024

Jaffe, D; Ray, J. (2007). Increase in surface ozone at rural sites in the western US. Atmos Environ 41: 5452-5463.

Jaffe, DA; Wigder, NL. (2012). Ozone production from wildfires: A critical review. Atmos Environ 51: 1-10. http://dx.doi.org/10.1016/j.atmosenv.2011.11.063

James, P; Stohl, A; Forster, C; Eckhardt, S; Seibert, P; Frank, A. (2003). A 15-year climatology of stratosphere-troposphere exchange with a Lagrangian particle dispersion model: 2. Mean climate and seasonal variability. J Geophys Res 108: D12. http://dx.doi.org/10.1029/2002JD002639

Jimenez, JL; Jayne, JT; Shi, Q; Kolb, CE; Worsnop, DR; Yourshaw, I; Seinfeld, JH; Flagan, RC; Zhang, X; Smith, KA. (2003). Ambient aerosol sampling using the Aerodyne Aerosol Mass Spectrometer. J Geophys Res 108: 8425.

Jo, WK; Park, JH. (2005). Characteristics of roadside air pollution in Korean metropolitan city (Daegu) over last 5 to 6 years: Temporal variations, standard exceedances, and dependence on meteorological conditions. Chemosphere 59: 1557-1573. http://dx.doi.org/10.1016/j.chemosphere.2004.12.021

Johnson, D; Jenkin, ME; Wirtz, K; Martin-Riviejo, M. (2004). Simulating the formation of secondary organic aerosol from the photooxidation of toluene. Environ Chem 1: 150-165.

Johnson, TR. (1995). Recent advances in the estimation of population exposure to mobile source pollutants. J Expo Sci Environ Epidemiol 5: 551-571.

Kang, D; Aneja, VP; Mathur, R; Ray, JD. (2003). Nonmethane hydrocarbons and ozone in three rural southeast United States national parks: A model sensitivity analysis and comparison to measurements. J Geophys Res 108: 4604. http://dx.doi.org/10.1029/2002JD003054

Kasibhatla, P; Chameides, WL. (2000). Seasonal modeling of regional ozone pollution in the eastern United States. Geophys Res Lett 27: 1415-1418. http://dx.doi.org/10.1029/1999GL011147

Kaynak, B; Hu, Y; Martin, RV; Russell, AG; Choi, Y; Wang, Y. (2008). The effect of lightning NOx production on surface ozone in the continental United States. Atmos Chem Phys 8: 5151-5159.

Kim, SW; Heckel, A; Mckeen, SA; Frost, GJ; Hsie, EY; Trainer, MK; Richter, A; Burrows, JP; Peckham, SE; Grell, GA. (2006). Satellite-observed US power plant NOx emission reductions and their impact on air quality. Geophys Res Lett 33: L22812. http://dx.doi.org/10.1029/2006GL027749

King, GM. (1999). Characteristics and significance of atmospheric carbon monoxide consumption by soils. Chemosphere 1: 53-63.

Kleffmann, J; Lorzer, JC; Wiesen, P; Kern, C; Trick, S; Volkamer, R; Rodenas, M; Wirtz, K. (2006). Intercomparison of the DOAS and LOPAP techniques for the detection of nitrous acid (HONO). Atmos Environ 40: 3640-3652.

Kleffmann, J; Wiesen, P. (2008). Technical note: Quantification of interferences of wet chemical HONO LOPAP measurements under simulated polar conditions. Atmos Chem Phys 8: 6813-6822.

Kleindienst, TE; Hudgens, EE; Smith, DF; McElroy, FF; Bufalini, JJ. (1993). Comparison of chemiluminescence and ultraviolet ozone monitor responses in the presence of humidity and photochemical pollutants. Air Waste 43: 213-222.

Lam, Y; Fu, J. (2010). Corrigendum to "A novel downscaling technique for the linkage of global and regional air quality modeling" published in Atmos. Chem. Phys., 9, 9169-9185, 2009. Atmos Chem Phys 10: 4013-4031. http://dx.doi.org/10.5194/acp-10-4013-2010

Langford, AO; Aikin, KC; Eubank, CS; Williams, EJ. (2009). Stratospheric contribution to high surface ozone in Colorado during springtime. Geophys Res Lett 36: L12801. http://dx.doi.org/10.1029/2009gl038367

Lee, J; Kim, KH; Kim, YJ; Lee, J. (2008b). Application of a long-path differential optical absorption spectrometer (LP-DOAS) on the measurements of NO(2), SO(2), O(3), and HNO(2) in Gwangju, Korea. J Environ Manage 86: 750-759. http://dx.doi.org/10.1016/j.jenvman.2006.12.044

Lefohn, AS; Shadwick, D; Oltmans, SJ. (2008). Characterizing long-term changes in surface ozone levels in the United States (1980-2005). Atmos Environ 42: 8252-8262. http://dx.doi.org/10.1016/j.atmosenv.2008.07.060

Lefohn, AS; Shadwick, D; Oltmans, SJ. (2010b). Characterizing changes in surface ozone levels in metropolitan and rural areas in the United States for 19802008 and 19942008. Atmos Environ 44: 5199-5210. http://dx.doi.org/10.1016/j.atmosenv.2010.08.049

Lefohn, AS; Wernli, H; Shadwick, D; Limbach, S; Oltmans, SJ; Shapiro, M. (2011). The importance of stratospherictropospheric transport in affecting surface ozone concentrations in the western and northern tier of the United States. Atmos Environ 45: 4845-4857. http://dx.doi.org/10.1016/j.atmosenv.2011.06.014

Leibensperger, EM; Mickley, LJ; Jacob, DJ. (2008). Sensitivity of US air quality to mid-latitude cyclone frequency and implications of 1980-2006 climate change. Atmos Chem Phys Discuss 8: 12253-12282. http://dx.doi.org/10.5194/acpd-8-12253-2008

Leston, AR; Ollinson, WM; Spicer, CW; Satola, J. (2005). Potential interference bias in ozone standard compliance monitoring. J Air Waste Manag Assoc 55: 1464-1472.

Li, Y; Lee, SR; Wu, CY. (2006c). UV-absorption-based measurements of ozone and mercury: An investigation on their mutual interferences. Aerosol Air Qual Res 6: 418-429.

Lin, M; Fiore, AM; Horowitz, LW; Cooper, OR; Naik, V; Holloway, J; Johnson, BJ; Middlebrook, AM; Oltmans, SJ; Pollack, IB; Ryerson, TB; Warner, JX; Wiedinmyer, C; Wilson, J; Wyman, B. (2012). Transport of Asian ozone pollution into surface air over the western United States in spring. J Geophys Res 117: D00V07. http://dx.doi.org/10.1029/2011JD016961

Liu, X; Chance, K; Sioris, CE; Kurosu, TP; Spurr, RJD; Martin, RV; Fu, TM; Logan, JA; Jacob, DJ; Palmer, PI; Newchurch, MJ; Megretskaia, IA; Chatfield, RB. (2006). First directly retrieved global distribution of tropospheric column ozone from GOME: Comparison with the GEOS-CHEM model. J Geophys Res 111: D02308. http://dx.doi.org/10.1029/2005JD006564

Liu, XH; Hegg, DA; Stoelinga, MT. (2001). Numerical simulation of new particle formation over the northwest Atlantic using the MM5 mesoscale model coupled with sulfur chemistry. J Geophys Res 106: 9697-9715.

Lockwood, AL; Shepson, PB; Fiddler, MN; Alaghmand, M. (2010). Isoprene nitrates: preparation, separation, identification, yields, and atmospheric chemistry. Atmos Chem Phys 10: 6169-6178. http://dx.doi.org/10.5194/acp-10-6169-2010

Lu, R; Turco, RP; Jacobson, MZ. (1997). An integrated air pollution modeling system for urban and regional scales: 1 Structure and performance. J Geophys Res 102: 6063-6079.

Luecken, DJ; Phillips, S; Sarwar, G; Jang, C. (2008). Effects of using the CB05 vs. SAPRC99 vs. CB4 chemical mechanism on model predictions: Ozone and gas-phase photochemical precursor concentrations. Atmos Environ 42: 5805-5820. http://dx.doi.org/10.1016/j.atmosenv.2007.08.056

Macintyre, HL; Evans, MJ. (2010). Sensitivity of a global model to the uptake of N2O5 by tropospheric aerosol. Atmos Chem Phys 10: 7409-7414. http://dx.doi.org/10.5194/acp-10-7409-2010

Macintyre, HL; Evans, MJ. (2011). Parameterisation and impact of aerosol uptake of HO2 on a global tropospheric model. Atmos Chem Phys 11: 10965-10974. http://dx.doi.org/10.5194/acp-11-10965-2011

Mahajan, AS; Shaw, M; Oetjen, H; Hornsby, KE; Carpenter, LJ; Kaleschke, L; Tian-Kunze, X; Lee, JD; Moller, SJ; Edwards, P. (2010). Evidence of reactive iodine chemistry in the Arctic boundary layer. J Geophys Res 115: D20303. http://dx.doi.org/10.1029/2009JD013665

Martin, RV; Fiore, AM; Van Donkelaar, A. (2004). Space-based diagnosis of surface ozone sensitivity to anthropogenic emissions. Geophys Res Lett 31: L06120. http://dx.doi.org/10.1029/2004GL019416

Maruo, YY. (2007). Measurement of ambient ozone using newly developed porous glass sensor. Sens Actuators B 126: 485-491. http://dx.doi.org/10.1016/j.snb.2007.03.041

Maruo, YY; Akaoka, K; Nakamura, J. (2010). Development and performance evaluation of ozone detection paper using azo dye orange I: Effect of pH. Sens Actuators B 143: 487-493. http://dx.doi.org/10.1016/j.snb.2009.09.042

Mathur, R. (2008). Estimating the impact of the 2004 Alaskan forest fires on episodic particulate matter pollution over the eastern United States through assimilation of satellite-derived aerosol optical depths in a regional air quality model. J Geophys Res 113: D17302. http://dx.doi.org/10.1029/2007JD009767

McDonald-Buller, EC; Allen, DT; Brown, N; Jacob, DJ; Jaffe, D; Kolb, CE; Lefohn, AS; Oltmans, S; Parrish, DD; Yarwood, G; Zhang, L. (2011). Establishing Policy Relevant Background (PRB) Ozone Concentrations in the United States. Environ Sci Technol 45: 9484-9497. http://dx.doi.org/10.1021/es2022818

McElroy, MB; Salawitch, RJ; Wofsy, SC; Logan, JA. (1986). Reductions of Antarctic ozone due to synergistic interactions of chlorine and bromine. Nature 321: 759-762.

Milford, JB; Gao, D; Sillman, S; Blossey, P; Russell, AG. (1994). Total reactive nitrogen (NOy) as an indicator of the sensitivity of ozone to reductions in hydrocarbon and NOx emissions. J Geophys Res 99: 3533-3542.

Millet, DB; Jacob, DJ; Boersma, KF; Fu, TM; Kurosu, TP; Chance, K; Heald, CL; Guenther, A. (2008). Spatial distribution of isoprene emissions from North America derived from formaldehyde column measurements by the OMI satellite sensor. J Geophys Res 113. http://dx.doi.org/10.1029/2007jd008950

Millet, DB; Jacob, DJ; Turquety, S; Hudman, RC; Wu, S; Fried, A; Walega, J; Heikes, BG; Blake, DR; Singh, HB; Anderson, BE; Clarke, AD. (2006). Formaldehyde distribution over North America: Implications for satellite retrievals of formaldehyde columns and isoprene emission. J Geophys Res 111: D24S02. http://dx.doi.org/10.1029/2005JD006853

Miwa, T; Maruo, YY; Akaoka, K; Kunioka, T; Nakamura, J. (2009). Development of colorimetric ozone detection papers with high ultraviolet resistance using ultraviolet absorbers. J Air Waste Manag Assoc 59: 801-808. http://dx.doi.org/10.3155/1047-3289.59.7.801

Mollner, AK; Valluvadasan, S; Feng, L; Sprague, MK; Okumura, M; Milligan, DB; Bloss, WJ; Sander, SP; Martien, PT; Harley, RA. (2010). Rate of gas phase association of hydroxyl radical and nitrogen dioxide. Science 330: 646-649. http://dx.doi.org/10.1126/science.1193030

Mueller, SF; Mallard, JW. (2011a). Contributions of natural emissions to ozone and PM 2.5 as simulated by the Community Multiscale Air Quality (CMAQ) model. Environ Sci Technol 45: 4817-4823. http://dx.doi.org/10.1021/es103645m

Mueller, SF; Mallard, JW. (2011b). Errata in 'Contributions of natural emissions to ozone and PM 2.5 as simulated by the Community Multiscale Air Quality (CMAQ) model' [Erratum]. Environ Sci Technol 45: 7950. http://dx.doi.org/10.1021/es2027086

Nassar, R; Logan, JA; Worden, HM; Megretskaia, IA; Bowman, KW; Osterman, GB; Thompson, AM; Tarasick, DW; Austin, S; Claude, H; Dubey, MK; Hocking, WK; Johnson, BJ; Joseph, E; Merrill, J; Morris, GA; Newchurch, M; Oltmans, SJ; Posny, F; Schmidlin, FJ; Vomel, H; Whiteman, DN; Witte, JC. (2008). Validation of Tropospheric Emission Spectrometer (TES) nadir ozone profiles using ozonesonde measurements. D15S17 (13 pp.). http://dx.doi.org/10.1029/2007jd008819

Newell, RE; Thouret, V; Cho, JYN; Stoller, P; Marenco, A; Smit, HG. (1999). Ubiquity of quasi-horizontal layers in the troposphere. Nature 398: 316-319. http://dx.doi.org/10.1038/18642

NOAA (National Oceanic and Atmospheric Administration). (2010). The Rapid Update Cycle (RUC). Available online at http://ruc.noaa.gov/ (accessed January 28, 2011).

Nolte, CG; Gilliland, AM; Hogrefe, C; Mickley, LJ. (2008). Linking global to regional models to assess future climate impacts on surface ozone levels in the United States. J Geophys Res 113: D14307. http://dx.doi.org/10.1029/2007JD008497

Nozière, B; González, NJD; Borg-karlson, AK; Pei, Y; Redeby, JP; Krejci, R; Dommen, J; Prevot, ASH; Anthonsen, T. (2011). Atmospheric chemistry in stereo: A new look at secondary organic aerosols from isoprene. Geophys Res Lett 38: L11807. http://dx.doi.org/10.1029/2011GL047323

NPS (U.S. National Park Service). (2011). Portable Ozone Monitoring Systems (POMS). Washington, DC. http://www.nature.nps.gov/air/studies/porto3.cfm

NRC (National Research Council). (1991). Rethinking the ozone problem in urban and regional air pollution. Washington, DC: The National Academies Press.

NRC (National Research Council). (2007). Models in environmental regulatory decision making. Washington, DC: National Academies Press. http://www.nap.edu/catalog/11972.html

NRC (National Research Council). (2009). Global sources of local pollution: An assessment of long-range transport of key air pollutants to and from the United States. Washington, DC: The National Academies Press. http://www.nap.edu/catalog.php?record_id=12743

Ohira, SI; Dasgupta, PK; Schug, KA. (2009). Fiber optic sensor for simultaneous determination of atmospheric nitrogen dioxide, ozone, and relative humidity. Anal Chem 81: 4183-4191. http://dx.doi.org/10.1021/ac801756z

Olaguer, EP; Rappenglück, B; Lefer, B; Stutz, J; Dibb, J; Griffin, R; Brune, WH; Shauck, M; Buhr, M; Jeffries, H; Vizuete, W; Pinto, JP. (2009). Deciphering the role of radical precursors during the Second Texas Air Quality Study. J Air Waste Manag Assoc 59: 1258-1277. http://dx.doi.org/10.3155/1047-3289.59.11.1258

Olszyna, KJ; Bailey, EM; Simonaitis, R; Meagher, JF. (1994). O3 and NOy relationships at a rural site. J Geophys Res 99: 14557-14563. http://dx.doi.org/10.1029/94JD00739

Oltmans, SJ; Lefohn, AS; Harris, JM; Shadwick, DS. (2008). Background ozone levels of air entering the west coast of the US and assessment of longer-term changes. Atmos Environ 42: 6020-6038. http://dx.doi.org/10.1016/j.atmosenv.2008.03.034

Park, RJ; Stenchikov, GL; Pickering; Dickerson, RR; Allen, DJ; Kondragunta, S. (2001). Regional air pollution and its radiative forcing: Studies with a single column chemical and radiation transport model. J Geophys Res 106: 28,751-728,770.

Parrish, DD. (2006). Critical evaluation of US on-road vehicle emission inventories. Atmos Environ 40: 2288-2300.

Parrish, DD; Millet, DB; Goldstein, AH. (2009). Increasing ozone in marine boundary layer inflow at the west coasts of North America and Europe. Atmos Chem Phys 9: 1303-1323. http://dx.doi.org/10.5194/acpd-8-13847-2008

Paulot, F; Crounse, JD; Kjaergaard, HG; Kroll, JH; Seinfeld, JH; Wennberg, PO. (2009). Isoprene photooxidation: New insights into the production of acids and organic nitrates. Atmos Chem Phys 9: 1479-1501. http://dx.doi.org/10.5194/acp-9-1479-2009

Peeters, J; Müller, JF. (2010). HOx radical regeneration in isoprene oxidation via peroxy radical isomerisations. II: Experimental evidence and global impact. Phys Chem Chem Phys 12: 14227-14235. http://dx.doi.org/10.1039/C0CP00811G

Peeters, J; Nguyen, TL; Vereecken, L. (2009). HOx radical regeneration in the oxidation of isoprene. Phys Chem Chem Phys 11: 5935. http://dx.doi.org/10.1039/B908511D

Pegues, AH; Cohan, DS; Digar, A; Douglass, C; Wilson, RS. (2012). Efficacy of recent state implementation plans for 8-hour ozone. J Air Waste Manag Assoc 62: 252-261. http://dx.doi.org/10.1080/10473289.2011.646049

Perring, AE; Bertram, TH; Wooldridge, PJ; Fried, A; Heikes, BG; Dibb, J; Crounse, JD; Wennberg, PO; Blake, NJ; Blake, DR; Brune, WH; Singh, HB; Cohen, RC. (2009). Airborne observations of total RONO2: New constraints on the yield and lifetime of isoprene nitrates. Atmos Chem Phys 9: 1451-1463.

Pfister, G; Hess, PG; Emmons, LK; Lamarque, JF; Wiedinmyer, C; Edwards, DP; Petron, G; Gille, JC; Sachese, GW. (2005). Quantifying CO emissions from the 2004 Alaskan wildfires using MOPITT CO data. Geophys Res Lett 32: L11809.

Pinto, JP; Lefohn, AS; Shadwick, DS. (2004). Spatial variability of PM2.5 in urban areas in the United States. J Air Waste Manag Assoc 54: 440-449.

Pokharel, SS; Bishop, GA; Stedman, DH. (2002). An on-road motor vehicle emissions inventory for Denver: An efficient alternative to modeling. Atmos Environ 36: 5177-5184.

Pokharel, SS; Bishop, GA; Stedman, DH. (2003). Emissions reductions as a result of automobile improvement. Environ Sci Technol 37: 5097-5101.

Pollack, AK; Lindhjem, C; Stoeckenius, TE; Tran, C; Mansell, G; Jimenez, M; Wilson, G; Coulter-Burke, S. (2004). Final Report: Evaluation of the US EPA MOBILE6 highway vehicle emission factor model. (CRC Project E-64). Novato, CA: ENVIRON International Corporation.

Poppe, D; Wallasch, M; Zimmermann, J. (1993). The dependence of the concentration of OH on its precursors under moderately polluted conditions: A model study. J Atmos Chem 16: 61-78.

Rao, ST; Ku, JY; Berman, S; Zhang, K; Mao, H. (2003). Summertime characteristics of the atmospheric boundary layer and relationships to ozone levels over the eastern United States. Pure Appl Geophys 160: 21-55.

Rappenglück, B; Dasgupta, PK; Leuchner, M; Li, Q; Luke, W. (2009). Formaldehyde and its relation to CO, PAN, and SO2 in the Houston-Galveston airshed. Atmos Chem Phys Discuss 9: 24193-24223. http://dx.doi.org/10.5194/acp-10-2413-2010

Rasmussen, DJ; Fiore, AM; Naik, V; Horowitz, LW; Mcginnis, SJ; Schultz, MG. (2012). Surface ozone-temperature relationships in the eastern US: A monthly climatology for evaluating chemistry-climate models. Atmos Environ 47: 142-153. http://dx.doi.org/10.1016/j.atmosenv.2011.11.021

Rastigejev, Y; Park, R; Brenner, MP; Jacob, DJ. (2010). Resolving intercontinental pollution plumes in global models of atmospheric transport. J Geophys Res 115: D02302. http://dx.doi.org/10.1029/2009JD012568

Real, E; Law, KS; Weinzierl, B; Fiebig, M; Petzold, A; Wild, O; Methven, J; Arnold, S; Stohl, A; Huntrieser, H; Roiger, A; Schlager, H; Stewart, D; Avery, M; Sachse, G; Browell, E; Ferrare, R; Blake, D. (2007). Processes influencing ozone levels in Alaskan forest fire plumes during long-range transport over the North Atlantic. J Geophys Res 112. http://dx.doi.org/10.1029/2006jd007576

Reid, N; Yap, D; Bloxam, R. (2008). The potential role of background ozone on current and emerging air issues: An overview. Air Qual Atmos Health 1: 19-29. http://dx.doi.org/10.1007/s11869-008-0005-z

Reidmiller, DR; Fiore, AM; Jaffe, DA; Bergmann, D; Cuvelier, C; Dentener, FJ; Duncan; Bryan, N; Folberth, G; Gauss, M; Gong, S; Hess, P; Jonson, JE; Keating, T; Lupu, A; Marmer, E; Park, R; Schultz, MG; Shindell, DT; Szopa, S; Vivanco, MG; Wild, O; Zuber, A. (2009). The influence of foreign vs. North American emissions on surface ozone in the US. Atmos Chem Phys 9: 5027-5042.

Reisinger, AR. (2000). Unidentified interference in DOAS measurements of ozone. Appl Spectros Rev 54: 72-79.

Richards, NAD; Osterman, GB; Browell, EV; Hair, JW; Avery, M; Qinbin, L. (2008). Validation of tropospheric emission spectrometer ozone profiles with aircraft observations during the intercontinental chemical transport experiment-B. J Geophys Res 113: D16S29. http://dx.doi.org/10.1029/2007jd008815

Riediker, M; Williams, R; Devlin, R; Griggs, T; Bromberg, P. (2003). Exposure to particulate matter, volatile organic compounds, and other air pollutants inside patrol cars. Environ Sci Technol 37: 2084-2093. http://dx.doi.org/10.1021/es026264y

Rodes, CE; Holland, DM. (1981). Variations of NO, NO2 and O3 concentrations downwind of a Los Angeles freeway. Atmos Environ 15: 243-250.

Russell, A; Dennis, R. (2000). NARSTO critical review of photochemical models and modeling [Review]. Atmos Environ 34: 2283-2324. http://dx.doi.org/10.1016/S1352-2310(99)00468-9

Ryerson, TB; Buhr, MP; Frost, GJ; Goldan, PD; Holloway, JS; Hubler, G; Jobson, BT; Kuster, WC; McKeen, SA; Parrish, DD; Roberts, JM; Sueper, DT; Trainer, M; Williams, J; Fehsenfeld, FC. (1998). Emissions lifetimes and ozone formation in power plant plumes. J Geophys Res 103: 22569-22583. http://dx.doi.org/10.1029/98JD01620

Ryerson, TB; Trainer, M; Holloway, JS; Parrish, DD; Huey, LG; Sueper, DT; Frost, GJ; Donnelly, SG; Schauffler, S; Atlas, EL; Kuster, WC; Goldan, PD; Hubler, G; Meagher, JF; Fehsenfeld, FC. (2001). Observations of ozone formation in power plant plumes and implications for ozone control strategies. Science 292: 719-723. http://dx.doi.org/10.1126/science.1058113

Sakugawa, H; Kaplan, IR. (1989). H2O2 and O3 in the atmosphere of Los Angeles and its vicinity: Factors controlling their formation and their role as oxidants of SO2. J Geophys Res 94: 12957-12973.

Sarwar, G; Roselle, SJ; Mathur, R; Appel, W; Dennis, RL; Vogel, B. (2008). A comparison of CMAQ HONO predictions with observations from the Northeast Oxidant and Particle Study. Atmos Environ 42: 5760-5770. http://dx.doi.org/10.1016/j.atmosenv.2007.12.065

Schichtel, BA; Husar, RB. (2001). Eastern North American transport climatology during high- and low-ozone days. Atmos Environ 35: 1029-1038. http://dx.doi.org/10.1016/S1352-2310(00)00370-8

Schnell, RC; Oltmans, SJ; Neely, RR; Endres, MS; Molenar, JV; White, AB. (2009). Rapid photochemical production of ozone at high concentrations in a rural site during winter. Nat Geosci 2: 120-122. http://dx.doi.org/10.1038/NGEO415

Seaman, NL. (2000). Meteorological modeling for air quality assessments. Atmos Environ 34: 2231-2259.

Seinfeld, JH; Pandis, SN. (1998). Atmospheric chemistry and physics: From air pollution to climate change. New York: John Wiley & Sons.

Sexton, KG; Jeffries, HE; Jang, M; Kamens, RM; Doyle, M; Voicu, I; Jaspers, I. (2004). Photochemical products in urban mixtures enhance inflammatory responses in lung cells. Inhal Toxicol 1: 107-114. http://dx.doi.org/10.1080/08958370490443196

Shapiro, MA. (1980). Turbulent mixing within tropopause folds as a mechanism for the exchange of chemical constituents between the stratosphere and troposphere. J Atmos Sci 37: 994-1004.

Sillman, S. (1995). The use of NOy, H2O2 and HNO3 as indicators for ozone-NOx-hydrocarbon sensitivity in urban locations. J Geophys Res 100: 14175-14188.

Sillman, S; He, D; Pippin, MR; Daum, PH; Imre, DG; Kleinman, LI; Lee, JH; Weinstein-Lloyd, J. (1998). Model correlations for ozone, reactive nitrogen, and peroxides for Nashville in comparison with measurements: implications for O3-NOx-hydrocarbon chemistry. J Geophys Res 103: 22629-22644.

Sillman, S; He, DY. (2002). Some theoretical results concerning O3-NOx-VOC chemistry and NOx-VOC indicators. J Geophys Res 107: 4659. http://dx.doi.org/10.1029/2001JD001123

Singh, HB; Anderson, BE; Brune, WH; Cai, C; Cohen, RC; Crawford, JH; Cubison, MJ; Czech, EP; Emmons, L; Fuelberg, HE. (2010b). Pollution influences on atmospheric composition and chemistry at high northern latitudes: Boreal and California forest fire emissions. Atmos Environ 44: 4553-4564. http://dx.doi.org/10.1016/j.atmosenv.2010.08.026

Singh, HB; Cai, C; Kaduwela, A; Weinheimer, A; Wisthaler, A. (2012). Interactions of fire emissions and urban pollution over California: Ozone formation and air quality simulations. Atmos Environ 56: 45-51. http://dx.doi.org/10.1016/j.atmosenv.2012.03.046

Spicer, CW; Joseph, DW; Ollison, WM. (2010). A re-examination of ambient air ozone monitor interferences. J Air Waste Manag Assoc 60: 1353-1364. http://dx.doi.org/10.3155/1047-3289.60.11.1353

Stavrakou, T; Müller, JF; Boersma, KF; De Smedt, I; Van Der a, RJ. (2008). Assessing the distribution and growth rates of NO x emission sources by inverting a 10-year record of NO 2 satellite columns. Geophys Res Lett 35: L10801. http://dx.doi.org/10.1029/2008GL033521

Stedman, DH; Daby, EE; Stuhl, F; Niki, H. (1972). Analysis of ozone and nitric oxide by a chemiluminescent method in laboratory and atmospheric studies of photochemical smog. J Air Waste Manag Assoc 22: 260-263.

Stevens, RK; Drago, RJ; Mamane, Y. (1993). A long path differential optical absorption spectrometer and EPA-approved fixed-point methods intercomparison. Atmos Environ 27: 231-236.

Stutz, J; Ackermann, R; Fast, JD; Barrie, L. (2002). Atmospheric reactive chlorine and bromine at the Great Salt Lake, Utah. Geophys Res Lett 29: 1380. http://dx.doi.org/10.1029/2002GL014812

Stutz, J; Oh, HJ; Whitlow, SI; Anderson, C; Dibb, JE; Flynn, JH; Rappengluck, B; Lefe, B. (2010). Simultaneous DOAS and mist-chamber IC measurements of HONO in Houston, TX. Atmos Environ 44: 40904098. http://dx.doi.org/10.1016/j.atmosenv.2009.02.003

Surratt, JD; Chan, AW; Eddingsaas, NC; Chan, M; Loza, CL; Kwan, AJ; Hersey, SP; Flagan, RC; Wennberg, PO; Seinfeld, JH. (2010). Reactive intermediates revealed in secondary organic aerosol formation from isoprene. PNAS 107: 6640-6645. http://dx.doi.org/10.1073/pnas.0911114107

Tang, Q; Prather, MJ; Hsu, J. (2011). Stratosphere-troposphere exchange ozone flux related to deep convection. Geophys Res Lett 38: L03806. http://dx.doi.org/10.1029/2010GL046039

Tanimoto, H; Mukai, H; Hashimoto, S; Norris, JE. (2006). Intercomparison of ultraviolet photometry and gas-phase titration techniques for ozone reference standards at ambient levels. J Geophys Res 111: D16313. http://dx.doi.org/10.1029/2005JD006983

Tarasick, DW; Slater, R. (2008). Ozone in the troposphere: Measurements, climatology, budget, and trends. Atmos Ocean 46: 93-115. http://dx.doi.org/10.3137/ao.460105

Taubman, BF; Hains, JC; Thompson, AM; Marufu, LT; Doddridge, BG; Stehr, JW; Piety, CA; Dickerson, RR. (2006). Aircraft vertical profiles of trace gas and aerosol pollution over the mid-Atlantic United States: Statistics and meteorological cluster analysis. J Geophys Res 111: D10S07. http://dx.doi.org/10.1029/2005JD006196

Taubman, BF; Marufu, LT; Piety, CA; Doddridge, BG; Stehr, JW; Dickerson, RR. (2004). Airborne characterization of the chemical, optical, and meteorological properties, and origins of a combined ozone-haze episode over the eastern United States. J Atmos Sci 61: 1781-1793.

Thompson, AM; Stone, JB; Witte, JC; Miller, SK; Oltmans, SJ; Kucsera, TL; Ross, KL; Pickering, KE; Merrill, JT; Forbes, G; Tarasick, DW; Joseph, E; Schmidlin, FJ; Mcmillan, WW; Warner, J; Hintsa, EJ; Johnson, JE. (2007). Intercontinental Chemical Transport Experiment Ozonesonde Network study (IONS) 2004: 2 Tropospheric ozone budgets and variability over northeastern North America. J Geophys Res 112: D12S13. http://dx.doi.org/10.1029/2006JD007670

Thornton, JA; Kercher, JP; Riedel, TP; Wagner, NL; Cozic, J; Holloway, JS; Dube, WP; Wolfe, GM; Quinn, PK; Middlebrook, AM; Alexander, B; Brown, SS. (2010). A large atomic chlorine source inferred from mid-continental reactive nitrogen chemistry. Nature 464: 271-274. http://dx.doi.org/10.1038/nature08905

Tonnesen, GS; Dennis, RL. (2000a). Analysis of radical propagation efficiency to assess ozone sensitivity to hydrocarbons and NO x: 1. Local indicators of instantaneous odd oxygen production sensitivity. J Geophys Res 105: 9213. http://dx.doi.org/10.1029/1999JD900371

Tonnesen, GS; Dennis, RL. (2000b). Analysis of radical propagation efficiency to assess ozone sensitivity to hydrocarbons and NOx: 2 Long-lived species as indicators of ozone concentration sensitivity. J Geophys Res 105: 9227-9241. http://dx.doi.org/10.1029/1999JD900372

Tonnesen, S; Jeffries, HE. (1994). Inhibition of odd oxygen production in the carbon bond four and generic reaction set mechanisms. Atmos Environ 28: 1339-1349. http://dx.doi.org/10.1016/1352-2310(94)90281-X

Trainer, M; Parrish, DD; Buhr, MP; Norton, RB; Fehsenfeld, FC; Anlauf, KG; Bottenheim, JW; Tang, YZ; Wiebe, HA; Roberts, JM; Tanner, RL; Newman, L; Bowersox, VC; Meagher, JF; Olszyna, KJ; Rodgers, MO; Wang, T; Berresheim, H; Demerjian, KL; Roychowdhury, UK. (1993). Correlation of ozone with NOy in photochemically aged air. J Geophys Res 98: 2917-2925.

Turner, AJ; Fiore, AM; Horowitz, LW; Naik, V; Bauer, M. (2012). Summertime cyclones over the Great Lakes Storm Track from 1860–2100: variability, trends, and association with ozone pollution. Atmos Chem Phys Discuss 12: 21679-21712. http://dx.doi.org/10.5194/acpd-12-21679-2012

U.S. Census Bureau. (2011). U.S. Census Bureau. Available online at http://www.census.gov/ (accessed January 28, 2011).

U.S. EPA (U.S. Environmental Protection Agency). (1979b). Transfer standards for the calibration of ambient air monitoring analyzers for ozone: Technical assistance document [EPA Report]. (EPA-600/4-79-056). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (1996a). Air quality criteria for ozone and related photochemical oxidants [EPA Report]. (EPA/600/P-93/004AF). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (2006b). Air quality criteria for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-05/004AF). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=149923

U.S. EPA (U.S. Environmental Protection Agency). (2008a). 2005 National Emissions Inventory data and documentation. Available online at http://www.epa.gov/ttn/chief/net/2005inventory.html (accessed November 1, 2010).

U.S. EPA (U.S. Environmental Protection Agency). (2008c). Integrated science assessment for oxides of nitrogen: Health criteria [EPA Report]. (EPA/600/R-08/071). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=194645

U.S. EPA (U.S. Environmental Protection Agency). (2008d). Integrated science assessment for sulfur oxides: Health criteria [EPA Report]. (EPA/600/R-08/047F). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=198843

U.S. EPA (U.S. Environmental Protection Agency). (2008e). National air quality: Status and trends through 2007 [EPA Report]. (EPA/454/R-08/006). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (2009d). Integrated science assessment for particulate matter [EPA Report]. (EPA/600/R-08/139F). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=216546

U.S. EPA (U.S. Environmental Protection Agency). (2009e). Risk and exposure assessment for review of the secondary National Ambient Air Quality Standards for oxides of nitrogen and oxides of sulfur [EPA Report]. (EPA/452/R-09/008A). Research Triangle Park, NC. http://www.epa.gov/ttnnaaqs/standards/no2so2sec/cr_rea.html

U.S. EPA (U.S. Environmental Protection Agency). (2010a). Air trends: Design values. Available online at http://epa.gov/airtrends/values.html (accessed February 1, 2011).

U.S. EPA (U.S. Environmental Protection Agency). (2010b). Biogenic Emissions Inventory System (BEIS) modeling. Available online at http://www.epa.gov/AMD/biogen.html (accessed January 28, 2011).

U.S. EPA (U.S. Environmental Protection Agency). (2010c). Integrated science assessment for carbon monoxide [EPA Report]. (EPA/600/R-09/019F). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=218686

U.S. EPA (U.S. Environmental Protection Agency). (2010d). MOBILE6 vehicle emission modeling software. Available online at http://www.epa.gov/otaq/m6.htm (accessed January 28, 2011).

U.S. EPA (U.S. Environmental Protection Agency). (2010e). Our nation's air: Status and trends through 2008 [EPA Report]. (EPA-454/R-09-002). Research Triangle Park, NC. http://www.epa.gov/airtrends/2010/report/fullreport.pdf

U.S. EPA (U.S. Environmental Protection Agency). (2010f). Transfer standards for calibration of air monitoring analyzers for ozone [EPA Report]. (EPA454/B10001). Research Triangle Park, NC. http://www.epa.gov/ttn/amtic/files/ambient/qaqc/OzoneTransferStandardGuidance.pdf

U.S. EPA (U.S. Environmental Protection Agency). (2011a). AirNow. Available online at http://www.airnow.gov/ (accessed January 28, 2011).

U.S. EPA (U.S. Environmental Protection Agency). (2011e). Map monitoring sites. Available online at http://www.epa.gov/airexplorer/monitor_kml.htm (accessed January 28, 2011).

U.S. EPA (U.S. Environmental Protection Agency). (2011f). MOVES (Motor Vehicle Emission Simulator). Available online at http://www.epa.gov/otaq/models/moves/index.htm (accessed January 28, 2011).

UNC (University of North Carolina). (2011). SMOKE (Version 2.7) [Computer Program]. Chapel Hill, NC: University of North Carolina at Chapel Hill, Center for Environmental Modeling for Policy Development. Retrieved from http://www.smoke-model.org/index.cfm

Univ of Leeds, NCAS, (University of Leeds, National Centre for Atmospheric Science). (2010). The master chemical mechanism. Available online at http://mcm.leeds.ac.uk/MCM/home.htt (accessed January 28, 2011).

Utembe, SR; Hansford, GM; Sanderson, MG; Freshwater, RA; Pratt, KFE; Williams, DE; Cox, RA; Jones, RL. (2006). An ozone monitoring instrument based on the tungsten trioxide (WO3) semiconductor. Sens Actuators B 114: 507-512. http://dx.doi.org/10.1016/j.snb.2005.04.049

van der Werf, GR; Randerson, JT; Giglio, L; Collatz, GJ; Kasibhatla, PS; Arellano, AF, Jr. (2006). Interannual variability in global biomass burning emissions from 1997 to 2004. Atmos Chem Phys 6: 3423-3441.

Vardoulakis, S; Lumbreras, J; Solazzo, E. (2009). Comparative evaluation of nitrogen oxides and ozone passive diffusion tubes for exposure studies. Atmos Environ 43: 2509-2517. http://dx.doi.org/10.1016/j.atmosenv.2009.02.048

Viallon, J; Moussay, P; Norris, JE; Guenther, FR; Wielgosz, RI. (2006). A study of systematic biases and measurement uncertainties in ozone mole fraction measurements with the NIST Standard Reference Photometer. Metrologia 43: 441-450. http://dx.doi.org/10.1088/0026-1394/43/5/016

Volz, A; Kley, D. (1988). Evaluation of the Montsouris series of ozone measurements made in the nineteenth century. Nature 332: 240-242. http://dx.doi.org/10.1038/332240a0

von Kuhlmann, R; Lawrence, MG; Poschl, U; Crutzen, PJ. (2004). Sensitivities in global scale modeling of isoprene. Atmos Chem Phys 4: 1-17.

Wang, HQ; Jacob, DJ; Le Sager, P; Streets, DG; Park, RJ; Gilliland, AB; van Donkelaar, A. (2009a). Surface ozone background in the United States: Canadian and Mexican pollution influences. Atmos Environ 43: 1310-1319. http://dx.doi.org/10.1016/j.atmosenv.2008.11.036

Wang, J; Christopher, SA; Nair, US; Reid, JS; Prins, EM; Szykman, J; Hand, JL. (2006). Mesoscale modeling of Central American smoke transport to the United States: 1. "Top-down" assessment of emission strength and diurnal variation impacts. J Geophys Res 111: D05S17. http://dx.doi.org/10.1029/2005JD006416

Weaver, CP; Cooter, E; Gilliam, R; Gilliland, A; Grambsch, A; Grano, D; Hemming, B; Hunt, SW; Nolte, C; Winner, DA; Liang, XZ; Zhu, J; Caughey, M; Kunkel, K; Lin, JT; Tao, Z; Williams, A; Wuebbles, DJ; Adams, PJ; Dawson, JP; Amar, P; He, S; Avise, J; Chen, J; Cohen, RC; Goldstein, AH; Harley, RA; Steiner, AL; Tonse, S; Guenther, A; Lamarque, JF; Wiedinmyer, C; Gustafson, WI; Leung, LR; Hogrefe, C; Huang, HC; Jacob, DJ; Mickley, LJ; Wu, S; Kinney, PL; Lamb, B; Larkin, NK; Mckenzie, D; Liao, KJ; Manomaiphiboon, K; Russell, AG; Tagaris, E; Lynn, BH; Mass, C; Salathé, E; O'neill, SM; Pandis, SN; Racherla, PN; Rosenzweig, C; Woo, JH. (2009). A Preliminary Synthesis of Modeled Climate Change Impacts on U.S. Regional Ozone Concentrations. Bull Am Meteorol Soc 90: 1843-1863. http://dx.doi.org/10.1175/2009BAMS2568.1

Webster, M; Nam, J; Kimura, Y; Jeffries, H; Vizuete, W; Allen, DT. (2007). The effect of variability in industrial emissions on ozone formation in Houston, Texas. Atmos Environ 41: 9580-9593. http://dx.doi.org/10.1016/j.atmosenv.2007.08.052

Wells, B; Wesson, K; Jenkins, S. (2012). Analysis of recent U.S. ozone air quality data to support the O3 NAAQS review and quadratic rollback simulations to support the first draft of the risk and exposure assessment. Wells, B; Wesson, K; Jenkins, S. http://www.epa.gov/ttnnaaqs/standards/o3/data/20120814wells.pdf

Wernli, H; Bourqui, M. (2002). A Lagrangian "1-year climatology" of (deep) cross-tropopause exchange in the extratropical Northern Hemisphere. J Geophys Res 107: 4021. http://dx.doi.org/10.1029/2001JD000812

Wild, O; Fiore, AM; Shindell, DT; Doherty, RM; Collins, WJ; Dentener, FJ; Schultz, MG; Gong, S; Mackenzie, IA; Zeng, G; Hess, P; Duncan, BN; Bergmann, DJ; Szopa, S; Jonson, JE; Keating, TJ; Zuber, A. (2012). Modelling future changes in surface ozone: a parameterized approach. Atmos Chem Phys 12: 2037-2054. http://dx.doi.org/10.5194/acp-12-2037-2012

Williams, EJ; Fehsenfeld, FC; Jobson, BT; Kuster, WC; Goldan, PD; Stutz, J; McClenny, WA. (2006). Comparison of ultraviolet absorbance, chemiluminescence, and DOAS instruments for ambient ozone monitoring. Environ Sci Technol 40: 57555762. http://dx.doi.org/10.1021/es0523542

Wilson, KL; Birks, JW. (2006). Mechanism and elimination of a water vapor interference in the measurement of ozone by UV absorbance. Environ Sci Technol 40: 6361-6367. http://dx.doi.org/10.1021/es052590c

Wimmers, AJ; Moody, JL; Browell, EV; Hair, JW; Grant, WB; Butler, CF; Fenn, MA; Schmidt, CC; Li, J; Ridley, BA. (2003). Signatures of tropopause folding in satellite imagery. J Geophys Res 108D4: 8360. http://dx.doi.org/10.1029/2001JD001358

Wise, EK; Comrie, AC. (2005). Meteorologically adjusted urban air quality trends in the Southwestern United States. Atmos Environ 39: 2969-2980. http://dx.doi.org/10.1016/j.atmosenv.2005.01.024

Worden, HM; Logan, JA; Worden, JR; Beer, R; Bowman, K; Clough, SA; Eldering, A; Fisher, BM; Gunson, MR; Herman, RL; Kulawik, SS; Lampel, MC; Luo, M; Megretskaia, IA; Osterman, GB; Shephard, MW. (2007a). Comparisons of Tropospheric Emission Spectrometer (TES) ozone profiles to ozonesondes: Methods and initial results. J Geophys Res 112: D03309. http://dx.doi.org/10.1029/2006JD007258

Worden, J; Liu, X; Bowman, K; Chance, K; Beer, R; Eldering, A; Gunson, M; Worden, H. (2007b). Improved tropospheric ozone profile retrievals using OMI and TES radiances. Geophys Res Lett 34: L01809. http://dx.doi.org/10.1029/2006GL027806

Wu, S; Mickley, LJ; Jacob, DJ; Logan, JA. (2007). Why are there large differences between models in global budgets of tropospheric ozone? J Geophys Res 112: D05302. http://dx.doi.org/10.1029/2006JD007801

Wu, S; Mickley, LJ; Leibensperger, EM; Jacob, DJ; Rind, D; Streets, DG. (2008b). Effects of 2000-2050 global change on ozone air quality in the United States. J Geophys Res 113: D06302. http://dx.doi.org/10.1029/2007JD008917

Yang, Q; Cunnold, DM; Choi, Y; Wang, Y; Nam, J; Wang, HJ; Froidevaux, L; Thompson, AM; Bhartia, PK. (2010). A study of tropospheric ozone column enhancements over North America using satellite data and a global chemical transport model. J Geophys Res 115: D08302. http://dx.doi.org/10.1029/2009JD012616

Yung, YL; Pinto, JP; Watson, RT; Sander, SP. (1980). Atmospheric bromine and ozone perturbations in the lower stratosphere. J Atmos Sci 37: 339-353.

Zhang, K; Wexler, A. (2008). Modeling urban and regional aerosols: Development of the UCD Aerosol Module and implementation in CMAQ model. Atmos Environ 42: 3166-3178.

Zhang, L; Jacob, DJ; Boersma, KF; Jaffe, DA; Olson, JR; Bowman, KW; Worden, JR; Thompson, AM; Avery, MA; Cohen, RC; Dibb, JE; Flock, FM; Fuelberg, HE; Huey, LG; McMillan, WW; Singh, HB; Weinheimer, AJ. (2008). Transpacific transport of ozone pollution and the effect of recent Asian emission increases on air quality in North America: An integrated analysis using satellite, aircraft, ozonesonde, and surface observations. Atmos Chem Phys 8: 6117-6136.

Zhang, L; Jacob, DJ; Downey, NV; Wood, DA; Blewitt, D; Carouge, CC; Van donkelaar, A; Jones, DBA; Murray, LT; Wang, Y. (2011). Improved estimate of the policy-relevant background ozone in the United States using the GEOS-Chem global model with 1/2 2/3 horizontal resolution over North America. Atmos Environ 45: 6769-6776. http://dx.doi.org/10.1016/j.atmosenv.2011.07.054

Zhang, L; Jacob, DJ; Logan, JA; Chance, K; Eldering, A; Bojkov, BR. (2010b). Intercomparison methods for satellite measurements of atmospheric composition: Application to tropospheric ozone from TES and OMI. Atmos Chem Phys 10: 4725-4739. http://dx.doi.org/10.5194/acpd-10-1417-2010

Zhang, Q; Jimenez, JL; Canagaratna, MR; Jayne, JT; Worsnop, DR. (2005). Time- and size-resolved chemical composition of submicron particles in Pittsburgh: Implications for aerosol sources and processes. J Geophys Res 110: 1-19.

Zhang, X; Zhuang, G; Guo, J; Yin, K; Zhang, P. (2007b). Characterization of aerosol over the Northern South China Sea during two cruises in 2003. Atmos Environ 41: 7821-7836.

Zhang, Y. (2005). Evaluation of three probing techniques in a three-dimensional air quality model. J Geophys Res 110: D02305. http://dx.doi.org/10.1029/2004JD005248

Ziemke, JR; Chandra, S; Duncan, BN; Froidevaux, L; Bhartia, PK; Levelt, PF; Waters, JW. (2006). Tropospheric ozone determined from Aura OMI and MLS: Evaluation of measurements and comparison with the Global Modeling Initiative's Chemical Transport Model. J Geophys Res 111: D19303. http://dx.doi.org/10.1029/2006JD007089

Zimmermann, J; Poppe, D. (1993). Nonlinear chemical couplings in the tropospheric NOx-HOx gas phase chemistry. J Atmos Chem 17: 141-155.

# 4 EXPOSURE TO AMBIENT OZONE

## 4.1 Introduction

The 2006 $O_3$ AQCD (U.S. EPA, 2006b) evaluated $O_3$ concentrations and exposures in multiple microenvironments, discussed methods for estimating personal and population exposure via monitoring and modeling, analyzed relationships between personal exposure and ambient concentrations, and discussed the implications of using ambient $O_3$ concentrations as an estimate of exposure in epidemiologic studies. This chapter presents new information regarding exposure to ambient $O_3$ which builds upon the body of evidence presented in the 2006 $O_3$ AQCD. A brief summary of findings from the 2006 $O_3$ AQCD is presented at the beginning of each section as appropriate.

Section 4.2 presents general exposure concepts describing the relationship between ambient pollutant concentrations and personal exposure. Section 4.3 describes exposure measurement techniques and studies that measured personal, ambient, indoor, and outdoor concentrations of $O_3$ and related pollutants. Section 4.4 presents material on parameters relevant to exposure estimation, including activity patterns, averting behavior, and population proximity to ambient monitors. Section 4.5 describes techniques for modeling local $O_3$ concentrations, air exchange rates, microenvironmental concentrations, and personal and population exposure. Section 4.6 discusses the implications of using ambient $O_3$ concentrations to estimate exposure in epidemiologic studies, including several factors that contribute to exposure error.

## 4.2 General Exposure Concepts

A theoretical model of personal exposure is presented to highlight measurable quantities and the uncertainties that exist in this framework. An individual's time-integrated total exposure to $O_3$ can be described based on a compartmentalization of the person's activities throughout a given time period:

$$\boldsymbol{E_T = \int C_j dt}$$

**Equation 4-1**

where $E_T$ = total exposure over a time-period of interest, $C_j$ = airborne $O_3$ concentration at microenvironment $j$, and $dt$ = portion of the time-period spent in microenvironment $j$. Equation 4-1 can be decomposed into a model that accounts for exposure to $O_3$, of ambient ($E_a$) and nonambient ($E_{na}$) origin of the form:

$$E_T = E_a + E_{na}$$

**Equation 4-2**

Ambient $O_3$ is formed through photochemical reactions involving $NO_X$, VOCs, and other compounds, as described in Chapter 3. Although nonambient sources of $O_3$ exist, such as $O_3$ generators and laser printers, these sources are specific to individuals and may not be important sources of population exposure. Ozone concentrations generated by ambient and nonambient sources are subject to spatial and temporal variability that can affect estimates of exposure and influence epidemiologic effect estimates. Exposure parameters affecting interpretation of epidemiologic studies are discussed in Section 4.6.

This assessment focuses on the ambient component of exposure because this is more relevant to the NAAQS review. Assuming steady-state outdoor conditions, $E_a$ can be expressed in terms of the fraction of time spent in various outdoor and indoor microenvironments (U.S. EPA, 2006c; Wilson et al., 2000):

$$E_a = \sum f_o C_o + \sum f_i F_{inf_i} C_{o,i}$$

**Equation 4-3**

where $f$ = fraction of the relevant time period (equivalent to $dt$ in Equation 4-1), subscript $o$ = index of outdoor microenvironments, subscript $i$ = index of indoor microenvironments, subscript $o,i$ = index of outdoor microenvironments adjacent to a given indoor microenvironment $i$, and $F_{\text{inf},i}$ = infiltration factor for indoor microenvironment $i$. Equation 4-3 is subject to the constraint $\Sigma f_o + \Sigma f_i = 1$ to reflect the total exposure over a specified time period, and each term on the right hand side of the equation has a summation because it reflects various microenvironmental exposures. Here, "indoors" refers to being inside any aspect of the built environment, e.g., home, office buildings, enclosed vehicles (automobiles, trains, buses), and/or recreational facilities (movie theaters, restaurants, bars). "Outdoor" exposure can occur in parks or yards, on sidewalks, and on bicycles or motorcycles. Assuming steady state ventilation conditions, the infiltration factor is a function of the penetration ($P$) of $O_3$ into the microenvironment, the air exchange rate ($a$) of the microenvironment, and the rate of $O_3$ loss ($k$) in the microenvironment; $F_{\text{inf}} = Pa/(a + k)$.

In epidemiologic studies, the central-site ambient concentration, $C_a$, is often used in lieu of outdoor microenvironmental data to represent these exposures based on the availability of data. Thus it is often assumed that $C_o = C_a$ and that the fraction of time spent outdoors can be expressed cumulatively as $f_o$; the indoor terms still retain a summation because infiltration differs among different microenvironments. If an epidemiologic study employs only $C_a$, then the assumed model of an individual's

exposure to ambient $O_3$, first given in Equation 4-3, is re-expressed solely as a function of $C_a$:

$$E_a = \left(f_o + \sum f_i F_{inf_i}\right) C_a$$

**Equation 4-4**

The spatial variability of outdoor $O_3$ concentrations due to meteorology, topography, varying precursor emissions and $O_3$ formation rates; the design of the epidemiologic study; and other factors determine whether or not Equation 4-4 is a reasonable approximation for Equation 4-3. These equations also assume steady-state microenvironmental concentrations. Errors and uncertainties inherent in use of Equation 4-4 in lieu of Equation 4-3 are described in Section 4.6 with respect to implications for interpreting epidemiologic studies. Epidemiologic studies often use concentration measured at a central site monitor to represent ambient concentration; thus $\alpha$, the ratio between personal exposure to ambient $O_3$ and the ambient concentration of $O_3$, is defined as:

$$\alpha = \frac{E_a}{C_a}$$

**Equation 4-5**

Combination of Equation 4-4 and Equation 4-5 yields:

$$\alpha = f_o + \sum f_i F_{inf_i}$$

**Equation 4-6**

*where* $\alpha$ varies between 0 and 1. If a person's exposure occurs in a single microenvironment, the ambient component of a microenvironmental $O_3$ concentration can be represented as the product of the ambient concentration and $F_{inf}$. The U.S.EPA (2006c) noted that time-activity data and corresponding estimates of $F_{inf}$ for each microenvironmental exposure are needed to compute an individual's $\alpha$ with accuracy. In epidemiologic studies, $\alpha$ is assumed to be constant in lieu of time-activity data and estimates of $F_{inf}$, which can vary with building and meteorology-related air exchange characteristics. If important local outdoor sources and sinks exist that are not captured by central site monitors, then the ambient component of the local outdoor concentration may be estimated using dispersion models, land use regression models, receptor models, fine scale CTMs or some combination of these techniques. These techniques are described in Section 4.5.

## 4.3 Exposure Measurement

This section describes techniques that have been used to measure microenvironmental concentrations of $O_3$ and personal $O_3$ exposures as well as results of studies using those techniques. Previous studies from the 2006 $O_3$ AQCD are described along with newer studies that evaluate indoor-outdoor concentration relationships, associations between personal exposure and ambient monitor concentration, and multipollutant exposure to other pollutants in conjunction with $O_3$. Tables are provided to summarize important study results.

### 4.3.1 Personal Monitoring Techniques

As described in the 2006 $O_3$ AQCD, passive samplers have been developed and deployed to measure personal exposure to $O_3$ (Grosjean and Hisham, 1992; Kanno and Yanagisawa, 1992). Widely used versions of these samplers utilize a filter coated with nitrite, which is converted to nitrate by $O_3$ and then quantified by a technique such as ion chromatography (Koutrakis et al., 1993). This method has been licensed and marketed by Ogawa, Inc., Japan (Ogawa & Co, 2007). The cumulative sampling and the detection limit of the passive badges makes them mainly suitable for monitoring periods of 24 hours or greater, which limits their ability to measure short-term daily fluctuations in personal $O_3$ exposure. Longer sampling periods give lower detection limits; use of the badges for a 6-day sampling period yields a detection limit of 1 ppb, while a 24-hour sampling period gives a detection limit of approximately 5-10 ppb. This can result in a substantial fraction of daily samples being below the detection limit (Sarnat et al., 2006a; Sarnat et al., 2005), which is a limitation of past and current exposure studies. Development of improved passive samplers capable of shorter-duration monitoring with lower detection limits would enable more precise characterization of personal exposure in multiple microenvironments with relatively low participant burden.

The nitrite-nitrate conversion reaction has also been used as the basis for an active sampler consisting of a nitrite-coated glass tube through which air is drawn by a pump operating at 65 mL/min (Geyh et al., 1999; Geyh et al., 1997). The reported detection limit is 10 ppb-h, enabling the quantification of $O_3$ concentrations measured over a few hours rather than a full day (Geyh et al., 1999).

A portable active $O_3$ monitor based on the UV photometric technique used for stationary monitors (Chapter 3) has recently been approved as a FEM (75 FR 22126). This monitor includes a Nafion tube in the inlet line to equilibrate humidity, reducing the effect of humidity changes in different microenvironments (Wilson and Birks, 2006). Its size and weight (approximately 10×20×30 cm; 2 kg) make it suitable for use in a backpack configuration. The monitors are currently used by the U.S. National Park service as stationary monitors to measure $O_3$ in several national parks (Chapter 3). Future improvements and continued miniaturization of real-time $O_3$

monitors can yield highly time-resolved personal measurements to further evaluate $O_3$ exposures in specific situations, such as near roadways or while in transit.

### 4.3.2 Indoor-Outdoor Concentration Relationships

Several studies summarized in the 2006 $O_3$ AQCD, along with some newer studies, have evaluated the relationship between indoor $O_3$ concentration and the $O_3$ concentration immediately outside the indoor microenvironment. These studies show that the indoor concentration is often substantially lower than the outdoor concentration unless indoor sources are present. Low indoor $O_3$ concentrations can be explained by reactions of $O_3$ with surfaces and airborne constituents. Studies have shown that $O_3$ is deposited onto indoor surfaces where reactions produce secondary pollutants such as formaldehyde (Reiss et al., 1995b; Reiss et al., 1995a). However, the indoor-outdoor relationship is greatly affected by the air exchange rate; under conditions of high air exchange rate, such as open windows, the indoor $O_3$ concentration may approach the outdoor concentration. Thus, in rooms with open windows, the indoor-outdoor (I/O) ratio may approach 1.0. Table 4-1 summarizes I/O ratios and correlations reported by older and more recent studies, with discussion of individual studies in the subsequent text. In general, I/O ratios range from about 0.1 to 0.4, with some evidence for higher ratios during the $O_3$ season when concentrations are higher.

Ozone concentrations near and below the monitor detection limit cause uncertainty in I/O ratios, because small changes in low concentration values cause substantial variation in resulting ratios. This problem is particularly acute in the non-ozone season when ambient $O_3$ concentrations are low. Further improvements in characterization of microenvironmental $O_3$ concentrations and I/O ratios will rely on improved monitoring. Until new monitoring techniques are available and can be used in the field, past studies summarized in the 2006 $O_3$ AQCD remain relevant to consider along with more recent studies in evaluating the relationship between indoor and outdoor $O_3$ concentrations.

## Table 4-1 Relationships between indoor and outdoor $O_3$ concentration.

| Study | Location | Years/ Season | Population | Sample duration | Ratio[a] | Correlation | Micro-environ-ment | Comment | Concentration/ Detection limit (ppb) |
|---|---|---|---|---|---|---|---|---|---|
| Geyh et al. (2000) | Upland, Southern California | June - Sept 1995 and May 1996 | Children | 6 days | 0.24 | NR | Home | Air-conditioned<br>Ratio: Indoor mean/ outdoor mean | Mean (SD) *Indoor*<br>11.8 (9.2)<br>*Outdoor*<br>48.2 (12.2) |
| | | Oct 1995-Apr 1996 | | | 0.15 | | | | *Indoor*<br>3.2 (3.9)<br>*Outdoor*<br>21.1 (10.7) |
| | Mountain Communities, Southern California | June - Sept 1995 and May 1996 | | | 0.36 | | | Window ventilation<br>Ratio: indoor mean/ outdoor mean | *Indoor*<br>21.4 (14.8)<br>*Outdoor*<br>60.1 (17.1) |
| | | Oct 1995-Apr 1996 | | | 0.08 | | | | *Indoor*<br>2.8 (4.2)<br>*Outdoor*<br>35.7 (9.3) |
| | Upland & Mountain | Entire period | | | | | | | LOD: 1.0<br>Fraction above LOD<br>*Indoor* 80.3%<br>*Outdoor* 99.95% |

| Study | Location | Years/ Season | Population | Sample duration | Ratio[a] | Correlation | Micro-environ-ment | Comment | Concentration/ Detection limit (ppb) |
|---|---|---|---|---|---|---|---|---|---|
| Avol et al. (1998a) | Southern California | Feb-Dec 1994 | NR | 24 h | 0.37<br>SD: 0.25<br>IQR: 0.07-0.45 | 0.58 | Home | Ratio: each pair of values | Mean (SD)<br>*Indoor*<br>13 (12)<br>*Outdoor*<br>37 (19)<br>LOD: 5 |
| | | Summer (late June – late Sept) | | | 0.43<br>SD: 0.29 | NR | | | |
| | | Non-summer | | | 0.32<br>SD: 0.21 | NR | | | |
| Romieu et al. (1998a) | Mexico City, Mexico | Sept 1993 - July 1994 | Children | 7 or 14 days | 0.20<br>SD: 0.18<br>0.15[b]<br>Range: 0.01-1.00 | NR | Home | Ratio: each pair of values | Mean (SD)<br>*Indoor*<br>7-day: 5 (4)<br>14-day: 7 (5)<br>*Outdoor*<br>7-day: 27 (10)<br>14-day: 37 (12)<br>LOD:<br>7-day: 2.4-2.9<br>14-day: 1.2-3.5 |
| Lee et al. (2004a) | Nashville, TN | Summer 1994 | Children | 1 week | 0.1<br>SD: 0.18 | NR | Home | Ratio: Indoor mean/ outdoor mean | *Indoor*<br>Range of Weekly Means: 1.6-3.1<br>Fraction above LOD, Range:<br>14-87%<br>*Outdoor*<br>Range of Weekly Means: 18.6-25.9<br>Fraction above LOD:<br>100%<br>LOD: 1.2 |

| Study | Location | Years/ Season | Population | Sample duration | Ratio[a] | Correlation | Micro-environ-ment | Comment | Concentration/ Detection limit (ppb) |
|---|---|---|---|---|---|---|---|---|---|
| Héroux et al. (2010) | Regina, Saskatchewa, Canada | Summer 2007 | All age groups | 5 days | 0.13 | NR | Home | Ratio: Indoor mean/ outdoor mean | Mean (SD)<br>*Indoor*<br>0.7 (0.8)<br>*Outdoor*<br>5.4 (1.3)<br>LOD: NR<br>Fraction above field LOD: Indoor: <50%<br>Outdoor: NR |
| Liu et al. (1995) | Toronto, Canada | Winter 1992 | All age groups | 1 week | 0.07<br>SD: 0.10 | NR | Home | Ratio: each pair of values | Mean (SD)<br>*Indoor*<br>1.6 (4.1)<br>*Outdoor*<br>15.4 (6)<br>LOD: 1.05 |
| | | Summer 1992 | | | 0.40<br>SD: 0.29 | | | | *Indoor*: NR<br>*Outdoor*: NR |
| | | Summer 1992 | | 12 h | 0.30<br>SD: 0.32 | | | Daytime<br>Ratio: each pair of values | *Indoor*<br>7.1 (12.6)<br>*Outdoor*<br>19.1 (10.8)<br>LOD: 14.7 |
| | | Summer 1992 | | | 0.43<br>SD: 0.54 | | | Nighttime<br>Ratio: each pair of values | *Indoor*<br>6.2 (9.5)<br>*Outdoor*<br>9.4 (10.2)<br>LOD: NR |

| Study | Location | Years/ Season | Population | Sample duration | Ratio[a] | Correlation | Micro-environ-ment | Comment | Concentration/ Detection limit (ppb) |
|---|---|---|---|---|---|---|---|---|---|
| | | | Children | 24 h/day, 14 days | 0.15 | | | Ratio: each pair of values | Mean (SD)<br>*Indoor*<br>6 (2.8)<br>LOD: 0.7-1.3<br>*Outdoor*<br>41 (8.2) |
| Romieu et al. (1998a) | Mexico City, Mexico | Sept 1993 - July 1994 | Children (during school hours) | 5 h/day, 5 days, 10 days | 0.30-0.40 | NR | School | Immediately outside the schools | *Indoor*<br>5-day: 22 (16.1)<br>10-day: 22 (16.0)<br>LOD:<br>5 day: 3.6-4.1<br>10 day: 0.3-1.6<br>*Outdoor*<br>5-day: 73 (21.5)<br>10-day: 56 (17.9) |
| Blondeau et al. (2005) | La Rochelle, France | Spring 2000 | Children | 2 weeks | Range: 0.00-0.45 | NR | School | No air conditioning<br>Ratio: Indoor mean/ outdoor mean | Range*<br>*Indoor*<br>0-57<br>*Outdoor*<br>0-68<br>LOD: 1<br>*Estimated from Figure 1, showing two of eight schools |

| Study | Location | Years/ Season | Population | Sample duration | Ratio[a] | Correlation | Micro-environ-ment | Comment | Concentration/ Detection limit (ppb) |
|---|---|---|---|---|---|---|---|---|---|
| López-Aparicio et al. (2011) | Prague, Czech Republic | July 2009 | All age groups | 1 month | 0.10 | NR | Historic Library | No heating or air conditioning<br>Ratio: Indoor mean/ outdoor mean | Mean*<br>*Indoor*: 2<br>*Outdoor*: 18.2 |
| | | Dec 2009 | | | 0.30 | | | | Mean*<br>*Indoor*: 1.3<br>*Outdoor*: 4.4<br>*Estimated from Fig. 2 |
| | | July 2009 – Mar 2010 Overall | | | NR | | | | Range<br>Indoor: 1-2.5<br>Outdoor: 4.1-21.9<br>LOD: 0.5 |
| Riediker et al. (2003) | North Carolina | Aug - Oct 2001 | Adults | 9 h | 0.51<br>p- value: 0.000 | NR | Vehicle | Ratio: Indoor mean/ outdoor mean | Mean (SD)<br>*In-vehicle*<br>11.7 (15.9)<br>*Roadside*<br>22.8 (13.3)<br>LOD: 10 |

[a]Mean value unless otherwise indicated

[b]Median

LOD = limit of detection; NR = not reported; SD = standard deviation

Geyh et al. (2000) measured 6-day indoor and outdoor concentrations at 116 homes in southern California, approximately equally divided between the community of Upland and several mountain communities. The extended sampling period resulted in a relatively low detection limit (1 ppb) for the passive samplers used and a large fraction of samples above the detection limit; over 80% of the indoor samples and virtually all of the outdoor samples were above the detection limit. The Upland homes were nearly all air-conditioned, while the mountain community homes were ventilated by opening windows. During the $O_3$ season, the indoor $O_3$ concentration averaged over all homes was approximately 24% of the overall mean outdoor concentration in Upland (11.8 versus 48.2 ppb), while in the mountain communities, the indoor concentration was 36% of the outdoor concentration (21.4 versus 60.1 ppb). This is consistent with the increased air exchange rate expected in homes using window ventilation. In the non-ozone season, when homes are likely to be more tightly closed to conserve heat, the ratios of indoor to outdoor concentration were 0.15 (3.2 versus 21.1 ppb) and 0.08 (2.8 versus 35.7 ppb) in Upland and the mountain communities, respectively. Avol et al. (1998a) observed a mean I/O ratio of 0.37 for 239 matched 24-hour samples collected between February and December at homes in the Los Angeles area. The I/O ratio during summer was somewhat higher than the non-summer I/O ratio (0.43 versus 0.32). The authors also reported a correlation of 0.58 between the 24-h avg indoor concentration and the outdoor concentration, which was only slightly higher than the correlation between the indoor concentration and the concentration at the neighborhood fixed-site monitor (0.49). Substantially higher summer I/O ratios were reported in a study in Toronto, Canada (Liu et al., 1995), which found summer I/O ratios of 0.30-0.43, in comparison with a winter I/O ratio of 0.07. Romieu et al. (1998a) reported a mean I/O ratio of 0.20 in 145 homes in Mexico City, Mexico, for 7- or 14-day cumulative samples, with the highest ratios observed in homes where windows were usually open during the day and where there was no carpeting or air filters. Studies conducted in Nashville, TN, and Regina, Saskatchewan (Canada) reported mean residential I/O ratios of approximately 0.1 (Héroux et al., 2010; Lee et al., 2004a).

Investigators have also measured I/O ratios for non-residential microenvironments, including schools and vehicles. Romieu et al. (1998a) reported that $O_3$ concentrations measured during school hours (10-day cumulative sample, 5 h/day) were 30-40% of concentrations immediately outside the schools, while overall I/O ratios (14-day cumulative sample, 24 h/day) were approximately 15%. The authors attribute this discrepancy to increased air exchange during the school day due to opening doors and windows. Air exchange was also identified as an important factor in the I/O ratios measured at eight French schools (Blondeau et al., 2005). In this study, the I/O ratios based on simultaneous continuous measurements ranged from 0-0.45, increasing with decreasing building tightness. A historical library building in Prague, Czech Republic with no heating or air conditioning (i.e., natural ventilation) was observed to have ratios of one-month indoor and outdoor concentrations ranging from 0.10-0.30 during a nine-month sampling campaign, with the highest ratios reported in Nov-Dec 2009 and the lowest ratios during July-Aug 2009 (López-Aparicio et al., 2011). Indoor concentrations were relatively constant (approximately 3-7 $\mu g/m^3$ or 2-3 ppb), while outdoor concentrations were lower in the winter

(9-10 µg/ $m^3$ or about 5 ppb) than in the summer (35-45 µg/ $m^3$ or about 20 ppb). This seasonal variation in outdoor concentrations coupled with homogeneous indoor concentrations, together with increased wintertime air exchange rate due to higher indoor-outdoor temperature differences, is likely responsible for the observed seasonal pattern in I/O ratios.

Exposures in near-road, on-road and in-vehicle microenvironments are likely to be more variable and lower in magnitude than those in other microenvironments due to reaction of $O_3$ with NO and other combustion emissions. Depending on wind direction, $O_3$ concentrations near the roadway have been found to be 20-80% of ambient concentrations at sites 400 meters or more distant from roads (Section 3.6.2.1). A study on patrol cars during trooper work shifts reported in-vehicle 9-h concentrations that were approximately 51% of simultaneously measured roadside concentrations (mean of 11.7 versus 22.8 ppb) (Riediker et al., 2003).

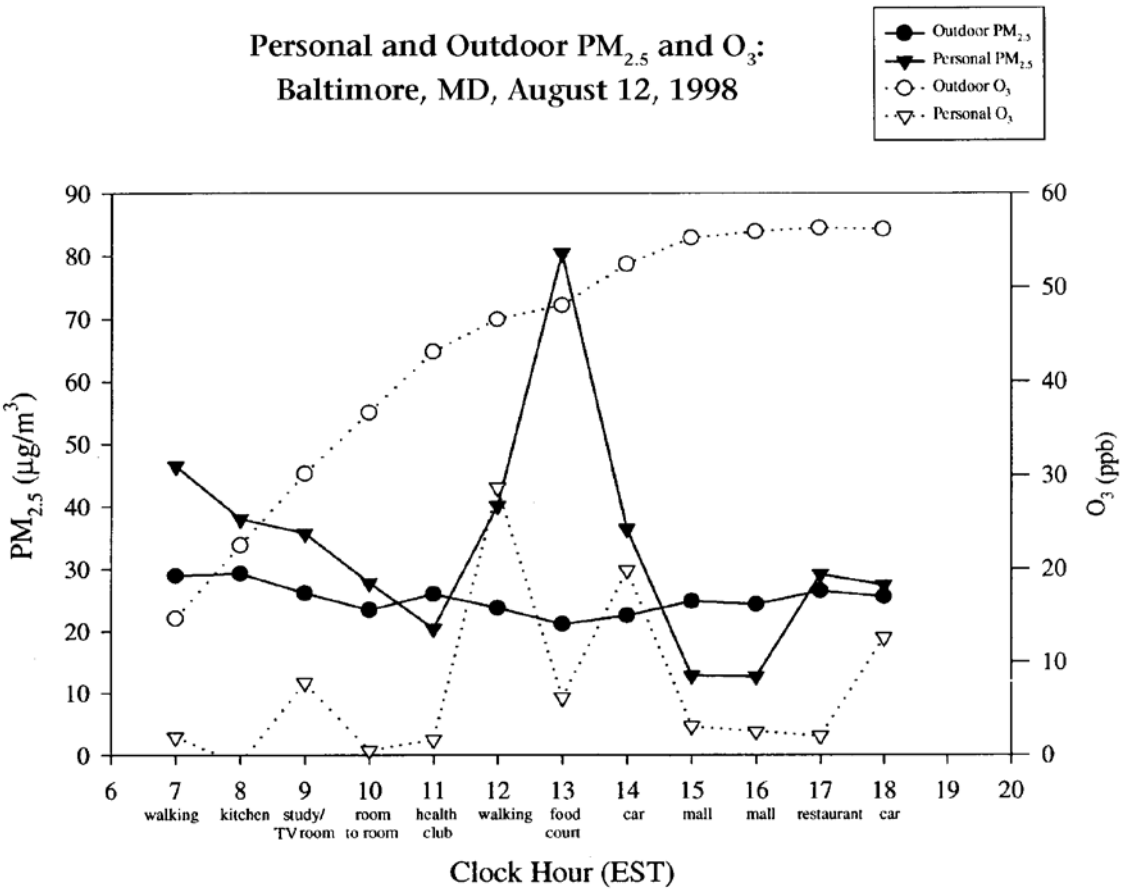
### 4.3.3 Personal-Ambient Concentration Relationships

Several factors influence the relationship between personal $O_3$ exposure and ambient concentration. Due to the lack of indoor $O_3$ sources, along with reduction of ambient $O_3$ that penetrates into enclosed microenvironments, indoor and in-vehicle $O_3$ concentrations are highly dependent on air exchange rate and therefore vary widely in different microenvironments. Ambient $O_3$ varies spatially due to reactions with other atmospheric species, especially near busy roadways where $O_3$ concentrations are decreased by reaction with NO (Section 3.6.2.1). This is in contrast with pollutants such as CO and $NO_X$, which show appreciably higher concentrations near the roadway than several hundred meters away (Karner et al., 2010). Ozone also varies temporally over multiple scales, with generally increasing concentrations during the daytime hours, and higher $O_3$ concentrations during summer than in winter. An example of this variability is shown in Figure 4-1, taken from a personal exposure study conducted by Chang et al. (2000).

In this figure, hourly personal exposures are seen to vary from a few ppb in some indoor microenvironments to tens of ppb in vehicle and outdoor microenvironments. The increase in ambient $O_3$ concentration during the day is apparent from the outdoor monitoring data. In comparison, ambient $PM_{2.5}$ exhibits less temporal variability over the day than $O_3$, although personal exposure to $PM_{2.5}$ also varies by microenvironment. This combined spatial and temporal variability for $O_3$ results in varying relationships between personal exposure and ambient concentration.

Correlations between personal exposure to $O_3$ and corresponding ambient concentrations, summarized in Table 4-2, exhibit a wide range (generally 0.3-0.8, although both higher and lower values have been reported), with higher correlations generally observed in outdoor microenvironments, high building ventilation conditions, and during the summer season. Low $O_3$ concentrations indoors and during the winter lead to a high proportion of personal exposures below the sampler detection limit, which may partially explain the low correlations observed in some

studies under those conditions. Studies report varying correlations over a range of averaging times, with no clear trend. Ratios of personal exposure to ambient concentration, summarized in Table 4-3, are generally lower in magnitude (typically 0.1-0.3), and are also variable, with increasing time spent outdoors associated with higher ratios. The next two subsections describe studies that have reported personal-ambient correlations and slopes for a variety of seasons, locations, and populations.




Note: the notation below each clock hour shows the location or activity during that hour.
Source: Reprinted with permission of Air and Waste Management Association (Chang et al., 2000).

**Figure 4-1 Variation in hourly personal and ambient concentrations of $O_3$ and $PM_{2.5}$ in various microenvironments during daytime hours.**

Ozone concentrations near and below the passive sampler detection limit lead to uncertainty in personal-ambient correlations and ratios. Correlations are reduced in magnitude by values below the detection limit because noise obscures the underlying signal in the data, while ratios tend to fluctuate widely at low concentration since small changes in measured values cause large relative changes in resulting ratios.

As with I/O ratios, this problem is particularly acute in the non-ozone season when ambient $O_3$ concentrations are low. Improved characterization of the relationship between personal exposure and ambient concentration will depend on the use of recent improved monitoring techniques to accurately capture low $O_3$ concentrations, preferably at high time resolution to facilitate evaluation of the effect of activity pattern on exposure (Section 4.3.1). While data from studies using new monitoring techniques become available, past studies summarized in the 2006 $O_3$ AQCD (U.S. EPA, 2006b) remain relevant to consider along with more recent studies in evaluating personal-ambient concentration relationships.

**Personal-Ambient Correlations.** Correlations between personal exposure and ambient $O_3$ concentrations have been evaluated in several research studies, many of which were conducted prior to 2005 and are discussed in the 2006 $O_3$ AQCD. Some studies evaluated subject-specific, or longitudinal correlations, which describe multiple daily measurements for a single individual. These studies indicate the inter-individual variability of personal-ambient correlations. Another type of correlation is a pooled correlation, which combines data from multiple individuals over multiple monitoring periods (e.g., days), providing an overall indicator of the personal-ambient relationship for all study subjects. A third type of correlation is a community-average correlation, which correlates average exposure across all study subjects with fixed-site monitor concentrations. Community-average correlations are particularly informative for interpreting time-series epidemiologic studies, in which ambient concentrations are used as a surrogate for community-average exposure. However, few studies report this metric; this represents another opportunity for improvement of future personal exposure studies. Table 4-2 summarizes studies reporting personal-ambient correlations, and the studies in the table are discussed in the subsequent text.

The results of these studies generally indicate that personal exposures are moderately well correlated with ambient concentrations, and that the ratio of personal exposure to ambient concentration is higher in outdoor microenvironments and during the summer season. In some situations, a low correlation was observed, and this may be due in part to a high proportion of personal measurements below the detection limit of the personal sampler [e.g., Sarnat et al. (2000)]. Apart from this, correlations do not appear to be strongly dependent on concentration. The effect of season is unclear, with mixed evidence on whether higher correlations are observed during the $O_3$ season. Chang et al. (2000) measured hourly personal exposures in multiple microenvironments and found that the pooled correlation between personal exposure and ambient concentration was highest for outdoor microenvironments ($r = 0.68 - 0.91$). In-vehicle microenvironments showed moderate to high correlations (0.57-0.72). Correlations in residential indoor microenvironments were very low ($r = 0.05 – 0.09$), with moderate correlations (0.34-0.46) in other indoor microenvironments such as restaurants and shopping malls. Liard et al. (1999) evaluated community-average correlations based on 4-day mean personal $O_3$ exposure measurements for adults and children and found a relatively high correlation ($r = 0.83$) with ambient concentrations, even with only 18-69% of the personal measurements above the detection limit. Sarnat et al. (2000) studied a

population of older adults in Baltimore, MD, and found that longitudinal correlations between 24-h personal exposure and ambient concentration varied by subject and season, with somewhat higher correlations observed in this study during summer (mean = 0.20) than in winter (mean = 0.06). Although the fraction of samples above the detection limit was not reported separately for the older adults in this study, in the larger study of which this population is a part, only a few percent of samples were above the detection limit, with less than 1% above the detection limit during the winter (see Table 4-3) (Koutrakis et al., 2005). This may account for the low observed correlations, particularly in winter. Some evidence was presented that subjects living in well-ventilated indoor environments have higher correlations than those living in poorly ventilated indoor environments, although exceptions to this were also observed. Ramírez-Aguilar et al. (2008) measured 48- to 72-h personal exposures of four groups of asthmatic children aged 6-14 in Mexico City, Mexico, during 1998-2000. A moderate pooled correlation (r = 0.35) was observed between these exposures and corresponding ambient concentrations.

## Table 4-2 Correlations between personal and ambient $O_3$ concentration.

| Study | Location | Years/Season | Population | Sample duration | Correlation | Study Type | Comment | Concentration (ppb) | Personal Detection limit (ppb) |
|---|---|---|---|---|---|---|---|---|---|
| Chang et al. (2000) | Baltimore, MD | Summer 1998 | Older adults | 1 h | 0.91 | Pooled | Outdoor near roadway | Summer 1998<br>*Personal*<br>Median (Range): 10.0 (-11.3-76)<br>Mean (SD): 15.0 (18.3)<br>*Ambient**<br>Range: 12.2-59.8 | Summer 1998 LOD: 17.2<br>Winter 1999 LOD: 12.0<br>Fraction above LOD: NR |
| | | Winter 1999 | | | 0.77 | | | | |
| | | Summer 1998 | | | 0.68 | | Outdoor away from road | | |
| | | Winter 1999 | | | 0.86 | | | | |
| | | Summer 1998 | | | 0.72 | | In vehicle | | |
| | | Winter 1999 | | | 0.57 | | | | |
| | | Summer 1998 | | | 0.09 | | Indoors-residence | Winter 1999<br>*Personal*<br>Median (Range): 0.5 (-0.3-12.2)<br>Mean (SD): 1.1 (1.7)<br>*Ambient**<br>Range: 12.2-24.6<br>*Estimated from Figure 3 | |
| | | Winter 1999 | | | 0.05 | | | | |
| | | Summer 1998 | | | 0.34 | | | | |
| | | Winter 1999 | | | 0.46 | | Indoors-other | | |
| Liard et al. (1999) | Paris, France | Summer 1996 | All age groups | 4 day | 0.83 | Community-averaged | | Range<br>Children<br>*Personal**<br>-0.1-3.9<br>*Ambient**<br>14.8-30.7<br>Adults<br>*Personal**<br>0.9-2.7<br>*Ambient**<br>19.8-27.5<br>*Estimated from Figure 3 | LOD: 1.5 ppb<br>Fraction above LOD:<br>Children: 15-69%<br>Adults: 18-34% |

| Study | Location | Years/Season | Population | Sample duration | Correlation | Study Type | Comment | Concentration (ppb) | Personal Detection limit (ppb) |
|---|---|---|---|---|---|---|---|---|---|
| Sarnat et al. (2000) | Baltimore, MD | Summer | Older adults | 24 h | 0.20<br>SD: 0.28<br>95% CI: 0.06, 0.34 | Longitudinal | | Mean (SD)<br>*Personal*<br>3.5 (3.0)<br>*Ambient*<br>37.3 (8.3) | LOD: 6.6<br>Fraction above LOD: NR |
| | | Winter | | | 0.06<br>SD: 0.34<br>95% CI: -0.88, 0.24 | | | *Personal*<br>0 (1.8)<br>*Ambient*<br>17.8 (10.3) | LOD: 5.5<br>Fraction above LOD: NR |
| Linn et al. (1996) | Southern California | All seasons from 1992 to 1993 | Children | 24 h | 0.61 | Community-averaged | | Mean (SD)<br>*Personal*<br>5 (3)<br>*Ambient*<br>23 (12) | LOD: NR<br>Fraction above LOD: NR |
| Brauer and Brook (1997) | Vancouver, Canada | Summers 1992 and 1993 | Health clinic workers | 24 h | 0.60 | Pooled | 0-25% of time outdoors | Range<br>*Personal**<br>0.3-10.4<br>*Ambient**<br>5.3-30.1 | LOD<br>24h: 17 ppb<br>12h: 12 ppb<br>Fraction above LOD: NR |
| | | | Camp counselors | 24 h | 0.42 | Pooled | 7.5-45% of time outdoors | *Personal**<br>0.1-13.8<br>*Ambient**<br>11.2-26.1 | |
| | | | Farm workers | 6-14 h (work shift) | 0.64 | Pooled | 100% of time outdoors | *Personal**<br>3.2-43<br>*Ambient**<br>8.6-49.0<br>*Estimated from Figure 2 | |

| Study | Location | Years/Season | Population | Sample duration | Correlation | Study Type | Comment | Concentration (ppb) | Personal Detection limit (ppb) |
|---|---|---|---|---|---|---|---|---|---|
| Ramírez-Aguilar et al. (2008) | Mexico City, Mexico | December 1998-April 2000 | Asthmatic children | 48 h to 72 h | 0.35 | Pooled | | Mean (Range)<br>*Personal*<br>7.8 (0.2-30.9)<br>*Ambient*<br>33.3 (12.5-64.6) | LOD: NR<br>Fraction above LOD: NR |
| Delfino et al. (1996) | San Diego, California | September and October 1993 | Asthmatic children | 12-h daytime | 0.45<br>Range: 0.35-0.69 | Pooled | | Mean (SD)<br>*Personal*<br>11.6 (11.2)<br>*Ambient*<br>12h: 43 (17)<br>1hr max: 68 (30) | LOD: 8.67<br>Fraction above LOD: 53% |

LOD = limit of detection; NR = not reported; SD = standard deviation

Consistent with hourly microenvironment-specific results from the Chang et al. (2000) study described above, studies have found moderate to high personal-ambient correlations for individuals spending time outdoors. A moderate pooled correlation of 0.61 was reported between 24-h avg personal and central-site measurements by Linn et al. (1996) for a population of southern California schoolchildren who spent an average of 101-136 minutes per day outdoors. The authors also report a correlation of 0.70 between central-site measurements and concentrations outside the children's schools. Although the average school outdoor concentration (34 ppb) was higher than the average central-site concentration (23 ppb) and the average personal exposure concentration was lower (5 ppb) than the central-site value, the similarity between the correlations in this study indicate that central-site monitor concentrations can represent personal exposures in addition to representing local outdoor concentrations. A study in Vancouver, BC provided another illustration of the effect of outdoor microenvironments on personal-ambient relationships by comparing three groups spending different amounts of time outdoors: health clinic workers (0-25% of sampling period outdoors), camp counselors (7.5-45% of sampling period outdoors), and farm workers (100% of sampling period outdoors) (Brauer and Brook, 1997). Health clinic workers and camp counselors were monitored 24 h/day, while farm workers were monitored during their work shift (6-14 hours). In this study, the pooled correlations between personal exposure and fixed-site concentration were not substantially different among the groups (r = 0.60, 0.42, and 0.64, respectively), even though the farm workers experienced the highest concentrations. The ratios of personal exposure to fixed-site monitor concentration increased among the groups with increasing amount of time spent outdoors (0.35, 0.53, and 0.96, respectively). This indicates that temporal variations in personal exposure to $O_3$ are driven by variations in ambient concentration, even for individuals that spend little time outdoors.

**Personal-Ambient Ratios.** Studies indicate that the ratio between personal $O_3$ exposure and ambient concentration varies widely, depending on activity patterns, housing characteristics, and season. Higher personal-ambient ratios are generally observed with increasing time spent outside, higher air exchange rate, and in seasons other than winter. Table 4-3 summarizes the results of several such studies discussed in the 2006 $O_3$ AQCD together with newer studies showing the same pattern of results.

O'Neill et al. (2003) studied a population of shoe cleaners working outdoors in Mexico City, Mexico, and presented a regression model indicating a 0.56 ppb increase in 6-h personal exposure for each 1 ppb increase in ambient concentration. Regression analyses by Sarnat et al. (2005) and Sarnat et al. (2001) for 24-hour data from mixed populations of children and older adults in Baltimore, MD (Sarnat et al., 2001), and Boston, MA (Sarnat et al., 2005), found differing results between the two cities, with Baltimore subjects showing a near-zero slope (0.01) during the summertime while Boston subjects showed a positive slope of 0.27 ppb personal exposure per 1 ppb ambient concentration. In both cities, the winter slope was near zero. The low slope observed in Baltimore may have been due to differences in time spent outdoors, residential ventilation conditions, or other factors. The intercept in

Baltimore was more than half of the median personal exposure (1.84 versus 2.5 ppb), and only 6.6% of the personal samples were above the detection limit, suggesting that noise in the data may also have contributed to the low observed regression slope. However, other studies with a relatively large fraction of personal values below the detection limit reported slopes of 0.27 or above (Sarnat et al., 2006a; Brauer and Brook, 1997). Xue et al. (2005) measured 6-day personal exposure of children in southern California and found that the average ratio of personal exposure to ambient concentration was relatively stable throughout the year at 0.3. These authors also regressed personal exposures on ambient concentration after adjusting for time-activity patterns and housing characteristics and found a slope of 0.54 ppb/ppb, with the regression $R^2$ value of 0.58. Unadjusted regression slopes were not presented. It should also be noted that the ratio and slope would not be expected to be identical unless the intercept and other regression parameters were effectively zero.

A few additional studies have been published since the 2006 $O_3$ AQCD comparing personal exposures with ambient concentrations, and these findings generally confirm the conclusions of the 2006 $O_3$ AQCD that ventilation conditions, activity pattern, and season may impact personal-ambient ratios. Sarnat et al. (2006a) measured 24-hour personal exposures for a panel of older adults in Steubenville, OH during summer and fall 2000. Subjects were classified as high-ventilation or low-ventilation based on whether they spent time in indoor environments with open windows. Regression of personal exposures on ambient concentration found a higher slope for high-ventilation subjects compared with low-ventilation subjects in both summer (0.18 versus 0.08) and fall (0.27 versus 0.20). Suh and Zanobetti (2010) reported an average 24-hour personal exposure of 2.5 ppb as compared to 24-hour ambient concentration of 29 ppb for a group of individuals with either recent MI or diagnosed COPD in Atlanta. A similar result was observed in Detroit, where the mean 24-hour personal exposure across 137 participants in summer and winter was 2.1 ppb, while the mean ambient concentration on sampling days was 25 ppb (Williams et al., 2009b). Although no personal exposures were measured, McConnell et al. (2006) found that average 24-hour home outdoor $O_3$ concentrations were within 6 ppb of $O_3$ concentrations measured at central-site monitors in each of three southern California communities, with a combined average home outdoor concentration of 33 ppb compared to the central-site average of 36 ppb. In Mexico City, Mexico, Ramírez-Aguilar et al. (2008) regressed 48- to 72-hour personal exposures of four groups of asthmatic children aged 6-14 with ambient concentrations and found slope of 0.17 ppb/ppb after adjustment for distance to the fixed-site monitor, time spent outdoors, an interaction term combining these two variables, and an interaction term representing neighborhood and study group.

## Table 4-3 Ratios of personal to ambient $O_3$ concentration.

| Study | Location | Years/ Season | Population | Sample duration | Ratio[a] | Slope | Inter-cept | Study Type | Comment | Concentration/ Detection limit (ppb) |
|---|---|---|---|---|---|---|---|---|---|---|
| Sarnat et al. (2001)<br>Koutrakis et al. (2005) | Baltimore, MD | Summer 1998 | Older adults and children | 24 h | NR | 0.01 | 1.84 | Longitudinal | t-value: 1.21 | Median (IQR)<br>*Personal**<br>2.5 (0-6.4)<br>LOD: 6.6<br>Fraction above LOD: 6.6%<br>*Ambient**<br>36 (31-43) |
| | | Winter 1999 | Older adults, children, and individuals with COPD | | NR | 0.00 | 0.46 | | t-value: 0.03 | *Personal**<br>1.1 (-0.6-1.9)<br>LOD: 5.5<br>Fraction above LOD: 0.2%<br>*Ambient**<br>18 (8.6-26)<br>*Estimated from Figure 1 |

| Study | Location | Years/ Season | Population | Sample duration | Ratio[a] | Slope | Inter-cept | Study Type | Comment | Concentration/ Detection limit (ppb) |
|---|---|---|---|---|---|---|---|---|---|---|
| Brauer and Brook (1997) | Vancouver, Canada | Summers 1992 and 1993 | Health clinic workers | 24 h | 0.35 | NR | NR | Pooled | 0-25% of time outdoors | Range<br>*Personal**<br>0.3-10.4<br>*Ambient**<br>5.3-30.1<br>LOD: 17 |
| | | | Camp counselors | 24 h | 0.53 | NR | NR | Pooled | 7.5-45% of time outdoors | *Personal**<br>0.1-13.8<br>*Ambient**<br>11.2-26.1<br>LOD: 17 |
| | | | Farm workers | 6-14 h (work shift) | 0.96 | NR | NR | Pooled | 100% of time outdoors | *Personal**<br>3.2-43<br>*Ambient**<br>8.6-49.0<br>LOD: 12<br>*Estimated from Figure 2 |
| O'Neill et al. (2003) | Mexico City, Mexico | April - July 1996 | Shoe cleaners | 6 h | 0.40<br>0.37[b]<br>SD: 0.22 | 0.56<br>95% CI: 0.43-0.69 | NR | Longitudinal | | Mean (SD)<br>*Personal*<br>34.4 (22.3)<br>*Ambient*<br>84.0 (24.8)<br>LOD: 21.1 (20.6) |

| Study | Location | Years/ Season | Population | Sample duration | Ratio[a] | Slope | Inter-cept | Study Type | Comment | Concentration/ Detection limit (ppb) |
|---|---|---|---|---|---|---|---|---|---|---|
| Sarnat et al. (2005) | Boston | Summer | Older adults and children | 24 h | NR | 0.27<br>95% CI: 0.18-0.37 | NR | Longitudinal | | Range of Means<br>*Personal*<br>4.8-7.6<br>LOD: 7.0<br>Fraction above LOD: 23.8%<br>*Ambient*<br>Mean (SD): 22.7-31.6 |
| | | Winter | | | NR | 0.04<br>95% CI: 0.00-0.07 | NR | | | Range of Means<br>*Personal*<br>0.1-2.5<br>LOD: 4.9<br>Fraction above LOD: 3.2%<br>*Ambient*<br>14.0-21.8 |
| Xue et al. (2005) | Southern California | June 1995 - May 1996 | Children | 6 day | 0.3<br>SD: 0.13 | NR | NR | Longitudinal | | $O_3$ season (May-Sept)<br>*Personal**<br>Median (IQR): 22 (14-30)<br>*Ambient**<br>Median (IQR): 53 (44-67)<br>Non-$O_3$ season (Oct-Apr)<br>*Personal**<br>Median (IQR): 6 (5-10)<br>*Ambient**<br>Median (IQR): 26 (14- 32)<br>LOD: NR<br>*Estimated from Figure 2 |

| Study | Location | Years/ Season | Population | Sample duration | Ratio[a] | Slope | Inter-cept | Study Type | Comment | Concentration/ Detection limit (ppb) |
|---|---|---|---|---|---|---|---|---|---|---|
| Sarnat et al. (2006a) | Steuben-ville, OH | | Older adults | 24 h | NR | 0.15<br>SE: 0.02<br>t-value: 7.21<br>$R^2$: 0.24 | NR | Longitudinal | All individuals | Mean (SD)<br>*Personal* 5.3 (5.2)<br>Fraction above LOD: 8.2%<br>*Ambient* 29.3 (13.4)<br>Fraction above LOD: 94%<br>LOD: 12.7 |
| | | Summer | | | NR | 0.18<br>SE: 0.03<br>t-value: 7.34<br>$R^2$: 0.27 | NR | | High-ventilation | |
| | | | | | NR | 0.08<br>SE: 0.04<br>t-value: 1.89<br>$R^2$: 0.19 | NR | | Low-ventilation | |
| | | | | | NR | 0.27<br>SE: 0.03<br>t-value: 8.64<br>$R^2$: 0.25 | NR | | All individuals | Mean (SD)<br>*Personal* 3.9 (4.4)<br>Fraction above LOD: 8.4%<br>*Ambient* 16.0 (8.1)<br>Fraction above LOD: 71%<br>LOD: 10.7 |
| | | Fall | | | NR | 0.27<br>SE: 0.04<br>t-value: 7.38<br>$R^2$: 0.33 | NR | | High-ventilation | |
| | | | | | NR | 0.20<br>SE: 0.05<br>t-value: 3.90<br>$R^2$: 0.12 | NR | | Low-ventilation | |

| Study | Location | Years/ Season | Population | Sample duration | Ratio[a] | Slope | Inter-cept | Study Type | Comment | Concentration/ Detection limit (ppb) |
|---|---|---|---|---|---|---|---|---|---|---|
| Ramírez-Aguilar et al. (2008) | Mexico City, Mexico | Dec.1998-Apr. 2000 | Asthmatic children | 48 h to 72 h | 0.23 | 0.17<br>SE: 0.02<br>95% CI: 0.13-0.21<br>p-value: 0.00 | | Pooled | | Mean (Range)<br>*Personal*<br>7.8 (0.2-30.9)<br>*Ambient*<br>33.3 (12.5-64.6)<br>LOD: NR |

[a] Mean value unless otherwise indicated

[b] Median

IQR = interquartile range; LOD = limit of detection; NR = not reported; SD = standard deviation

### 4.3.4 Co-exposure to Other Pollutants and Environmental Stressors

Exposure to ambient $O_3$ occurs in conjunction with exposure to a complex mixture of ambient pollutants that varies over space and time. Multipollutant exposure is an important consideration in evaluating health effects of $O_3$ since these other pollutants have either known or potential health effects that may impact health outcomes due to $O_3$. The co-occurrence of high $O_3$ concentrations with high heat and humidity may also contribute to health effects. This section presents data on relationships between overall personal $O_3$ exposure and exposure to other ambient pollutants, as well as co-exposure relationships for near-road $O_3$ exposure.

#### 4.3.4.1 Personal Exposure to Ozone and Copollutants

Personal exposure to $O_3$ shows variable correlation with personal exposure to other pollutants, with differences in correlation depending on factors such as instrument detection limit, season, city-specific characteristics, time scale, and spatial variability of the copollutant. Suh and Zanobetti (2010) reported Spearman rank correlation coefficients during spring and fall between 24-h avg $O_3$ measurements and copollutants of 0.14, 0.00, and -0.03 for $PM_{2.5}$, EC, and $NO_2$, respectively. Titration of $O_3$ near roadways is likely to contribute to the low or slightly negative correlations with the traffic-related pollutants EC and $NO_2$. The somewhat higher correlation with $PM_{2.5}$ may reflect the influence of air exchange rate and time spent outdoors on co-exposures to ambient $PM_{2.5}$ and $O_3$. Overall, the copollutant correlations are quite small, which may be due to the very low personal exposures observed in this study (2-3 ppb), likely to be near or below the detection limit of the passive sampler over a 24-hour period. Chang et al. (2000) measured hourly personal exposures to $PM_{2.5}$ and $O_3$ in summer and winter in Baltimore, Maryland. Correlations between $PM_{2.5}$ and $O_3$ were 0.05 and -0.28 in summer and winter, respectively. Results indicate personal $O_3$ exposures were not significantly associated with personal $PM_{2.5}$ exposures in either summer or winter. These non-significant correlations may be attributed in part to the relatively low personal $O_3$ exposures observed in this study; in both summer and winter, the mean personal $O_3$ exposure was below the calculated limit of detection.

Studies conducted in Baltimore, MD (Sarnat et al., 2001), and Boston, MA (Sarnat et al., 2005), found differing results for the correlation between 24-h avg personal $O_3$ and personal $PM_{2.5}$ exposures, particularly during the winter season. Sarnat et al. (2001) found a positive slope when regressing personal exposures of both total $PM_{2.5}$ (0.21) and $PM_{2.5}$ of ambient origin (0.22) against personal $O_3$ exposures during the summer season, but negative slopes (-0.05 and -0.18, respectively) during the winter season. The summertime slope for personal $PM_{2.5}$ exposure versus personal $O_3$ exposure was much higher for children (0.37) than for adults (0.07), which may be

the result of different activity patterns. This team of researchers also found a positive, although higher, summer slope between 24-h avg personal $O_3$ and personal $PM_{2.5}$ in Boston (0.72) (Sarnat et al., 2005). However, the winter slope was positive (1.25) rather than negative, as in Baltimore. In both cities during both seasons, there was a wide range of subject-specific correlations between personal $O_3$ and personal $PM_{2.5}$ exposures, with some subjects showing relatively strong positive correlations (>0.75) and others showing strong negative correlations (<-0.50). The median correlation in both cities was slightly positive in the summer and near zero (Boston) or slightly negative (Baltimore) in the winter. These results indicate the potential effects of city-specific characteristics, such as housing stock and building ventilation patterns, on relationships between $O_3$ and copollutants.

The lack of long-term exposure assessment studies limits evaluation of long-term correlations between $O_3$ exposure and copollutant exposure. Although some long-term epidemiologic studies have reported copollutant correlations for fixed-site monitor concentrations or city-wide averages used as exposure metrics, no clear pattern is apparent. Correlations with PM concentrations range from less than 0.2 to nearly 0.9. For example, the long-term correlation between 30-yr mean (1973-1992) $PM_{10}$ and the 30-yr mean of 8-h avg (9 am to 5 pm) $O_3$ was 0.88 for participants in the Adventist Health Smog study (AHSMOG) (McDonnell et al., 1999a). Jerrett et al. (2009) reported a moderate correlation of 0.56 between two-year average $O_3$ and $PM_{2.5}$ in 86 U.S. metropolitan areas. In the Southern California Children's Health Study, the correlation between 1994-2000 average $O_3$ and $PM_{2.5}$ was 0.33 for 1-h daily max $O_3$, but only 0.18 for the 1994-2000 mean of 8-h avg (10 am – 6 pm) $O_3$ concentrations (Gauderman et al., 2004). Similar correlations were reported in this study between these $O_3$ metrics and $PM_{10}$. For children participating in the National Health Interview Survey and living in U.S. metropolitan areas, the correlation between 2000-2004 $O_3$ concentrations and $PM_{2.5}$ and $PM_{10}$ concentrations was 0.29 and 0.55, respectively (Akinbami et al., 2010). For $NO_2$, near-zero or negative correlations have been reported, consistent with atmospheric chemistry involving $NO_2$ and $O_3$. Correlations with $NO_2$ in the Children's Health Study were 0.10 and -0.11 for 1994-2000 mean 1-h daily max $O_3$ and 8-h avg $O_3$, respectively (Gauderman et al., 2004). This is similar to the value of -0.05 reported by Akinbami et al. (2010). However, the AHSMOG study reported a correlation of 0.61 between 30-yr averages of $O_3$ and $NO_2$, possibly reflecting similar overall levels of air pollution experienced by the participants (McDonnell et al., 1999a). This lack of a consistent pattern makes it difficult to draw conclusions regarding long-term correlations between $O_3$ and copollutants.

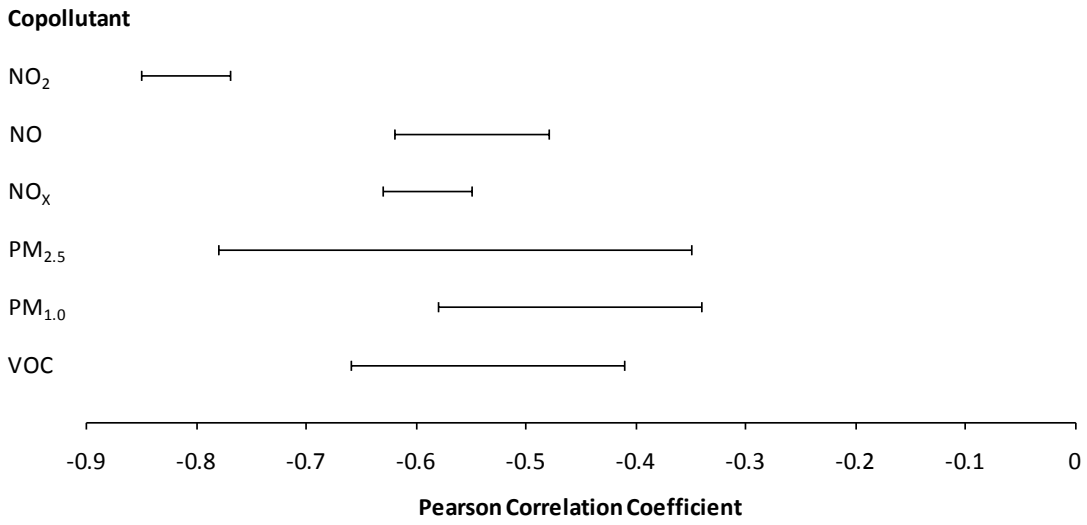
To the extent that short-term concentrations drive long-term patterns, some insight may be provided by an analysis of short-term correlations between $O_3$ and other criteria pollutants, such as is provided in Section 3.6.4. Warm-season 8-h daily max $O_3$ concentrations are generally positively correlated with co-located 24-h avg measurements of other criteria pollutants (Figure 3-57). Median correlations range from approximately 0.15 to 0.55 for CO, $SO_2$, $NO_2$, $PM_{10}$, and $PM_{2.5}$, in that order. In contrast, year-round 8-h daily max $O_3$ data show negative median correlations with CO and $NO_2$, positive correlations with $PM_{10}$ and $PM_{2.5}$, and essentially zero

correlation with $SO_2$. This reflects mostly negative correlations between $O_3$ and all pollutants during wintertime, as shown in Figure 3-56. Titration of $O_3$ near roadways also likely contributes to overall negative correlations with $NO_2$ and CO. Positive correlations between $O_3$ and $PM_{2.5}$ during the summertime can be partly explained by meteorological conditions favoring increased formation of both secondary PM and $O_3$. Strong positive correlations can influence the interpretation of epidemiologic results, potentially complicating the ability to identify the independent effect of a pollutant.

#### 4.3.4.2 Near-Road Exposure to Ozone and Copollutants

Beckerman et al. (2008) measured both 1-week and continuous concentrations of $O_3$, NO, $NO_2$, $NO_X$, $PM_{2.5}$, $PM_{1.0}$, and several VOCs (the BTEX compounds, MTBE, hexane, and THC) in the vicinity of heavily traveled (annual average daily traffic [AADT] >340,000) roadways in Toronto, Canada. Passive samplers were deployed for one week in August 2004. Ozone concentrations were negatively correlated with all pollutants, with the exception of VOCs at one of the monitoring sites which were suspected of being influenced by small area sources. Site specific correlations are given in Figure 4-2. Correlations were -0.77 to -0.85 for $NO_2$, -0.48 to -0.62 for NO, and -0.55 to -0.63 for $NO_X$. Pooled correlations using data from both sites were somewhat lower in magnitude. $PM_{2.5}$ and $PM_{1.0}$ correlations were -0.35 to -0.78 and -0.34 to -0.58, respectively. At the monitoring site not influenced by small area sources, $O_3$-VOC correlations ranged from -0.41 to -0.66.

Beckerman et al. (2008) also made on-road measurements of multiple pollutants with a instrumented vehicle. Concentrations were not reported, but correlations between $O_3$ and other pollutants were negative and somewhat greater in magnitude (i.e., more negative) than the near-road correlations. $SO_2$, CO, and BC were measured in the mobile laboratory, although not at the roadside, and they all showed negative correlations with $O_3$ when the data were controlled for site. Correlations for continuous concentrations between $O_3$ and copollutants were somewhat lower than the 1-week correlations, except for $O_3$-$PM_{2.5}$ correlations. Correlations were -0.90, -0.66, -0.77, and -0.89 for $NO_2$, NO, $NO_X$, and $PM_{1.0}$ respectively. The continuous $O_3$-$PM_{2.5}$ correlation was -0.62, which is in the range of the 1-week correlation.




Source: Data from: Beckerman et al. (2008)

**Figure 4-2 Correlations between 1-week concentrations of $O_3$ and copollutants measured near roadways.**

### 4.3.4.3 Indoor Exposure to Ozone and Copollutants

Ambient $O_3$ that infiltrates indoors reacts with organic compounds and other chemicals to form oxidized products, as described in Section 3.2.3.1 as well as the 2006 $O_3$ AQCD. It is anticipated that individuals are exposed to these reaction products, although no evidence was identified regarding personal exposures. The reactions are similar to those occurring in the ambient air, as summarized in Chapter 3. For example, $O_3$ can react with terpenes and other compounds from cleaning products, air fresheners, and wood products both in the gas phase and on surfaces to form particulate and gaseous species, such as formaldehyde (Chen et al., 2011; Shu and Morrison, 2011; Aoki and Tanabe, 2007; Reiss et al., 1995b). Ozone has also been shown to react with material trapped on HVAC filters and generate airborne products (Bekö et al., 2007; Hyttinen et al., 2006). Potential oxygenated reaction products have been found to act as irritants (Anderson et al., 2007), indicating that these reaction products may have health effects separate from those of $O_3$ itself (Weschler and Shields, 1997). Ozone may also react to form other oxidants, which then go on to participate in additional reactions. White et al. (2010) found evidence that HONO, or other oxidants, may have been present during experiments to estimate indoor OH concentrations; indicating complex indoor oxidant chemistry. Rates of these reactions are dependent on indoor $O_3$ concentration, temperature, and air exchange rate, making estimation of exposures to reaction products difficult.

## 4.4 Exposure-Related Metrics

In this section, parameters are discussed that are relevant to the estimation of exposure, but are not themselves direct measures of exposure. Time-location-activity patterns, including behavioral changes to avoid exposure, have a substantial influence on exposure and dose. Proximity of populations to ambient monitors may influence how well their exposure is represented by measurements at the monitors, although factors other than distance play an important role as well.

### 4.4.1 Activity Patterns

The activity pattern of individuals is an important determinant of their exposure. Variation in $O_3$ concentrations among various microenvironments means that the amount of time spent in each location, as well as the level of activity, will influence an individual's exposure to ambient $O_3$. The effect of activity pattern on exposure is explicitly accounted for in Equation 4-3 by the fraction of time spent in different microenvironments.

Activity patterns vary both among and within individuals, resulting in corresponding variations in exposure across a population and over time. Large-scale human activity databases, such as those developed for the National Human Activity Pattern Survey (NHAPS) (Klepeis et al., 2001) or the Consolidated Human Activity Database (CHAD) (McCurdy et al., 2000), which includes NHAPS data together with other activity study results, have been designed to characterize exposure patterns among much larger population subsets than can be examined during individual panel studies. The complex human activity patterns across the population (all ages) are illustrated in Figure 4-3 (Klepeis et al., 2001), which is presented to illustrate the diversity of daily activities among the entire population as well as the proportion of time spent in each microenvironment. For example, about 25% of the individuals reported being outdoors or in a vehicle between 2:00 and 3:00 p.m., when daily $O_3$ levels are peaking, although about half of this time was spent in or near a vehicle, where $O_3$ concentrations are likely to be lower than ambient concentrations.

Time spent in different locations has also been found to vary by age. Table 4-4 summarizes NHAPS data reported for four age groups, termed Very Young (0-4 years), School Age (5-17 years), Working (18-64 years), and Retired (65+ years) (Klepeis et al., 1996). The working population spent the least time outdoors, while the school age population spent the most time outdoors. NHAPS respondents aged 65 and over spent somewhat more time outdoors than adults aged 18-64, with a greater fraction of time spent outdoors at a residence. Children aged 0-4 also spent most of their outdoor time in a residential outdoor location. On average, the fraction of time spent outdoors by school age respondents was 2.62 percentage points higher than working respondents, corresponding to approximately 38 minutes more time outdoors per day. Although not accounting for activity level, this increased time

spent outdoors is consistent with evidence in Chapter 8 suggesting that younger and older age groups are more at risk for $O_3$-related health effects.

**Table 4-4 Mean fraction of time spent in outdoor locations by various age groups in the NHAPS study.**

| Age Group | Residential-Outdoor | Other Outdoor | Total Outdoors |
|---|---|---|---|
| 0-4 yr | 5.38% | 0.96% | 6.34% |
| 5-17 yr | 5.05% | 2.83% | 7.88% |
| 18-64 yr | 2.93% | 2.33% | 5.26% |
| 65+ yr | 4.48% | 1.27% | 5.75% |

Source: Data from Klepeis et al. (1996).

Together with location, exertion level is an important determinant of exposure. Table 4-5 summarizes ventilation rates for different age groups at several levels of activity as presented in Table 6-2 of the EPA's *Exposure Factors Handbook* (U.S. EPA, 2011b). Most of the age-related variability is seen for moderate and high intensity activities, except for individuals under 1 year. For moderate intensity, ventilation rate increases with age through childhood and adulthood until age 61, after which a moderate decrease is observed. Ventilation rate is most variable for high intensity activities. Children aged 1 to <11 years have ventilation rates of approximately 40 L/min, while children aged 11+ and adults have ventilation rates of approximately 50 L/min. The peak is observed for the 51 to <61 age group, at 53 L/min, with lower ventilation rates for older adults.

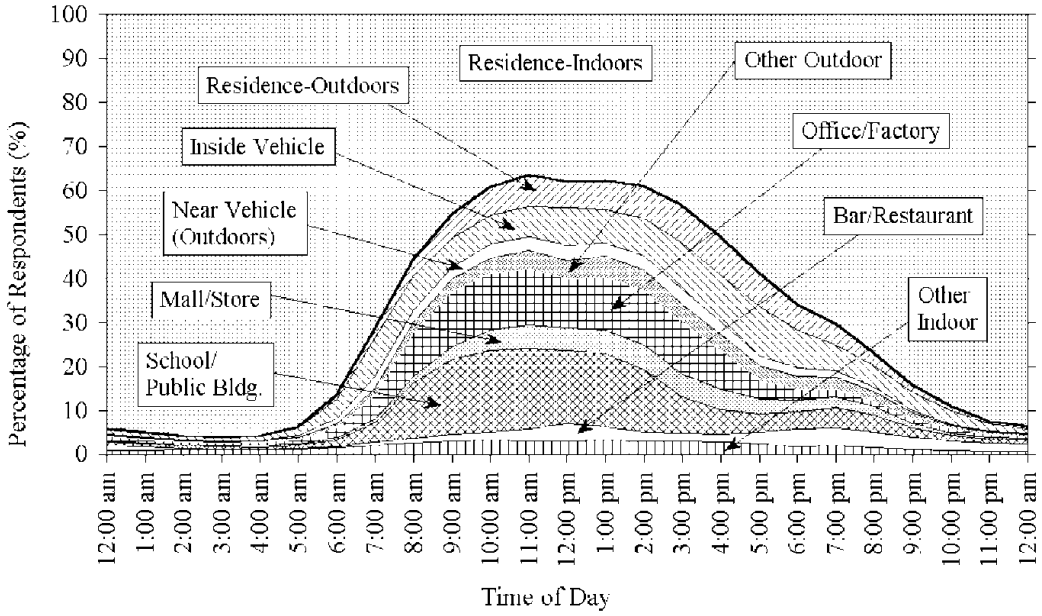
A dramatic increase in ventilation rate occurs as exercise intensity increases. For children and adults <31 years, high intensity activities result in nearly double the ventilation rate for moderate activity, which itself is nearly double the rate for light activity. Children have other important differences in ventilation compared to adults. As discussed in Chapter 5, children tend to have a greater oral breathing contribution than adults, and they breathe at higher minute ventilations relative to their lung volumes. Both of these factors tend to increase dose normalized to lung surface area.

**Table 4-5 Mean ventilation rates (L/min) at different activity levels for different age groups.**

| Age Group | Sleep or Nap | Sedentary/Passive | Light Intensity | Moderate Intensity | High Intensity |
|---|---|---|---|---|---|
| Birth to <1 yr | 3.0 | 3.1 | 7.6 | 14 | 26 |
| 1 to <2 yr | 4.5 | 4.7 | 12 | 21 | 38 |
| 2 to <3 yr | 4.6 | 4.8 | 12 | 21 | 39 |
| 3 to <6 yr | 4.3 | 4.5 | 11 | 21 | 37 |
| 6 to <11 yr | 4.5 | 4.8 | 11 | 22 | 42 |
| 11 to <16 yr | 5.0 | 5.4 | 13 | 25 | 49 |
| 16 to <21 yr | 4.9 | 5.3 | 12 | 26 | 49 |
| 21 to <31 yr | 4.3 | 4.2 | 12 | 26 | 50 |
| 31 to <41 yr | 4.6 | 4.3 | 12 | 27 | 49 |
| 41 to <51 yr | 5.0 | 4.8 | 13 | 28 | 52 |
| 51 to <61 yr | 5.2 | 5.0 | 13 | 29 | 53 |
| 61 to <71 yr | 5.2 | 4.9 | 12 | 26 | 47 |
| 71 to <81 yr | 5.3 | 5.0 | 12 | 25 | 47 |
| 81+ yr | 5.2 | 4.9 | 12 | 25 | 48 |

Source: Data from *Exposure Factors Handbook* (U.S. EPA, 2011b).

Longitudinal activity pattern information is also an important determinant of exposure, as different people may exhibit different patterns of time spent outdoors over time due to age, sex, employment, and lifestyle-dependent factors. These differences may manifest as higher mean exposures or more frequent high-exposure episodes for some individuals. The extent to which longitudinal variability in individuals contributes to the population variability in activity and location can be quantified by the ratio of between-person variance to total variance in time spent in different locations and activities (the intraclass correlation coefficient, ICC). Xue et al. (2004) quantified ICC values in time-activity data collected by Harvard University for 160 children aged 7–12 years in Southern California (Geyh et al., 2000). For time spent outdoors, the ICC was approximately 0.15, indicating that 15% of the variance in outdoor time was due to between-person differences. The ICC value might be different for other population groups.




Source: Reprinted with permission of Nature Publishing Group (Klepeis et al., 2001).

**Figure 4-3 Distribution of time that NHAPS respondents spent in ten microenvironments based on smoothed 1-min diary data.**

The EPA's National Exposure Research Laboratory (NERL) has consolidated many of the most important human activity databases into one comprehensive database called the Consolidated Human Activity Database (CHAD). The current version of CHAD contains data from nineteen human activity pattern studies (including NHAPS), which were evaluated to obtain over 33,000 person-days of 24-hour human activities in CHAD (McCurdy et al., 2000). The surveys include probability-based recall studies conducted by EPA and the California Air Resources Board, as well as real-time diary studies conducted in individual U.S. metropolitan areas using both probability-based and volunteer subject panels. All ages of both sexes are represented in CHAD. The data for each subject consist of one or more days of sequential activities, in which each activity is defined by start time, duration, activity type, and microenvironment classification (i.e., location). Activities vary from one minute to one hour in duration, with longer activities being subdivided into clock-hour durations to facilitate exposure modeling. CHAD also provides information on the level of exertion associated with each activity, which can be used by exposure models, including the APEX model (Section 4.5.3), to estimate ventilation rate and pollutant dose.

### 4.4.2 Ozone-Averting Behavior

Individuals can reduce their exposure to $O_3$ by altering their behaviors, such as by staying indoors, scheduling outdoor activity during periods of low $O_3$ concentration, and by reducing activity levels or time spent being active outdoors on high-$O_3$ days. To assist the public in avoiding exposure to air pollution on days with high pollutant concentrations, EPA has developed an information tool known as the Air Quality Index (AQI) to provide information to the public on ambient levels of pollutants and the potential for individuals to experience health effects (U.S. EPA, 2011a). The AQI describes the potential for health effects from $O_3$ (and other individual pollutants) in six color-coded categories of air-quality, ranging from good (green), moderate (yellow), unhealthy for sensitive groups (orange), unhealthy (red), very unhealthy (purple), and hazardous (maroon). The levels are associated with descriptors of the likelihood of health effects and the populations most likely to be affected. For example, the orange level indicates that the general population is not likely to be at risk, but susceptible groups may experience health effects. These advisories explicitly state that children, older adults, people with lung disease, and those who are active outdoors may be at greater risk from exposure to air pollution. Forecasted and actual conditions typically are reported to the public during high-$O_3$ months through local media outlets, using various versions of this air-quality categorization scheme. People are advised to change their behavior to reduce exposure depending on predicted $O_3$ concentrations and the likelihood of risk. Behavioral recommendations include moving outdoor activities to times when air quality is better, and reducing activity levels or the time spent being active outdoors on high-$O_3$ days. Staying indoors to reduce exposure is only recommended when the AQI is at or above the very unhealthy range.

Evidence of individual averting behaviors in response to advisories has been found in several studies, especially for potentially susceptible populations, such as children, older adults, and asthmatics. Reduced time spent outdoors was reported in an activity diary study in 35 U.S. cities (Mansfield et al., 2006), which found that asthmatic children who spent at least some time outdoors reduced their total time spent outdoors by an average of 30 min on a code red $O_3$ day relative to a code green, yellow, or orange day; however, the authors noted that there was appreciable variation in both the overall amount of time spent outdoors and the reduction in outdoor time on high $O_3$ days among asthmatic children. Bresnahan et al. (1997) examined survey data collected during 1985-86 from a panel of adults in the Los Angeles area, many of whom had compromised respiratory function, by an averting behavior model. A regression analysis indicated that individuals with smog-related symptoms spent about 12 minutes less time outdoors over a two-day period for each 10 ppb increase in $O_3$ concentration above 120 ppb. Considering that the average daily maximum $O_3$ concentration at the time was approximately 180 ppb on days when the then-current standard (1-h max of 120 ppb) was exceeded, this implies that those individuals spent about 40 minutes less time outside per day on a typical high $O_3$ day compared to days with $O_3$ concentrations below the standard. However, the behavior was not specifically linked to exceedances or air quality alerts.

The fraction of individuals who reduce time spent outdoors, or restrict their children's outdoor activity, has been found to vary based on health status. In the Bresnahan et al. (1997) study, 40 percent of respondents reported staying indoors on days when air quality was poor. Individuals who reported experiencing smog-related symptoms were more likely to take the averting actions, although the presence of asthma or other chronic respiratory conditions did not have a statistically significant effect on behavior. A study of parents of asthmatic children (McDermott et al., 2006) suggests that parents are aware of the hazard of outdoor air pollution and the official alerts designed to protect them and their children. It also suggests that a majority of parents (55%) comply with recommendations of the alerts to restrict children's outdoor activity, with more parents of asthmatics reporting awareness and responsiveness to alerts. However, only 7% of all parents complied with more than one-third of the advisories issued (McDermott et al., 2006). Wen et al. (2009) analyzed data from the 2005 Behavioral Risk Factor Surveillance System (BRFSS) and indicated that people with asthma are about twice as likely as people without asthma to reduce their outdoor activities based on either media alerts of poor air quality (31% versus 16%) or individual perception of air quality (26% versus 12%). Respondents who had received advice from a health professional to reduce outdoor activity when air quality is poor were more likely to report a reduction based on media alerts, both for those with and without asthma. In a study of randomly selected individuals in Houston, TX and Portland, OR, Semenza et al. (2008) found that a relatively small fraction of survey respondents (9.7% in Houston, 10.5% in Portland) changed their behaviors during poor air quality episodes. This fraction is appreciably lower than the fraction reported for people with asthma in the Wen et al. (2009) study, although it is similar to the fraction reported in that study for those without asthma. Most of the people in the Semenza et al. (2008) study reported that their behavioral changes were motivated by self-perception of poor air quality rather than an air quality advisory. It should be noted that the McDermott et al. (2006), Wen et al. (2009), and Semenza et al. (2008) studies evaluated air quality in general and therefore are not necessarily specific to $O_3$.

Commuting behavior does not seem to change based on air quality alerts. A study in the Atlanta area showed that advisories can raise awareness among commuters but do not necessarily result in a change in an individual's travel behavior (Henry and Gordon, 2003). This finding is consistent with a survey for 1,000 commuters in Denver, Colorado, which showed that the majority (76%) of commuters heard and understood the air quality advisories, but did not alter their commuting behavior (Blanken et al., 2001).

Some evidence is available for other behavioral changes in response to air quality alerts. Approximately 40 percent of the respondents in the Los Angeles study by Bresnahan et al. (1997) limited or rearranged leisure activities, and 20 percent increased use of air conditioners. As with changes in time spent outdoors, individuals who reported experiencing smog-related symptoms, but not those with asthma or chronic respiratory conditions, were more likely to take the averting actions. Other factors influencing behavioral changes, such as increased likelihood of averting behavior among high school graduates, are also reported in the study. In a separate

Southern California study, attendance at two outdoor facilities (i.e., a zoo and an observatory) was reduced by 6-13% on days when smog alerts were announced, with greater decreases observed among children and older adults (Neidell, 2010, 2009).

The studies discussed in this section indicate that averting behavior is dependent on several factors, including health status and lifestage. People with asthma and those experiencing smog-related symptoms reduce their time spent outdoors and are more likely to change their behavior than those without respiratory conditions. Children and older adults appear more likely to change their behavior than the general population. Commuters, even when aware of air quality advisories, tend not to change their commuting behavior.

### 4.4.3 Population Proximity to Fixed-Site Ozone Monitors

The distribution of $O_3$ monitors across urban areas varies between cities (Section 3.6.2.1), and the population living near each monitor varies as well. Monitoring sites in rural areas are generally located in national or state parks and forests, and these monitors may be relevant for exposures of exercising visitors as well as those who live in similar locations. They also serve as an important source of data for evaluating ecological effects of $O_3$ (Chapter 9). Rural monitors tend to be less affected than urban monitors by strong and highly variable anthropogenic sources of species participating in the formation and destruction of $O_3$ (e.g., onroad mobile sources) and more highly influenced by regional transport of $O_3$ or $O_3$ precursors (Section 3.6.2.2). This may contribute to less diel variability in $O_3$ concentration than is observed in urban areas.

A variety of factors determine the siting of the $O_3$ monitors that are part of the SLAMS network reporting to AQS. As discussed in Section 3.5.6, the number and location of required $O_3$ monitors in an urban area depend on $O_3$ concentration and population, among other factors. Areas classified as serious, severe, or extreme nonattainment have additional monitoring requirements. Generally, high-$O_3$ urban areas with a population of 50,000 or greater are required to have at least one monitor; in low- or moderate-concentration areas, the minimum population for a required monitor is 350,000. Most large U.S. cities have several monitors, as shown in Figure 3-76 through Figure 3-95.

As an illustration of the location of $O_3$ monitors and their concentrations with respect to population density, Figure 4-4 through Figure 4-6 present this information for Atlanta, Boston, and Los Angeles, the three cities selected for detailed analysis in Chapter 3. They represent a cross-section with respect to geographic distribution, $O_3$ concentration, layout, geographic features, and other factors. The maps show the location of $O_3$ monitors, identified by the same letters as in Chapter 3 to facilitate intercomparisons, along with the 2007-2009 mean 8-h daily max $O_3$ concentration for perspective on the variation in $O_3$ concentration across the urban area. Population density at the census block group level is also presented on the maps.

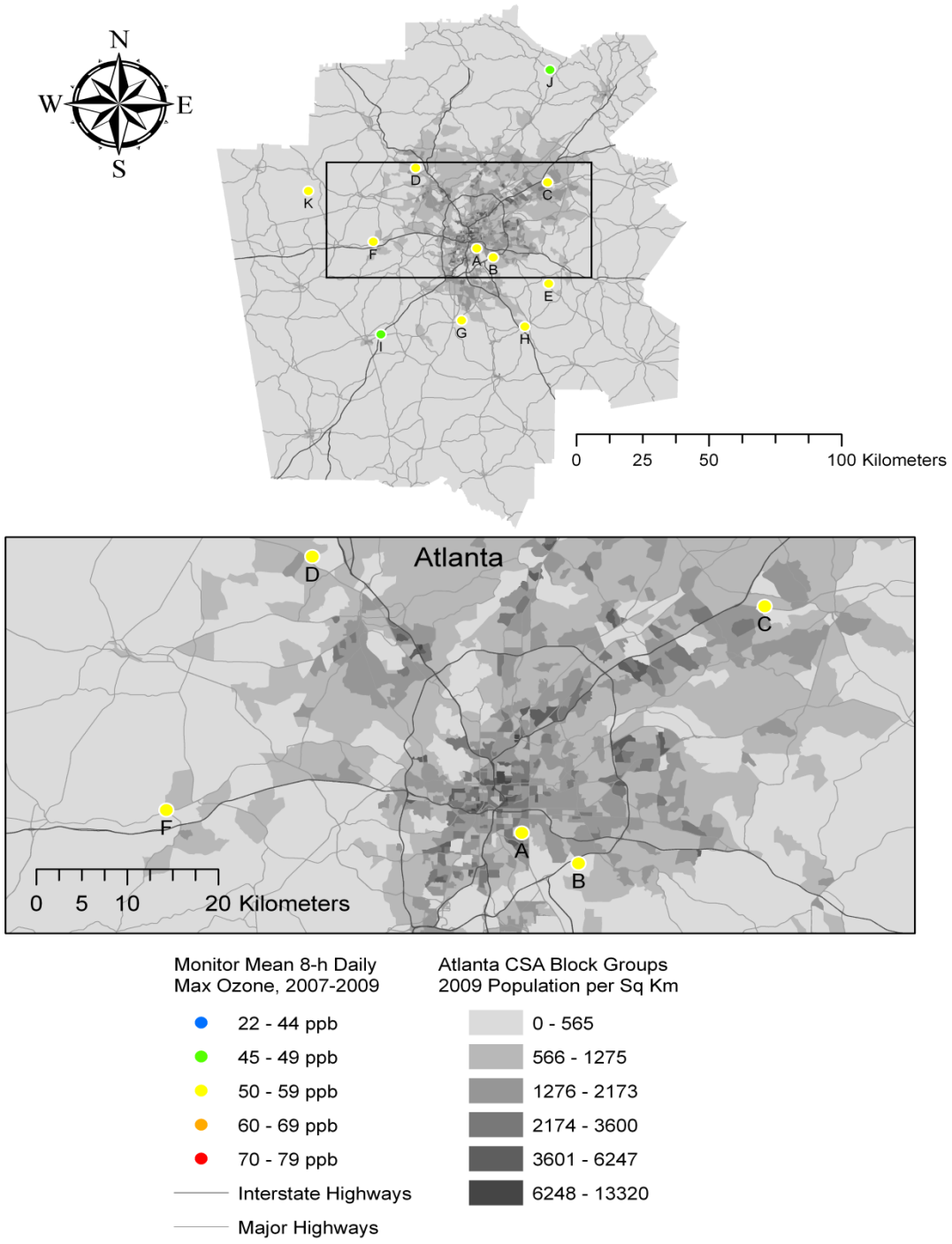



**Figure 4-4 Map of the Atlanta CSA including $O_3$ monitor locations and major roadways with respect to census block group population density estimates for 2009.**

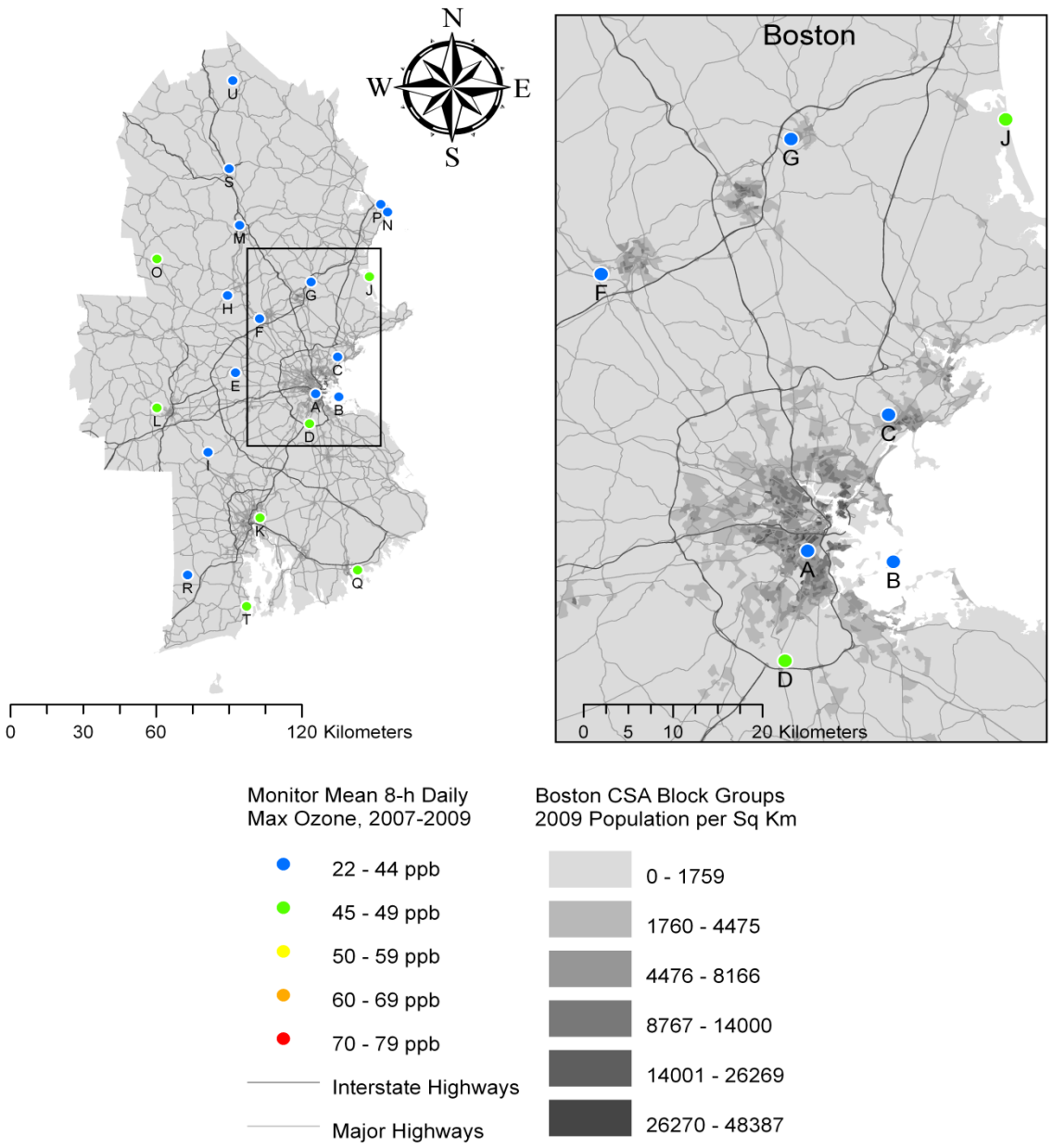



**Figure 4-5** **Map of the Boston CSA including $O_3$ monitor locations and major roadways with respect to census block group population density estimates for 2009.**

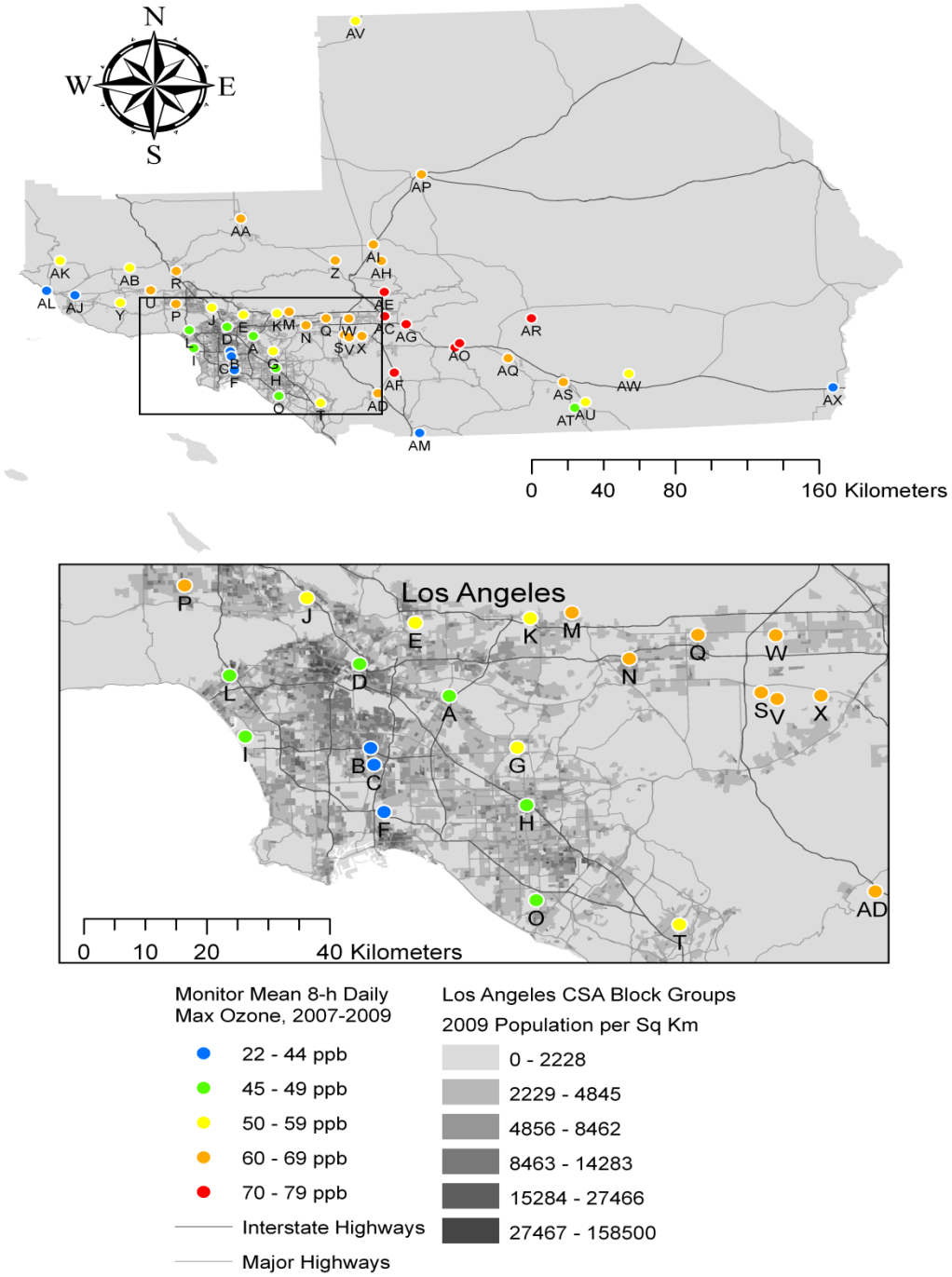



**Figure 4-6** **Map of the Los Angeles CSA including $O_3$ monitor locations and major roadways with respect to census block group population density estimates for 2009.**

Similarities and differences are apparent among the cities. The spatial distribution of monitor locations in Atlanta and Boston is similar, with one site (site A) near the high population density area and other monitors in surrounding areas of lower population density. In Atlanta, the monitors near the city all have similar concentrations, while somewhat lower concentrations are observed at sites I and J, which are located >50 km from the city center. Boston shows a different spatial concentration pattern, with some low and some high concentrations in urban and less-populated areas. The differences in spatial concentration profiles between the two cities may be due to more consistent terrain in Atlanta compared with Boston, which has a coastline, along with the downwind influence of New York and other northeastern cities contributing to concentration variability.

Los Angeles has a much more complex spatial pattern of monitors, population, and geography. There are a large number of monitors located in multiple levels of population density across the entire CSA, which includes substantial rural areas. Most monitors are near at least moderate population density areas, but there are some high-density areas without a monitor. Concentrations increase in a somewhat radial or west-east pattern from the city, with lower concentrations near the port of Long Beach (monitors B, C, and F). The highest concentrations are located near the San Bernadino forest (e.g., monitors AG, AO, and AR), which have lower population density, but more potential for ecological impacts. Low concentrations in highly populated areas near the coast likely reflect titration by $NO_X$ and other atmospheric constituents, while high downwind concentrations reflect lack of local $NO_X$ sources and increased photochemical processing time.

The location of these monitors relative to the location of dense population centers varies among urban areas. NCore sites, a subset of the overall monitoring network, are designed with population exposure as a monitoring objective, and the monitoring requirements in 40 CFR Part 58, Appendix D include population density as one of several factors that would be considered in designing the $O_3$ monitoring program for an area. At least one site for each MSA is designed to be a maximum concentration site, which could presumably represent the location with the maximum exposure potential in the city. Sites may also be required upwind and downwind of high-concentration urban areas.

All three cities have some high population density areas without an $O_3$ monitor. The siting considerations for NCore monitors generally target the neighborhood (0.5-4 km) or urban (4-50 km) scale to provide representative concentrations throughout the metropolitan area; however, a middle-scale (0.1-0.5 km) site may be acceptable in cases where the site can represent many such locations throughout a metropolitan area. In other words, a monitor could potentially represent exposures in other similar areas of the city if land use and atmospheric chemistry conditions are similar. This is supported by the correlation analyses in Chapter 3. For example, in Los Angeles, monitors H and L are located in medium-density areas and show moderately high correlation (R = 0.78), although they are some 50 km apart.

Although proximity to a monitor does not determine the degree to which that monitor represents an individual's ambient exposure, it is one indicator. One way to calculate

monitor representativeness is to calculate the fraction of the urban population living within a certain radius of a monitor. Table 4-6 presents the fraction of the population in selected cities living within 1, 5, 10, and 20 km of an $O_3$ monitor. Values are presented for both total population and for those under 18 years of age, a potentially susceptible population to the effects of $O_3$. The data indicate that relatively few people live within 1 km of an $O_3$ monitor, while nearly all of the population in most cities lives within 20 km of a monitor. Looking at the results for a 5-km radius, corresponding roughly to the neighborhood scale (Section 3.5.6.1), generally 20-30% of the population lives within this distance from an $O_3$ monitor. Some cities have a greater population in this buffer, such as Salt Lake City, while others have a lower percentage, such as Minneapolis and Seattle. Percentages for children are generally similar to the total population, with no clear trend.

Another approach is to divide the metropolitan area into sectors surrounding each monitor such that every person in the sector lives closer to that monitor than any other. This facilitates calculation of the fraction of the city's population represented (according to proximity) by each monitor. In Atlanta, for example, the population fraction represented by each of the 11 monitors in the city ranged from 2.9-22%. The two monitors closest to the city center (sites A and B on Figure 4-4) accounted for 16% and 8% of the population, respectively. Site B has two listed monitoring objectives, highest concentration and population exposure. The other monitor in Atlanta with a listed objective of highest concentration is Site C, which represents the largest fraction of the population (22%). The eight monitors with a primary monitoring objective of population exposure account for 2.9-17% of the population per monitor.

**Table 4-6 Fraction of the 2009 population living within a specified distance of an $O_3$ monitor in selected U.S. cities.**

| | Population | | Within 1 km | | Within 5 km | | Within 10 km | | Within 20 km | |
|---|---|---|---|---|---|---|---|---|---|---|
| **City** | **Total** | **<18 yr** | **Total** | **<18 yr** | **Total** | **<18 yr** | **Total** | **<18 yr** | **Total** | **<18 yr** |
| Atlanta, GA, CSA | 5,901,670 | 1,210,932 | 0.3% | 0.3% | 8% | 9% | 28% | 29% | 75% | 77% |
| Baltimore, MD, CSA | 8,421,016 | 1,916,106 | 1.3% | 1.1% | 25% | 24% | 57% | 55% | 89% | 89% |
| Birmingham, AL, CSA | 1,204,399 | 281,983 | 1.4% | 1.6% | 22% | 24% | 56% | 59% | 73% | 74% |
| Boston, MA, CSA | 7,540,533 | 1,748,918 | 0.9% | 0.9% | 17% | 16% | 49% | 47% | 85% | 85% |
| Chicago, IL, CSA | 9,980,113 | 2,502,454 | 1.5% | 1.5% | 28% | 29% | 63% | 65% | 89% | 91% |
| Dallas, TX, CSA | 6,791,942 | 1,530,877 | 0.4% | 0.4% | 13% | 13% | 45% | 44% | 87% | 87% |
| Denver, CO, CSA | 3,103,801 | 675,380 | 1.7% | 1.6% | 35% | 36% | 66% | 68% | 92% | 93% |
| Detroit, MI, CSA | 5,445,448 | 1,411,875 | 0.8% | 0.9% | 15% | 17% | 42% | 44% | 77% | 78% |
| Houston, TX, CSA | 5,993,633 | 1,387,851 | 1.5% | 1.8% | 26% | 28% | 54% | 57% | 83% | 84% |
| Los Angeles, CA, CSA | 18,419,720 | 4,668,441 | 1.6% | 1.7% | 28% | 29% | 77% | 79% | 98% | 98% |
| Minneapolis, MN, CSA | 3,652,490 | 872,497 | 0.3% | 0.3% | 5% | 4% | 16% | 16% | 57% | 56% |
| New York, NY, CSA | 22,223,406 | 5,284,875 | 1.5% | 1.7% | 23% | 23% | 51% | 50% | 91% | 91% |
| Philadelphia, PA, CSA | 6,442,836 | 1,568,878 | 0.9% | 1.0% | 22% | 24% | 55% | 56% | 89% | 89% |
| Phoenix, AZ, CBSA | 4,393,462 | 873,084 | 2.0% | 2.4% | 35% | 41% | 74% | 79% | 96% | 97% |
| Pittsburgh, PA, CSA | 2,471,403 | 563,309 | 1.5% | 1.4% | 22% | 21% | 52% | 50% | 88% | 88% |
| Salt Lake City, UT, CSA | 1,717,045 | 460,747 | 3.0% | 3.0% | 41% | 38% | 79% | 79% | 95% | 95% |
| San Antonio, TX, CBSA | 2,061,147 | 484,473 | 0.5% | 0.5% | 12% | 12% | 42% | 43% | 78% | 80% |
| San Francisco, CA, CSA | 7,497,443 | 1,675,711 | 2.6% | 2.9% | 41% | 40% | 81% | 81% | 98% | 98% |
| Seattle, WA, CSA | 4,181,278 | 918,309 | 0.3% | 0.3% | 5% | 5% | 18% | 16% | 43% | 39% |
| St. Louis, MO, CSA | 2,914,754 | 720,746 | 1.3% | 1.5% | 17% | 18% | 52% | 53% | 80% | 82% |

Atlanta population fractions for children (<18 years of age) are similar to those for the general population, but other populations show a different pattern of monitor representativeness. Older adults (age 65 and up) were somewhat differently distributed with respect to the monitors, with most monitors showing a difference of more than a percentage point compared to the general population. Based on 2000 population data, the fraction of older adults closest to the two city center monitors (A and B) was 4% higher and 2% lower, respectively, than the fraction for the population as a whole. Site C showed the highest differential, with 21% of the total population but only 15% of the older adult population. This indicates the potential for monitors to differentially represent potentially susceptible populations.

## 4.5 Exposure Modeling

In the absence of personal exposure measurements, modeling techniques are used to estimate exposures, particularly for large populations for which individual-level measurements would be impractical. Model estimates may be used as inputs to epidemiologic studies or as stand-alone assessments of the level of exposure likely to be experienced by a population under certain air quality conditions. This section describes approaches used to improve exposure estimates, including concentration surface modeling, which calculates local outdoor concentrations over a geographic area; air exchange rate modeling, which estimates building ventilation based on housing characteristics and meteorological parameters; and microenvironment-based exposure modeling, which combines air quality data with demographic information and activity pattern simulations to estimate time-weighted exposures based on concentrations in multiple microenvironments. These models each have strengths and limitations, as summarized in Table 4-7. The remainder of this section provides more detail on specific modeling approaches, as well as results of applying the models.

**Table 4-7 Characteristics of exposure modeling approaches.**

| Model Type | Model | Description | Strengths | Limitations |
|---|---|---|---|---|
| Concentration Surface | Spatial Interpolation (e.g., Inverse Distance Weighting, Kriging) | Measured concentrations are interpolated across an area to yield local outdoor concentration estimates | High concentration resolution; uses available data; requires low to moderate resources for implementation | Spatial heterogeneity not fully captured; a single high-concentration monitor can skew results; no location-activity information |
| | Chemistry-transport (e.g., CMAQ) | Grid-based $O_3$ concentrations are calculated from precursor emissions, meteorology, and atmospheric chemistry and physics | First-principles characterization of physical and chemical processes influencing $O_3$ formation | Grid cell resolution; resource-intensive; no location-activity information |
| | Land-use regression (LUR) | Merges concentration data with local-scale variables such as land use factors to yield local concentration surface | High concentration resolution | Reactivity and small-scale spatial variability of $O_3$; location-specific, limiting generalizability; no location-activity information |
| Air Exchange Rate | Mechanistic (LBL, LBLX) | Uses database on building leakage tests to predict AER based on building characteristics and meteorological variables (including natural ventilation in LBLX) | Physical characterization of driving forces for air exchange | Moderate resource requirement; no location-activity information |
| | Empirical | Predicts AER based on factors such as building age and floor area | Low input data requirements | Cannot account for meteorology; no location-activity information |
| Integrated Microenvironmental Exposure and Dose | Population (APEX, SHEDS) | Stochastic treatment of air quality data, demographic variables, and activity pattern to generate estimates of microenvironmental concentrations, exposures, and doses | Probabilistic estimates of exposure and dose distributions for specific populations; consideration of nonambient sources; small to moderate uncertainty for exercising asthmatic children (APEX) | Resource-intensive; evaluation with measured exposures; underestimation of multiple high-exposure events in an individual (APEX) |

### 4.5.1 Concentration Surface Modeling

One approach to improve exposure estimates in urban areas involves construction of a concentration surface over a geographic area, with the concentration at locations between monitors estimated using a model to compensate for missing data. The calculated $O_3$ concentration surface can then be used to estimate exposures outside residences, schools, workplaces, roadways, or other locations of interest. This technique does not estimate exposure directly because it does not account for activity patterns or concentrations in different microenvironments. This is an important consideration in the utility of these methods for exposure assessment; while improved local-scale estimates of outdoor concentrations may contribute to better assignment of exposures, information on activity patterns is needed to produce

estimates of personal exposure. There are three main types of approaches: spatial interpolation of measured concentrations; statistical models using meteorological variables, pollutant concentrations, and other predictors to estimate concentrations at receptors in the domain; and rigorous first-principle models, such as chemistry-transport models or dispersion models incorporating $O_3$ chemistry. Some researchers have developed models that combine these techniques. The models may be applied over urban, regional, or national spatial scales, and can be used to estimate daily concentrations or longer-term averages. This discussion will focus on short-term concentrations estimated across urban areas.

The 2006 $O_3$ AQCD (U.S. EPA, 2006b) discussed concentration surface models, focusing on chemistry-transport models as well as geospatial and spatiotemporal interpolation techniques (e.g., Christakos and Vyas, 1998a, b; Georgopoulos et al., 1997). Recent research has continued to refine and extend the modeling approaches. A few recent papers have compared different approaches for the same urban area.

Marshall et al. (2008) compared four spatial interpolation techniques for estimation of $O_3$ concentrations in Vancouver, BC. The investigators assigned a daily average $O_3$ concentration to each of the 51,560 postal-code centroids using one of the following techniques: (1) the concentration from the nearest monitor within 10 km; (2) the average of all monitors within 10 km; (3) the inverse-distance-weighted (IDW) average of all monitors in the area; and (4) the IDW average of the 3 closest monitors within 50 km. Method 1 (the nearest-monitor approach) and Method 4 (IDW-50 km) had similar mean and median estimated annual- and monthly-average concentrations, although the 10th-90th percentile range was smaller for IDW-50. This is consistent with the averaging of extreme values inherent in IDW methods. The Pearson correlation coefficient between the two methods was 0.93 for monthly-average concentrations and 0.78 for annual-average concentrations. Methods 2 and 3 were considered sub-optimal and were excluded from further analysis. In the case of Method 2, a single downtown high-concentration monitor skewed the results in the vicinity, partially as a result of the asymmetric layout of the coastal city of Vancouver. Method 3 was too spatially homogenous because it assigned most locations a concentration near the regional average, except for locations immediately adjacent to a monitoring site. CMAQ concentration estimates using a 4 km×4 km grid were also compared to the interpolation techniques in this study. Mean and median concentrations from CMAQ were approximately 50% higher than Method 1 and Method 4 estimates for both annual and monthly average concentrations. This may be due in part to the CMAQ grid size, which was too coarse to reveal near-roadway decrements in $O_3$ concentration due to titration by NO. The IQR for the annual average was similar between CMAQ and the interpolation techniques, but the monthly average CMAQ IQR was approximately twice as large, indicating a seasonal effect.

Bell (2006) compared CMAQ estimates for northern Georgia with nearest-monitor and spatial interpolation techniques, including IDW and kriging. The area-weighted concentration estimates from CMAQ indicated areas of spatial heterogeneity that were not captured by approaches based on the monitoring network. The author

concluded that some techniques, such as spatial interpolation, were not suitable for estimation of exposure in certain situations, such as for rural areas. Using the concentration from the nearest monitor resulted in an overestimation of exposure relative to model estimates.

Land use regression (LUR) models have been developed to estimate levels of air pollutants, predominantly $NO_2$, as a function of several land use factors, such as land use designation, traffic counts, home heating usage, point source strength, and population density (Ryan and LeMasters, 2007; Gilliland et al., 2005; Briggs et al., 1997). LUR, initially termed regression mapping (Briggs et al., 1997), is a regression derived from monitored concentrations as a function of data from a combination of the land use factors. The regression is then used for predicting concentrations at multiple locations based on the independent variables at those particular locations without monitors. Hoek et al. (2008) warn of several limitations of LUR, including distinguishing real associations between pollutants and covariates from those of correlated copollutants, limitations in spatial resolution from monitor data, applicability of the LUR model under changing temporal conditions, and introduction of confounding factors when LUR is used in epidemiologic studies. These limitations may partially explain the lack of LUR models that have been developed for $O_3$ at the urban scale. Brauer et al. (2008) evaluated the use of LUR and IDW-based spatial-interpolation models in epidemiologic analyses for several different pollutants in Vancouver, BC and suggested that LUR is appropriate for directly-emitted pollutants with high spatial variability, such as NO and BC, while IDW is appropriate for secondary pollutants such as $NO_2$ and $PM_{2.5}$ with less spatial variability. Although $O_3$ is also a secondary pollutant, its reactivity and high small-scale spatial variability near high-traffic roadways indicates this conclusion may not apply for $O_3$.

At a much larger spatial scale, EU-wide, Beelen et al. (2009) compared a LUR model for $O_3$ with ordinary kriging and universal kriging, which incorporated meteorological, topographical, and land use variables to characterize the underlying trend. The LUR model performed reasonably well at rural locations (5-km resolution), explaining a higher percentage of the variability ($R^2 = 0.62$) than for other pollutants. However, at the urban scale (1-km resolution), only one variable was selected into the $O_3$ LUR model (high-density residential land use), and the $R^2$ value was very low (0.06). Universal kriging was the best method for the large-scale composite EU concentration map, for $O_3$ as well as for $NO_2$ and $PM_{10}$, with an $R^2$ value for $O_3$ of 0.70. The authors noted that these methods were not designed to capture spatial variation in concentrations that are known to occur within tens of meters of roadways (Section 3.6.2.1), which could partially explain poor model performance at the urban scale.

Titration of $O_3$ with NO emitted by motor vehicles tends to reduce $O_3$ concentrations near roadways. McConnell et al. (2006) developed a regression model to predict residential $O_3$ concentrations in southern California using estimates of residential $NO_X$ calculated from traffic data with the CALINE4 line source dispersion model. The annual average model results were well-correlated ($R^2 = 0.97$) with multi-year

average monitoring data. The authors estimated that local traffic contributes 18% of $NO_X$ concentrations measured in the study communities, with the remainder coming from regional background. Their regression model indicates that residential $NO_X$ reduces residential $O_3$ concentrations by 0.51 ppb (SE 0.11 ppb) $O_3$ per 1 ppb $NO_X$, and that a 10th-90th percentile increase in local $NO_X$ results in a 7.5 ppb decrease in local $O_3$ concentrations. This intra-urban traffic-related variability in $O_3$ concentrations suggests that traffic patterns are an important factor in the relationship between central site monitor and residential $O_3$, and that differences in traffic density between the central site monitor and individual homes could result in either an overestimate or underestimate of residential $O_3$.

A substantial number of researchers have used geostatistical methods and chemistry-transport models to estimate $O_3$ concentrations at urban, regional, national, and continental scales, both in the U.S. and in other countries (Section 3.3). In addition to short-term exposure assessment for epidemiologic studies, such models may also be used for long-term exposure assessment, $O_3$ forecasts, or evaluating emission control strategies. However, as discussed at the beginning of this section, caveats regarding the importance of activity pattern information in estimating personal and population exposure should be kept in mind.

### 4.5.2 Residential Air Exchange Rate Modeling

The residential air exchange rate (AER), which is the airflow into and out of a home, is an important mechanism for entry of ambient $O_3$. As described in Section 4.3.2, the indoor-outdoor relationship is greatly affected by the AER. Since studies show that people spend approximately 66% of their time indoors at home (Leech et al., 2002; Klepeis et al., 2001), the residential AER is a critical parameter for exposure models, such as APEX, SHEDS, and EMI (discussed in Section 4.5.3) (U.S. EPA, 2011c, 2009b; Burke et al., 2001). Since the appropriate AER measurements may not be available for exposure models, mechanistic and empirical (i.e., regression-based) AER models can be used for exposure assessments. The input data for the AER models can include building characteristics (e.g., age, number of stories, wind sheltering), occupant behavior (e.g., window opening), climatic region, and meteorology (e.g., local temperature and wind speed). Mechanistic AER models use these meteorological parameters to account for the physical driving forces of the airflows due to pressure differences across the building envelope from wind and indoor-outdoor temperature differences (ASHRAE, 2009). Empirical AER models do not consider the driving forces from the wind and indoor-outdoor temperature differences. Instead, a scaling constant can be used based on factors such as building age and floor area (Chan et al., 2005b).

Single-zone mechanistic models represent a whole-building as a single, well-mixed compartment. These AER models, such as the Lawrence Berkeley Laboratory (LBL) model, can predict residential AER using input data from whole-building pressurization tests (Sherman and Grimsrud, 1980), or leakage area models (Breen et

al., 2010; Sherman and McWilliams, 2007). Recently, the LBL air infiltration model was linked with a leakage area model using population-level census and residential survey data (Sherman and McWilliams, 2007) and individual-level questionnaire data (Breen et al., 2010). The LBL model, which predicts the AER from air infiltration (i.e., small uncontrollable openings in the building envelope) was also extended to include airflow from natural ventilation (LBLX), and evaluated using window opening data (Breen et al., 2010). The AER predictions from the LBL and LBLX models were compared to daily AER measurements on seven consecutive days during each season from detached homes in central North Carolina (Breen et al., 2010). For the individual model-predicted and measured AER, the median absolute difference was 43% (0.17 $h^{-1}$) and 40% (0.17 $h^{-1}$) for the LBL and LBLX models, respectively. Given the uncertainty of the AER measurements (accuracy of 20-25% for occupied homes), these results demonstrate the feasibility of using these AER models for both air infiltration (e.g., uncontrollable openings) and natural ventilation (e.g., window opening) to help reduce the AER uncertainty in exposure models. The capability of AER models could help support the exposure modeling needs, as described in Section 4.5.3, which includes the ability to predict indoor concentrations of ambient $O_3$ that may be substantial for conditions of high AER such as open windows.

### 4.5.3 Microenvironment-Based Models

Population-based methods, such as the Air Pollution Exposure (APEX) and Stochastic Human Exposure and Dose Simulation (SHEDS) integrated microenvironmental exposure and dose models, involve stochastic treatment of the model inputs (U.S. EPA, 2009b; Burke et al., 2001). These are described in detail in the 2008 $NO_X$ ISA (U.S. EPA, 2008c), in AX3.6.1. Stochastic models utilize distributions of pollutant-related and individual-level variables, such as ambient and local $O_3$ concentration contributions and breathing rate respectively, to compute the distribution of individual exposures across the modeled population. The models also have the capability to estimate received dose through a dosimetry model. Using distributions of input parameters in the model framework rather than point estimates allows the models to incorporate uncertainty and variability explicitly into exposure estimates (Zidek et al., 2007). These models estimate time-weighted exposure for modeled individuals by summing exposure in each microenvironment visited during the exposure period.

The initial set of input data for population exposure models is ambient air quality data, which may come from a monitoring network or model estimates. Estimates of concentrations in a set of microenvironments are generated either by mass balance methods, which can incorporate AER models (Section 4.5.2), or microenvironmental factors. Microenvironments modeled include indoor residences; other indoor locations, such as schools, offices, and public buildings; and vehicles. The sequence of microenvironments and exertion levels during the exposure period is determined from characteristics of each modeled individual. The APEX model does this by

generating a profile for each simulated individual by sampling from distributions of demographic variables such as age, sex, and employment; physiological variables such as height and weight; and situational variables such as living in a house with a gas stove or air conditioning. Activity and location (microenvironmental) patterns from a database such as CHAD are assigned to the simulated individual in a longitudinal manner, using age, sex, and biometric characteristics (U.S. EPA, 2009a; Glen et al., 2008). Breathing rates for each individual are calculated for each activity based on predicted energy expenditures, and the corresponding dose may then be computed. APEX has an algorithm to estimate $O_3$ dose and changes in $FEV_1$ resulting from $O_3$ exposure. Summaries of individual- and population-level metrics are produced, such as maximum exposure or dose, number of individuals exceeding a specified exposure/dose, and number of person-days at or above benchmark exposure levels. The models also consider the nonambient contribution to total exposure. Nonambient source terms are added to the infiltration of ambient pollutants to calculate the total concentration in the microenvironment. Output from model runs with and without nonambient sources can be compared to estimate the ambient contribution to total exposure and dose.

Georgopoulos et al. (2005) used a version of the SHEDS model as the exposure component of a modeling framework known as MENTOR (Modeling Environment for Total Risk Studies) in a simulation of $O_3$ exposure in Philadelphia over a 2-week period in July 1999. Five hundred (500) individuals were sampled from CHAD in each of 482 census tracts to match local demographic characteristics from U.S. Census data. Outdoor concentrations over the modeling domain were calculated from interpolation of photochemical modeling results and fixed-site monitor concentrations. These concentrations were then used as input data for SHEDS. Median microenvironmental concentrations predicted by SHEDS for nine simulated microenvironments were strongly correlated with outdoor concentrations, a result consistent with the lack of indoor $O_3$ sources in the model. A regression of median microenvironmental concentrations against outdoor concentrations indicated that the microenvironmental concentrations were appreciably lower than outdoor concentrations (regression slope = 0.26). 95th percentile microenvironmental concentrations were also well correlated with outdoor concentrations and showed a regression slope of 1.02, although some microenvironmental concentrations were well below the outdoor values. This suggests that in most cases the high-end concentrations were associated with outdoor microenvironments. Although the authors did not report exposure statistics for the population, their dose calculations also indicated that $O_3$ dose due to time spent outdoors dominated the upper percentiles of the population dose distribution. They found that both the 50th and 95th percentile $O_3$ concentrations were correlated with census-tract level outdoor concentrations estimated by photochemical modeling combined with spatiotemporal interpolation, and attributed this correlation to the lack of indoor sources of $O_3$. Relationships between exposure and concentrations at fixed-site monitors were not reported.

An analysis has been conducted for the APEX model to evaluate the contribution of uncertainty in input parameters and databases to the uncertainty in model outputs

(Langstaff, 2007). The Monte Carlo analysis indicates that the uncertainty in model exposure estimates for asthmatic children during moderate exercise is small to moderate, with 95% confidence intervals of at most ± 6 percentage points at exposures above 60, 70, and 80 ppb (8-h avg). However, APEX appears to substantially underestimate the frequency of multiple high-exposure events for a single individual. The two main sources of uncertainty identified were related to the activity pattern database and the spatial interpolation of fixed-site monitor concentrations to other locations. Additional areas identified in the uncertainty analysis for potential improvement include: further information on children's activities, including longitudinal patterns in the activity pattern database; improved information on spatial variation of $O_3$ concentrations, including in near-roadway and indoor microenvironments; and data from personal exposure monitors with shorter averaging times to capture peak exposures and lower detection limits to capture low indoor concentrations. A similar modeling approach has been developed for panel epidemiologic studies or for controlled human exposure studies, in which activity pattern data specific to the individuals in the study can be collected. Time-activity data is combined with questionnaire data on housing characteristics, presence of indoor or personal sources, and other information to develop a personalized set of model input parameters for each individual. This model, the Exposure Model for Individuals, has been developed by EPA's National Exposure Research Laboratory (U.S. EPA, 2011c; Zartarian and Schultz, 2010).

## 4.6 Implications for Epidemiologic Studies

Exposure measurement error, which refers to the uncertainty associated with using exposure metrics to represent the actual exposure of an individual or population, can be an important contributor to variability in epidemiologic study results. Time-series studies assess the daily health status of a population of thousands or millions of people over the course of multiple years (i.e., thousands of days) across an urban area by estimating their daily exposure using a short monitoring interval (hours to days). In these studies, the community-averaged concentration of an air pollutant measured at central-site monitors is typically used as a surrogate for individual or population ambient exposure. In addition, panel studies, which consist of a relatively small sample (typically tens) of study participants followed over a period of days to months, have been used to examine the health effects associated with short-term exposure to ambient concentrations of air pollutants (Delfino et al., 1996). Panel studies may also apply a microenvironmental model to represent exposure to an air pollutant. A longitudinal cohort epidemiologic study, such as the ACS cohort study, typically involves hundreds or thousands of subjects followed over several years or decades (Jerrett et al., 2009). Concentrations are generally aggregated over time and by community to estimate exposures.

Exposure error can under- or over-estimate epidemiologic associations between ambient pollutant concentrations and health outcomes by biasing effect estimates toward or away from the null, and tends to widen confidence intervals around those

estimates (Sheppard et al., 2005; Zeger et al., 2000). Exposure misclassification can also tend to obscure the presence of potential thresholds for health effects, as demonstrated by a simulation study of nondifferential exposure misclassification (Brauer et al., 2002). The importance of exposure misclassification varies with study design and is dependent on the spatial and temporal aspects of the design. For example, the use of a community-averaged $O_3$ concentration in a time-series epidemiologic study may be adequate to represent the day-to-day temporal concentration variability used to evaluate health effects, but may not capture differences in the magnitude of exposure due to spatial variability. Other factors that could influence exposure estimates include nonambient exposure, topography of the natural and built environment, meteorology, measurement errors, use of ambient $O_3$ concentration as a surrogate for ambient $O_3$ exposure, and the presence of $O_3$ in a mixture of pollutants. The following sections will consider various sources of error and how they affect the interpretation of results from epidemiologic studies of different designs.

### 4.6.1 Non-Ambient Ozone Exposure

For other criteria pollutants, nonambient sources can be an important contributor to total personal exposure. There are relatively few indoor sources of $O_3$; as a result, personal $O_3$ exposure is expected to be dominated by ambient $O_3$ in outdoor microenvironments and in indoor microenvironments with high air exchange rates (e.g., with open windows). Even in microenvironments where nonambient exposure is substantial, such as in a room with an $O_3$ generator, this nonambient exposure is unlikely to be temporally correlated with ambient $O_3$ exposure (Wilson and Suh, 1997), and therefore would not affect epidemiologic associations between $O_3$ and a health effect (Sheppard et al., 2005). In simulations of a nonreactive pollutant, Sheppard et al. (2005) concluded that nonambient exposure does not influence the health outcome effect estimate if ambient and nonambient concentrations are independent. Since personal exposure to ambient $O_3$ is some fraction of the ambient concentration, it should be noted that effect estimates calculated based on personal exposure rather than ambient concentration will be increased in proportion to the ratio of ambient concentration to ambient exposure, and daily fluctuations in this ratio can widen the confidence intervals in the ambient concentration effect estimate, but uncorrelated nonambient exposure will not bias the effect estimate (Sheppard et al., 2005; Wilson and Suh, 1997).

### 4.6.2 Spatial and Temporal Variability

Spatial and temporal variability in $O_3$ concentrations can contribute to exposure error in epidemiologic studies, whether they rely on central-site monitor data or concentration modeling for exposure assessment. Spatial variability in the magnitude of concentrations may affect cross-sectional and large-scale cohort studies by

undermining the assumption that intra-urban concentration and exposure differences are less important than inter-urban differences. This issue may be less important for time-series studies, which rely on day-to-day temporal variability in concentrations to evaluate health effects. Low inter-monitor correlations contribute to exposure error in time-series studies, including bias toward the null and increased confidence intervals.

#### 4.6.2.1 Spatial Variability

Spatial variability of $O_3$ concentrations is highly dependent on spatial scale; in effect, $O_3$ is a regional pollutant subject to varying degrees of local variability. In the immediate vicinity of roadways, $O_3$ concentrations are reduced due to reaction with NO and other species (Section 4.3.4.2); over spatial scales of a few kilometers, $O_3$ may be more homogeneous due to its formation as a secondary pollutant; over scales of tens of kilometers, atmospheric processing can result in higher concentrations downwind of an urban area than in the urban core. Local-scale variations have a large impact on the relative magnitude of concentrations among urban monitors, while conditions favoring high or low rates of $O_3$ formation (e.g., temperature) vary over large spatial scales. This suggests that neighborhood monitors are likely to track one another temporally, but miss small-scale spatial variability in magnitude. This is supported by an analysis in Atlanta, GA, that found correlations greater than 0.8 for daily $O_3$ concentration metrics (1-h max, 8-h max, and 24-h avg) measured at monitors 10-60 km apart (Darrow et al., 2011a). In rural areas, a lower degree of fluctuation in $O_3$ precursors such as NO and VOCs is likely to make the diel concentration profile less variable than in urban areas, resulting in more sustained ambient levels. Spatial variability contributes to exposure error if the ambient $O_3$ concentration measured at the central site monitor is used as an ambient exposure surrogate and differs from the actual ambient $O_3$ concentration outside a subject's residence and/or worksite (in the absence of indoor $O_3$ sources). Averaging data from a large number of samplers will dampen intersampler variability, and use of multiple monitors over smaller land areas may allow for more variability to be incorporated into an epidemiologic analysis.

Community exposure may not be well represented when monitors cover large areas with several subcommunities having different sources and topographies, such as the Los Angeles, CA, CSA (Section 3.6.2.1 and Section 4.4.3). Ozone monitors in Los Angeles had a much wider range of intermonitor correlations (-0.06 to 0.97) than Atlanta, GA, (0.61 to 0.96) or Boston, MA, (0.56 to 0.97) using 2007-2009 data. Although the negative and near-zero correlations in Los Angeles were observed for monitors located some distance apart (>150 km), some closer monitor pairs had low positive correlations, likely due to changes in land use, topography, and airflow patterns over short distances. Lower COD values, which indicate less variability among monitors in the magnitude of $O_3$ concentrations, were observed in Atlanta (0.05-0.13) and Boston (0.05-0.19) than Los Angeles (0.05-0.56), although a single monitor (AM) was responsible for all Los Angeles COD values above 0.40.

The spatial and temporal variability in $O_3$ concentration in 24 MSAs across the U.S. was also examined in the 2006 $O_3$ AQCD (U.S. EPA, 2006b) by using Pearson correlation coefficients, values of the 90th percentile of the absolute difference in $O_3$ concentrations, and CODs. No clear discernible regional differences across the U.S. were found in the ranges of parameters analyzed.

An analysis of the impact of exposure error due to spatial variability and instrument imprecision on time-series epidemiologic study results indicated that $O_3$ has relatively low exposure error compared to other routinely monitored pollutants, and that the simulated impact on effect estimates is minor. Goldman et al. (2011) computed population-weighted scaled semivariances and Pearson correlation coefficients for daily concentration metrics of twelve pollutants measured at multiple central-site monitors in Atlanta, GA. The 8-h daily max $O_3$ exhibited the lowest semivariance and highest correlation of any of the pollutants. Although this indicates some degree of urban-scale homogeneity for $O_3$, the analysis did not account for near-road effects on $O_3$ concentrations.

Studies evaluating the influence of monitor selection on epidemiologic study results have found that $O_3$ effect estimates are similar across different spatial averaging scales and monitoring sites. A study in Italy compared approaches for using fixed-site monitoring data in a case-crossover epidemiologic study of daily $O_3$ and mortality (Zauli Sajani et al., 2011). Ozone effect estimates were found to be similar whether the nearest monitor was used, or whether single-city, three-city, or six-city regional averages were used for exposure assessment. In contrast, effect estimates for $PM_{10}$ and $NO_2$ increased with increasing scale of spatial averaging. Confidence intervals increased with increasing spatial scale for all pollutants. The authors attributed the consistency of $O_3$ effect estimates to the relative spatial homogeneity of $O_3$ over multi-km spatial scales, and pointed to the high (0.85-0.95) inter-monitor correlations to support this. The use of background monitors rather than monitors influenced by local sources in this study suggests that local-scale spatial variation in $O_3$, such as that due to titration by traffic emissions, was not captured in the analyses. A multi-city U.S. study of asthmatic children found comparable respiratory effect estimates when restricting the analysis to the monitors closest to the child's zip code centroid as when using the average of all monitors in the urban area (Mortimer et al., 2002), suggesting little impact of monitor selection. Sarnat et al. (2010) studied the spatial variability of $O_3$, along with $PM_{2.5}$, $NO_2$, and CO, in the Atlanta, GA, metropolitan area and evaluated how spatial variability affects interpretation of epidemiologic results, using time-series data for circulatory disease ED visits. The authors found that associations with ambient 8-h daily max $O_3$ concentration were similar among all sites tested, including multiple urban sites and a rural site some 38 miles from the city center. This result was also observed for 24-hour $PM_{2.5}$ concentrations. In contrast, hourly CO and $NO_2$ showed different associations for the rural site than the urban sites, although the urban site associations were similar to one another for CO. This suggests that the choice of monitor may have little impact on the results of $O_3$ time-series studies, consistent with the moderate to high inter-monitor correlations observed in Atlanta (Chapter 3).

One potential explanation for this finding from the study by Sarnat et al. (2010) is that although spatial variability at different scales contributes to a complicated pattern of variations in the magnitude of $O_3$ concentrations between near-road, urban core, and urban downwind sites, day-to-day fluctuations in concentrations may be reflected across multiple urban microenvironments. In addition, time-averaging of $O_3$ and $PM_{2.5}$ concentrations may smooth out some of the intra-day spatial variability observed with the hourly CO and $NO_2$ concentrations. However, some uncertainty in observed effect estimates due to spatial variability and associated exposure error is expected to remain, including a potential bias toward the null.

#### 4.6.2.2 Seasonality

The relationship between personal exposure and ambient concentration has been found to vary by season, with at least three factors potentially contributing to this variation: differences in building ventilation (e.g., air conditioning or heater use versus open window ventilation), higher $O_3$ concentrations during the $O_3$ season contributing to increased exposure and improved detection by personal monitors; and changes in activity pattern resulting in more time spent outside. Evidence has been presented in studies conducted in several cities regarding the effect of ventilation on personal-ambient and indoor-outdoor $O_3$ relationships (see Section 4.3.2 and Section 4.3.3). More limited evidence is available regarding the specific effects of $O_3$ detection limits and activity pattern changes on $O_3$ relationships.

Several studies have found increased summertime correlations or ratios between personal exposure and ambient concentration (Sarnat et al., 2005; Sarnat et al., 2000) or between indoor and outdoor $O_3$ concentrations (Geyh et al., 2000; Avol et al., 1998a). However, others have found higher ratios in fall than in summer (Sarnat et al., 2006a) or equivalent, near-zero ratios in winter and summer (Sarnat et al., 2001), possibly because summertime use of air conditioners decreases building air exchange rates. It should be noted that $O_3$ concentrations during winter are generally much lower than summertime concentrations, possibly obscuring wintertime relationships due to detection limit issues. Studies specifically evaluating the effect of ventilation conditions on $O_3$ relationships have found increased correlations or ratios for individuals or buildings experiencing higher air exchange rates (Sarnat et al., 2006a; Geyh et al., 2000; Sarnat et al., 2000; Romieu et al., 1998a).

Increased correlations or ratios between personal exposure and ambient concentration, or between indoor and outdoor concentration, are likely to reduce error in exposure estimates used in epidemiologic studies. This suggests that studies conducted during the $O_3$ season or in periods when communities are likely to have high air exchange rates (e.g., during mild weather) may be less prone to exposure error than studies conducted only during winter. Year-round studies that include both the $O_3$ and non-$O_3$ seasons may have an intermediate level of exposure error.

### 4.6.3 Exposure Duration

Epidemiologic studies of health effects associated with short-term and long-term exposures use different air pollution metrics and thus have different sources of exposure error. The following subsections discuss the impact of using different short-term and long-term exposure metrics on epidemiologic results.

#### 4.6.3.1 Short-Term Exposure

The averaging time of the daily exposure metrics used to evaluate daily aggregated health data (e.g., 1-h or 8-h daily max, versus 24-h avg concentration) may also impact epidemiologic results, since different studies report different daily metrics. Correlations between 1-h daily max, 8-h daily max, and 24-h avg concentrations for U.S. monitoring sites are presented in Section 3.6.1 (Figure 3-23 and accompanying text). The two daily peak values (1-h max and 8-h max) are well correlated, with a median (IQR) correlation of 0.97 (0.96-0.98). The correlation between the 8-h max and 24-h avg are somewhat less well correlated with a median (IQR) correlation of 0.89 (0.86-0.92). While this may complicate quantitative comparisons between epidemiologic studies using different daily metrics, as well as the interpretation of studies using metrics other than the current 8-hour standard, the high inter-metric correlations suggest it is a relatively small source of uncertainty in comparing the results of studies using different metrics. This is supported by a study comparing each of these metrics in a time-series study of respiratory ED visits (Darrow et al., 2011a), which found positive associations for all metrics, with the strongest association for the 8-h daily max exposure metric (Section 6.2.7.3).

The ratios of 1-h daily max, 8-h daily max, and 24-h avg concentrations to one another have been found to differ across communities and across time within individual communities (Anderson and Bell, 2010). For example, 8:24 hour ratios ranged from 1.23-1.83, with a median of 1.53. Lower ratios were generally observed in the spring and summer compared to fall and winter. Ozone concentration was identified as the most important predictor of $O_3$ metric ratios, with higher overall $O_3$ concentrations associated with lower ratios. In communities with higher long-term $O_3$ concentrations, the lower 8:24 hour ratio is attributed to high baseline $O_3$, which results in elevated 24-h average values. Differences in the representativeness of $O_3$ metrics introduces uncertainty into the interpretation of epidemiologic results and complicates comparison of studies using different metrics. Preferably, studies will report results using multiple metrics. In cases where this does not occur, the results of the study by Anderson and Bell (2010) can inform the uncertainty associated with using a standard increment to adjust effect estimates based on different metrics so that they are comparable (Chapter 6).

A study compared measures of spatial and temporal variability for 1-h daily max and 24-h daily avg $O_3$ concentrations in Brazil (Bravo and Bell, 2011). The 1-h daily max value was found to have higher correlation between monitors (i.e., lower

temporal variability) and lower COD (a measure of spatiotemporal variability which incorporates differences in concentration magnitude, with lower values indicating lower variability; see Chapter 3) than the 24-h avg value. The range of correlation coefficients and COD values was similar between the two metrics, although the variation was lower for the 1-h daily max, as indicated by the $R^2$ value for the regression of correlation coefficient on inter-monitor distance.

#### 4.6.3.2 Long-Term Exposure

A study in Canada suggests that an exposure metric based on a single year can represent exposure over a multi-decade period. The authors compared exposure assessment methods for long-term $O_3$ exposure and found that the annual average concentration in the census tract of a subject's residence during 1980 and 1994 was well-correlated (0.76 and 0.82, respectively) with a concentration metric accounting for movement among census subdivisions during 1980-2002 (Guay et al., 2011). This may have been due in part to a relatively low rate of movement, with subjects residing on average for 71% of the 22-year period in the same census subdivision they were in during 1980.

Analysis of the exposure assessment methodology in a recent study of mortality associated with long-term $O_3$ exposure (Jerrett et al., 2009) is illustrative. In this study, the authors computed quarterly averages of the daily 1-h max $O_3$ concentration, averaged the two summer quarters together to produce an annual value, then calculated a 23-year average value for each city in the study. Producing a single value for each city enables a comparison of relatively cleaner cities with relatively more polluted cities. In this case, the average was calculated using the 1-h daily max value; if the 24-h avg value had been used, concentrations would have been lower and potentially more variable, based on analyses in Chapter 3. According to Table 3-7, the 2007-2009, 3-year average 1-h daily max value during the warm season was approximately 50% higher than the corresponding 24-avg value on a nationwide basis. Correlation between the two metrics varies by site, indicating the differential influence of the overnight period on 24-h avg concentrations. The median correlation between 1-h daily max and 24-h avg is 0.83, with an IQR of 0.78-0.88. It is not clear, however, that a different exposure assignment method would yield different results.

Long-term $O_3$ trends, as discussed in Chapter 3, show gradually decreasing concentrations. Figure 3-48 shows that concentrations have decreased most for the 90th percentile, with relatively little change among the 10th percentile monitors. The decrease has been greater in the eastern U.S. than in the western part of the country (excluding California). For the most part, the rank order of regions in terms of $O_3$ concentration has remained the same, as shown in Figure 3-50, with the Northeast, Southeast, and California exhibiting the highest concentrations. The decreasing trend is consistent across nearly all monitors in the U.S., with only 1-2% of monitors reporting an increase of more than 5 ppb between the 2001-2003

and 2008-2010 time periods (Figure 3-52 and Figure 3-53). This figure provides some evidence that epidemiologic studies of long-term exposure are not affected by drastic changes in $O_3$ concentration, such as a relatively clean city becoming highly polluted or the reverse.

A few epidemiologic studies have evaluated the impact of distance to monitor on associations between long-term $O_3$ concentration and reproductive outcomes, as discussed in Chapter 7. It is not clear from this evidence whether using a local monitor for these multi-month concentration averages improves exposure assessment. Jalaludin et al. (2007) found somewhat higher effect estimates for women living within 5 km of a fixed-site $O_3$ monitor than for all women in the Sydney, Australia, metropolitan area, suggesting that increased monitor proximity reduced exposure misclassification. In contrast, Darrow et al. (2011b) found no substantial difference between effect estimates for those living within 4 mi of a fixed-site monitor and those living in the five-county area around Atlanta, GA. This result could be due to spatial variability over smaller scales than the 4-mi radius evaluated, time spent away from the residence impacting $O_3$ exposure, or similarity in monitor location and representativeness across the urban area (see Figure 4-4). At this time, the effect of exposure error on long-term exposure epidemiologic studies is unclear.

### 4.6.4 Relationship between Personal Exposure and Ambient Concentration

Personal exposure is generally moderately correlated with ambient $O_3$ concentration, although the magnitude of personal exposures is often much lower than the magnitude of ambient concentrations (Section 4.3.3). Moderate correlation between personal exposure and ambient concentration indicates that concentration-based exposure metrics are capturing the variability in exposure needed for epidemiologic studies, particularly for time-series and panel studies. Low personal-ambient correlations reported in the literature are strongly influenced by high detection limits of personal samplers. This results in a high fraction of personal samples below the detection limit that include substantial random variation and are thus unable to provide a signal that could correlate with variations in ambient concentration. Low correlations in situations with a high proportion of samples below the detection limit should not be interpreted as evidence for the lack of a relationship between personal exposure and ambient $O_3$ concentrations. To the extent that true correlations are less than one, epidemiologic effect estimates based on ambient concentration will be biased toward the null (Zeger et al., 2000). High detection limits are less of an issue for ratios of personal exposure to ambient concentration, for which a low personal sample value likely represents an actual low exposure, and thus appropriately leads to a low ratio. Low ratios result from low penetration and high reaction of $O_3$ in indoor microenvironments where people spend most of their time. This results in attenuation of the magnitude of the exposure-based effect estimate or response function relative to the ambient concentration-based response function

(see Equation 4-5), although if the ratio is approximately constant over time, the strength of the statistical association would be similar for concentration- and exposure-based effect stimates (Sheppard, 2005; Sheppard et al., 2005).

In addition to the effect of the correlations and ratios themselves, spatial variation in their values across urban areas also impacts epidemiologic results. In this case, the exposure error is not likely to cause substantial bias, but tends more toward widening confidence intervals, thus reducing the precision of the effect estimate (Zeger et al., 2000). This loss of precision is due to the Berkson-like nature of this spatial variation, in which individual or subpopulation correlations and ratios tend to vary about the overall population mean.

Long-term $O_3$ exposure studies are not available that permit evaluation of the relationship between long-term $O_3$ concentrations and personal or population exposure. The value of short-term exposure data for evaluating long-term concentration-exposure relationships is uncertain. If the longer averaging time (annual, versus daily or hourly) smooths out short-term fluctuations, long-term concentrations may be well-correlated with long-term exposures. However, lower correlation between long-term exposures and ambient concentration could occur if important exposure determinants change over a period of several years, including activity pattern and residential air exchange rate.

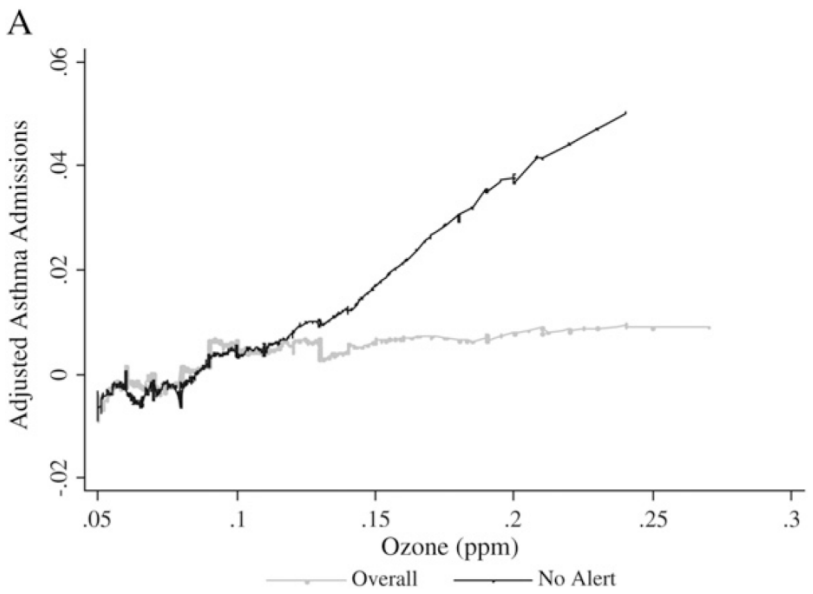
### 4.6.5 Exposure to Copollutants and Ozone Reaction Products

Although indoor $O_3$ concentrations are usually well below ambient concentrations, the same reactions that reduce $O_3$ indoors form particulate and gaseous species, including other oxidants, as summarized in Section 4.3.4.3. Exposures to these reaction products would therefore be expected to be correlated with ambient $O_3$ concentrations, although no evidence was identified regarding personal exposures. Such exposure could potentially contribute to health effects observed in epidemiologic studies.

### 4.6.6 Averting Behavior

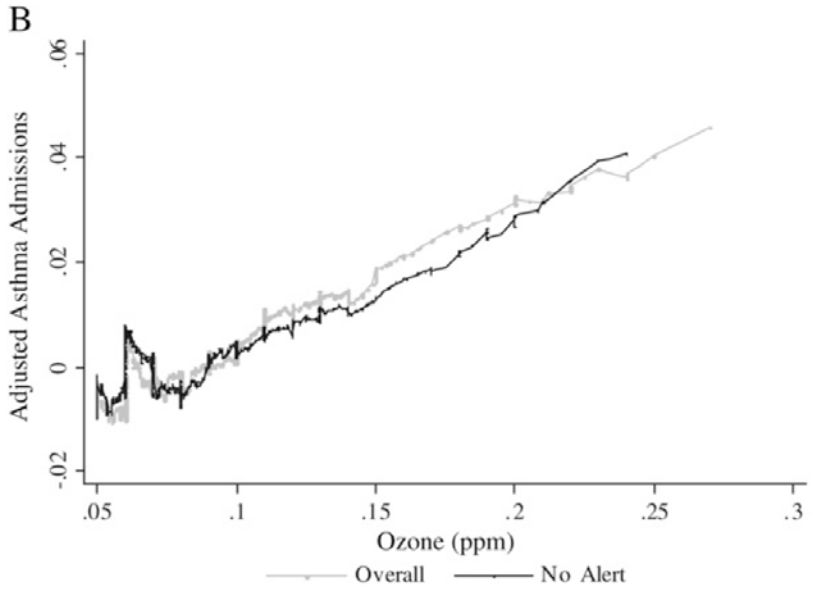
As described in Section 4.4.2, several recent studies indicate that some lifestages and populations alter their behavior on high $O_3$ days to avoid exposure. Such behavioral responses to information about forecasted air quality may introduce systematic measurement error in air pollution exposure, leading to biased estimates of the impact of air pollution on health. For example, studies have hypothesized that variation in time spent outdoors may be a driving factor behind the considerable heterogeneity in $O_3$ mortality impacts across communities (Bell et al., 2004). If averting behavior reduces outdoor $O_3$ exposure, then studies that do not account for averting behavior may produce effect estimates that are biased toward the null (Section 6.2.7.2).

This is supported by an epidemiologic study that examined the association between exposure to ambient $O_3$ concentrations and asthma hospitalizations in Southern California during 1989-1997, which indicates that controlling for avoidance behavior increases the effect estimate for both children and older adults, but not for adults aged 20-64 (Neidell and Kinney, 2010; Neidell, 2009). Figure 4-7 and Figure 4-8, reproduced from Neidell (2009), show covariate-adjusted asthma hospital admissions as a function of daily maximum 1-hour $O_3$ concentration for all days (gray line) and days when no $O_3$ alert was issued (black line). Stage 1 smog alerts were issued by the State of California for days when ambient $O_3$ concentrations were forecast to be above 0.20 ppm; however, the concentration-response functions are based on measured $O_3$ concentrations. For children aged 5-19 (Figure 4-7), hospital admissions were higher on high-$O_3$ days when no alert was issued, especially on days with $O_3$ concentrations above 0.15 ppm (150 ppb). The concentration-response curves for all days and days with no alert diverge at measured $O_3$ concentrations between 0.10 and 0.15 ppm because smog alerts begin to be issued more frequently in this range. This suggests that in the absence of information that would enable averting behavior, children experience higher $O_3$ exposure and subsequently a greater number of asthma hospital admissions than on alert days with similar $O_3$ concentrations. The lower rate of admissions observed when alert days were included in the analysis suggests that averting behavior reduced $O_3$ exposure and asthma hospital admissions. In both cases, $O_3$ was found to be associated with asthma hospital admissions, although the strength of the association is underestimated when not accounting for averting behavior. A different result was observed when examining associations for adults aged 20-64 (Figure 4-8), who had similar rates of hospital admissions on non-alert days as on all days. The lack of change for adults aged 20-64, which is primary employment age, may reflect lower response to air quality alerts due to the increased opportunity cost of behavior change. The finding that air quality information reduces the daily asthma hospitalization rate in these populations provides additional support for a link between $O_3$ and health effects.




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**Figure 4-7 Adjusted asthma hospital admissions by age on lagged $O_3$ by alert status, ages 5-19 years old.**




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**Figure 4-8 Adjusted asthma hospital admissions by age on lagged $O_3$ by alert status, ages 20-64 years old.**

### 4.6.7 Exposure Estimation Methods in Epidemiologic Studies

Epidemiologic studies use a variety of methods to assign exposure. Study design, data availability, and research objectives are all important factors for epidemiologists when selecting an exposure assessment method. Common methods for assigning exposure using monitoring data include using a single fixed-site monitor to represent population exposure, averaging concentrations from multiple monitors, and selecting the closest monitor. Investigators may also use statistical adjustment methods, such as trimming extreme values, to prepare the concentration data set. Panel or small-scale cohort studies involving dozens of individuals may use more individualized concentration measurements, such as personal exposures, residential indoor or outdoor measurements, or concentration data from local study-specific monitors. For long-term epidemiologic studies, the lack of personal exposure data or dedicated measurements means that investigators must rely on fixed-site monitoring data. These data may be used directly, averaged across counties or other geographic areas, or used to construct geospatial or regression models to assign concentrations to unmonitored locations. Longer-term averages (months to years) are typically used (e.g., in studies discussed in Section 7.3.1.1). Chapters 6 and 7 describe the exposure assessment methods used in the epidemiologic studies described therein, providing additional detail on studies with innovative or expanded techniques designed to improve exposure assessment and reduce exposure error.

The use of $O_3$ measurements from central ambient monitoring sites is the most common method for assigning exposure in epidemiologic studies. However, fixed-site measurements do not account for the effects of spatial variation in $O_3$ concentration, ambient and non-ambient concentration differences, and varying activity patterns on personal exposures (Brown et al., 2009; Chang et al., 2000; Zeger et al., 2000). Inter-individual variability in exposure error across a population will be minimal when: (1) $O_3$ concentrations are uniform across the region; (2) personal activity patterns are similar across the population; and (3) housing characteristics, such as air exchange rate and indoor reaction rate, are constant over the study area. To the extent that these factors vary by location and population, there will be errors in the magnitude of total exposure based solely on ambient monitoring data.

Modeled concentrations can also be used as exposure surrogates in epidemiologic studies, as discussed in Section 4.5. Geostatistical spatial interpolation techniques, such as IDW and kriging, can provide finer-scale estimates of local concentration over urban areas. A microenvironmental modeling approach simulates exposure using empirical distributions of concentrations in specific microenvironments together with human activity pattern data. The main advantage of the modeling approach is that it can be used to estimate exposures over a wide range of population and scenarios. However, this probabilistic, distribution-based approach is not well-suited to estimate exposures for specific individuals, such as might be needed for

cohort or panel epidemiologic studies. Another main disadvantage of the modeling approach is that the results of modeling exposure assessment must be compared to an independent set of measured exposure levels (Klepeis, 1999). In addition, resource-intensive development of validated and representative model inputs is required, such as human activity patterns, distributions of air exchange rate, and deposition rate. Therefore, modeled exposures are used much less frequently in epidemiologic studies.

## 4.7 Summary and Conclusions

This section will briefly summarize and synthesize the main points of the chapter, with particular attention to the relevance of the material for the interpretation of epidemiologic studies.

Passive badge samplers are the most widely used technique for measuring personal $O_3$ exposure (Section 4.3.1). The detection limit of the badges for a 24-hour sampling period is approximately 5-10 ppb, with lower detection limits at longer sampling durations. In low-concentration conditions this may result in an appreciable fraction of 24-hour samples being below the detection limit. The use of more sensitive portable active monitors, including some that have recently become available, may help overcome this issue and improve personal monitoring in the future.

Since there are relatively few indoor sources of $O_3$, indoor $O_3$ concentrations are often substantially lower than outdoor concentrations due to reactions of $O_3$ with indoor surfaces and airborne constituents (Section 4.3.2). Air exchange rate is a key determinant of the I/O ratio, which is generally in the range of 0.1-0.4 (Table 4-1), with some evidence for higher ratios during the $O_3$ season when concentrations are higher.

Personal exposure is moderately correlated with ambient $O_3$ concentration, as indicated by studies reporting correlations generally in the range of 0.3-0.8 (Table 4-2). Hourly concentration correlations are more variable than those averaged over 24 hours or longer, with correlations in outdoor microenvironments (0.7-0.9) much higher than those in residential indoor (0.1) or other indoor (0.3-0.4) microenvironments. Some studies report substantially lower personal-ambient correlations, a result attributable in part to low air exchange rate and $O_3$ concentrations below the sampler detection limit, conditions often encountered during wintertime. Low correlations may also occur for individuals or populations spending substantial time indoors.

The ratio between personal exposure and ambient concentration varies widely depending on activity patterns, housing characteristics, and season, with higher personal-ambient ratios generally observed with increasing time spent outside, higher air exchange rate, and in seasons other than winter (Table 4-3). Personal-ambient ratios are typically 0.1-0.3, although individuals spending substantial time outdoors

(e.g., outdoor workers) may have much higher ratios (0.5-0.9). Low personal-ambient ratios result in attenuation of the magnitude of the exposure-based effect estimate or response function relative to the concentration-based response function, although the statistical association is similar for concentration- and exposure-based effect estimates if the ratio is approximately constant over time.

Personal exposure to other pollutants shows variable association with personal exposure to $O_3$, with differences in copollutant relationships depending on factors such as season, city-specific characteristics, activity pattern, and spatial variability of the copollutant (Section 4.3.4). In near-road and on-road microenvironments, correlations between $O_3$ and traffic-related pollutants are moderately to strongly negative, with the most strongly negative correlations observed for $NO_2$ (-0.8 to -0.9). This is consistent with the chemistry of NO oxidation, in which $O_3$ is consumed to form $NO_2$. The more moderate negative correlations observed for $PM_{2.5}$, $PM_{1.0}$, and VOC may reflect reduced concentrations of $O_3$ in polluted environments due to other scavenging reactions. A similar process occurs indoors, where infiltrated $O_3$ reacts with airborne or surface-associated materials to form secondary compounds, such as formaldehyde. Although such reactions decrease indoor $O_3$ exposure, they result in increasing exposure to other species which may themselves have health effects.

Variations in ambient $O_3$ concentrations occur over multiple spatial and temporal scales. Near roadways, $O_3$ concentrations are reduced due to reaction with NO and other species (Section 4.3.4.2). Over spatial scales of a few kilometers and away from roads, $O_3$ may be somewhat more homogeneous due to its formation as a secondary pollutant, while over scales of tens of kilometers, additional atmospheric processing can result in higher concentrations downwind of an urban area. Although local-scale variability impacts the magnitude of $O_3$ concentrations, $O_3$ formation rates are influenced by factors that vary over larger spatial scales, such as temperature (Section 3.2), suggesting that urban monitors may track one another temporally but miss small-scale variability in magnitude. The resulting uncertainty in exposure contributes to exposure measurement error in epidemiologic studies.

Another factor that may influence epidemiologic results is the tendency for people to avoid $O_3$ exposure by altering their behavior (e.g., reducing time spent outdoors) on high-$O_3$ days. Activity pattern has a substantial effect on ambient $O_3$ exposure, with time spent outdoors contributing to increased exposure (Section 4.4.2). Averting behavior has been predominantly observed among children, older adults, and people with respiratory problems. Such effects are less pronounced in the general population, possibly due to the opportunity cost of behavior modification. Evidence from one recent epidemiologic study indicates increased asthma hospital admissions among children and older adults when $O_3$ alert days were excluded from the analysis (presumably thereby eliminating averting behavior based on high $O_3$ forecasts). The lower rate of admissions observed when alert days were included in the analysis suggests that estimates of health effects based on concentration-response functions which do not account for averting behavior may be biased toward the null.

The range of personal-ambient correlations reported by most studies (0.3-0.8) is similar to that for $NO_2$ (U.S. EPA, 2008c) and somewhat lower than that for $PM_{2.5}$ (U.S. EPA, 2009d). To the extent that relative changes in central-site monitor concentration are associated with relative changes in exposure concentration, this indicates that ambient monitor concentrations are representative of day-to-day changes in average total personal exposure and in personal exposure to ambient $O_3$. The lack of indoor sources of $O_3$, in contrast to $NO_2$ and $PM_{2.5}$, is partly responsible for low indoor-outdoor ratios (generally 0.1-0.4) and low personal-ambient ratios (generally 0.1-0.3), although it contributes to increased personal-ambient correlations. The lack of indoor sources also suggests that fluctuations in ambient $O_3$ may be primarily responsible for changes in personal exposure, even under low-ventilation, low-concentration conditions. Nevertheless, low personal-ambient correlations are a source of exposure error for epidemiologic studies, tending to obscure the presence of potential thresholds, bias effect estimates toward the null, and widen confidence intervals, and this impact may be more pronounced among populations spending substantial time indoors. The impact of this exposure error may tend more toward widening confidence intervals than biasing effect estimates, since epidemiologic studies evaluating the influence of monitor selection indicate that effect estimates are similar across different spatial averaging scales and monitoring sites.

## References


Akinbami, LJ; Lynch, CD; Parker, JD; Woodruff, TJ. (2010). The association between childhood asthma prevalence and monitored air pollutants in metropolitan areas, United States, 2001-2004. Environ Res 110: 294-301. http://dx.doi.org/10.1016/j.envres.2010.01.001

Anderson, GB; Bell, ML. (2010). Does one size fit all? The suitability of standard ozone exposure metric conversion ratios and implications for epidemiology. J Expo Sci Environ Epidemiol 20: 2-11. http://dx.doi.org/10.1038/jes.2008.69

Anderson, SE; Wells, JR; Fedorowicz, A; Butterworth, LF; Meade, BJ; Munson, AE. (2007). Evaluation of the contact and respiratory sensitization potential of volatile organic compounds generated by simulated indoor air chemistry. Toxicol Sci 97: 355-363. http://dx.doi.org/10.1093/toxsci/kfm043

Aoki, T; Tanabe, S. (2007). Generation of sub-micron particles and secondary pollutants from building materials by ozone reaction. Atmos Environ 41: 3139-3150. http://dx.doi.org/10.1016/j.atmosenv.2006.07.053

ASHRAE (American Society of Heating, Refrigerating and Air-Conditioning Engineers). (2009). The 2009 ASHRAE handbook: Fundamentals. Atlanta, GA: American Society of Heating, Refrigerating and Air-Conditioning Engineers Inc. http://www.ashrae.org/publications/page/2283

Avol, EL; Navidi, WC; Colome, SD. (1998a). Modeling ozone levels in and around southern California homes. Environ Sci Technol 32: 463-468.

Beckerman, B; Jerrett, M; Brook, JR; Verma, DK; Arain, MA; Finkelstein, MM. (2008). Correlation of nitrogen dioxide with other traffic pollutants near a major expressway. Atmos Environ 42: 275-290. http://dx.doi.org/10.1016/j.atmosenv.2007.09.042

Beelen, R; Hoek, G; Pebesma, E; Vienneau, D; de Hoogh, K; Briggs, DJ. (2009). Mapping of background air pollution at a fine spatial scale across the European Union. Sci Total Environ 407: 1852-1867. http://dx.doi.org/10.1016/j.scitotenv.2008.11.048

Bekö, G; Clausen, G; Weschler, CJ. (2007). Further studies of oxidation processes on filter surfaces: Evidence for oxidation products and the influence of time in service. Atmos Environ 41: 5202-5212. http://dx.doi.org/10.1016/j.atmosenv.2006.07.063

Bell, ML. (2006). The use of ambient air quality modeling to estimate individual and population exposure for human health research: A case study of ozone in the Northern Georgia region of the United States. Environ Int 32: 586-593.

Bell, ML; McDermott, A; Zeger, SL; Samet, JM; Dominici, F. (2004). Ozone and short-term mortality in 95 US urban communities, 1987-2000. JAMA 292: 2372-2378. http://dx.doi.org/10.1001/jama.292.19.2372

Blanken, PD; Dillon, J; Wismann, G. (2001). The impact of an air quality advisory program on voluntary mobile source air pollution reduction. Atmos Environ 35: 2417-2421.

Blondeau, P; Iordache, V; Poupard, O; Genin, D; Allard, F. (2005). Relationship between outdoor and indoor air quality in eight French schools. Indoor Air 15: 2-12.

Brauer, M; Brook, JR. (1997). Ozone personal exposures and health effects for selected groups residing in the Fraser Valley. Atmos Environ 31: 2113-2121.

Brauer, M; Brumm, J; Vedal, S; Petkau, AJ. (2002). Exposure misclassification and threshold concentrations in time series analyses of air pollution health effects. Risk Anal 22: 1183-1193.

Brauer, M; Lencar, C; Tamburic, L; Koehoorn, M; Demers, P; Karr, C. (2008). A cohort study of traffic-related air pollution impacts on birth outcomes. Environ Health Perspect 116: 680-686. http://dx.doi.org/10.1289/ehp.10952

Bravo, MA; Bell, ML. (2011). Spatial heterogeneity of PM10 and O3 in Sao Paulo, Brazil, and implications for human health studies. J Air Waste Manag Assoc 61: 69-77.

Breen, MS; Breen, M; Williams, RW; Schultz, BD. (2010). Predicting residential air exchange rates from questionnaires and meteorology: Model evaluation in central North Carolina. Environ Sci Technol 44: 9349-9356. http://dx.doi.org/10.1021/es101800k

Bresnahan, BW; Dickie, M; Gerking, S. (1997). Averting behavior and urban air pollution. Land Econ 73: 34-57.

Briggs, DJ; Collins, S; Elliott, P; Fischer, P; Kingham, S; Lebret, E; Pryl, K; Van Reeuwijk, H; Smallbone, K; Van Der Veen, A. (1997). Mapping urban air pollution using GIS: A regression-based approach. Int J Geogr Inform Sci 11: 699-718.

Brown, K; Sarnat, J; Suh, H; Coull, B; Koutrakis, P. (2009). Factors influencing relationships between personal and ambient concentrations of gaseous and particulate pollutants. Sci Total Environ 407: 3754-3765. http://dx.doi.org/10.1016/j.scitotenv.2009.02.016

Burke, JM; Zufall, MJ; Ozkaynak, H. (2001). A population exposure model for particulate matter: Case study results for PM2.5 in Philadelphia, PA. J Expo Sci Environ Epidemiol 11: 470-489.

Chan, WR; Nazaroff, WW; Price, PN; Sohn, MD; Gadgil, AJ. (2005b). Analyzing a database of residential air leakage in the United States. Atmos Environ 39: 3445-3455.

Chang, LT; Koutrakis, P; Catalano, PJ; Suh, HH. (2000). Hourly personal exposures to fine particles and gaseous pollutants--Results from Baltimore, Maryland. J Air Waste Manag Assoc 50: 1223-1235.

Chen, X; Hopke, PK; Carter, WP. (2011). Secondary organic aerosol from ozonolysis of biogenic volatile organic compounds: Chamber studies of particle and reactive oxygen species formation. Environ Sci Technol 45: 276-282. http://dx.doi.org/10.1021/es102166c

Christakos, G; Vyas, VM. (1998a). A composite space/time approach to studying ozone distribution over eastern United States. Atmos Environ 32: 2845-2857. http://dx.doi.org/10.1016/S1352-2310(98)00407-5

Christakos, G; Vyas, VM. (1998b). A novel method for studying population health impacts of spatiotemporal ozone distribution. Soc Sci Med 47: 1051-1066.

Darrow, LA; Klein, M; Sarnat, JA; Mulholland, JA; Strickland, MJ; Sarnat, SE; Russell, AG; Tolbert, PE. (2011a). The use of alternative pollutant metrics in time-series studies of ambient air pollution and respiratory emergency department visits. J Expo Sci Environ Epidemiol 21: 10-19. http://dx.doi.org/10.1038/jes.2009.49

Darrow, LA; Klein, M; Strickland, MJ; Mulholland, JA; Tolbert, PE. (2011b). Ambient air pollution and birth weight in full-term infants in Atlanta, 1994-2004. Environ Health Perspect 119: 731-737. http://dx.doi.org/10.1289/ehp.1002785

Delfino, RJ; Coate, BD; Zeiger, RS; Seltzer, JM; Street, DH; Koutrakis, P. (1996). Daily asthma severity in relation to personal ozone exposure and outdoor fungal spores. Am J Respir Crit Care Med 154: 633-641.

Gauderman, WJ; Avol, E; Gilliland, F; Vora, H; Thomas, D; Berhane, K; McConnell, R; Kuenzli, N; Lurmann, F; Rappaport, E; Margolis, H; Bates, D; Peters, J. (2004). The effect of air pollution on lung development from 10 to 18 years of age. N Engl J Med 351: 1057-1067.

Georgopoulos, PG; Purushothaman, V; Chiou, R. (1997). Comparative evaluation of methods for estimating potential human exposure to ozone: Photochemical modeling and ambient monitoring. J Expo Sci Environ Epidemiol 7: 191-215.

Georgopoulos, PG; Wang, SW; Vyas, VM; Sun, Q; Burke, J; Vedantham, R; McCurdy, T; Ozkaynak, H. (2005). A source-to-dose assessment of population exposures to fine PM and ozone in Philadelphia, PA, during a summer 1999 episode. J Expo Sci Environ Epidemiol 15: 439-457.

Geyh, AS; Roberts, PT; Lurmann, FW; Schoell, BM; Avol, EL. (1999). Initial field evaluation of the Harvard active ozone sampler for personal ozone monitoring. J Expo Sci Environ Epidemiol 9: 143-149.

Geyh, AS; Wolfson, JM; Koutrakis, P; Mulik, JD; Avol, EL. (1997). Development and evaluation of a small active ozone sampler. Environ Sci Technol 31: 2326-2330.

Geyh, AS; Xue, J; Ozkaynak, H; Spengler, JD. (2000). The Harvard Southern California chronic ozone exposure study: Assessing ozone exposure of grade-school-age children in two southern California communities. Environ Health Perspect 108: 265-270.

Gilliland, F; Avol, E; Kinney, P; Jerrett, M; Dvonch, T; Lurmann, F; Buckley, T; Breysse, P; Keeler, G; de Villiers, T; Mcconnell, R. (2005). Air pollution exposure assessment for epidemiologic studies of pregnant women and children: Lessons learned from the Centers for Children's Environmental Health and Disease Prevention Research. Environ Health Perspect 113: 1447-1454. http://dx.doi.org/10.1289/ehp.7673

Glen, G; Smith, L; Isaacs, K; Mccurdy, T; Langstaff, J. (2008). A new method of longitudinal diary assembly for human exposure modeling. J Expo Sci Environ Epidemiol 18: 299-311. http://dx.doi.org/10.1038/sj.jes.7500595

Goldman, GT; Mulholland, JA; Russell, AG; Strickland, MJ; Klein, M; Waller, LA; Tolbert, PE. (2011). Impact of exposure measurement error in air pollution epidemiology: Effect of error type in time-series studies. Environ Health Global Access Sci Source 10: 61. http://dx.doi.org/10.1186/1476-069X-10-61

Grosjean, D; Hisham, MWM. (1992). A passive sampler for atmospheric ozone. J Air Waste Manag Assoc 42: 169-173.

Guay, M; Stieb, DM; Smith-Doiron, M. (2011). Assessment of long-term exposure to air pollution in a longitudinal national health survey. J Expo Sci Environ Epidemiol 21: 337-342. http://dx.doi.org/10.1038/jes.2010.37

Henry, GT; Gordon, CS. (2003). Driving less for better air: Impacts of a public information campaign. J Policy Anal Manage 22: 45-63. http://dx.doi.org/10.1002/pam.10095

Héroux, ME; Clark, N; Van Ryswyk, K; Mallick, R; Gilbert, NL; Harrison, I; Rispler, K; Wang, D; Anastassopoulos, A; Guay, M; MacNeill, M; Wheeler, AJ. (2010). Predictors of indoor air concentrations in smoking and non-smoking residences. Int J Environ Res Public Health 7: 3080-3099. http://dx.doi.org/10.3390/ijerph7083080

Hoek, G; Beelen, R; de Hoogh, K; Vienneau, D; Gulliver, J; Fischer, P; Briggs, D. (2008). A review of land-use regression models to assess spatial variation of outdoor air pollution [Review]. Atmos Environ 42: 7561-7578.

Hyttinen, M; Pasanen, P; Kalliokoski, P. (2006). Removal of ozone on clean, dusty and sooty supply air filters. Atmos Environ 40: 315-325.

Jalaludin, B; Mannes, T; Morgan, G; Lincoln, D; Sheppeard, V; Corbett, S. (2007). Impact of ambient air pollution on gestational age is modified by season in Sydney, Australia. Environ Health 6: 16. http://dx.doi.org/10.1186/1476-069X-6-16

Jerrett, M; Burnett, RT; Pope, CA, III; Ito, K; Thurston, G; Krewski, D; Shi, Y; Calle, E; Thun, M. (2009). Long-term ozone exposure and mortality. N Engl J Med 360: 1085-1095. http://dx.doi.org/10.1056/NEJMoa0803894

Kanno, S; Yanagisawa, Y. (1992). Passive ozone/oxidant sampler with coulometric determination using iodine/nylon-6 charge-transfer complex. Environ Sci Technol 26: 744-749. http://dx.doi.org/10.1021/es00028a012

Karner, AA; Eisinger, DS; Niemeier, DA. (2010). Near-roadway air quality: Synthesizing the findings from real-world data. Environ Sci Technol 44: 5334-5344. http://dx.doi.org/10.1021/es100008x

Klepeis, NE. (1999). An introduction to the indirect exposure assessment approach: Modeling human exposure using microenvironmental measurements and the recent National Human Activity Pattern Survey. Environ Health Perspect 107: 365-374.

Klepeis, NE; Nelson, WC; Ott, WR; Robinson, JP; Tsang, AM; Switzer, P; Behar, JV; Hern, SC; Engelmann, WH. (2001). The National Human Activity Pattern Survey (NHAPS): A resource for assessing exposure to environmental pollutants. J Expo Anal Environ Epidemiol 11: 231-252.

Klepeis, NE; Tsang, AM; Behar, JV. (1996). Analysis of the national human activity pattern survey (NHAPS) respondents from a standpoint of exposure assessment [EPA Report]. (EPA/600/R-96/074). Washington, DC: U.S. Environmental Protection Agency.

Koutrakis, P; Suh, HH; Sarnat, JA; Brown, KW; Coull, BA; Schwartz, J. (2005). Characterization of particulate and gas exposures of sensitive subpopulations living in Baltimore and Boston (pp. 1-65; discussion 67-75). (131). Boston, MA: Health Effects Institute. http://pubs.healtheffects.org/view.php?id=91

Koutrakis, P; Wolfson, JM; Bunyaviroch, A; Froehlich, SE; Hirano, K; Mulik, JD. (1993). Measurement of ambient ozone using a nitrite-coated filter. Anal Chem 65: 209-214.

Langstaff, JE. (2007). Analysis of uncertainty in ozone population exposure modeling [technical memorandum]. Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Air Quality Planning and Standards.

Lee, K; Parkhurst, WJ; Xue, J; Ozkaynak, H; Neuberg, D; Spengler, JD. (2004a). Outdoor/indoor/personal ozone exposures of children in Nashville, Tennessee. J Air Waste Manag Assoc 54: 352-359.

Leech, JA; Nelson, WC; Burnett, RT; Aaron, S; Raizenne, ME. (2002). It's about time: A comparison of Canadian and American time-activity patterns. J Expo Anal Environ Epidemiol 12: 427-432. http://dx.doi.org/10.1038/sj.jea.7500244

Liard, R; Zureik, M; Le Moullec, Y; Soussan, D; Glorian, M; Grimfeld, A; Neukirch, F. (1999). Use of personal passive samplers for measurement of NO2, NO, and O3 levels in panel studies. Environ Res 81: 339-348.

Linn, WS; Shamoo, DA; Anderson, KR; Peng, RC; Avol, EL; Hackney, JD; Gong, H, Jr. (1996). Short-term air pollution exposures and responses in Los Angeles area schoolchildren. J Expo Sci Environ Epidemiol 6: 449-472.

Liu, LJS; Koutrakis, P; Leech, J; Broder, I. (1995). Assessment of ozone exposures in the greater metropolitan Toronto area. J Air Waste Manag Assoc 45: 223-234.

López-Aparicio, S; Smolík, J; Mašková, L; Součková, M; Grøntoft, T; Ondráčková, L; Stankiewicz, J. (2011). Relationship of indoor and outdoor air pollutants in a naturally ventilated historical building envelope. Build Environ 46: 1460-1468. http://dx.doi.org/10.1016/j.buildenv.2011.01.013

Mansfield, CA; Johnson, FR; Van Houtven, GL. (2006). The missing piece: Valuing averting behavior for children's ozone exposures. Resource Energ Econ 28: 215-228. http://dx.doi.org/10.1016/j.reseneeco.2006.02.002

Marshall, JD; Nethery, E; Brauer, M. (2008). Within-urban variability in ambient air pollution: Comparison of estimation methods. Atmos Environ 42: 1359-1369. http://dx.doi.org/10.1016/j.atmosenv.2007.08.012

McConnell, R; Berhane, K; Yao, L; Lurmann, FW; Avol, E; Peters, JM. (2006). Predicting residential ozone deficits from nearby traffic. Sci Total Environ 363: 166-174. http://dx.doi.org/10.1016/j.scitotenv.2005.06.028

McCurdy, T; Glen, G; Smith, L; Lakkadi, Y. (2000). The National Exposure Research Laboratory's consolidated human activity database. J Expo Sci Environ Epidemiol 10: 566-578.

McDermott, M; Srivastava, R; Croskell, S. (2006). Awareness of and compliance with air pollution advisories: A comparison of parents of asthmatics with other parents. J Asthma 43: 235-239. http://dx.doi.org/10.1080/02770900600567114

McDonnell, WF; Abbey, DE; Nishino, N; Lebowitz, MD. (1999a). Long-term ambient ozone concentration and the incidence of asthma in nonsmoking adults: the Ahsmog study. Environ Res 80: 110-121.

Mortimer, KM; Neas, LM; Dockery, DW; Redline, S; Tager, IB. (2002). The effect of air pollution on inner-city children with asthma. Eur Respir J 19: 699-705. http://dx.doi.org/10.1183/09031936.02.00247102

Neidell, M. (2009). Information, avoidance behavior, and health: The effect of ozone on asthma hospitalizations. Journal of Human Resources 44: 450-478.

Neidell, M. (2010). Air quality warnings and outdoor activities: Evidence from Southern California using a regression discontinuity design. J Epidemiol Community Health 64: 921-926. http://dx.doi.org/10.1136/jech.2008.081489

Neidell, M; Kinney, PL. (2010). Estimates of the association between ozone and asthma hospitalizations that account for behavioral responses to air quality information. Environ Sci Pol 13: 97-103. http://dx.doi.org/10.1016/j.envsci.2009.12.006

O'Neill, MS; Ramirez-Aguilar, M; Meneses-Gonzalez, F; Hernandez-Avila, M; Geyh, AS; Sienra-Monge, JJ; Romieu, I. (2003). Ozone exposure among Mexico City outdoor workers. J Air Waste Manag Assoc 53: 339-346.

Ogawa & Co (Ogawa & Company). (2007). Ambient air passive sampler for NO-NO2, NOx, SO2, O3, NH3. Pompano Beach, FL: Ogawa & Company USA, Inc. http://www.ogawausa.com/passive.html

Ramírez-Aguilar, M; Barraza-Villarreal, A; Moreno-Macías, H; Winer, AM; Cicero-Fernández, P; Vélez-Márquez, MG; Cortez-Lugo, M; Sienra-Monge, JJ; Romieu, I. (2008). Assessment of personal exposure to ozone in asthmatic children residing in Mexico City. Salud Publica Mex 50: 67-75.

Reiss, R; Ryan, PB; Koutrakis, P; Tibbetts, SJ. (1995a). Ozone reactive chemistry on interior latex paint. Environ Sci Technol 29: 1906-1912.

Reiss, R; Ryan, PB; Tibbetts, SJ; Koutrakis, P. (1995b). Measurement of organic acids, aldehydes, and ketones in residential environments and their relation to ozone. J Air Waste Manag Assoc 45: 811-822.

Riediker, M; Williams, R; Devlin, R; Griggs, T; Bromberg, P. (2003). Exposure to particulate matter, volatile organic compounds, and other air pollutants inside patrol cars. Environ Sci Technol 37: 2084-2093. http://dx.doi.org/10.1021/es026264y

Romieu, I; Lugo, MC; Colome, S; Garcia, AM; Avila, MH; Geyh, A; Velasco, SR; Rendon, EP. (1998a). Evaluation of indoor ozone concentration and predictors of indoor-outdoor ratio in Mexico City. J Air Waste Manag Assoc 48: 327-335.

Ryan, PH; LeMasters, GK. (2007). A review of land-use regression models for characterizing intraurban air pollution exposure [Review]. Inhal Toxicol 19: 127.

Sarnat, JA; Brown, KW; Schwartz, J; Coull, BA; Koutrakis, P. (2005). Ambient gas concentrations and personal particulate matter exposures: Implications for studying the health effects of particles. Epidemiology 16: 385-395. http://dx.doi.org/10.1097/01.ede.0000155505.04775.33

Sarnat, JA; Koutrakis, P; Suh, HH. (2000). Assessing the relationship between personal particulate and gaseous exposures of senior citizens living in Baltimore, MD. J Air Waste Manag Assoc 50: 1184-1198.

Sarnat, JA; Schwartz, J; Catalano, PJ; Suh, HH. (2001). Gaseous pollutants in particulate matter epidemiology: Confounders or surrogates? Environ Health Perspect 109: 1053-1061.

Sarnat, SE; Coull, BA; Schwartz, J; Gold, DR; Suh, HH. (2006a). Factors affecting the association between ambient concentrations and personal exposures to particles and gases. Environ Health Perspect 114: 649-654.

Sarnat, SE; Klein, M; Sarnat, JA; Flanders, WD; Waller, LA; Mulholland, JA; Russell, AG; Tolbert, PE. (2010). An examination of exposure measurement error from air pollutant spatial variability in time-series studies. J Expo Sci Environ Epidemiol 20: 135-146. http://dx.doi.org/10.1038/jes.2009.10

Semenza, JC; Wilson, DJ; Parra, J; Bontempo, BD; Hart, M; Sailor, DJ; George, LA. (2008). Public perception and behavior change in relationship to hot weather and air pollution. Environ Res 107: 401-411. http://dx.doi.org/10.1016/j.envres.2008.03.005

Sheppard, L. (2005). Acute air pollution effects: consequences of exposure distribution and measurements. J Toxicol Environ Health A 68: 1127-1135.

Sheppard, L; Slaughter, JC; Schildcrout, J; L-JS, L; Lumley, T. (2005). Exposure and measurement contributions to estimates of acute air pollution effects. J Expo Sci Environ Epidemiol 15: 366-376.

Sherman, M; McWilliams, J. (2007). Air leakage of U.S. homes: Model prediction. (LBNL-62078). Berkeley, CA: Lawrence Berkeley National Laboratory. http://epb.lbl.gov/publications/lbnl-62078.pdf

Sherman, MH; Grimsrud, DT. (1980). Infiltration-pressurization correlation: Simplified physical modeling. In ASHRAE Transactions. Berkeley, CA: Lawrence Berkeley Laboratory. http://eetd.lbl.gov/ie/pdf/LBL-10163.pdf

Shu, S; Morrison, GC. (2011). Surface reaction rate and probability of ozone and alpha-terpineol on glass, polyvinyl chloride, and latex paint surfaces. Environ Sci Technol 45: 4285-4292. http://dx.doi.org/10.1021/es200194e

Suh, HH; Zanobetti, A. (2010). Exposure error masks the relationship between traffic-related air pollution and heart rate variability [Erratum]. J Occup Environ Med 52: 1138. http://dx.doi.org/10.1097/JOM.0b013e3181fd2632

U.S. EPA (U.S. Environmental Protection Agency). (2006c). Estimating contributions of outdoor fine particles to indoor concentrations and personal exposures: Effects of household characteristics and personal activities [EPA Report]. (EPA/600/R-06/023). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (2008c). Integrated science assessment for oxides of nitrogen: Health criteria [EPA Report]. (EPA/600/R-08/071). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=194645

U.S. EPA (U.S. Environmental Protection Agency). (2009a). Consolidated Human Activity Database. Available online at http://www.epa.gov/chadnet1/ (accessed August 27, 2009).

U.S. EPA (U.S. Environmental Protection Agency). (2009b). Human exposure modeling: Air pollutants exposure model (APEX/TRIM.Expo Inhalation). Available online at http://www.epa.gov/ttn/fera/human_apex.html (accessed June 13, 2012).

U.S. EPA (U.S. Environmental Protection Agency). (2009d). Integrated science assessment for particulate matter [EPA Report]. (EPA/600/R-08/139F). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=216546

U.S. EPA (U.S. Environmental Protection Agency). (2011a). AirNow. Available online at http://www.airnow.gov/ (accessed January 28, 2011).

U.S. EPA (U.S. Environmental Protection Agency). (2011b). Exposure factors handbook 2011 edition (final) [EPA Report]. (EPA/600/R-09/052F). http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=236252

U.S. EPA (U.S. Environmental Protection Agency). (2011c). Exposure model for individuals. Available online at http://www.epa.gov/heasd/products/emi/emi.html (accessed June 11, 2012).

Wen, XJ; Balluz, L; Mokdad, A. (2009). Association between media alerts of air quality index and change of outdoor activity among adult asthma in six states, BRFSS, 2005. J Community Health 34: 40-46. http://dx.doi.org/10.1007/s10900-008-9126-4

Weschler, CJ; Shields, HC. (1997). Potential reactions among indoor pollutants. Atmos Environ 31: 3487-3495.

White, IR; Martin, D; Muñoz, MP; Petersson, FK; Henshaw, SJ; Nickless, G; Lloyd-Jones, GC; Clemitshaw, KC; Shallcross, DE. (2010). Use of reactive tracers to determine ambient OH radical concentrations: Application within the indoor environment. Environ Sci Technol 44: 6269-6274. http://dx.doi.org/10.1021/es901699a

Williams, R; Rea, A; Vette, A; Croghan, C; Whitaker, D; Stevens, C; Mcdow, S; Fortmann, R; Sheldon, L; Wilson, H; Thornburg, J; Phillips, M; Lawless, P; Rodes, C; Daughtrey, H. (2009b). The design and field implementation of the Detroit exposure and aerosol research study. J Expo Sci Environ Epidemiol 19: 643-659. http://dx.doi.org/10.1038/jes.2008.61

Wilson, KL; Birks, JW. (2006). Mechanism and elimination of a water vapor interference in the measurement of ozone by UV absorbance. Environ Sci Technol 40: 6361-6367. http://dx.doi.org/10.1021/es052590c

Wilson, WE; Mage, DT; Grant, LD. (2000). Estimating separately personal exposure to ambient and nonambient particulate matter for epidemiology and risk assessment: Why and how. J Air Waste Manag Assoc 50: 1167-1183.

Wilson, WE; Suh, HH. (1997). Fine particles and coarse particles: Concentration relationships relevant to epidemiologic studies. J Air Waste Manag Assoc 47: 1238-1249.

Xue, J; Liu, SV; Ozkaynak, H; Spengler, JD. (2005). Parameter evaluation and model validation of ozone exposure assessment using Harvard Southern California Chronic Ozone Exposure Study data. J Air Waste Manag Assoc 55: 1508-1515.

Xue, J; McCurdy, T; Spengler, J; Ozkaynak, H. (2004). Understanding variability in time spent in selected locations for 7-12-year old children. J Expo Anal Environ Epidemiol 14: 222-233. http://dx.doi.org/10.1038/sj.jea.7500319

Zartarian, VG; Schultz, BD. (2010). The EPA's human exposure research program for assessing cumulative risk in communities. J Expo Sci Environ Epidemiol 20: 351-358. http://dx.doi.org/10.1038/jes.2009.20

Zauli Sajani, S; Hänninen, O; Marchesi, S; Lauriola, P. (2011). Comparison of different exposure settings in a case-crossover study on air pollution and daily mortality: Counterintuitive results. J Expo Sci Environ Epidemiol 21: 385-394. http://dx.doi.org/10.1038/jes.2010.27

Zeger, SL; Thomas, D; Dominici, F; Samet, JM; Schwartz, J; Dockery, D; Cohen, A. (2000). Exposure measurement error in time-series studies of air pollution: Concepts and consequences. Environ Health Perspect 108: 419-426.

Zidek, JV; Shaddick, G; Meloche, J; Chatfield, C; White, R. (2007). A framework for predicting personal exposures to environmental hazards. Environ Ecol Stat 14: 411-431.

# 5 DOSIMETRY, MODE OF ACTION, AND SPECIES HOMOLOGY

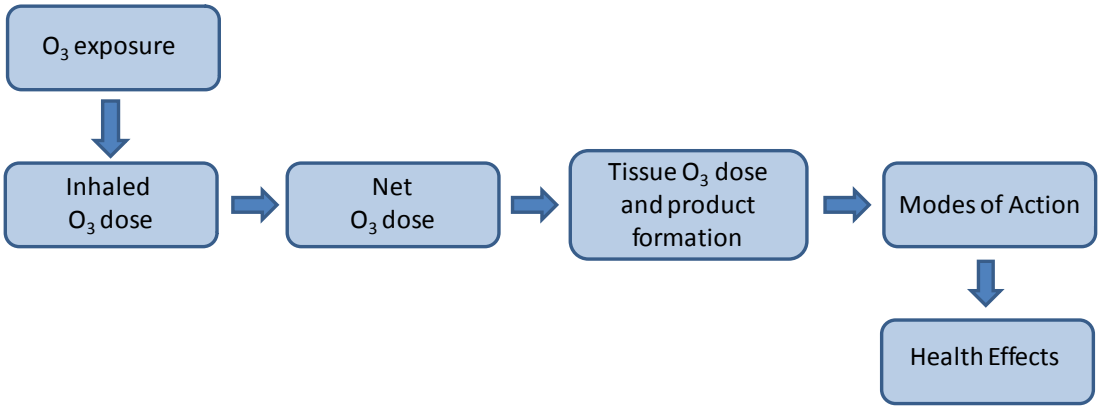
## 5.1 Introduction

This chapter has three main purposes. The first is to describe the principles that underlie the dosimetry of $O_3$ and to discuss factors that influence it. The second is to describe the modes of action leading to the health effects that will be presented in Chapters 6 and 7. The third is to discuss the homology of responses in animals and humans exposed to $O_3$ and the interspecies differences that may affect these responses. This chapter is not intended to be a comprehensive overview, but rather, it updates the basic concepts derived from $O_3$ literature presented in previous documents (U.S. EPA, 2006b, 1996a) and introduces the recent relevant literature.

In Section 5.2, particular attention is given to dosimetric factors influencing individual risk of developing effects from $O_3$ exposure. As there have been few $O_3$ dosimetry studies published since the last AQCD, the reader is referred to previous documents (U.S. EPA, 2006b, 1996a) for more detailed discussion of the past literature. Evaluation of the progress in the interpretation of past dosimetry studies, as well as studies published since 2005, in the areas of uptake, reactions, and models for $O_3$ dosimetry, is discussed.

Section 5.3 highlights findings of studies published since the 2006 $O_3$ AQCD, which provide insight into the biological pathways by which $O_3$ exerts its actions. Since common mechanisms lead to health effects from both short- and long-term exposure to $O_3$, these pathways are discussed in Chapter 5 rather than in later chapters. The related sections of Chapters 6 and 7 are indicated. Earlier studies that represent the current state of the science are also discussed. Studies conducted at more environmentally-relevant concentrations of $O_3$ are of greater interest, since mechanisms responsible for effects at low $O_3$ concentrations may not be identical to those occurring at high $O_3$ concentrations. Some studies at higher concentrations are included if they were early demonstrations of key mechanisms or if they are recent demonstrations of potentially important new mechanisms. The topics of dosimetry and mode of action are bridged by reactions of $O_3$ with components of the extracellular lining fluid (ELF), which play a role in both $O_3$ uptake and biological responses (Figure 5-1).

In addition, this chapter discusses interindividual variability in responses, and issues related to species comparison of doses and responses (Section 5.4 and Section 5.5). These topics are included in this chapter because they are influenced by both dosimetric and mechanistic considerations.




Note: Ozone transport follows a path from exposure concentration, to inhaled dose, to net dose, to the local tissue dose. Chapter 5 discusses the concepts of dose and modes of action that result in the health effects discussed in Chapters 6 and 7.

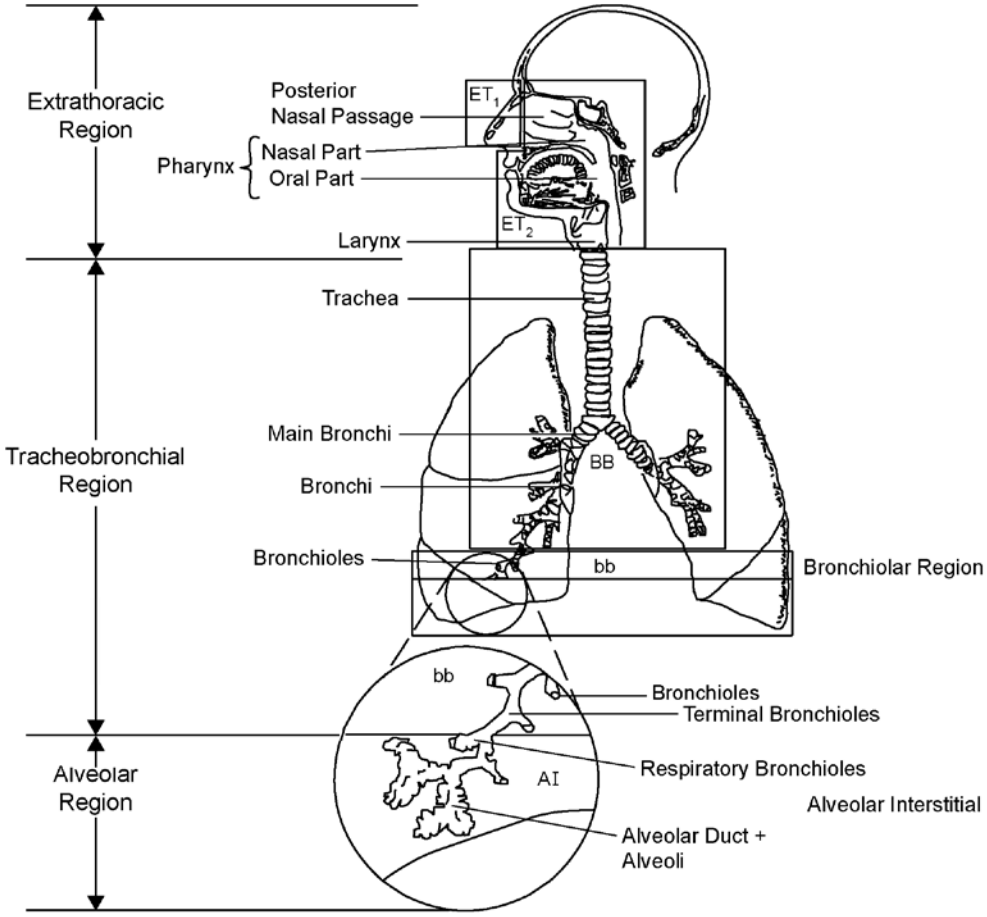
**Figure 5-1 Schematic of the $O_3$ exposure and response pathway.**

## 5.2 Human and Animal Ozone Dosimetry

### 5.2.1 Introduction

Dosimetry refers to the measurement or estimation of the quantity of or rate at which a chemical and/or its reaction products are absorbed and retained at target sites. Figure 5-1 illustrates the transport of $O_3$ or its reaction products from exposure to dose to the development of health effects. Ozone exposure has been defined in Section 4.2 and consists of contact between the human or animal and $O_3$ at a specific concentration for a specified period of time (i.e., exposure = concentration × time). The amount of $O_3$ present in a given volume of air for which animals and individuals are exposed is termed exposure concentration. Ozone exposure will result in some amount (dose) of $O_3$ crossing an exposure surface to enter a target area. The initial measure of dose after $O_3$ enters the respiratory tract (RT) is inhaled dose and is the amount or rate of $O_3$ that crosses the outer RT surface before crossing the ELF and is effectively $C \times t \times \dot{V}_E$, where C is concentration, t is time, and $\dot{V}_E$ is minute ventilation. Ozone may then cross from the gas phase across the ELF interface where net dose may be measured. Net dose is the amount or rate of entry of $O_3$ across the gas/ELF interface. In modeling studies, the dose rate is often expressed as a flux per unit of surface area of a region of respiratory epithelium. Finally, $O_3$ or its reaction products may reach the tissues and tissue dose of $O_3$ can be reported. Tissue dose is the amount of $O_3$ or its reaction products absorbed and available for reacting with tissues and is difficult and rarely measured. In the literature, the exposure concentration and various measures of dose (i.e., net dose and inhaled dose) are often used as surrogates for tissue dose. However, ambient or exposure concentrations are not a true measure of dose so understanding the relationship between ambient

concentrations and tissue dose allows for a greater appreciation of the dose-response from $O_3$ exposure.




Note: Structures are anterior nasal passages, $ET_1$; oral airway and posterior nasal passages, $ET_2$; bronchial airways, BB; bronchioles, bb; and alveolar interstitial, AI.

Source: Based on ICRP (1994).

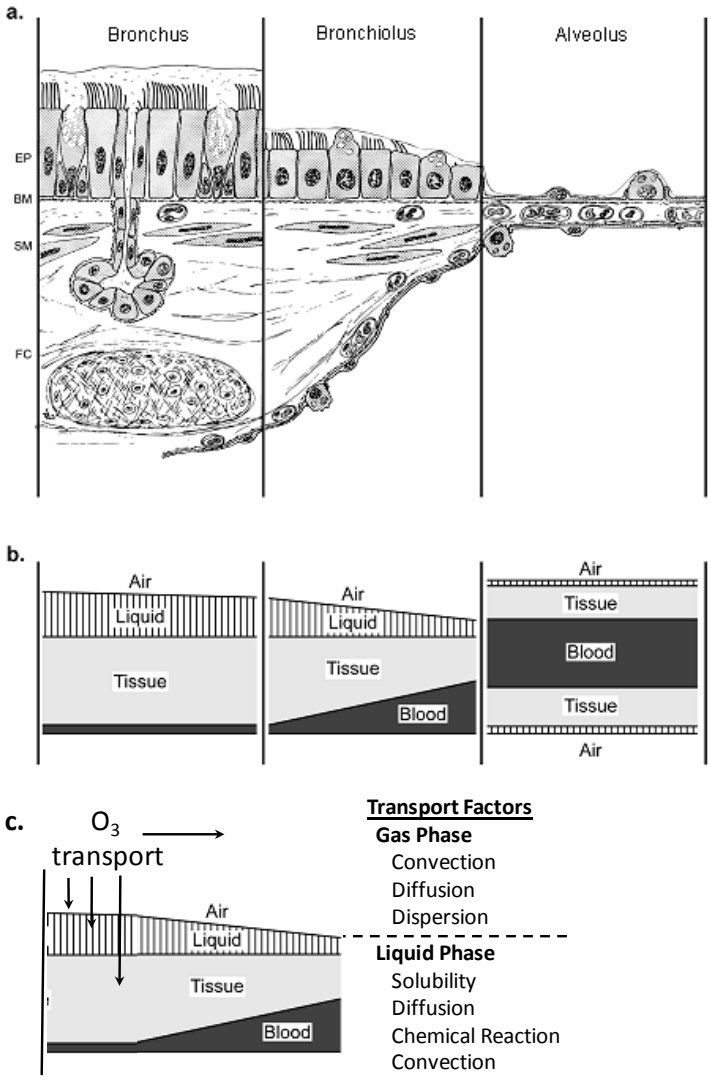
**Figure 5-2 Representation of respiratory tract regions in humans.**

Ozone is a highly reactive, though poorly water soluble, gas at physiological temperature. The latter feature is believed to be the reason why it is able to penetrate into targets in the lower respiratory tract (LRT). Figure 5-2 presents the basic structure of the human RT. The lung can be divided into three major regions: the extrathoracic (ET) region or upper respiratory tract (URT, from the nose/mouth to the end of the larynx); the tracheobronchial (TB) tree (from trachea to the terminal bronchioles); and the alveolar or pulmonary region (from the respiratory bronchioles to the terminal alveolar sacs). The latter two regions comprise the LRT. Although the structure varies, the illustrated anatomic regions are common to all mammalian

species with the exception of the respiratory bronchioles. Respiratory bronchioles, the transition region between ciliated and fully alveolated airways, are found in humans, dogs, ferrets, cats, and monkeys. Respiratory bronchioles are absent in rats and mice and abbreviated in hamsters, guinea pigs, sheep, and pigs. The branching structure of the ciliated bronchi and bronchioles also differs between species from being a rather symmetric and dichotomous branching network of airways in humans to a more monopodial branching network in other mammals.

Figure 5-3 illustrates the structure of the LRT with progression from the large airways in the TB region to the alveolus in the alveolar region. The fact that $O_3$ is so chemically reactive has suggested to some that its tissue dose at the target sites exists in the form of oxidation products such as aldehydes and peroxides (see Section 5.2.3). Reaction products are formed when $O_3$ interacts with components of the ELF such as lipids and antioxidants. The ELF varies throughout the length of the RT with the nasal airways through the bronchial tree lined with a thicker layer of ELF than the alveolar region (Figure 5-3b). Ozone dose is directly related to the coupled diffusion and chemical reactions occurring in ELF, a process termed "reactive absorption." Thus, the $O_3$ dose depends on both the concentration of $O_3$ as well as the availability of substrates within the ELF.

Ozone dose is affected by complex interactions between a number of other major factors including RT morphology, breathing route, frequency, and volume, physicochemical properties of the gas, physical processes of gas transport, as well as the physical and chemical properties of the ELF and tissue layers (Figure 5-3c). The role of these processes varies throughout the length of the RT and as $O_3$ moves from the gas to liquid compartments of the RT.




Note: (a) Illustrates basic airway anatomy. Structures are epithelial cells, EP; basement membrane, BM; smooth muscle cells, SM; and fibrocartilaginous coat, FC. (b) Illustrates the relative amounts of liquid, tissue, and blood with distal progression. In the bronchi there is a thick surface lining over a relatively thick layer of tissues. With distal progress, the lining diminishes allowing increased access of compounds crossing the air-liquid interface to the tissues and the blood. (c) Presents the factors acting in the gas and liquid phases of $O_3$ transport.

Source: Panel (a) reprinted with permission of McGraw-Hill (Weibel, 1980).

**Figure 5-3 Structure of lower airways with progression from the large airways to the alveolar region.**

Two types of measurements have been used to arrive at the $O_3$ dose to target sites during breathing: (1) measurement of removal of $O_3$ from the air stream (termed "uptake"); and (2) measurement of chemical reactions in tissues or with biomolecules known to be present in tissues (termed "reactants"). The results of the above measurements have been incorporated into mathematical models for the purpose of explaining, predicting, and extrapolating $O_3$ dose in different exposure

scenarios. Few new studies have investigated the uptake of $O_3$ in the RT since the last $O_3$ assessment (U.S. EPA, 2006b). The studies that have been conducted generally agree with the results presented in the past and do not change the dosimetry conclusions of the last document.

### 5.2.2 Ozone Uptake

Past AQCDs provide information on the majority of literature relevant to understanding the state of the science in $O_3$ dosimetry. Measurements of $O_3$ dose have been inferred from simultaneous measurements of airflow and $O_3$ concentration at the airway opening of the nose or mouth (Nodelman and Ultman, 1999; Wiester et al., 1996a) as well as at internal sampling catheters (Gerrity et al., 1995; Gerrity et al., 1988). One method of quantifying $O_3$ dose is to measure the amount of $O_3$ removed from the air stream during breathing (termed "uptake"). The difference in the amount of $O_3$ inhaled and exhaled relative to the amount of inhaled $O_3$ is termed fractional absorption. Uptake efficiency is also reported and refers to the $O_3$ absorbed in a region expressed as a fraction of the total amount of $O_3$ entering the given region. Uptake studies have utilized bolus and continuous $O_3$ breathing techniques as well as modeling to investigate these measures of uptake and the distribution of $O_3$ uptake between the URT and LRT. A number of the studies that have measured the fractional absorption and uptake efficiency of $O_3$ in the human RT, URT, and LRT are presented in Table 5-1. For studies that reported fractional absorption of $O_3$ boluses, the equivalent fractional absorption of a continuous inhalation of $O_3$ was estimated as the sum of the products of the experimental bolus absorption and incremental volume of a bolus into a breath divided by the tidal volume of the breath, or, where available, was taken from Table 1A of Schlesinger et al. (1997).

## Table 5-1 Human respiratory tract uptake efficiency data.

| Reference | Mouth/ Nose[a] | Inspiratory Flow (mL/sec) | $V_T$ (mL) | $f_B$ (bpm)[b] | Uptake Efficiency | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | URT, complete breath | URT, inspiration | LRT, complete breath | Total RT, tidal breath |
| **Continuous Exposure** | | | | | | | | |
| Gerrity et al. (1988) | OR | 509 | 832 | 18 | | 0.40 | 0.91 | |
| | N | 456 | 754 | 18 | | 0.36 | 0.91 | |
| | OR/N | 500 | 800 | 18 | | 0.43 | 0.91 | |
| | OR/N | 350 | 832 | 12 | | 0.41 | 0.93 | |
| | OR/N | 634 | 778 | 24 | | 0.38 | 0.89 | |
| Gerrity et al. (1994) | OR | 1,360 | 1,650 | 25 | | 0.37 | 0.43 | 0.81 |
| | OR | 1,360 | 1,239 | 35 | | 0.41 | 0.36 | 0.78 |
| Gerrity et al. (1995) | OR | 330 | 825 | 12 | | 0.27 | 0.95 | 0.91 |
| Wiester et al. (1996a) | OR | 539 | 631 | 16 | | | | 0.76 |
| | N | 514 | 642 | 16 | | | | 0.73 |
| Rigas et al. (2000) | Face mask | 480 | 1,100 | 27.6 | | | | 0.86 |
| Santiago et al. (2001) | N | 50 | | | | 0.80[c] | | |
| | N | 250 | | | | 0.33 | | |
| **Bolus Exposure** | | | | | | | | |
| Hu et al. (1992) | Mouth-piece | 250 | 500 | | 0.46 | | | 0.88 |
| Kabel et al. (1994) | Mouth-piece | 250 | 500 | | 0.50 | | | 0.88 |
| | Mouth-piece | 250 | 500 | | 0.53 | | | 0.88 |
| | N | 250 | 500 | | 0.78 | | | 0.94 |
| Hu et al. (1994) | Mouth-piece | 150 | 500 | | 0.65 | | | 0.91 |
| | Mouth-piece | 250 | 500 | | 0.51 | | | 0.87 |
| | Mouth-piece | 500 | 500 | | 0.26 | | | 0.82 |
| | Mouth-piece | 750 | 500 | | 0.16 | | | 0.78 |
| | Mouth-piece | 1,000 | 500 | | 0.11 | | | 0.76 |
| Ultman et al. (1994) | Mouth-piece | 250 | 500[d] | 15 | 0.30 | | | |
| | Mouth-piece | 250 | 500 | 15 | 0.47 | | | |
| Bush et al. (1996) | Mouth-piece | 250 | 500 | | 0.51 | | | 0.89 |

| Reference | Mouth/ Nose[a] | Inspiratory Flow (mL/sec) | $V_T$ (mL) | $f_B$ (bpm)[b] | Uptake Efficiency | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | URT, complete breath | URT, inspiration | LRT, complete breath | Total RT, tidal breath |
| Nodelman and Ultman (1999) | Nasal Cannula | 150 | 500 | 18 | 0.90 | | | 0.92 |
| | Nasal Cannula | 1,000 | 500 | 120 | 0.50 | | | 0.84 |
| | Mouth-piece | 150 | 500 | 18 | 0.77 | | | 0.91 |
| | Mouth-piece | 1,000 | 500 | 120 | 0.25 | | | 0.75 |
| Ultman et al. (2004) | OR | 490 | 450[d] | 32.7 | | | | 0.87 |
| | OR | 517 | 574 | 27 | | | | 0.91 |

[a]OR = oral exposure during spontaneous breathing; N = nasal exposure during spontaneous breathing; OR/N = pooled data from oral and nasal exposure; mouthpiece = exposure by mouthpiece.

[b]$f_B$ is either measured or is computed from flows and $V_T$.

[c] $F_{URT}$ from Santiago et al. (2001) represents nasal absorption ($F_{nose}$).

[d]$V_T$ is computed from flow and $f_B$.

### 5.2.2.1 Gas Transport Principles

The three-dimensional transport of $O_3$ in the lumen of an airway is governed by diffusion associated with the Brownian motion of gas molecules and convection that depends on local velocity patterns. Simultaneously, $O_3$ is absorbed from the gas stream into the ELF where it undergoes simultaneous radial diffusion and chemical reaction.

When air flows through an airway, $O_3$ located near the tube center moves faster than $O_3$ near the tube wall where frictional forces retard the flow. This non-uniformity in the radial profile of velocity gives rise to an axial spreading or dispersion of the $O_3$ that operates in parallel with bulk flow and axial diffusion The shape of the velocity profile is affected by the flow direction through bifurcating airway branches (Schroter and Sudlow, 1969). The velocity profile is nearly parabolic during inhalation but quite flat during exhalation. Thus, there tends to be greater axial dispersion during inhalation than during exhalation. Dispersion also depends on the nature of the flow, that is, whether it is laminar (i.e., streamlined) or turbulent (i.e., possessing random velocity fluctuations). Because turbulent flow flattens velocity profiles, it may actually diminish dispersion. In humans, turbulent flow persists only a few generations into the RT. The persistence of turbulence into the RT also varies by species and flow rates. For example, airflow is nonturbulent in the rat nose at any physiologic flow rate but may be highly turbulent in the human nose during exercise (Miller, 1995).

The relative importance of axial convection, diffusion, and dispersion varies among RT regions for a given level of ventilation. In the URT and major bronchi, axial convection and dispersion tend to be the predominant mechanisms. Moving into more distal areas of the RT, the summed cross-sectional area of the airways rapidly increases and linear velocities decrease, leading to a greater role for molecular diffusion. The principal mechanism of gas mixing in the lung periphery is molecular diffusion (Engel, 1985).

Absorption of $O_3$ at the airway wall depends on a concentration boundary layer on the gas side of the airway wall as well as simultaneous radial diffusion and chemical reaction within the ELF (Figure 5-3c) (Miller, 1995). The boundary layer caused by slowly moving gas near the airway wall can be an important component of the radial diffusion resistance to $O_3$ absorption. This diffusive resistance increases with distal penetration into the RT with one study reporting that the gas boundary layer contributes 53% of the overall diffusive resistance in the URT, 78% in the proximal LRT, and 87% in the distal LRT (Hu et al., 1994). The geometry of airway surfaces also affects local $O_3$ absorption. For example, nasal and lung regions receive different $O_3$ exposures or doses (Miller and Kimbell, 1995); and larger surface-to-volume ratio of the smaller airways in women enhances local $O_3$ uptake and reduces the distal penetration volume of $O_3$ into the RT of women relative to men (Ultman et al., 2004).

#### 5.2.2.2 Target Sites for Ozone Dose

A primary uptake site of $O_3$ delivery to the lung epithelium is believed to be the centriacinar region (CAR). The CAR refers to the zone at the junction of the TB airways and the gas exchange region. This area is also termed the proximal alveolar region (PAR) and is defined as the first generation distal to the terminal bronchioles. Contained within the CAR, the respiratory bronchioles were confirmed as the site receiving the greatest $O_3$ dose ($^{18}O$ mass/lung weight) in resting $O_3$ exposed rhesus monkeys, when not considering the nose (Plopper et al., 1998). Furthermore, the greatest cellular injury occurred in the vicinity of the respiratory bronchioles and was dependent on the delivered $O_3$ dose to these tissues (see also Section 5.4.1). However, $^{18}O$ label was detected to a lesser extent in other regions of the TB airway tree, showing that $O_3$ is delivered to these compartments as well, although in a smaller dose. These studies agree with earlier model predictions showing that the tissue $O_3$ dose ($O_3$ flux to liquid-tissue interface) was low in the trachea, increased to a maximum in the terminal bronchioles and the CAR, and then rapidly decreased in the alveolar region (Miller et al., 1985). It was also predicted that the net $O_3$ dose ($O_3$ flux to air-liquid interface) gradually decreased with distal progression from the trachea to the end of the TB region and then rapidly decreased in the alveolar region. Despite the exclusion of the URT and appreciable $O_3$ reactions with ELF constituents after the 16th generation, the results from the model agree with experimental results showing that the greatest $O_3$ tissue dose was received in the CAR (Miller et al., 1985).

Inhomogeneity in the RT structure may affect the dose delivered to this target site. Models have predicted that the farther the PAR is from the trachea, the less the $O_3$ tissue dose to the region. Ultman and Anjilvel (1990) and Overton and Graham (1989) predicted approximately a 50 to 300% greater PAR dose for the shortest path relative to the longest path in humans and rats, respectively. In addition, Mercer et al. (1991) found that both path distance and ventilatory unit size affected dose. The variation of $O_3$ dose among anatomically equivalent ventilatory units was predicted to vary as much as 6-fold, as a function of path length from the trachea. This could have implications in regional damage to the LRT, such that even though the average LRT dose may be at a level where health effects would not be predicted, local regions of the RT may receive considerably higher than average doses and therefore be at greater risk of effects.

Since the URT is the first part of the RT to be exposed to $O_3$, the nasal membranes are another target site at risk of injury from inhaled $O_3$. Injury to the nasal epithelium has been shown to be site-specific (see Section 5.3.7) and studies have found that the location of reactive gas-induced nasal lesions may be attributable to the local dose of gas reaching that area (Garcia et al., 2009a; Morgan and Monticello, 1990). Similar to the LRT, inhomogeneity of the nasal anatomy, nasal fluid composition, and ventilation and airflow patterns affects the uptake of $O_3$ into the nasal passageways.

#### 5.2.2.3 Upper Respiratory Tract Ozone Removal and Dose

Total $O_3$ uptake in the entire RT in rats and guinea pigs is 40-54% efficient (Hatch et al., 1989; Wiester et al., 1988; Wiester et al., 1987), while in humans at rest it ranges from 80-95% efficient (Hu et al., 1992). The URT provides a defense against $O_3$ entering the lungs by removing half of the $O_3$ that will be absorbed from the airstream. In both animals and humans, about 50% of the $O_3$ that was absorbed in the RT was removed in the head (nose, mouth, and pharynx), about 7% in the larynx/trachea, and about 43% in the lungs (Hu et al., 1992; Hatch et al., 1989; Miller et al., 1979). However, experimental studies in dogs have reported 75-100% uptake in the URT (Yokoyama and Frank, 1972; Vaughan et al., 1969). The fraction of $O_3$ taken up was inversely related to flow rate and to inlet $O_3$ concentration (Yokoyama and Frank, 1972; Vaughan et al., 1969). URT absorption is relatively high due in part to the large surface area of the nasal airways. The limiting factors in nasal $O_3$ uptake were simultaneous diffusion and chemical reaction of $O_3$ in the nasal ELF (Santiago et al., 2001). The ELF layer in the nose is thicker than in the rest of the RT, and mathematical estimates predicted that $O_3$ penetrates less than the thickness of the ELF layer; reaction products are likely the agents damaging the nasal tissue and not $O_3$ itself. It was hypothesized that the nasal non-linear kinetics of $O_3$ uptake fraction result from the depleting substrates in the nasal ELF becoming the limiting factor of the reaction (Santiago et al., 2001).

Uptake efficiencies have been measured for various segments of the URT (Table 5-1). Gerrity et al. (1995) reported unidirectional uptake efficiencies of $O_3$

inhaled from a mouthpiece; of 0.18 from the mouth to vocal cords, 0.095 from the vocal cords to the upper trachea (totaling 0.27), 0.084 from the upper trachea to the main bifurcation carina (total cumulative efficiency from the mouth of 0.36), and essentially zero between the carina and the bronchus intermedius (total cumulative efficiency from the mouth of 0.33). These values are lower than those calculated by Hu et al. (1992) that reported cumulative uptake efficiencies of 0.21, 0.36, 0.44, and 0.46 during a complete breath in which an $O_3$ bolus penetrated between the mouth and the vocal cords, the upper trachea, the main bifurcation carina, and the bronchus intermedius, respectively. The lower efficiencies seen in Gerrity et al. (1995) may have resulted because these investigators measurements were based on inhalation alone or were caused by $O_3$ scrubbing by the mouthpiece.

Past studies investigating nasal uptake of $O_3$ have shown that the nose partially protects the LRT from damage from inspired $O_3$ (Santiago et al., 2001; Gerrity et al., 1988). Sawyer et al. (2007) further investigated nasal uptake of $O_3$ in healthy adults during exercise. Fractional $O_3$ uptake, acoustic rhinometry (AR), and nasal NO measurements were taken in ten adults (8 women, 2 men) exposed to 200 ppb $O_3$ before and after moderate exercise at two flow rates (10 and 20 L/min). The percent nasal uptake of $O_3$ was ~50% greater at 10 L/min compared to 20 L/min both pre- and post-exercise. However, the inhaled $O_3$ dose delivered to the LRT (i.e., flow rate × exposure concentration × (1 - nasal absorbed fraction)) was 1.6-fold greater at the higher flow than at the lower flow (2.5 compared to 0.9 ppm·L/min). Prior exercise did not affect $O_3$ uptake at either flow rate, but did significantly increase nasal volume (Vn) and AR measurements of nasal cross-sectional area (minimum cross-sectional area (MCA) that corresponds to the nasal valve, CSA2 that corresponds to the anterior edge of the nasal turbinates, and CSA3 that corresponds to the posterior edge of the nasal turbinates) ($p \leq 0.05$) (Sawyer et al., 2007). Conversely, exercise decreased nasal resistance (Rn) ($p < 0.01$) and NO production (nonsignificant, $p > 0.05$). The change in Vn and CSA2:MCA ratio was correlated with the percent change in nasal uptake, however the overall effect was small and sensitive to elimination of outliers and sex segregation.

Overall, the majority of studies suggest that the URT removes about half of the $O_3$ that will be absorbed by reactions in the nasal ELF. The exact uptake efficiency is dependent on variations in flow rate and inhaled concentration.

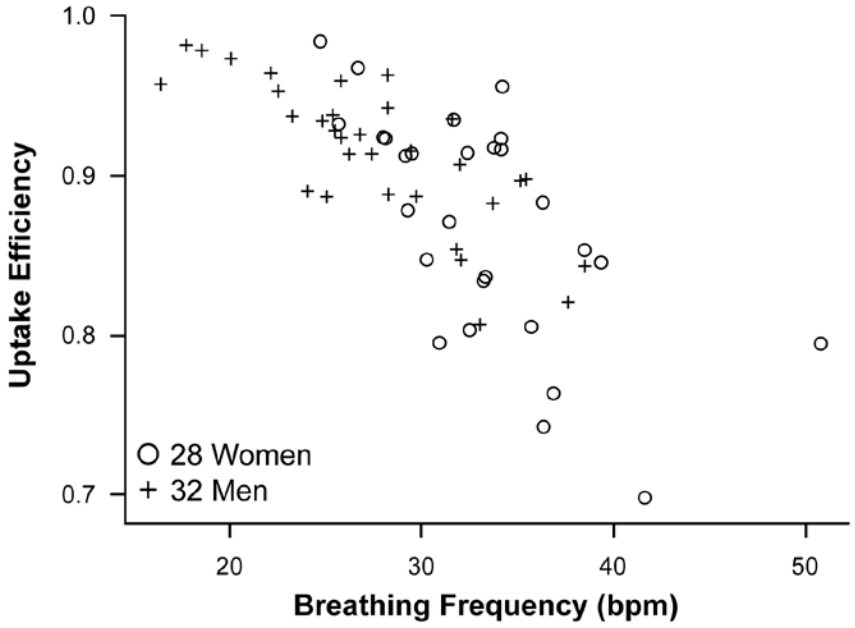
#### 5.2.2.4 Lower Respiratory Tract Ozone Uptake and Dose

Approximately 43% of the $O_3$ absorption occurs in the LRT of both humans and animals. Models predicted that the net $O_3$ dose decreases distally from the trachea toward the end of the TB region and then rapidly decreases in the alveolar region (Miller et al., 1985). Further, these models predicted low tissue $O_3$ dose in the trachea and large bronchi.

Uptake efficiency depends on a number of variables, including $O_3$ exposure concentration, exposure time, and breathing pattern. For breaths of similar

waveforms, respiratory patterns are uniquely described by breathing frequency ($f_B$) and tidal volume ($V_T$); by minute ventilation ($\dot{V}_E = f_B \times V_T$) and $f_B$; or by $\dot{V}_E$ and $V_T$. Simulations from the Overton et al. (1996) single-path anatomical respiratory tract model, where the upper and lower respiratory tracts were modeled but uptake by the URT was not considered, predicted that fractional uptake and PAR $O_3$ dose increased with $V_T$ when $f_B$ was held constant. Likewise, experimental studies found that $O_3$ uptake was positively correlated with changes in $V_T$ (Ultman et al., 2004; Gerrity et al., 1988). Also, $O_3$ exposure led to a reflex mediated increase in $f_B$ and reduction in $V_T$, hypothesized to be protective by decreasing the dose delivered to the lung at a particular $\dot{V}_E$ (Gerrity et al., 1994). Nasal $O_3$ uptake efficiency was inversely proportional to flow rate (Santiago et al., 2001), so that an increase in $\dot{V}_E$ will increase $O_3$ delivery to the lower airways. At a fixed $\dot{V}_E$, increasing $V_T$ (corresponding to decreasing $f_B$) drove $O_3$ deeper into the lungs and increased total respiratory uptake efficiency (Figure 5-4) (Ultman et al., 2004; Wiester et al., 1996a; Gerrity et al., 1988). Modeling predicted a decrease in fractional uptake with increased $f_B$ when $V_T$ was held constant, but an increase in PAR dose with increased $f_B$ (Overton et al., 1996). Similarly, increased $f_B$ (80 - 160 bpm) and shallow breathing in rats decreased midlevel tracheal $^{18}O$ content and an increased $^{18}O$ content in the mainstem bronchi (Alfaro et al., 2004). This dependence may be a result of frequency-induced alterations in contact time that affects the first-order absorption rate for $O_3$ (Postlethwait et al., 1994). Also, an association of $O_3$ uptake efficiency was found with $\dot{V}_E$ and exposure time.

Increasing flow leads to deeper penetration of $O_3$ into the lung, such that a smaller fraction of $O_3$ is absorbed in the URT and uptake shifts to the TB airways and respiratory airspaces (Nodelman and Ultman, 1999; Hu et al., 1994; Ultman et al., 1994). Hu et al. (1994) and Ultman et al. (1994) found that $O_3$ absorption increased with volumetric penetration (Vp) of a bolus of $O_3$ into the RT. Ozone uptake efficiency and Vp were not affected by bolus $O_3$ concentration (Kabel et al., 1994; Hu et al., 1992), indicating that under these experimental conditions $O_3$ uptake was a linear absorption process, where the diffusion and chemical reaction rates of $O_3$ were proportional to the $O_3$ concentration. The absorption relationship would not be linear once interfacial mass transfer was saturated. As mentioned above, a weak negative relationship between $O_3$ concentration and uptake efficiency was reported for the nasal cavities by Santiago et al. (2001). Rigas et al. (2000) also found a weak but significant negative dependence of $O_3$ concentration on RT uptake efficiency in exercising individuals. This study also found that exposure time had a small but significant influence on uptake efficiency; however, this negative dependence may be an artifact of progressive depletion of reactive substrates from the ELF.




Note: Subjects breathed 250 ppb $O_3$ oronasally via a breathing mask. The uptake efficiency was well correlated with breathing frequency (r = -0.723, p <0.001) and tidal volume (not illustrated; r = 0.490, p <0.001).

Source: Reprinted with permission of Health Effects Institute (Ultman et al., 2004).

**Figure 5-4 Total $O_3$ uptake efficiency as a function of breathing frequency at a constant minute ventilation of 30 L/min.**

Past studies have shown that $O_3$-induced epithelial damage to the lung occurs with a reproducible pattern of severity between daughter branches of individual bifurcations that is dependent on the $O_3$ concentration-time profile of the inhaled gas. A 3-D computational fluid dynamics model was created to investigate the $O_3$ transport in a single airway bifurcation (Taylor et al., 2007). The model consisted of one parent branch and two symmetrical daughter branches with a branching angle of 90° and a sharp carinal ridge. Various flow scenarios were simulated using Reynolds numbers (Re) ranging from 100 to 500. The Reynolds number that corresponds to a certain airway generation is dependent upon both lung size and $\dot{V}_E$, such that the range in Reynolds numbers, from 100-500, would encompass generations 1-5, 3-7, and 6-10 for an adult during quiet breathing, light exertion, and heavy exercise, respectively, whereas the same Reynolds number range corresponds to generations 0-4, 1-6, and 4-8 for a 4-year-old child. This model predicted velocity distributions that were consistent with earlier work of Schroter and Sudlow (1969), and also reported $O_3$ concentration and wall uptake distributions. The model predicted that during inspiration, the velocity and $O_3$ concentration distribution were axisymmetric throughout the parent branch, but skewed toward the inner wall within the daughter branches. During expiration, the model predicted that the velocity and $O_3$ concentration distribution was slightly skewed toward the outer walls of the daughter branches. Hot spots of wall flux existed at the carina during inspiration and

expiration with Re >100. Additional hot spots were found during expiration on the parent branch wall downstream of the branching region.

Overall $O_3$ inhalation uptake in humans is over 80% efficient, but the exact efficiency that determines how much $O_3$ is available at longitudinally distributed compartments in the lung is sensitive to changes in $V_T$, $f_B$, and to a minor extent, exposure time.

#### 5.2.2.5 Mode of Breathing

Ozone uptake and distribution is sensitive to the mode of breathing. Variability in TB airways volume had a weaker influence on $O_3$ absorption during nasal breathing compared to oral breathing. This could be a result of $O_3$ scrubbing in the nasal passageways that are bypassed by oral breathing. Studies by Ultman and colleagues, using bolus inhalation in humans, demonstrated that $O_3$ uptake fraction into the upper airways was greater during nasal breathing than during oral breathing (e.g., 0.90 during nasal breathing and 0.80 during oral breathing at 150 mL/sec and 0.45 during nasal breathing and 0.25 during oral breathing at 1,000 mL/sec) (Nodelman and Ultman, 1999; Kabel et al., 1994; Ultman et al., 1994). Therefore, oral breathing results in deeper penetration of $O_3$ into the RT with a higher absorbed fraction in the TB and alveolar airways (Nodelman and Ultman, 1999). Similar results were also obtained from $O_3$ uptake studies in dogs (Yokoyama and Frank, 1972). Earlier human studies suggested that oral or oronasal breathing results in a higher $O_3$ uptake efficiency than nasal breathing (Wiester et al., 1996a; Gerrity et al., 1988). Overall, the mode of breathing may have a seemingly small effect on the RT uptake efficiency; however, it does play an important role in the distribution of $O_3$ deposited in the distal airways.

#### 5.2.2.6 Interindividual Variability in Dose

Similarly exposed individuals vary in the amount of actual dose delivered to the LRT (Santiago et al., 2001; Rigas et al., 2000; Bush et al., 1996). Interindividual variability accounted for between 10-50% of the absolute variability in $O_3$ uptake measurements (Santiago et al., 2001; Rigas et al., 2000). When concentration, time, and $\dot{V}_E$ were held constant, fractional absorption ranged from 0.80 to 0.91 (Rigas et al., 2000). It has been hypothesized that interindividual variation in $O_3$ induced responses such as $FEV_1$ is the result of interindividual variation in net dose or regional $O_3$ uptake among exposed individuals.

Recent studies have reiterated the importance of intersubject variation in $O_3$ uptake. The intersubject variability in nasal $O_3$ uptake determined by Sawyer et al. (2007) ranged from 26.8 to 65.4% (pre- and post-exercise). A second study investigating the use of the $CO_2$ expirogram to quantify pulmonary responses to $O_3$ found that

intersubject variability accounted for 50% of the overall variance in the study (Taylor et al., 2006).

Variability in net or tissue dose may be attributed to differences in the pulmonary physiology, anatomy, and biochemistry. Since the URT and TB airways remove the majority of inhaled $O_3$ before it reaches the gas exchange region, the volume and surface area of these airways will influence $O_3$ uptake. Models predicted that fractional $O_3$ uptake and PAR dose (flux of $O_3$ to the PAR surfaces divided by exposure concentration) increase with decreasing TB volume and decreasing TB region expansion. On the contrary, alveolar expansion had minimal effect on uptake efficiency as relatively little $O_3$ reaches the peripheral lung (Bush et al., 2001; Overton et al., 1996). Ozone uptake was virtually complete by the time $O_3$ reaches the alveolar spaces of the lung (Postlethwait et al., 1994). Experimental studies have found that differences in TB volumes may account for 75% of the variation in absorption between subjects (Ultman et al., 2004). In support of this concept, regression analysis showed that $O_3$ absorption was positively correlated with anatomical dead space ($V_D$) and TB volume (i.e., $V_D$ minus $V_{URT}$), but not total lung capacity (TLC), forced vital capacity (FVC), or functional residual capacity (FRC) (Ultman et al., 2004; Bush et al., 1996; Hu et al., 1994; Postlethwait et al., 1994). Variability in $V_D$ was correlated more with the variability in the TB volume than the URT volume. Similarly, uptake was correlated with changes in individual bronchial cross-sectional area, indicating that changes in cross-sectional area available for gas diffusion are related to overall $O_3$ retention (Reeser et al., 2005; Ultman et al., 2004). When coupled, these results suggest that the larger surface-to-volume ratio associated with the smaller airways in women enhances local $O_3$ uptake, thereby reducing the distal penetration volume of $O_3$ into the female respiratory system. When absorption data were normalized to $Vp/V_D$, variability attributed to sex differences were not distinguishable (Bush et al., 1996). These studies provide support to the RT anatomy, especially the TB volume and surface area, playing a key role in variability of $O_3$ uptake between individuals.

In addition, variability between individuals is influenced by age. Overton and Graham (1989) predicted that the total mass of $O_3$ absorbed per minute (in units of: µg/min per [$\mu g/m^3$ of ambient $O_3$]) increased with age from birth to adulthood. This model predicted that during quiet breathing the LRT distribution of absorbed $O_3$ and the CAR $O_3$ tissue dose were not sensitive to age. However, during heavy exercise or work $O_3$ uptake was dependent on age. A physiologically based pharmacokinetic model simulating $O_3$ uptake predicted that regional extraction of $O_3$ was relatively insensitive to age, but extraction per unit surface area was 2-fold to 8-fold higher in infants compared to adults, due to the fact that children under age 5 have much a much smaller airway surface area in the extrathoracic (nasal) and alveolar regions (Sarangapani et al., 2003). Additionally, children tend to have a greater oral breathing contribution than adults at rest and during exercise (Bennett et al., 2008; Becquemin et al., 1999; James et al., 1997). Even after adjusting for differences in surface area, the dose rate to the lower airways of children compared to adults is increased further because children breathe at higher minute ventilations relative to their lung volumes.

Smoking history, with its known increase in mucus production, was not found to affect the fractional uptake of a bolus of $O_3$ in apparently healthy smokers with limited smoking history (Bates et al., 2009). Despite similar internal $O_3$ dose distribution, the smokers exhibited greater pulmonary responses to $O_3$ bolus exposures, measured as $FEV_1$ decrements and increases in the normalized slope of the alveolar plateau ($S_N$). This was contrary to previous studies conducted in smokers with a greater smoking history that found decreased $O_3$ induced decrements in $FEV_1$ in smokers during continuous $O_3$ exposure (Frampton et al., 1997a; Emmons and Foster, 1991).

#### 5.2.2.7 Physical Activity

Exercise increases the overall exposure of the lung to inhaled contaminants due, in most part, to the increased intake of air. Thus, human studies have used exercise, at a variety of activity levels, to enhance the effects of $O_3$ (Table 5-2). Further explanation of the effects of physical activity on ventilation can be found in Chapters 4 and 6. Table 4-5 presents the mean ventilation rates at different activity levels for different age groups. Table 6-1 provides activity levels as detailed in specific human exposure studies.

**Table 5-2 General adult human inhalation rates by activity levels.**

| Activity Level | Inhalation Rate |
|---|---|
| Light | 2 to 3 × resting $\dot{V}_E$ [a] |
| Moderate | 4 to 6 × resting $\dot{V}_E$ |
| Heavy | 7 to 8 × resting $\dot{V}_E$ |
| Very Heavy | >9 × resting $\dot{V}_E$ |

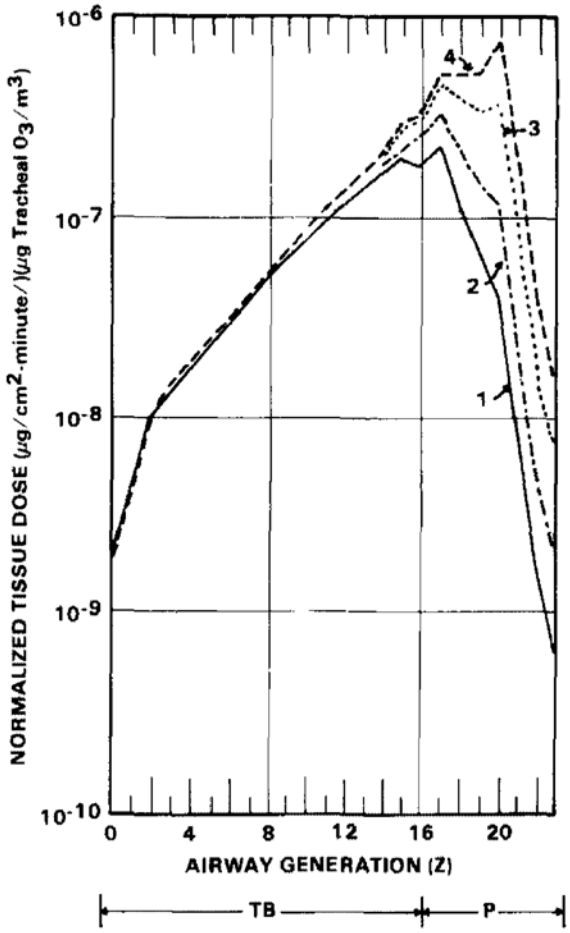
[a]Resting $\dot{V}_E$ approximates 8 L/min
Source: U.S. EPA (1986).

As exercise increases from a light to moderate level, $V_T$ increases. This increase in $V_T$ is achieved by encroaching upon both the inspiratory and expiratory reserve volumes of the lung (Dempsey et al., 1990). After $V_T$ reaches about 50% of the vital capacity, generally during heavy exercise, further increases in ventilation are achieved by increasing $f_B$. Ventilatory demands of very heavy exercise require airway flow rates that often exceed 10 times resting levels and $V_T$ that approach 5 times resting levels (Dempsey et al., 2008).

In addition to increasing the bulk transport of $O_3$ into the lung, exercise also leads to a switch from nasal to oronasal breathing. Higher ventilatory demand necessitates a lower-resistance path through the mouth. The contribution of nasal breathing to the $\dot{V}_E$ varies as a function of age, sex, and race. Children tended to have a lesser nasal contribution to breathing than adults at rest and during exercise at matched percent

maximum work (Bennett et al., 2008). Males had less nasal contribution to breathing at rest and during exercise at matched percent maximum work compared with females (Bennett et al., 2003). The difference between the sexes may be explained by the difference in $\dot{V}_E$ at a given percent maximal workload. Females had a lower $\dot{V}_E$ than males so had to augment breathing orally at higher work efforts. Caucasians had a lesser nasal contribution than African-Americans at rest and during exercise at matched percent maximum work (Bennett et al., 2003).

This increase in $V_T$ and flow associated with exercise in humans shifts the net $O_3$ dose further into the periphery of the RT causing a disproportionate increase in distal lung tissue dose. Modeling heavy exercise by increasing ventilatory parameters from normal respiration levels predicted a 10-fold increase in total mass uptake of $O_3$ (Miller et al., 1985). This model also predicted that as exercise and ventilatory demand increased, the maximum tissue dose, the $O_3$ reaching the tissues, moved distally into the RT (Figure 5-5). When the flow was increased to what is common in moderate or heavy exercise (respiratory flow = 45-60 L/min compared to 15 L/min), the URT absorbed a smaller fraction of the $O_3$ (0.10 at high flow rate to ~0.50 at low flow rate); however, the trachea and more distal TB airways received higher doses during higher flow rates than at lower flow rates (0.65 absorbed in the lower TB airways, and 0.25 absorbed in the alveolar zone with high flow compared to 0.5 in the TB with almost no $O_3$ reaching the alveolar zone at low flow) (Hu et al., 1994). The same shift in the $O_3$ dose distribution more distally in the lung occurred in other studies mimicking the effects of exercise (Nodelman and Ultman, 1999). Also, LRT uptake efficiency was sensitive to age only under exercise conditions (Overton and Graham, 1989). The total mass of $O_3$ absorbed per minute (µg/min per [µg/$m^3$ of ambient $O_3$]) was predicted to increase with age during heavy work or exercise. A recent study by Sawyer et al. (2007) approximated that doubling minute ventilation led to only a 1.6-fold higher delivered dose rate of $O_3$ to the lung (delivered dose was calculated as: flow rate × [$O_3$ ppm] × (100-percent nasal $O_3$ uptake)) due to a decrease in URT uptake with increasing flow rate. Past models have predicted the increase in uptake during exercise is distributed unevenly in the RT compartments and regions. Tissue and net dose in the TB region increased ~1.4-fold during heavy exercise compared to resting conditions, whereas the alveolar surface layer and tissue uptake increased by factors of 5.2 and 13.6, respectively (Miller et al., 1985).




Note: Curve 1: $V_T$ = 500 mL; $f_B$ = 15 breaths/min. Curve 2: $V_T$ = 1,000 mL; $f_B$ = 15 breaths/min. Curve 3: $V_T$ = 1,750 mL; $f_B$ = 20.3 breaths/min. Curve 4: $V_T$ = 2,250 mL; $f_B$ = 30 breaths/min. TB = tracheobronchial region; P = pulmonary region.

Source: Reprinted with permission of Elsevier (Miller et al., 1985).

**Figure 5-5 Modeled effect of exercise on tissue dose of the LRT.**

### 5.2.2.8 Summary

In summary, $O_3$ uptake is affected by complex interactions between a number of factors including RT morphology, breathing route, frequency, and volume, physicochemical properties of the gas, physical processes of gas transport, as well as the physical and chemical properties of the ELF and tissue layers. The role of these processes varies throughout the length of the RT and as $O_3$ moves from the gas into liquid compartments of the RT.

About half of the $O_3$ that will be absorbed from the airstream is removed in the URT, which provides a defense against $O_3$ entering the lungs. However, the local dose to the URT tissue is site-specific and dependent on the nasal anatomy, nasal fluid composition, and ventilation and airflow patterns of the nasal passageways.

The primary uptake site of $O_3$ delivery to the LRT epithelium is believed to be the CAR, however, similar to the URT, inhomogeneity in the RT structure may affect the dose delivered to this target site with larger path lengths leading to smaller locally delivered doses. This could have implications in regional damage to the RT, such that even though the average RT dose may be at a level where health effects would not be predicted, local regions of the RT may receive considerably higher than average doses and therefore be at greater risk of effects. Recent studies have provided evidence for hot spots of $O_3$ flux around bifurcations in airways. Experimental studies and models have suggested that the net $O_3$ dose gradually decreases distally from the trachea toward the end of the TB region and then rapidly decreases in the alveolar region. However, the tissue $O_3$ dose is low in the trachea, increases to a maximum in the terminal bronchioles and the CAR, and then rapidly decreases distally into the alveolar region.

Ozone uptake efficiency is sensitive to a number of factors. Fractional absorption will decrease with increased flow and increase proportional to $V_T$, so that at a fixed $\dot{V}_E$, increasing $V_T$ (or decreasing $f_B$) drives $O_3$ deeper into the lungs and increases total respiratory uptake efficiency. Individual total airway $O_3$ uptake efficiency is also sensitive to large changes in $O_3$ concentration, exposure time, and $\dot{V}_E$. Major sources of variability in absorption of $O_3$ include $O_3$ concentration, exposure time, $f_B$, $\dot{V}_E$, and $V_T$, but the interindividual variation is the greatest source of variability uptake efficiency. The majority of this interindividual variability is due to differences in TB volume and surface area.

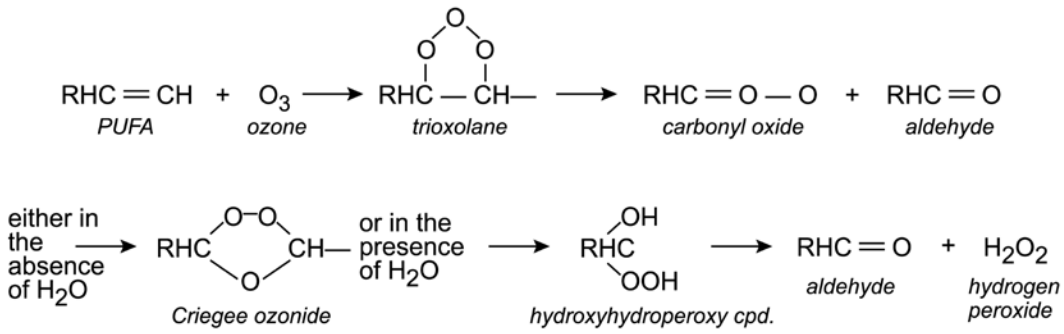
An increase in $V_T$ and $f_B$ are both associated with increased physical activity. These changes and a switch to oronasal breathing during exercise results in deeper penetration of $O_3$ into the lung with a higher absorbed fraction in the ET, TB, and alveolar airways. For these reasons, increased physical activity acts to move the maximum tissue dose of $O_3$ distally into the RT and into the alveolar region.

### 5.2.3 Ozone Reactions and Reaction Products

Ozone dose is affected by the chemical reactions or the products of these reactions that result from $O_3$ exposure. The process by which $O_3$ moves from the airway lumen into the ELF is related to the coupled diffusion and chemical reactions occurring in ELF and is called “reactive absorption.” Ozone is chemically reactive with a wide spectrum of biomolecules and numerous studies have evaluated the loss of specific molecules such as GSH and the appearance of plausible products such as nonanal. Both in vitro and in vivo studies contribute to the understanding of $O_3$ reactions and reaction products.

Ozone may interact with many of the components in the ELF including phospholipids, neutral lipids like cholesterol, free fatty acids, proteins, and low molecular weight antioxidants as has been demonstrated in in vitro studies (Perez-Gil, 2008; Uppu et al., 1995). It was estimated that 88% of the $O_3$ that does not come in contact with antioxidants will react with unsaturated fatty acids in the ELF

including those contained within phospholipids or neutral lipids (Uppu et al., 1995). Ozone reacts with the double bond of unsaturated fatty acids to form stable and less reactive ozonide, aldehyde, and hydroperoxide reaction products via chemical reactions such as the Criegee ozonolysis mechanism (Figure 5-6) (Pryor et al., 1991). Lipid ozonation products, such as the aldehydes hexanal, heptanal, and nonanal, have been recovered after $O_3$ exposure in human BAL fluid (BALF), rat BALF, isolated rat lung, and in vitro systems (Frampton et al., 1999; Postlethwait et al., 1998; Pryor et al., 1996). Adducts of the aldehyde 4-hydroxynonenal were found in human alveolar macrophages after $O_3$ exposure in vivo (Hamilton et al., 1998). Polyunsaturated fatty acid (PUFA) reactions are limited by the availability of $O_3$ since lipids are so abundant in the ELF. Yields of $O_3$-induced aldehydes were increased by the decrease in other substrates such as ascorbic acid ($AH_2$) (Postlethwait et al., 1998). Free radicals are also generated during $O_3$-mediated oxidation reactions with PUFA (Pryor, 1994). These reactions are reduced by the presence of the lipid-soluble free radical scavenger α-tocopherol (α-TOH) (Pryor, 1994; Fujita et al., 1987; Pryor, 1976). PUFA reactions may not generate sufficient bioactive materials to account for acute cell injury, however only modest amounts of products may be necessary to induce cytotoxicity (Postlethwait and Ultman, 2001; Postlethwait et al., 1998).




Note: Not all secondary reaction products are shown.
Source: U.S. EPA (2006b).

**Figure 5-6 Schematic overview of $O_3$ interaction with PUFA in ELF and lung cells.**

Cholesterol is the most abundant neutral lipid in human ELF. Reaction of cholesterol with $O_3$ results in biologically active cholesterol products such as the oxysterols, β-epoxide and 6-oxo-3,5-diol (Murphy and Johnson, 2008; Pulfer et al., 2005; Pulfer and Murphy, 2004). Product yields depend on ozonolysis conditions, however cholesterol ozonolysis products form in similar abundance to phospholipid-derived ozonolysis products in rat ELF (Pulfer and Murphy, 2004).

The ELF also contains proteins derived from blood plasma as well as proteins secreted by surface epithelial cells. Ozone reactions with proteins have been studied by their in vitro reactions as well as reactions of their constituent amino acids (the most reactive of which are cysteine, histidine, methionine, tyrosine, and tryptophan). Ozone preferentially reacts with biomolecules in the following order: thiosulfate >ascorbate >cysteine ≈ methionine >glutathione (Kanofsky and Sima, 1995). Rate constants for the reaction of amino acids with $O_3$ vary between studies due to differing reaction conditions and assumptions; however aliphatic amino acids were consistently very slow to react with $O_3$ (e.g., alanine: 25-100 moles/L/sec) (Kanofsky and Sima, 1995; Ignatenko and Cherenkevich, 1985; Pryor et al., 1984; Hoigné and Bader, 1983). Uppu et al. (1995) predicted that 12% of inhaled $O_3$ that does not react with antioxidants will react with proteins in the ELF.

Reactions of $O_3$ with low molecular weight antioxidants have been extensively studied. The consumption of antioxidants such as uric acid (UA), ascorbate ($AH_2$), and reduced glutathione (GSH) by $O_3$ was linear with time and positively correlated with initial substrate concentration and $O_3$ concentration (Mudway and Kelly, 1998; Mudway et al., 1996). Endogenous antioxidants are present in relatively high concentrations in the ELF of the human airways (obtained as BALF) and display high (but not equal) intrinsic reactivities toward $O_3$. In individual and in limited composite mixtures, UA was the most reactive antioxidant tested, followed by $AH_2$ (Mudway and Kelly, 1998). GSH was consistently less reactive than UA or $AH_2$ (Mudway and Kelly, 1998; Mudway et al., 1996; Kanofsky and Sima, 1995). To quantify these reactions, Kermani et al. (2006) evaluated the interfacial exposure of aqueous solutions of UA, $AH_2$, and GSH (50-200 µM) with $O_3$ (1-5 ppm). Similar to the results of Mudway and Kelly (1998), this study found the hierarchy in reactivity between $O_3$ and these antioxidants to be UA≥ $AH_2$>>GSH. UA and $AH_2$ shared a 1:1 stoichiometry with $O_3$, whereas 2.5 moles of GSH were consumed per mole of $O_3$. Using these stoichiometries, reaction rate constants were derived ($5.8\times10^4$ $M^{-1}$ $sec^{-1}$, $5.5\times10^4$ $M^{-1}$ $sec^{-1}$, and 57.5 $M^{-0.75}$ $sec^{-1}$ [20.9 $M^{-1}$ $sec^{-1}$] for the reaction of $O_3$ with UA, $AH_2$, and GSH, respectively). Other studies report reactive rate constants that are two to three orders of magnitude larger, however these studies used higher concentrations of $O_3$ and antioxidants under less physiologically relevant experimental conditions (Kanofsky and Sima, 1995; Giamalva et al., 1985; Pryor et al., 1984). Since $O_3$ acts through competition kinetics, the effective concentration of the reactants present in the ELF will determine the reactions that occur in vivo. For example, the pKa of GSH is about 8.7 so that at physiological pH very little is in the reactive form of thiolate ($GS^-$). On the other hand, ascorbic acid has a pKa of about 4.2 so it exists almost entirely as ascorbate ($AH^-$) in the ELF. Thus, the effective concentration of GSH that is available to react with $O_3$ will be much lower than that of ascorbate in ELF.

A series of studies used new techniques to investigate the reaction products resulting from initial air-liquid interface interactions of $O_3$ with ELF components (e.g., antioxidants and proteins) in ~1 millisecond (Enami et al., 2009a, b, c, 2008a, b). Solutions of aqueous UA, $AH_2$, GSH, α-TOH, and protein cysteines (CyS) were sprayed as microdroplets in $O_3/N_2$ mixtures at atmospheric pressure and analyzed by

electrospray mass spectrometry. These recent studies in which the large surface to volume ratio of microdroplets promote an interfacial reaction demonstrated different reactivity toward $AH_2$, UA, and GSH by $O_3$ compared to previous studies using bulk liquid phase bioreactors. This artificial system does not recapitulate the lung surface so caution must be taken in translating the results of these studies to in vivo conditions.

As was seen in previous studies (Kermani et al., 2006; Kanofsky and Sima, 1995), the hierarchy of reactivity of these ELF components with $O_3$ was determined to be $AH_2 \approx$ UA > CyS >GSH. There was some variance between the reaction rates and product formation of UA, $AH_2$, and GSH with $O_3$ as investigated by Enami et al. (2009a, b, c, 2008a, b) versus $O_3$ reacting with bulk liquid phase bioreactors as described previously. UA was more reactive than $AH_2$ toward $O_3$ in previous studies, but in reactions with $O_3$ using microdroplets, these antioxidants had equivalent reactivity (Enami et al., 2008b). As $O_3$ is a kinetically slow one-electron acceptor but very reactive O-atom donor, products of the interaction of $O_3$ with UA, $AH_2$, GSH, CyS, and α-TOH result from addition of *n* O-atoms (*n* = 1-4). These products included epoxides (e.g., U-$O^-$), peroxides (e.g., U-$O_2^-$), and ozonides (e.g., U-$O_3^-$). For instance, GSH was oxidized to sulfonates ($GSO_3^-$/$GSO_3^{2-}$), not glutathione disulfide (GSSG) by $O_3$ (Enami et al., 2009b). However, it is possible that other oxidative species are oxidizing GSH in vivo, since sulfonates are not detected in $O_3$ exposed ELF whereas GSSG is. This is also supported by the fact that $O_3$ is much less reactive with GSH than other antioxidants, such that <3% of $O_3$ will be scavenged by GSH when in equimolar amounts with $AH_2$ (Enami et al., 2009b).

This series of studies also demonstrated that ozonolysis product yields and formation were affected by pH. Acidified conditions (pH ≈ 3-4), such as those that may result from acidic particulate exposure or pathological conditions like asthma (pH ≈ 6), decreased the scavenging ability of UA and GSH for $O_3$; such that at low pH, the scavenging of $O_3$ must be taken over by other antioxidants, such as $AH_2$ (Enami et al., 2009b, 2008b). Also, under acidic conditions (pH ≈ 5), the ozonolysis products of $AH_2$ shifted from the innocuous dehydro-ascorbic acid to the more persistent products, $AH_2$ ozonide and threonic acid (Enami et al., 2008a). It is possible that the acidification of the ELF by acidic copollutant exposure will increase the toxicity of $O_3$ by preventing some antioxidant reactions and shifting the reaction products to more persistent compounds.

Since ELF exists as a complex mixture, it is important to look at $O_3$ reactivity in substrate mixtures. Individual antioxidant consumption rates decreased as the substrate mixture complexity increased (e.g., antioxidant mixtures and albumin addition) (Mudway and Kelly, 1998). However, $O_3$ reactions with $AH_2$ predominated over the reaction with lipids, when exposed to substrate solution mixtures (Postlethwait et al., 1998). It was suggested that $O_3$ may react with other substrates once $AH_2$ concentrations within the reaction plane fall sufficiently. Additionally, once $AH_2$ was consumed, the absorption efficiency diminished, allowing inhaled $O_3$ to be distributed to more distal airways (Postlethwait et al., 1998). Multiple studies have concluded $O_3$ is more reactive with $AH_2$ and UA than

with the weakly reacting GSH (or cysteine or methionine) or with amino acid residues and protein thiols (Kanofsky and Sima, 1995; Cross et al., 1992).

In a red blood cell (RBC) based system, $AH_2$ augmented the in vitro uptake of $O_3$ by 6-fold, as computed by the mass balance across the exposure chamber (Ballinger et al., 2005). However, estimated in vitro $O_3$ uptake was not proportional to the production of $O_3$-derived aldehydes from exposing $O_3$ to RBC membranes (Ballinger et al., 2005). In addition, $O_3$ induced cell membrane oxidation that required interactions with $AH_2$ and GSH, but not UA or the vitamin E analog Trolox. Further, aqueous phase reactions between $O_3$ and bovine serum albumin did not result in membrane oxidation (Ballinger et al., 2005). The presence of UA or bovine serum albumin protected against lipid and protein oxidation resulting from the reaction of $O_3$ and $AH_2$ (Ballinger et al., 2005). This study provided evidence that antioxidants may paradoxically facilitate $O_3$-mediated damage. This apparent contradiction should be viewed in terms of the concentration-dependent role of the ELF antioxidants. Reactions between $O_3$ and antioxidant species exhibited a biphasic concentration response, with oxidation of protein and lipid occurring at lower, but not higher, concentrations of antioxidant. In this way, endogenous reactants led to the formation of secondary oxidation products that were injurious and also led to quenching reactions that were protective. Moreover, the formation of secondary oxidation products mediated by some antioxidants was opposed by quenching reactions involving other antioxidants.

Alterations in ELF composition can result in alterations in $O_3$ uptake. Bolus $O_3$ uptake in human subjects can be decreased by previous continuous $O_3$ exposure (120-360 ppb), possibly due to depletion of compounds able to react with $O_3$ (Rigas et al., 1997; Asplund et al., 1996). Conversely, $O_3$ (360 ppb) bolus uptake was increased with prior $NO_2$ (360-720 ppb) or $SO_2$ (360 ppb) exposure (Rigas et al., 1997). It was hypothesized that this increased fractional absorption of $O_3$ could be due to increased production of reactive substrates in the ELF due to oxidant-induced airway inflammation.

Besides $AH_2$, GSH and UA, the ELF contains numerous antioxidant substances that appear to be an important cellular defense against $O_3$ including α-TOH, albumin, ceruloplasmin, lactoferrin, mucins, and transferrin (Mudway et al., 2006; Freed et al., 1999). The level and type of antioxidant present in ELF varies between species, regions of the RT, and can be altered by $O_3$ exposure. Mechanisms underlying the regional variability are not well-understood. It is thought that both plasma ultrafiltrate and locally secreted substances contribute to the antioxidant content of the ELF (Mudway et al., 2006; Freed et al., 1999). In the case of UA, the major source appears to be the plasma (Peden et al., 1995). Repletion of UA in nasal lavage fluid was demonstrated during sequential nasal lavage in human subjects (Mudway et al., 1999a). When these subjects, exercising at a moderate level, were exposed to 200 ppb $O_3$ for 2 hours, nasal lavage fluid UA was significantly decreased while plasma UA levels were significantly increased (Mudway et al., 1999a). The finding that UA, but not $AH_2$ or GSH, was depleted in nasal lavage fluid indicated that UA was the predominant antioxidant with respect to $O_3$ reactivity in the nasal cavity

(Mudway et al., 1999a). However, in human BALF samples, the mean consumption of $AH_2$ was greater than UA (Mudway et al., 1996). In addition, concentrations of UA were increased by cholinergic stimulation of the airways in human subjects, which suggested that increased mucosal gland secretions were an important source (Peden et al., 1993). Using the $O_3$-specific antioxidant capacity assay on human nasal lavage samples, Rutkowski et al. (2011) concluded that about 30% of the antioxidant capacity of the nasal ELF was attributed to UA activity. Additionally, more than 50% of the subject-to-subject differences in antioxidant capacity were driven by differences in UA concentration. However, day-to-day within-subject variations in measured antioxidant capacity were not related to the corresponding variations in UA concentration in the nasal lavage fluid. Efforts to identify the predominant antioxidant(s) in other RT regions besides the nasal cavity have failed to yield definitive results.

Regulation of $AH_2$, GSH and α-TOH concentrations within the ELF is less clear than that of UA (Mudway et al., 2006). In a sequential nasal lavage study in humans, wash-out of $AH_2$ and GSH occurred, indicating the absence of rapidly acting repletion mechanisms (Mudway et al., 1999a). Other studies demonstrated increases in BALF GSH and decreases in BALF and plasma $AH_2$ levels several hours following $O_3$ exposure (200 ppb for 2 h, while exercising at a moderate level) (Mudway et al., 2001; Blomberg et al., 1999; Mudway et al., 1999b). Studies with rats exposed to 0.4-1.1 ppm $O_3$ for 1-6 hours have shown consumption of $AH_2$ that correlates with $O_3$ exposure (Gunnison and Hatch, 1999; Gunnison et al., 1996; Vincent et al., 1996b). Further, cellular uptake of oxidized $AH_2$ by several cell types followed by intracellular reduction and export of reduced $AH_2$ has been demonstrated in vitro (Welch et al., 1995).

A body of evidence suggests that reaction of $O_3$ within the ELF limits its diffusive transport through the ELF; direct contact of $O_3$ with the apical membranes of the underlying epithelial cells therefore might be negligible in many regions of the RT (Ballinger et al., 2005; Connor et al., 2004; Postlethwait and Ultman, 2001; Pryor, 1992). This conclusion is based on computational analyses and in vitro studies. Direct confirmation using in vivo studies is limited. Nevertheless, when predicting exposure-related outcomes across species and anatomic sites, whether $O_3$ directly contacts the apical membranes of the epithelial cells is an important consideration, given that the extracellular surface milieu of the RT appreciably varies in terms of the types and concentrations of the substrates present and the thickness of the ELF.

For $O_3$ or its reaction products to gain access to the underlying cellular compartments, $O_3$ must diffuse at the air-liquid interface of the airway surface and travel through the ELF layer. In vitro experiments have shown that $O_3$ disappearance from the gas phase depends on the characteristics of the ELF substrates (Postlethwait et al., 1998; Hu et al., 1994). The ELF is comprised of the airway surface lining, which includes the periciliary sol layer and overlying mucus gel layer, and the alveolar surface lining, which includes the subphase of liquid and vesicular surfactant and the continuous surfactant monolayer (Bastacky et al., 1995). There is a progressive decrease in ELF thickness and increase in interfacial surface with

progression from the TB region to the alveolus (Mercer et al., 1992). The progressive thinning of the ELF while moving further down the RT decreases the radial distance $O_3$ or its reaction products must travel to reach the cells lining the RT.

Taking into account the high reactivity and low water solubility of $O_3$, Pryor (1992) estimated the distance that $O_3$ can penetrate into an ELF layer before it reacts with endogenous substrates to form other more long-lived reactive species, thus initiating a reaction cascade. These calculations utilize the Einstein-Smoluchowski equation to compare the time ($t_{diff}$) for $O_3$ to diffuse a distance (d) to the half-life ($t_{rx}$) of $O_3$ in its simultaneous reaction with substrates (Equation 5-1).

$$t_{diff} = d^2/2D_{O_3} \quad and \quad t_{rx} = ln2/k_sC_s$$

**Equation 5-1**

where $D_{O3}$ is the $O_3$ diffusion coefficient in ELF, $k_s$ is the bimolecular reaction rate constant of $O_3$ with a reactive substrate (s) in ELF, and $C_s$ is the molar substrate concentration. Importantly, it is assumed in the derivation of $t_{rx}$ that the substrate is far in excess of $O_3$ so that $C_s$ is spatially uniform in the ELF. To within some proportionality constant, the distance that $O_3$ penetrates can be estimated by equating $t_{diff}$ to $t_{rx}$ such that

$$d \propto \left(D_{O_3}/k_sC_s\right)^{1/2}$$

**Equation 5-2**

There is reasonable certainty that the $O_3$ diffusion coefficient anywhere in the ELF is in the range of $D_{O3}$~$10^{-5}$ – $10^{-6}$ $cm^2$/sec, but values of the $k_sC_s$ product for the reaction of $O_3$ with specific substrates are much less reliable. Moreover, it is unknown which substrates make the most important contributions to $k_sC_s$ and how these contributions vary from airway region to airway region. By asserting that polyunsaturated fatty acids are the primary reactive substrate, Miller et al. (1985) estimated that $k_sC_s$ = 1,198 $sec^{-1}$ in airway surface lining fluid and $k_sC_s$ = 21.4 $sec^{-1}$ in alveolar surface lining fluid. Pryor (1992) estimated the value of $k_sC_s$ = $10^{-6}$ $sec^{-1}$, by assuming reduced glutathione is the primary substrate in airway surface lining fluid. A value of $k_sC_s$ =2.5 × $10^5$ $sec^{-1}$ was extracted from in vivo measurements of $O_3$ uptake into the airway surface lining fluid of the nasal cavities (Santiago et al., 2001). These studies suggest that there is an uncertainty in the magnitude of $k_sC_s$ within airway surface lining fluid by a factor of 1,000, and that $k_sC_s$ may be more than 100 times greater in airway surface lining fluid than in alveolar surface lining fluid.

With their estimates of $k_sC_s$ = $10^6$ $sec^{-1}$ and $D_{O3}$ = $10^{-6}$ $cm^2$/sec, Pryor (1992) concluded that $O_3$ could not penetrate an airway surface lining layer even as thin as 0.1 µm. Comparable computations with the $k_sC_s$ = 1,198 $sec^{-1}$ value from Miller et

al. (1985) would indicate that $O_3$ penetrates an airway surface lining layer as thick as 3 µm. Since airway surface lining layer thickness is on the order of 10 µm in large airways and 0.1 µm in small airways, results using different estimates of $k_sC_s$ have entirely different implications regarding the direct role of $O_3$ in damage to underlying epithelium versus the role of toxic reaction products.

In the nasal passages, in particular, a diffusion analysis of in vivo $O_3$ uptake measurements made at different air flows indicated that the $O_3$ penetration distance (0.5 µm) is considerably less than the thickness of the nasal surface lining layer (10 µm) (Santiago et al., 2001). A computational fluid dynamics model was able to predict experimentally measured $O_3$ uptake when the presences of a nasal surface lining layer thickness was considered (Cohen-Hubal et al., 1996), further indicating the need to properly account for the reaction-diffusion processes in the surface lining layer.

Despite calculations and in vitro studies suggesting that reactions of $O_3$ with underlying epithelial cells may be negligible in some regions of the RT, there is some evidence that suggests direct interaction of $O_3$ with epithelial cells is possible. While moving distally in the lung, the ELF thickness decreases and becomes ultra thin in the alveolar region, possibly allowing for direct interaction of $O_3$ with the underlying epithelial cells. One definitive study conducted in excised rat lung measured alveolar surface lining layer thickness over relatively flat portions of the alveolar wall to be 0.14 µm, to be 0.89 µm at the alveolar wall junctions, and 0.09 µm over the protruding features (Bastacky et al., 1995). The area-weighted average thickness of the alveolar surface lining fluid was found to be about 0.2 µm and the alveolar surface lining layer was continuous over the entire alveolar surface measured. The surface appeared smooth; and no epithelial surface features or macrophage features protruded above the air-liquid interface. It was noted that measurements of alveolar surface lining layer thickness were made in lungs prepared in a state of roughly 80% of total lung capacity, and as a result, the values reported would be approaching the lowest values possible during the respiratory cycle. However, 4% of the surface area in the alveolar compartment was covered by alveolar lining fluid layer of less than 20 nm (Bastacky et al., 1995), suggesting the possibility that unreacted $O_3$ could penetrate to the cell layer in this region. Further it remains a possibility that airways macrophages may protrude into the gas phase, allowing for direct contact between $O_3$ and airways epithelial cells.

Still, direct reaction of $O_3$ with alveolar epithelial cells or macrophages may be limited by the presence of dipalmitoyl phosphatidylcholine (DPPC), the major component of surfactant, which has been shown in vitro to inhibit uptake of $O_3$ into an aqueous compartment containing ascorbate, glutathione, and uric acid (Connor et al., 2004). Further, the amount of $O_3$ available to the alveolar compartment may be limited by uptake of $O_3$ in nasal and TB compartments. In fact, the amount of $^{18}O$ reaction product was lower in the alveolar tissues than in TB tissues of rhesus monkeys immediately following a 2 hour exposure to $^{18}O$-labeled $O_3$ (0.4 and 1 ppm) (Plopper et al., 1998). These considerations illustrate the difficulty in determining whether $O_3$ reacts directly with cells in the alveolar compartment.

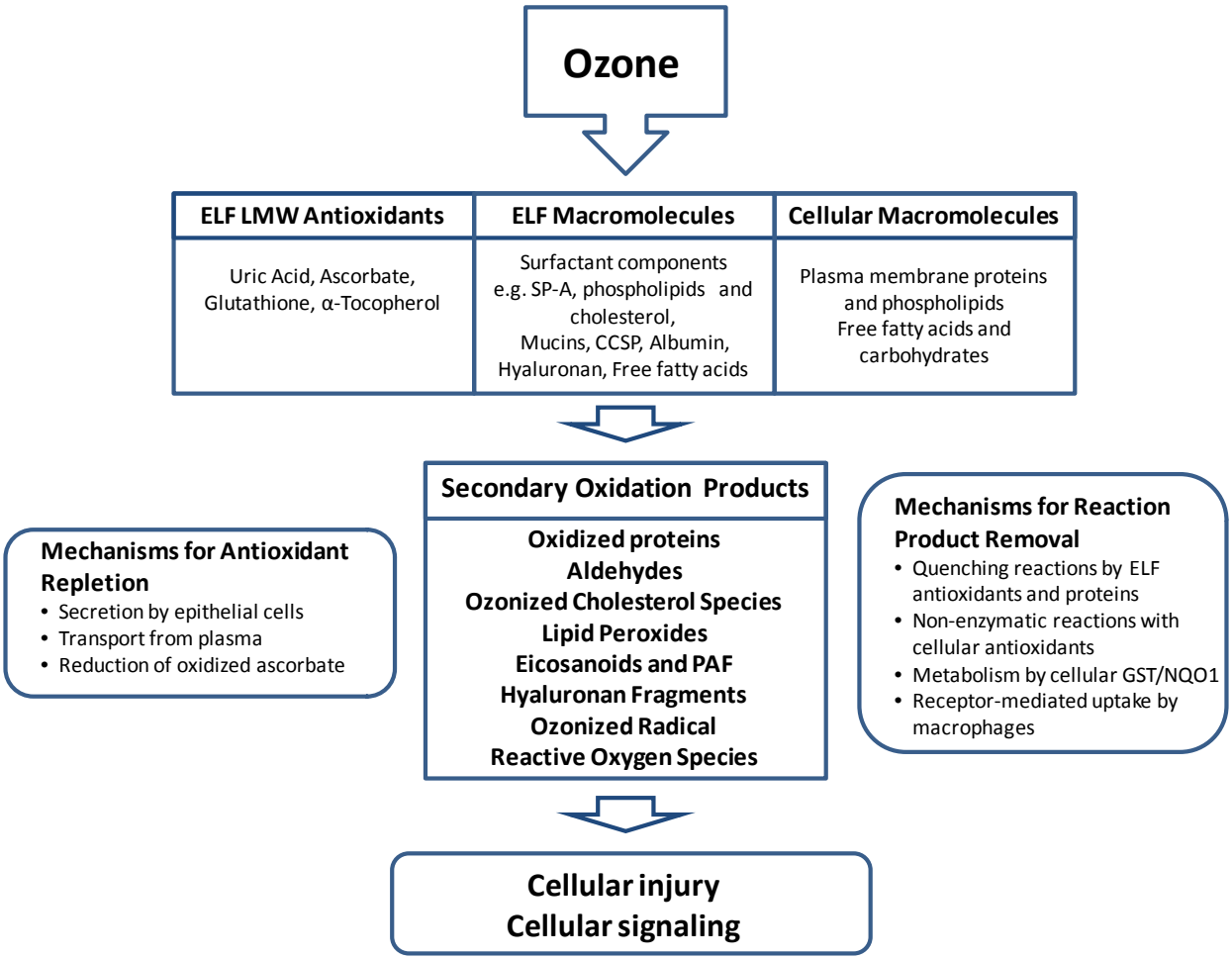
In some cases, however, with regard to the initiating mechanisms of cellular perturbations, the precise reactive species that encounters the epithelia might or might not have specificity to $O_3$ per se or to its secondary oxidants. Many of the measurable products formed as a consequence of $O_3$ exposure have limited specificity to $O_3$, such as 4-hydroxynonenal that is formed by autoxidation, an event that can be initiated by $O_3$ but also by a multitude of other oxidants. Although some classes of lipid oxidation products (e.g., specific aldehydes, cholesterol products) are specific to $O_3$, measurement in either BALF or in tissue does not necessarily provide insight regarding the compartment in which they were formed (i.e., the ELF, cell membrane, intracellular space) because the ELF is a dynamic compartment and, once formed, hydrophobic species can partition. Oxidation of membrane components might produce similar cellular outcomes regardless of the initiating oxidant. Lipid ozonides, which could be generated either within the ELF or from ozonation of cell membrane unsaturated lipids, could bind to receptors, activate signaling cascades, and act in other ways, making differences between pure extracellular reaction and direct membrane reaction indistinguishable. Thus, in some cases documenting whether $O_3$ per se reacts directly with cellular constituents might be essential (despite the challenges of in vivo demonstrations), while in other cases precisely where $O_3$ reacts might be of less concern with regard to characterizing mechanisms of health outcomes.

Thus, components of the ELF are major targets for $O_3$ and the resulting secondary oxidation products are key mediators of toxicity in the airways. The role of reaction products in $O_3$-induced toxicity is discussed in Section 5.3. The reaction cascade resulting from the interaction of $O_3$ with ELF substrates can then carry the oxidative burden deeper into cells lining the RT to elicit the health effects observed.

#### 5.2.3.1 Summary

The ELF is a complex mixture of lipids, proteins, and antioxidants that serve as the first barrier and target for inhaled $O_3$ (Figure 5-7). The thickness of the airways and alveolar surface lining layers is an important determinant of the dose of $O_3$ to the tissues. The progressive decrease in ELF thickness and increase in interfacial surface with progression from the TB region to the alveolus decreases the radial distance $O_3$ or its reaction products must travel to reach the cells lining the RT. The antioxidant substances present in the ELF appear in most cases to limit interaction of $O_3$ with underlying tissues and to prevent penetration of $O_3$ deeper into the lung. However, as the ELF thickness decreases and becomes ultra thin in the alveolar region, it may be possible for direct interaction of $O_3$ with the underlying epithelial cells to occur. The formation of secondary oxidation products is likely related to the concentration of antioxidants present and the quenching ability of the lining fluid. Mechanisms are present to replenish the antioxidant substrate pools as well as to remove secondary reaction products and prevent tissue interactions. Important differences exist in the reaction rates for $O_3$ and these ELF biomolecules and the reactivity of the resulting products. Overall, studies suggest that UA and $AH_2$ are more reactive with $O_3$ than

GSH, proteins, or lipids. In addition to contributing to the driving force for $O_3$ uptake, formation of secondary oxidation products may lead to increased cellular injury and cell signaling (discussed in Section 5.3). Studies indicate that the antioxidants might be participating in reactions where the resulting secondary oxidation products might penetrate into the tissue layer and lead to perturbations.




Note: Contents of this figure not discussed in Section 5.2 will be discussed in Section 5.3. Low molecular weight, LMW; Clara cell secretory protein, CCSP; Surfactant Protein-A, SP-A; Platelet activating factor, PAF. Ozone will react with components of the ELF to produce reaction products that may lead to cellular injury and cell signaling as discussed in Section 5.3.

**Figure 5-7 Details of the $O_3$ interaction with the airway ELF to form secondary oxidation products.**

## 5.3 Possible Pathways/Modes of Action

### 5.3.1 Introduction

Mode of action refers to a sequence of key events and processes that result in a given toxic effect (U.S. EPA, 2005). Elucidation of mechanisms provides a more detailed understanding of these key events and processes (U.S. EPA, 2005). Moreover, toxicity pathways describe the processes by which perturbation of normal biological processes produce changes sufficient to lead to cell injury and subsequent events such as adverse health effects (U.S. EPA, 2009f). The purpose of this section of Chapter 5 is to describe the key events and toxicity pathways that contribute to health effects resulting from short-term and long-term exposures to $O_3$. The extensive research carried out over several decades in humans and in laboratory animals has yielded numerous studies on mechanisms by which $O_3$ exerts its effects. This section will discuss some of the representative studies with particular emphasis on studies published since the 2006 $O_3$ AQCD (U.S. EPA, 2006b) and on studies in humans that inform biological mechanisms underlying responses to $O_3$.

It is well-appreciated that secondary oxidation products, which are formed as a result of $O_3$ exposure, initiate numerous responses at the cellular, tissue and whole organ level of the respiratory system. These responses include the activation of neural reflexes, initiation of inflammation, alteration of epithelial barrier function, sensitization of bronchial smooth muscle, modification of innate/adaptive immunity and airways remodeling, as will be discussed below. These have the potential to result in effects on other organ systems such as the cardiovascular, central nervous, hepatic and reproductive systems or result in developmental effects. It has been proposed that lipid ozonides and other secondary oxidation products, which are bioactive and cytotoxic in the respiratory system, are responsible for systemic effects. However it is not known whether they gain access to the vascular space (Chuang et al., 2009). Recent studies in animal models show that inhalation of $O_3$ results in systemic oxidative stress. The following subsections describe the current understanding of potential pathways and modes of action responsible for the pulmonary and extrapulmonary effects of $O_3$ exposure.

### 5.3.2 Activation of Neural Reflexes

Acute $O_3$ exposure results in reversible effects on lung function parameters through activation of neural reflexes. The involvement of bronchial C-fibers, a type of nociceptive sensory nerve, has been demonstrated in dogs exposed through an endotracheal tube to 2-3 ppm $O_3$ for 20-70 minutes (Coleridge et al., 1993; Schelegle et al., 1993). This vagal afferent pathway was found to be responsible for $O_3$-mediated rapid shallow breathing and other changes in respiratory mechanics in $O_3$-exposed dogs (Schelegle et al., 1993). Ozone also triggers neural reflexes that

stimulate the autonomic nervous system and alter electrophysiologic responses of the heart. For example, bradycardia, altered HRV and arrhythmia have been demonstrated in rodents exposed for several hours to 0.1-0.6 ppm $O_3$ (Hamade and Tankersley, 2009; Watkinson et al., 2001; Arito et al., 1990). Another effect is hypothermia, which in rodents occurred subsequent to the activation of neural reflexes involving the parasympathetic nervous system (Watkinson et al., 2001). Vagal afferent pathways originating in the RT may also be responsible for $O_3$-mediated activation of nucleus tractus solitarius neurons that resulted in neuronal activation in stress-responsive regions of the central nervous system (CNS) (rats, 0.5-2.0 ppm $O_3$ for 1.5-120 hours) (Gackière et al., 2011).

Recent studies in animals provide new information regarding the effects of $O_3$ on reflex responses mediated by bronchopulmonary C-fibers. In ex vivo mouse lungs, $O_3$ exposure (30 µM solubilized) selectively activated a subset of C-fiber receptors that are TRPA1 ion channels (Taylor-Clark and Undem, 2010). TRPA1 ion channels are members of the TRP family of ion channels, which are known to mediate the responses of sensory neurons to inflammatory mediators (Caceres et al., 2009). In addition to TRPA1 ion channels possibly playing a key role in $O_3$-induced decrements in pulmonary function, they may mediate allergic asthma (Caceres et al., 2009). Activation of TRPA1 ion channels following $O_3$ exposure is likely initiated by secondary oxidation products such as aldehydes and prostaglandins (Taylor-Clark and Undem, 2010) through covalent modification of cysteine and lysine residues (Trevisani et al., 2007). Ozonation of unsaturated fatty acids in the ELF was found to result in the generation of aldehydes (Frampton et al., 1999) such as 4-hydroxynonenal and 4-oxononenal (Taylor-Clark et al., 2008; Trevisani et al., 2007). 4-oxononenal is a stronger electrophile than 4-hydroxynonenal and exhibits greater potency toward the TRPA1 channels (Taylor-Clark et al., 2008; Trevisani et al., 2007). In addition, $PGE_2$ is known to sensitize TRPA1 channels (Bang et al., 2007).

In humans exercising at a moderate level, the response to $O_3$ (500 ppb for 2 h) was characterized by substernal discomfort, especially on deep inspiration, accompanied by involuntary truncation of inspiration (Hazucha et al., 1989). This latter response led to decreased inspiratory capacity and to decreased forced vital capacity (FVC) and forced expiratory volume in one second ($FEV_1$), as measured by spirometry. These changes, which occurred during $O_3$ exposure, were accompanied by decreased $V_T$ and increased respiratory frequency in human subjects. Spirometric changes in $FEV_1$ and FVC were not due to changes in respiratory muscle strength (Hazucha et al., 1989). In addition, parasympathetic involvement in the $O_3$-mediated decreases in lung volume was minimal (Mudway and Kelly, 2000), since changes in FVC or symptoms were not modified by treatment with bronchodilators such as atropine in human subjects exposed to 400 ppb $O_3$ for 2 hours while exercising at a heavy level (Beckett et al., 1985). However, the loss of vital capacity was reversible with intravenous administration of the rapid-acting opioid agonist, sufentanyl, in human subjects exercising at a moderate level and exposed to 420 ppb $O_3$ for 2 hours, which indicated the involvement of opioid receptor-containing nerve fibers and/or more central neurons (Passannante et al., 1998). The effects of sufentanyl may be

attributed to blocking C-fiber stimulation by $O_3$ since activation of opioid receptors downregulated C-fiber function (Belvisi et al., 1992). Thus, nociceptive sensory nerves, presumably bronchial C-fibers, are responsible for $O_3$-mediated responses in humans (Passannante et al., 1998). This vagal afferent pathway is responsible for pain-related symptoms and inhibition of maximal inspiration in humans (Hazucha et al., 1989).

There is some evidence that eicosanoids (see Section 5.3.3) play a role in the neural reflex since cyclooxygenase inhibition with indomethacin (Alexis et al., 2000; Schelegle et al., 1987) or ibuprofen, which also blocks some lipoxygenase activity (Hazucha et al., 1996), before exposure to $O_3$ significantly blunted the spirometric responses. These studies involved exposures of 1-2 hours to 350-400 ppb $O_3$ in human subjects exercising at light, moderate, and heavy levels. In the latter study, ibuprofen treatment resulted in measurable decreases in BALF levels of $PGE_2$ and $TXB_2$ at 1-hour postexposure (Hazucha et al., 1996). Although an earlier study demonstrated that $PGE_2$ stimulated bronchial C-fibers (Coleridge et al., 1993; Coleridge et al., 1976) and suggested that $PGE_2$ mediated $O_3$-induced decreases in pulmonary function, no correlation was observed between the degree of ibuprofen-induced inhibition of BALF $PGE_2$ levels and blunting of the spirometric response to $O_3$ (Hazucha et al., 1996). These results point to the involvement of a lipoxygenase product. Further, as noted above, $PGE_2$ may play a role in the neural reflex by sensitizing TRPA1 channels. A recent study in human subjects exercising at a moderate to high level and exposed for 1 hour to 350 ppb $O_3$ also provided evidence that arachidonic acid metabolites, as well as oxidative stress, contribute to human responsiveness to $O_3$ (Alfaro et al., 2007).

In addition to the spirometric changes, mild airways obstruction occurred in human subjects exercising at a moderate level during $O_3$ exposure (500 ppb for 2 hours) (Hazucha et al., 1989). This pulmonary function decrement is generally measured as specific airway resistance (sRaw) which is the product of airway resistance and thoracic gas volume. In several studies involving human subjects exercising at a moderate to heavy level and exposed for 1-4 hours to 200-300 ppb $O_3$, changes in sRaw correlated with changes in inflammatory and injury endpoints measured 18-hours postexposure, but did not follow the same time course or change to the same degree as spirometric changes (i.e., $FEV_1$, FVC) measured during exposure (Balmes et al., 1996; Aris et al., 1993; Schelegle et al., 1991). In addition, a small but persistent increase in airway resistance associated with narrowing of small peripheral airways (measured as changes in isovolumetric $FEF_{25-75}$) was demonstrated in $O_3$-exposed human subjects (350 ppb for 130 minutes, moderate exercise level) (Weinmann et al., 1995c; Weinmann et al., 1995b). A similar study (400 ppb $O_3$ for 2 hours in human subjects exercising at a heavy level) found decreases in $FEF_{25-75}$ concomitant with increases in residual volume, which is suggestive of small airways dysfunction (Kreit et al., 1989). In separate studies, a statistically significant increase in residual volume (500 ppb for 2 hours) (Hazucha et al., 1989) and a statistically significant decrease in $FEF_{25-75}$ (160 ppb for 7.6 hours) (Horstman et al., 1995) were observed following $O_3$ exposure in human subjects exercising at moderate and light

levels, respectively, providing further support for an $O_3$-induced effect on small airways.

Mechanisms underlying this rapid increase in airway resistance following $O_3$ exposure are incompletely understood. Pretreatment with atropine decreased baseline sRaw and prevented $O_3$-induced increases in sRaw in human subjects exercising at a heavy level (400 ppb for 0.5 hours) (Beckett et al., 1985), indicating the involvement of muscarinic cholinergic receptors of the parasympathetic nervous system. Interestingly, atropine pretreatment partially blocked the decrease in $FEV_1$, but had no effect on the decrease in FVC, breathing rate, tidal volume or respiratory symptoms (Beckett et al., 1985). Using a β-adrenergic agonist, it was shown that smooth muscle contraction, not increased airway mucus secretion, was responsible for $O_3$-induced increases in airway resistance (Beckett et al., 1985). Thus, pulmonary function decrements measured as $FEV_1$ may reflect both restrictive (such as decreased inspiratory capacity) and obstructive (such as bronchoconstriction) type changes in airway responses. This is consistent with findings of McDonnell et al. (1983) who observed a relatively strong correlation between sRaw and $FEV_1$ ($r = -0.31$, $p = 0.001$) and a far weaker correlation between sRaw and FVC ($r = -0.16$, $p = 0.10$) in human subjects exercising at a heavy level and exposed for 2.5 hours to 120-400 ppb $O_3$.

Furthermore, tachykinins may contribute to $O_3$-mediated increases in airway resistance. In addition to stimulating CNS reflexes, bronchopulmonary C-fibers mediate local axon responses by releasing neuropeptides such as substance P (SP), neurokinin (NK) A and calcitonin gene-related peptide (CGRP). Tachykinins bind to NK receptors resulting in responses such as bronchoconstriction. Recent studies in animals demonstrated that NK-1 receptor blockade had no effect on $O_3$-stimulated physiologic responses such as $V_T$ and $f_B$ in rats over the 8 hour exposure to 1 ppm $O_3$ (Oslund et al., 2008). However, SP and NK receptors contributed to vagally-mediated bronchoconstriction in guinea pigs 3 days after a single 4-hour exposure to 2 ppm $O_3$ (Verhein et al., 2011). In one human study in which bronchial biopsies were performed and studied by immunohistochemistry, SP was substantially diminished in submucosal sensory nerves 6 hours following $O_3$ exposure (200 ppb for 2 hours, light exercise) (Krishna et al., 1997). A statistically significant correlation was observed between loss of SP immunoreactivity from neurons in the bronchial mucosa and changes in $FEV_1$ measured 1-hour postexposure (Krishna et al., 1997). Another study found that SP was increased in lavage fluid of human subjects immediately after $O_3$ challenge (250 ppb for 1 hour, heavy exercise) (Hazbun et al., 1993). These results provide evidence that the increased airway resistance observed following $O_3$ exposure is due to vagally-mediated responses and possibly by local axon reflex responses through bronchopulmonary C-fiber-mediated release of SP.

A role for antioxidant defenses in modulating neural reflexes has been proposed given the delay in onset of $O_3$-induced pulmonary function responses that has been noted in numerous studies. Recently, this delay was characterized in terms of changes in $f_B$ (Schelegle et al., 2007). In humans exposed for 1-2 hours to

120-350 ppb $O_3$ while exercising at a high level, no change in $f_B$ was observed until a certain cumulative inhaled dose of $O_3$ had been reached. Subsequently, the magnitude of the change in $f_B$ was correlated with the inhaled dose rate (Schelegle et al., 2007). These investigators proposed that initial reactions of $O_3$ with ELF resulted in a time-dependent depletion of ELF antioxidants, and that activation of neural reflexes occurred only after the antioxidant defenses were overwhelmed (Schelegle et al., 2007).

### 5.3.3 Initiation of inflammation

As described previously (Section 5.2.3), $O_3$ mainly reacts with components of the ELF and cellular membranes resulting in the generation of secondary oxidation products. Higher concentrations of these products may directly injure RT epithelium. Subsequent airways remodeling may also occur (Section 5.3.7) (Mudway and Kelly, 2000). Lower concentrations of secondary oxidation products may initiate cellular responses including cytokine generation, adhesion molecule expression, and modification of tight junctions leading to inflammation and increased permeability across airway epithelium (Section 5.3.4) (Dahl et al., 2007; Mudway and Kelly, 2000).

An important hallmark of acute $O_3$ exposure in humans and animals is neutrophilic airways inflammation. Neutrophil influx into nasal airways has been demonstrated in human subjects (400 ppb $O_3$ 2 hours, heavy exercise) (Graham and Koren, 1990) and in rats (0.8 ppm $O_3$, 6 hours) (Hotchkiss et al., 1989). Many studies of neutrophil influx have focused on the lower airways (Hazucha et al., 1996; Aris et al., 1993). The time course of this response in the lower airways and its resolution appears to be slower than that of the decrements in pulmonary function in exercising human subjects (Hazucha et al., 1996). In several studies, airways neutrophilia was observed by 1-3 hours, peaked by 6 hours and was returning to baseline levels at 18-24 hours in human subjects exercising at a heavy level and exposed for 1-2 hours to 300-400 ppb $O_3$ (Schelegle et al., 1991; Koren et al., 1989; Seltzer et al., 1986). Neutrophils are thought to be injurious and a study in guinea pigs demonstrated that the influx and persistence of neutrophils in airways following $O_3$ exposure correlated with the temporal profile of epithelial injury (0.26-1 ppm $O_3$, 72 hours) (Hu et al., 1982). However, neutrophils have also been shown to contribute to repair of $O_3$-injured epithelium in rats exposed for 8 hours to 1 ppm $O_3$, possibly by removing necrotic epithelial cells (Mudway and Kelly, 2000; Vesely et al., 1999). Nonetheless, the degree of airways inflammation due to $O_3$ is thought to have more important long-term consequences than the more quickly resolving changes in pulmonary function since airways inflammation is often accompanied by tissue injury (Balmes et al., 1996).

Ozone exposure results in alterations in other airways inflammatory cells besides neutrophils, including lymphocytes, macrophages, monocytes and mast cells. Influx of some of these cells accounts for the later (i.e., 18-20 hours) phase of inflammation

following $O_3$ exposure. Numbers of lymphocytes and total cells in BALF were decreased early after $O_3$ exposure in human subjects exercising at a light to moderate level and exposed for 2 hours to 200 ppb $O_3$, which preceded the neutrophil influx (Mudway and Kelly, 2000; Blomberg et al., 1999; Krishna et al., 1997). The decrease in total cells was thought to reflect decreases in macrophages, although it was not clear whether the cells were necrotic or whether membrane adhesive properties were altered making them more difficult to obtain by lavage (Mudway and Kelly, 2000; Blomberg et al., 1999; Mudway et al., 1999b; Frampton et al., 1997b; Pearson and Bhalla, 1997). A recent study in human subjects exercising at a moderate level and exposed for 6.6 hours to 80 ppb $O_3$ demonstrated an increase in numbers of sputum monocytes and dendritic-like cells with increased expression of innate immune surface proteins and antigen presentation markers (Alexis et al., 2010). An increase in submucosal mast cells was observed 1.5 hours after a 2 hour-exposure to 200 ppb $O_3$ (Blomberg et al., 1999) and an increase in BAL mast cell number was observed 18 hours after a 4-hour exposure to 220 ppb $O_3$ exposure in human subjects exercising at a moderate level (Frampton et al., 1997b). Mast cells may play an important role in mediating neutrophil influx since they are an important source of several pro-inflammatory cytokines and since their influx preceded that of neutrophils in human subjects exercising at a moderate level and exposed for 2 hours to 200 ppb $O_3$ (Stenfors et al., 2002; Blomberg et al., 1999). Further, a study using mast cell-deficient mice demonstrated decreased neutrophilic inflammation in response to $O_3$ (1.75 ppm, 3 hours) compared with wild type mice (Kleeberger et al., 1993). Influx of these inflammatory cell types in the lung is indicative of $O_3$-mediated activation of innate immunity as will be discussed in Section 5.3.6.

Much is known about the cellular and molecular signals involved in inflammatory responses to $O_3$ exposure (U.S. EPA, 2006b). Eicosanoids are one class of secondary oxidation products that may be formed rapidly following $O_3$ exposure and that may mediate inflammation. Eicosanoids are metabolites of arachidonic acid—a 20-carbon PUFA—that are released from membrane phospholipids by phospholipase A2-mediated catalysis. Activation of phospholipase A2 occurs by several cell signaling pathways and may be triggered by $O_3$-mediated lipid peroxidation of cellular membranes (Rashba-Step et al., 1997). Additionally, cellular phospholipases A2, C and D may be activated by lipid ozonation products (Kafoury et al., 1998). While the conversion of arachidonic acid to prostaglandins, leukotrienes and other eicosanoid products is generally catalyzed by cyclooxygenases and lipoxygenases, non-enzymatic reactions also occur during oxidative stress leading to the generation of a wide variety of eicosanoids and reactive oxygen species. Further, the release of arachidonic acid from phospholipids is accompanied by the formation of lysophospholipids that are precursors for platelet activating factors (PAFs). Thus, formation of eicosanoids, reactive oxygen species and PAFs accompanies $O_3$-mediated lipid peroxidation.

In addition, secondary reaction products may stimulate macrophages to produce cytokines such as IL-1, IL-6, and TNF-α that in turn activate IL-8 production by epithelial cells. Although IL-8 has been proposed to play a role in neutrophil chemotaxis, measurements of IL-8 in BALF from humans exposed to $O_3$ found

increases that were too late to account for this effect (Mudway and Kelly, 2000). The time-course profiles of $PGE_2$ and IL-6 responses suggest that they may play a role in neutrophil chemotaxis in humans (Mudway and Kelly, 2000). However, pretreatment with ibuprofen attenuated $O_3$-induced increases in BALF $PGE_2$ levels, but had no effect on neutrophilia in human subjects exercising at a heavy level and exposed for 2 hour to 400 ppb $O_3$ (Hazucha et al., 1996).

One set of studies in humans focused on the earliest phase of airways inflammation (1-2 hours following exposure). Human subjects, exercising at a moderate level, were exposed to 200 ppb $O_3$ for 2 hours and bronchial biopsy tissues were obtained 1.5 and 6 hours after exposure (Bosson et al., 2009; Bosson et al., 2003; Stenfors et al., 2002; Blomberg et al., 1999). Results demonstrated upregulation of vascular endothelial adhesion molecules P-selectin and ICAM-1 at both 1.5 and 6 hours (Stenfors et al., 2002; Blomberg et al., 1999). Submucosal mast cell numbers were increased at 1.5 hours in the biopsy samples without an accompanying increase in neutrophil number (Blomberg et al., 1999). Pronounced neutrophil infiltration was observed at 6 hours in the bronchial mucosa (Stenfors et al., 2002). Surprisingly, suppression of the NF-κB and AP-1 pathways at 1.5 hours and a lack of increased IL-8 at 1.5 or 6 hours in bronchial epithelium were observed (Bosson et al., 2009). The authors suggested that vascular endothelial adhesion molecules, rather than redox sensitive transcription factors, play key roles in early neutrophil recruitment in response to $O_3$.

Increases in markers of inflammation occurred to a comparable degree in human subjects with mild (least sensitive) and more remarkable (more sensitive) spirometric responses to $O_3$ (200 ppb, 4 hours, moderate exercise) (Balmes et al., 1996). Two other studies (200 ppb for 4 hours with moderate exercise and 300 ppb for 1 hour with heavy exercise) found that acute spirometric changes were not positively correlated with cellular and biochemical indicators of inflammation (Aris et al., 1993; Schelegle et al., 1991). However inflammation was correlated with changes in sRaw (Balmes et al., 1996). In another study, pretreatment with ibuprofen had no effect on neutrophilia although it blunted the spirometric response in human subjects exercising at heavy level and exposed for 2 hours to 400 ppb $O_3$ (Hazucha et al., 1996). Taken together, results from these studies indicate different mechanisms underlying the spirometric and inflammatory responses to $O_3$.

A common mechanism underlying both inflammation and impaired pulmonary function was suggested by Krishna et al. (1997). This study, conducted in human subjects exercising at a light level and exposed to 200 ppb $O_3$ for 2 hours, demonstrated a correlation between loss of SP immunoreactivity from neurons in the bronchial mucosa and numbers of neutrophils and epithelial cells (shed epithelial cells are an index of injury) in the BALF 6-hours postexposure. Furthermore, the loss of SP immunoreactivity was correlated with the observed changes in $FEV_1$. Another study found that SP was increased in lavage fluid of exercising human subjects immediately after $O_3$ challenge (250 ppb, 1 hour, heavy exercise) (Hazbun et al., 1993). SP is a neuropeptide released by sensory nerves which mediates neurogenic edema and bronchoconstriction (Krishna et al., 1997). Collectively, these findings

suggest that $O_3$-mediated stimulation of sensory nerves that leads to activation of central and local axon reflexes is a common effector pathway leading to impaired pulmonary function and inflammation.

Studies in animal models have confirmed many of these findings and provided evidence for additional mechanisms involved in $O_3$-induced inflammation. A study in mice (2 ppm $O_3$, 3 hours) demonstrated that PAF may be important in neutrophil chemotaxis (Longphre et al., 1999), while ICAM-1 and macrophage inflammatory protein-2 (MIP-2), the rodent IL-8 homologue, have been implicated in a rat model (1 ppm $O_3$, 3 hours) (Bhalla and Gupta, 2000). Another study found that TNF receptor, NF-κB and JNK1 mediated lung inflammation induced by $O_3$ in mice (0.3 ppm $O_3$, 6 and 24 hours) (Cho et al., 2007). Key roles for CXCR2, a receptor for keratinocyte-derived chemokine (KC) and MIP-2, and for IL-6 in $O_3$-mediated neutrophil influx were demonstrated in mice (1 ppm $O_3$, 3 hours) (Johnston et al., 2005a; Johnston et al., 2005b). Activation of JNK and p38 pathways and cathepsin-S were also found to be important in this response (3 ppm $O_3$, 3 hours) (Williams et al., 2009a; Williams et al., 2008a; Williams et al., 2007a). Matrix metalloproteinase-9 (MMP-9) appeared to confer protection against $O_3$-induced airways inflammation and injury in mice (0.3 ppm $O_3$, 6-72 hours) (Yoon et al., 2007). Interleukin-10 (IL-10) also appeared to be protective since IL-10 deficient mice responded to $O_3$ exposure (0.3 ppm, 24-72 hours) with enhanced numbers of BAL neutrophils, enhanced NF-κB activation and MIP-2 levels compared with IL-10 sufficient mice (Backus et al., 2010).

In addition, lung epithelial cells may release ATP in response to $O_3$ exposure (Ahmad et al., 2005). ATP and its metabolites (catalyzed by ecto-enzymes) can bind to cellular purinergic receptors resulting in activation of cell signaling pathways (Picher et al., 2004). One such metabolite, adenine, is capable of undergoing oxidation leading to the formation of UA which, if present in high concentrations, could activate inflammasomes and result in caspase 1 activation and the maturation and secretion of IL-1β and IL-18 (Dostert et al., 2008). A recent study in human subjects exercising at a moderate level and exposed for 2 hours to 400 ppb $O_3$ demonstrated a correlation between ATP metabolites and inflammatory markers (Esther et al., 2011), which provides some support for this mechanism.

Several recent studies have focused on the role of Toll-like receptor (TLR) and its related adaptor protein MyD88 in mediating $O_3$-induced neutrophilia. Hollingsworth et al. (2004) demonstrated airways neutrophilia that was TLR4-independent following acute (2 ppm, 3 hours) and subchronic (0.3 ppm, 72 hours) $O_3$ exposure in a mouse model. However, Williams et al. (2007b) found that MyD88 was important in mediating $O_3$-induced neutrophilia in mice (3 ppm, 3 hours), with TLR4 and TLR2 contributing to the speed of the response. Moreover, MyD88, TLR2 and TLR4 contributed to inflammatory gene expression in this model and $O_3$ upregulated MyD88, TLR4 and TLR4 gene expression (Williams et al., 2007a). Neutrophilic inflammation was also found to be partially dependent on MyD88 in mice exposed to 1 ppm $O_3$ for 3 hours (Li et al., 2011).

Hyaluronan was found to mediate a later phase (24 hours) of $O_3$-induced inflammation in mice (Garantziotis et al., 2010; Garantziotis et al., 2009). Hyaluronan is an extracellular matrix component that is normally found in the ELF as a large polymer. Exposure to 2 ppm $O_3$ for 3 hours resulted in elevated levels of soluble low molecular weight hyaluronan in the BALF 24-hours postexposure (Garantziotis et al., 2010; Garantziotis et al., 2009). Similar results were found in response to 3 hour exposure to 1 ppm $O_3$ (Li et al., 2011). Ozone may have caused the depolymerization of hyaluronan to soluble fragments that are known to be endogenous ligands of the CD44 receptor and TLR4 in the macrophage (Jiang et al., 2005). Binding of hyaluronan fragments to the CD44 receptor activates hyaluronan clearance, while binding to TLR4 results in signaling through MyD88 to produce chemokines that stimulate the influx of inflammatory cells (Jiang et al., 2005). Activation of NF-κB occurred in both airway epithelia and alveolar macrophages 24-hours postexposure to $O_3$. Increases in BALF pro-inflammatory factors KC, IL-1β, MCP-1, TNF-α and IL-6 observed 24 hours following $O_3$ exposure were found to be partially dependent on TLR4 (Garantziotis et al., 2010) while increases in BAL inflammatory cells, which consisted mainly of macrophages, were dependent on CD44 (Garantziotis et al., 2009). BAL inflammatory cells number and injury markers following $O_3$ exposure were similar in wild-type and TLR4-deficient animals (Garantziotis et al., 2010).

Since exposure to $O_3$ leads to airways inflammation characterized by neutrophilia, and since neutrophil-derived oxidants often consume ELF antioxidants, concentrations of ELF antioxidants have been examined during airways neutrophilia (Long et al., 2001; Gunnison and Hatch, 1999; Mudway et al., 1999b). In human subjects exercising at a moderate level and exposed to 200 ppb $O_3$ for 2 hours, UA, GSH and α-TOH levels remained unchanged in BALF 6-hours postexposure while $AH_2$ was decreased significantly in both BALF and plasma (Mudway et al., 1999b). A second study involving the same protocol reported a loss of $AH_2$ from bronchial wash fluid and BALF, representing proximal and distal airway ELF respectively, as well as an increase in oxidized GSH in both compartments (Mudway et al., 2001). No change was observed in ELF UA levels in response to $O_3$ (Mudway et al., 2001). Further, $O_3$ exposure (0.8 ppm, 4 hours) in female rats resulted in a 50% decrease in BALF $AH_2$ immediately postexposure (Gunnison and Hatch, 1999). These studies suggested a role for $AH_2$ and GSH in protecting against the oxidative stress associated with inflammation.

The relationship between inflammation, antioxidant status and $O_3$ dose has also been investigated. The degree of inflammation in rats has been correlated with $^{18}O$-labeled $O_3$ dose markers in the lower lung. In female rats exposed to 0.8 ppm $O_3$ for 4 hours, BAL neutrophil number and $^{18}O$ reaction product were directly proportional (Gunnison and Hatch, 1999). Kari et al. (1997) observed that a 3-week caloric restriction (75%) in rats abrogated the toxicity of $O_3$ (2 ppm, 2 hours), measured as BALF increases in protein, fibronectin and neutrophils, that was seen in normally fed rats. Accompanying this resistance to $O_3$ toxicity was a reduction (30%) in the accumulation of $^{18}O$ reaction product in the lungs. These investigations also demonstrated an inverse relationship between $AH_2$ levels and $O_3$ dose and provided

evidence for $AH_2$ playing a protective role following $O_3$ exposure in these studies. Pregnant and lactating rats had lower $AH_2$ content in BALF and exhibited a greater increase in accumulation of $^{18}O$ reaction products compared with pre-pregnant rats in response to $O_3$ exposure (Gunnison and Hatch, 1999). In the calorie restricted model, a 30% higher basal BALF $AH_2$ concentration and a rapid accumulation of $AH_2$ into the lungs to levels 60% above normal occurred as result of $O_3$ exposure (Kari et al., 1997). However, this relationship between $AH_2$ levels and $O_3$ dose did not hold up in every study. Aging rats (9 and 24 months old) had 49% and 64% lower $AH_2$ in lung tissue compared with month-old rats but the aging-induced $AH_2$ loss did not increase the accumulation of $^{18}O$ reaction products following $O_3$ exposure (0.4-0.8 ppm, 2-6 hours) (Vincent et al., 1996b).

A few studies have examined the dose- or concentration-responsiveness of airways neutrophilia in $O_3$-exposed humans (Holz et al., 1999; Devlin et al., 1991). No concentration-responsiveness was observed in healthy human subjects exposed for 1 hour to 125-250 ppb $O_3$ while exercising at a light level and a statistically significant increase in sputum neutrophilia was observed only at the higher concentration (Holz et al., 1999). However, concentration-dependent and statistically significant increases in BAL neutrophils and the inflammatory mediator IL-6 were reported following exposure to 80 and 100 ppb $O_3$ for 6.6 hours in human subjects exercising at a moderate level (Devlin et al., 1991). Additional evidence is provided by a meta-analysis of the $O_3$ dose-inflammatory response in controlled human exposure studies involving exposure to 80-600 ppb $O_3$ for 60-396 minutes and exercise levels ranging from light to heavy (Mudway and Kelly, 2004b). Results demonstrated a linear relationship between inhaled $O_3$ dose (determined as the product of concentration, ventilation and time) and BAL neutrophils at 0-6 hours and 18-24 hours following $O_3$ exposure (Mudway and Kelly, 2004b).

### 5.3.4 Alteration of Epithelial Barrier Function

Following $O_3$ exposure, injury and inflammation can lead to altered airway barrier function. Histologic analysis has demonstrated damage to tight junctions between epithelial cells, suggesting an increase in epithelial permeability. In addition, the presence of shed epithelial cells in the BALF and increased epithelial permeability, which is measured as the flux of small solutes, have been observed and are indicative of epithelial injury. This could potentially lead to the loss of ELF solutes that could diffuse down their concentration gradient from the lung to the blood. Increases in vascular permeability, as measured by BALF protein and albumin, have also been demonstrated (Costa et al., 1985; Hu et al., 1982).

An early study in sheep measured changes in airway permeability as the flux of inhaled radiolabeled histamine into the plasma (Abraham et al., 1984). Exposure of sheep to 0.5 ppm $O_3$ for 2 hours via an endotracheal tube resulted in an increased rate of histamine appearance in the plasma at 1 day postexposure. Subsequently, numerous studies have measured epithelial permeability as the flux of the small

solute $^{99m}$Tc-DTPA that was introduced into the air spaces in different regions of the RT. Increased pulmonary epithelial permeability, measured as the clearance of $^{99m}$Tc-DTPA from lung to blood, was demonstrated in humans 1-2 hours following a 2-hour exposure to 400 ppb $O_3$ while exercising at a heavy level (Kehrl et al., 1987). Another study in human subjects found increased epithelial permeability 19-hours postexposure to 240 ppb $O_3$ for 130 minutes while exercising at moderate level (Foster and Stetkiewicz, 1996). Increased bronchial permeability was also observed in dogs 1-day postexposure (0.4 ppm $O_3$ by endotracheal tube for 6 hours) and did not resolve for several days (Foster and Freed, 1999).

A role for tachykinins in mediating airway epithelial injury and decreased barrier function has been suggested. Nishiyama et al. (1998) demonstrated that capsaicin, which depletes nerve fibers of substance P, blocked the $O_3$-induced increase in permeability of guinea pig tracheal mucosa (0.5-3 ppm $O_3$, 0.5 hours). Pretreatment with propranolol or atropine failed to inhibit this response, suggesting that adrenergic and cholinergic pathways were not involved. In another study, tachykinins working through NK-1 and CGRP receptors were found to contribute to airway epithelial injury in $O_3$-exposed rats (1 ppm, 8 hours) (Oslund et al., 2009, 2008).

Kleeberger et al. (2000) evaluated genetic susceptibility to $O_3$-induced altered barrier function in recombinant inbred strains of mice. Lung hyperpermeability, measured as BALF protein, was evaluated 72 hours after exposure to 0.3 ppm $O_3$ and found to be associated with a functioning *Tlr4* gene. This study concluded that *Tlr4* was a strong candidate gene for susceptibility to hyperpermeability in response to $O_3$ (Kleeberger et al., 2000). A subsequent study by these same investigators found that *Tlr4* modulated mRNA levels of the *Nos2* genes and suggested that the protein product of *Nos2*, iNOS, plays an important role in $O_2$-induced lung hyperpermeability (0.3 ppm, 72 hours) (Kleeberger et al., 2001). More recently, HSP70 was identified as part of the TLR4 signaling pathway (0.3 ppm, 6-72 hours) (Bauer et al., 2011).

Antioxidants have been shown to confer resistance to $O_3$-induced injury. In a recent study, lung hyperpermeability in response to $O_3$ (0.3 ppm, 48 hours) was unexpectedly reduced in mice deficient in the glutamate-cysteine ligase modifier subunit gene compared with sufficient mice (Johansson et al., 2010). Since the lungs of these mice exhibited 70% glutathione depletion, protection against $O_3$-induced injury was unexpected (Johansson et al., 2010). However it was found that several other antioxidant defenses, including metallothionein, were upregulated in response to $O_3$ to a greater degree in the glutathione-deficient mice compared with sufficient mice (Johansson et al., 2010). The authors suggested that resistance to $O_3$-induced lung injury was due to compensatory augmentation of antioxidant defenses (Johansson et al., 2010). Antioxidant effects have also been attributed to Clara cell secretory protein (CCSP) and surfactant protein A (SP-A). CCSP was found to modulate the susceptibility of airway epithelium to injury in mice exposed to $O_3$ (0.2 or 1 ppm for 8 hours) by an unknown mechanism (Plopper et al., 2006). SP-A appeared to confer protection against $O_3$-induced airways inflammation and injury in mice (2 ppm, 3 hours) (Haque et al., 2007).

Increased epithelial permeability has been proposed to play a role in allergic sensitization (Matsumura, 1970), in activation of neural reflexes and in stimulation of smooth muscle receptors (Dimeo et al., 1981). Abraham et al. (1984) reported a correlation between airway permeability and airways hyperresponsiveness (AHR) in $O_3$-exposed sheep. However a recent study in human subjects exposed to 220 ppb $O_3$ for 135 minutes while exercising at a light to moderate level did not find a relationship between $O_3$-induced changes in airway permeability and AHR (Que et al., 2011).

### 5.3.5 Sensitization of Bronchial Smooth Muscle

Bronchial reactivity is generally determined in terms of a response to a challenge agent. Non-specific bronchial reactivity in humans is assessed by measuring the effect of inhaling increasing concentrations of a bronchoconstrictive drug on lung mechanics (sRaw or $FEV_1$). Methacholine is most commonly employed but histamine and other agents are also used. Specific bronchial reactivity is assessed by measuring effects in response to an inhaled allergen in individuals (or animals) already sensitized to that allergen. An increase in sRaw in response to non-specific or specific challenge agents indicates AHR.

In addition to causing mild airways obstruction as discussed above, acute $O_3$ exposure results in reversible increases in bronchial reactivity by mechanisms that are not well understood. In one study, bronchial reactivity of healthy subjects was significantly increased 19-hours postexposure to $O_3$ (120-240 ppb $O_3$ for 2 hours with moderate exercise) (Foster et al., 2000). These effects may be more considerable in human subjects with already compromised airways (Section 5.4.2.2).

Ozone may sensitize bronchial smooth muscle to stimulation through an exposure-related effect on smooth muscle or through effects on the sensory nerves in the epithelium or on the motor nerves innervating the smooth muscle (O'Byrne et al., 1984; O'Byrne et al., 1983; Holtzman et al., 1979). It is also recognized that increased bronchial reactivity can be both a rapidly occurring and a persistent response to $O_3$ (Foster and Freed, 1999). Tachykinins and secondary oxidation products of $O_3$ have been proposed as mediators of the early response and inflammation-derived products have been proposed as mediators of the later response (Foster and Freed, 1999). Furthermore, bronchial reactivity may be increased as a result of $O_3$-mediated generation of ROS.

Ozone-induced increases in epithelial permeability, which could improve access of agonist to smooth muscle receptors, may be one mechanism of sensitization through a direct effect on bronchial smooth muscle (Holtzman et al., 1979). As noted above, a correlation between airway permeability and AHR has been reported in $O_3$-exposed sheep (Abraham et al., 1984) but not in $O_3$-exposed human subjects (Que et al., 2011).

Neurally-mediated sensitization has been demonstrated. In human subjects exposed for 2 hours to 600 ppb $O_3$ while exercising at a light level, pretreatment with atropine inhibited $O_3$-induced AHR, suggesting the involvement of cholinergic postganglionic pathways (Holtzman et al., 1979). Animal studies have demonstrated that $O_3$-induced AHR involved vagally-mediated responses (rabbits, 0.2 ppm $O_3$, 72 hours) (Freed et al., 1996) and local axon reflex responses through bronchopulmonary C-fiber-mediated release of SP (guinea pigs, 0.8 ppm $O_3$, 2 hours) (Joad et al., 1996). Further, pretreatment with capsaicin to deplete nerve fibers of SP blocked $O_3$-mediated AHR (guinea pigs, 1-2 ppm $O_3$, 2-2.25 hours) (Tepper et al., 1993). Other investigators demonstrated that SP released from airway nociceptive neurons in ferrets contributed to $O_3$-induced AHR (2 ppm $O_3$, 3 hours) (Wu et al., 2008c; Wu et al., 2003).

Some evidence suggests the involvement of arachidonic acid metabolites and neutrophils in mediating $O_3$-induced AHR (Seltzer et al., 1986; Fabbri et al., 1985). Increased BAL neutrophils and cyclooxygenase products were found in one study demonstrating AHR in human subjects exercising at a heavy level immediately postexposure to 600 ppb $O_3$ for 2 hours (Seltzer et al., 1986). Another study found that ibuprofen pretreatment had no effect on AHR in human subjects exercising at a heavy level following exposure to 400 ppb $O_3$ for 2 hours, although spirometric responses were blunted (Hazucha et al., 1996). This study measured arachidonic acid metabolites and provided evidence that that the arachidonic acid metabolites whose generation was blocked by ibuprofen, (i.e., prostaglandins, thromboxanes and some leukotrienes) did not play a role in AHR. Experiments in dogs exposed for 2 hours to 2.1 ppm $O_3$ demonstrated a close correlation between $O_3$-induced AHR and airways neutrophilic inflammation measured in tissue biopsies (Holtzman et al., 1983). Furthermore, the increased AHR observed in dogs following $O_3$ exposure (3 ppm, 2 hours) was inhibited by neutrophil depletion (O'Byrne et al., 1983) and by pre-treatment with inhibitors of arachidonic acid metabolism. In one of these studies, indomethacin pre-treatment did not prevent airways neutrophilia in response to $O_3$ (3 ppm, 2 hours) providing evidence that the subset of arachidonic acid metabolites whose generation was inhibitable by the cyclooxygenase inhibitor indomethacin (i.e., prostaglandins and thromboxanes) was not responsible for neutrophil influx (O'Byrne et al., 1984). It should be noted that these studies did not measure whether the degree to which the inhibitor blocked arachidonic acid metabolism and thus their results should be interpreted with caution. Taken together, these findings suggest that arachidonic acid metabolites may be involved in the AHR response following $O_3$ exposure in dogs. Studies probing the role of neutrophils in mediating the AHR response have provided inconsistent results (Al-Hegelan et al., 2011).

Evidence for cytokine and chemokine involvement in the AHR response to $O_3$ has been described. Some studies have suggested a role for TNF-α (mice, 0.5 and 2 ppm $O_3$, 3 hours) (Cho et al., 2001; Shore et al., 2001) and IL-1 (mice and ferrets, 2 ppm $O_3$, 3 hours) (Wu et al., 2008c; Park et al., 2004). The latter study found that SP expression in airway neurons was upregulated by IL-1 that was released in response to $O_3$. Other studies in mice have demonstrated a key role for CXCR2, the chemokine receptor for the neutrophil chemokines KC and MIP-2, but not for IL-6 in

$O_3$-mediated AHR (1 ppm $O_3$, 3 hours) (Johnston et al., 2005a; Johnston et al., 2005b). In contrast, CXCR2 and IL-6 were both required for neutrophil influx in this model (Johnston et al., 2005a; Johnston et al., 2005b), as discussed above. Williams et al. (2008b) demonstrated that the Th2 cytokine IL-13 contributed to AHR, as well as to airways neutrophilia, in mice (3 ppm $O_3$, 3 hours).

Other studies have focused on the role of TLR4. Hollingsworth et al. (2004) measured AHR, as well as airways neutrophilia, in mice 6 and 24 hours following acute (2 ppm $O_3$ for 3 hours) and subchronic (0.3 ppm for 3 days) exposure to $O_3$. TLR4 is a key component of the innate immune system and is responsible for the immediate inflammatory response seen following challenge with endotoxin and other pathogen-associated substances. In this study, a functioning TLR4 was required for the full AHR response following $O_3$ exposure but not for airways neutrophilia (Hollingsworth et al., 2004). These findings are complemented by an earlier study demonstrating that $O_3$ effects on lung hyperpermeability required a functioning TLR4 (mice, 0.3 ppm $O_3$, 72 hours) (Kleeberger et al., 2000). Williams et al. (2007b) found that TLR2, TLR4 and the TLR adaptor protein MyD88 contributed to AHR in mice (3 ppm $O_3$, 3 hours). Ozone was also found to upregulate MyD88, TLR4 and TLR4 gene expression in this model (Williams et al., 2007b). Furthermore, a recent study reported $O_3$-induced AHR that required TLR4 and MyD88 in mice exposed to 1 ppm $O_3$ for 3 hours (Li et al., 2011).

A newly recognized mechanistic basis for $O_3$-induced AHR is provided by studies focusing on the role of hyaluronan following $O_3$ exposure in mice (Garantziotis et al., 2010; Garantziotis et al., 2009). Hyaluronan is an extracellular matrix component that is normally found in the ELF as a large polymer. Briefly, TLR4 and CD44 were found to mediate AHR in response to $O_3$ and hyaluronan. Exposure to 2 ppm $O_3$ for 3 hours resulted in enhanced AHR and elevated levels of soluble low molecular weight hyaluronan in the BALF 24-hours postexposure (Garantziotis et al., 2010; Garantziotis et al., 2009). Ozone may have caused the depolymerization of hyaluronan to soluble fragments that are known to be endogenous ligands of the CD44 receptor and TLR4 in the macrophage (Jiang et al., 2005). In the two recent studies, $O_3$-induced AHR was attenuated in CD44 and TLR4-deficient mice (Garantziotis et al., 2010; Garantziotis et al., 2009). Hyaluronan fragment-mediated stimulation of AHR was found to require functioning CD44 receptor and TLR4 (Garantziotis et al., 2010; Garantziotis et al., 2009). In contrast, high-molecular-weight hyaluronan blocked AHR in response to $O_3$ (Garantziotis et al., 2009). In another study high-molecular-weight hyaluronan enhanced repair of epithelial injury (Jiang et al., 2005). These studies provide a link between innate immunity and the development of AHR following $O_3$ exposure, and indicate a role for TLR4 in increasing airways responsiveness. While TLR4-dependent responses usually involve activation of NF-κB and the upregulation of proinflammatory factors, the precise mechanisms leading to AHR are unknown (Al-Hegelan et al., 2011).

In guinea pigs, AHR was found to be mediated by different pathways at 1- and 3-days postexposure to a single exposure of $O_3$ (2 ppm for 4 hours) (Verhein et al., 2011; Yost et al., 2005). At 1 day, AHR was due to activation of airway

parasympathetic nerves rather than to an exposure-related effect on smooth muscle (Yost et al., 2005). This effect occurred as a result of $O_3$-stimulated release of major basic protein from eosinophils (Yost et al., 2005). Major basic protein is known to block inhibitory M2 muscarinic receptors that normally dampen acetylcholine release from parasympathetic nerves (Yost et al., 2005). The resulting increase in acetylcholine release caused an increase in smooth muscle contraction following $O_3$ exposure (Yost et al., 2005). Eosinophils played a different role 3-days postexposure to $O_3$ in guinea pigs (Yost et al., 2005). Ozone-mediated influx of eosinophils into lung airways resulted in a different population of cells present 3-days postexposure compared to those present at 1 day (Yost et al., 2005). At this time point, eosinophil-derived major basic protein increased smooth muscle responsiveness to acetylcholine which also contributed to AHR (Yost et al., 2005). However, the major effect of eosinophils was to protect against vagal hyperreactivity (Yost et al., 2005). The authors suggested that these beneficial effects were due to the production of nerve growth factor (Yost et al., 2005). Further work by these investigators demonstrated a key role for IL-1β in mediating AHR 3-days postexposure to $O_3$ (Verhein et al., 2011). In this study, IL-1β increased nerve growth factor and SP that acted through the NK1 receptor to cause vagally-mediated bronchoconstriction (Verhein et al., 2011). The mechanism by which SP caused acetylcholine release from parasympathetic nerves following $O_3$ exposure was not determined (Verhein et al., 2011). Taken together, the above study results indicate that mechanisms involved in $O_3$-mediated AHR can vary over time postexposure and that eosinophils and SP can play a role. Results of this animal model may provide some insight into allergic airways disease in humans that is characterized by eosinophilia (Section 5.4.2.2).

### 5.3.6 Modification of Innate/Adaptive Immune System Responses

Host defense depends on effective barrier function and on innate immunity and adaptive immunity (Al-Hegelan et al., 2011). The effects of $O_3$ on barrier function in the airways were discussed above (Section 5.3.4). This section focuses on the mechanisms by which $O_3$ impacts innate and adaptive immunity. Both tissue damage and foreign pathogens are triggers for the activation of the innate immune system. This results in the influx of inflammatory cells such as neutrophils, mast cells, basophils, eosinophils, monocytes and dendritic cells and the generation of cytokines such as TNF-α, IL-1, IL-6, KC and IL-17. Further, innate immunity encompasses the actions of complement and collections, and the phagocytic functions of macrophages, neutrophils and dendritic cells. Airway epithelium also contributes to innate immune responses. Innate immunity is highly dependent on cell signaling networks involving TLR4. Adaptive immunity provides immunologic memory through the actions of B and T-cells. Important links between the two systems are provided by dendritic cells and antigen presentation. Recent studies demonstrate that exposure to $O_3$ modifies cells and processes which are required for innate immunity, contributes to innate-adaptive immune system interaction and primes pulmonary immune responses to endotoxin.

Ozone exposure of human subjects resulted in recruitment of activated innate immune cells to the airways. Healthy individuals were exposed to 80 ppb $O_3$ for 6.6 hours while exercising at a moderate level and airways inflammation was characterized in induced sputum 18-hours postexposure (Alexis et al., 2010). Previous studies demonstrated that induced sputum contains liquid and cellular constituents of the ELF from central conducting airways (Alexis et al., 2001b) and also identified these airways as a site of preferential $O_3$ absorption during exercise (Hu et al., 1994). Ozone exposure resulted in increased numbers of neutrophils, airway monocytes and dendritic-like cells in sputum (Alexis et al., 2010). In addition, increased expression of cell surface markers characteristic of innate immunity and antigen presentation (i.e., CD-14 and HLA-DR) was demonstrated on airway monocytes (Alexis et al., 2010). Enhanced antigen presentation contributes to exaggerated T-cell responses and promotes Th2 inflammation and an allergic phenotype (Lay et al., 2007). Upregulation of pro-inflammatory cytokines was also demonstrated in sputum of $O_3$-exposed subjects (Alexis et al., 2010). One of these cytokines, IL-12p70, correlated with numbers of dendritic-like cells in the sputum, and is an indicator of dendritic cell activation (Alexis et al., 2010). These authors have previously reported that exposure of human subjects exercising at a light to moderate level to 400 ppb $O_3$ for 2 hours resulted in activation of monocytes and macrophages (Lay et al., 2007), which could play a role in exacerbating existing asthma by activating allergen-specific memory T-cells. The current study confirms these findings and extends them by suggesting a potential mechanism whereby $O_3$-activated dendritic cells could stimulate naïve T-cells to promote the development of asthma (Alexis et al., 2010). A companion study by these same investigators (described in detail in Section 5.4.2.1) provides evidence of dendritic cell activation, measured as increased expression of HLA-DR, in a subset of the human subjects (GSTM1 null) exposed to 400 ppb $O_3$ for 2 hours while exercising at a light to moderate level (Alexis et al., 2009). Since dendritic cells are a link between innate and adaptive immunity, these studies provide evidence for an $O_3$-mediated interaction between the innate and adaptive immune systems.

Another recent study linked $O_3$-mediated activation of the innate immune system to the development of non-specific AHR in a mouse model (Pichavant et al., 2008). Repeated exposure to 1 ppm $O_3$ for 3 hours (3 days over a 5 day period) induced non-specific AHR measured 24 hours following the last exposure (Pichavant et al., 2008). This response was found to require NKT-cells, which are effector lymphocytes of innate immunity, as well as IL-17 and airways neutrophilia (Pichavant et al., 2008). Since glycolipids such as galactosyl ceramide are ligands for the invariant CD1 receptor on NKT-cells and serve as endogenous activators of NKT-cells, a role for $O_3$-oxidized lipids in activating NKT-cells was proposed (Pichavant et al., 2008). The authors contrasted this innate immunity pathway with that of allergen-provoked specific AHR which involves adaptive immunity, the cytokines IL-4, IL -13, IL-17, and airways eosinophilia (Pichavant et al., 2008). Interestingly, NKT-cells were required for both the specific AHR provoked by allergen and the non-specific AHR provoked by $O_3$ (Pichavant et al., 2008). Different cytokine profiles of the NKT-cells from allergen and $O_3$-exposed mice were proposed to account for the different pathways (Pichavant et al., 2008). More

recently, NKT-cells have been found to function in both innate and adaptive immunity (Vivier et al., 2011).

An interaction between allergen and $O_3$ in the induction of nonspecific AHR was shown in another animal study (Larsen et al., 2010). Mice were sensitized with the aerosolized allergen OVA on 10 consecutive days followed by exposure to $O_3$ (0.1-0.5 ppm for 3 hours) (Larsen et al., 2010). While allergen sensitization alone did not alter airways responsiveness to a nonspecific challenge, $O_3$ exposure of sensitized mice resulted in nonspecific AHR at 6- and 24-hours postexposure (Larsen et al., 2010). The effects of $O_3$ on AHR were independent of airways eosinophilia and neutrophilia (Larsen et al., 2010). However, OVA pretreatment led to goblet cell metaplasia which was enhanced by $O_3$ exposure (Larsen et al., 2010). It should be noted that OVA sensitization using only aerosolized antigen in this study is less common than the usual procedure for OVA sensitization achieved by one or more initial systemic injections of OVA and adjuvant followed by repeated inhalation exposure to OVA. This study also points to an interaction between innate and adaptive immune systems in the development of the AHR response.

Furthermore, $O_3$ was found to act as an adjuvant for allergic sensitization (Hollingsworth et al., 2010). Oropharyngeal aspiration of OVA on day 0 and day 6 failed to lead to allergic sensitization unless mice were first exposed to 1 ppm $O_3$ for 2 hours (Hollingsworth et al., 2010). The $O_3$-mediated response involved Th2 (IL-4, IL-5 and IL-9) and Th17 cytokines (IL-17) and was dependent on a functioning TLR4 (Hollingsworth et al., 2010). Ozone exposure also activated OVA-bearing dendritic cells in the thoracic lymph nodes, as measured by the presence of the CD86 surface marker, which suggests naïve T-cell stimulation and the involvement of Th2 pathways (Hollingsworth et al., 2010). Thus the adjuvant effects of $O_3$ may be due to activation of both innate and adaptive immunity.

Priming of the innate immune system by $O_3$ was reported by Hollingsworth et al. (2007). In this study, exposure of mice to 2 ppm $O_3$ for 3 hours led to nonspecific AHR at 24- and 48-hours postexposure, an effect which subsided by 72 hours (Hollingsworth et al., 2007). However, in mice treated with aerosolized endotoxin immediately following $O_3$ exposure, AHR was greatly enhanced at 48-and 72-hours postexposure (Hollingsworth et al., 2007). In addition, $O_3$ pre-exposure was found to reduce the number of inflammatory cells in the BALF, to increase cytokine production and total protein in the BALF and to increase systemic IL-6 following exposure to endotoxin (Hollingsworth et al., 2007). Furthermore, $O_3$ stimulated the apoptosis of alveolar macrophages 24-hours postexposure, an effect which was greatly enhanced by endotoxin treatment. Apoptosis of circulating blood monocytes was also observed in response to the combined exposures (Hollingsworth et al., 2007). Ozone pre-exposure enhanced the response of lung macrophages to endotoxin (Hollingsworth et al., 2007). Taken together, these findings demonstrated that $O_3$ exposure increased innate immune responsiveness to endotoxin. The authors attributed these effects to the increased surface expression of TLR4 and increased signaling in macrophages observed in the study (Hollingsworth et al., 2007). It was proposed that the resulting decrease in airway inflammatory cells could account for

$O_3$-mediated decreased clearance of bacterial pathogens observed in numerous animal models (Hollingsworth et al., 2007).

More recently, these authors demonstrated that hyaluronan contributed to the $O_3$-primed response to endotoxin (Li et al., 2010). In this study, exposure of mice to 1 ppm $O_3$ for 3 hours resulted in enhanced responses to endotoxin, which was mimicked by intratracheal instillation of hyaluronan fragments (Li et al., 2010). Hyaluronan, like $O_3$, was also found to induce TLR4 receptor peripheralization in the macrophage membrane (Li et al., 2010; Hollingsworth et al., 2007), an effect which is associated with enhanced responses to endotoxin. This study and previous ones by the same investigators showed elevation of BALF hyaluronan in response to $O_3$ exposure (Garantziotis et al., 2010; Li et al., 2010; Garantziotis et al., 2009), providing evidence that the effects of $O_3$ on innate immunity are at least in part mediated by hyaluronan fragments. The authors note that excessive TLR4 signaling can lead to lung injury and suggest that $O_3$ may be responsible for an exaggerated innate immune response which may underlie lung injury and decreased host defense (Li et al., 2010).

Activation or upregulation of the immune system has not been reported in all studies. Impaired antigen-specific immunity was demonstrated following subacute $O_3$ exposure (0.6 ppm, 10 h/day for 15 days) in mice (Feng et al., 2006). Specifically, $O_3$ exposure altered the lymphocyte subset and cytokine profile and impacted thymocyte early development leading to immune dysfunction. Further, recent studies demonstrated SP-A oxidation in mice exposed for 3-6 hours to 2 ppm $O_3$. SP-A is an important innate immune protein which plays a number of roles in host defense including acting as opsonin for the recognition of some pathogens (Haque et al., 2009). These investigations found that $O_3$-mediated carbonylation of purified SP-A was associated with impaired macrophage phagocytosis in vitro (Mikerov et al., 2008c). In addition, $O_3$ exposure (2 ppm for 3 hours) in mice was found to increase susceptibility to pneumonia infection in mice through an impairment of SP-A dependent phagocytosis (Mikerov et al., 2008b; Mikerov et al., 2008a). Furthermore, early life exposure to $O_3$ in infant monkeys followed by a recovery period led to hyporesponsiveness to endotoxin (Maniar-Hew et al., 2011), as discussed below and in Section 5.4.2.4 and Section 7.2.3.2.

Taken together, results of recent studies provide evidence that $O_3$ alters host immunologic response and leads to immune system dysfunction through its effects on innate and adaptive immunity.

### 5.3.7 Airways Remodeling

The nasal airways, conducting airways and distal airways (i.e., respiratory bronchioles or CAR depending on the species) have all been identified as sites of $O_3$-mediated injury and inflammation (Mudway and Kelly, 2000). At all levels of the RT, loss of sensitive epithelial cells, degranulation of secretory cells, proliferation of resistant epithelial cells and neutrophilic influx have been observed as a result of $O_3$

exposure (Mudway and Kelly, 2000; Cho et al., 1999). An important study (Plopper et al., 1998) conducted in adult rhesus monkeys (0.4 and 1.0 ppm $O_3$ for 2 hours at rest) found that 1 ppm $O_3$ resulted in the greatest epithelial injury in the respiratory bronchioles immediately postexposure although injury was observed at all of the RT sites studied except for the lung parenchyma. Exposure to 0.4 ppm $O_3$ resulted in epithelial injury only in the respiratory bronchioles. Initial cellular injury correlated with site-specific $O_3$ dose since the respiratory bronchioles received the greatest $O_3$ dose ($^{18}O$ mass/lung weight) and sustained the greatest initial cellular injury. The respiratory bronchioles were also the site of statistically significant GSH reduction. In addition, a study in isolated perfused rat lungs found greater injury in conducting airways downstream of bifurcations where local doses of $O_3$ were higher (Postlethwait et al., 2000).

In addition to the degree of initial injury, the degree of airways inflammation due to $O_3$ may have important long-term consequences since airways inflammation may lead to tissue injury (Balmes et al., 1996). Persistent inflammation and injury, observed in animal models of chronic and intermittent exposure to $O_3$, are associated with airways remodeling, including mucous cell metaplasia of nasal transitional epithelium (Harkema et al., 1999; Hotchkiss et al., 1991) and bronchiolar metaplasia of alveolar ducts (Mudway and Kelly, 2000). In a nonhuman primate model, hyperplasia of both URT and LRT epithelium resulted from chronic exposure to $O_3$ concentrations as low as 0.15 ppm and 0.3 ppm (Harkema et al., 1993, 1987b). Fibrotic changes such as deposition of collagen in the airways and sustained lung function decrements especially in small airways have also been demonstrated as a response to chronic $O_3$ exposure (Mudway and Kelly, 2000; Chang et al., 1992). These effects, described in detail in Section 7.2.3.2, have been demonstrated in rats exposed to levels of $O_3$ as low as 0.25 ppm. Mechanisms responsible for the resolution of inflammation and the repair of injury remain to be clarified and there is only a limited understanding of the biological processes underlying long-term morphological changes. However, a recent study in mice demonstrated a key role for the TGF-β signaling pathway in the deposition of collagen in the airways wall following chronic intermittent exposure to 0.5 ppm $O_3$ (Katre et al., 2011). Studies in infant monkeys have also documented effects of chronic intermittent exposure to 0.5 ppm $O_3$ on the developing lung and immune system. Extensive discussion of this topic is found in Section 5.4.2.4 (Lifestage) and in Section 7.2.3.2.

It should be noted that repeated exposure to $O_3$ results in attenuation of some $O_3$-induced responses, including those associated with the activation of neural reflexes (e.g., decrements in pulmonary function), as discussed in Section 5.3.2. However, numerous studies demonstrate that some markers of injury and inflammation remain increased during multi-day exposures to $O_3$. Mechanisms responsible for attenuation, or the lack thereof, are incompletely understood.

### 5.3.8 Systemic Inflammation and Oxidative/Nitrosative Stress

Extrapulmonary effects of $O_3$ have been noted for decades (U.S. EPA, 2006b). It has been proposed that lipid oxidation products resulting from reaction of $O_3$ with lipids in the ELF are responsible for systemic effects, however it is not known whether they gain access to the vascular space (Chuang et al., 2009). Alternatively, extrapulmonary release of diffusible mediators may initiate or propagate inflammatory responses in the vascular or systemic compartments (Cole and Freeman, 2009). A role for $O_3$ in modulating endothelin, a potent vasoconstrictor, has also been proposed. Studies in rats found that exposure to 0.4 and 0.8 ppm $O_3$ induced endothelin system genes in the lung and increased circulating levels of endothelin (Thomson et al., 2006; Thomson et al., 2005). Systemic oxidative stress may be suggested by studies in humans which reported associations between $O_3$ exposure and levels of plasma 8-isoprostanes and the presence of peripheral blood lymphocyte micronuclei (Chen et al., 2007a; Chen et al., 2006a). However, plasma isoprostanes are not a direct measure of systemic oxidative stress since they are stable and can be generated in any compartment before diffusion into the vascular space. Evidence of $O_3$-mediated systemic oxidative stress is better provided by new animal studies described below.

Ozone-induced perturbations of the cardiovascular system were recently investigated in young mice and monkeys (Chuang et al., 2009) and in rats (Kodavanti et al., 2011; Perepu et al., 2010) (see Section 6.3.3 and Section 7.3.1.2). These are the first studies to suggest that systemic oxidative stress and inflammation play a mechanistic role in $O_3$-induced effects on the systemic vasculature and heart. Exposure to 0.5 ppm $O_3$ for 5 days resulted in oxidative/nitrosative stress, vascular dysfunction and mitochondrial DNA damage in the aorta (Chuang et al., 2009). Chronic exposure to 0.8 ppm $O_3$ resulted in an enhancement of inflammation and lipid peroxidation in the heart following an ischemia-reperfusion challenge (Perepu et al., 2010). In addition, chronic intermittent exposure to 0.4 ppm $O_3$ increased aortic levels of mRNA for biomarkers of oxidative stress, thrombosis, vasoconstriction and proteolysis and aortic lectin-like oxidized-low density lipoprotein receptor-1 (LOX-1) mRNA and protein levels (Kodavanti et al., 2011). The latter study suggests a role for circulating oxidized lipids in mediating the effects of $O_3$.

Two recent controlled human exposure studies also demonstrated cardiovascular effects in response to short-term $O_3$ exposure (see Section 6.3.1). Changes in high frequency heart rate variability (HRV) were reported, albeit effects were in opposing directions (Devlin et al., 2012; Fakhri et al., 2009). Differences in study design may account for this discrepancy; the increase in high frequency HRV was observed following relatively low $O_3$ exposure (120 ppb for 2 hours during rest) and the decrease in high frequency HRV was observed following higher $O_3$ exposures (300 ppb for 2 hours while exercising at a high rate). These changes in cardiac function provide evidence of $O_3$-induced modulation of the autonomic nervous system, potentially through the activation of neural reflexes in the lung. Devlin et al. (2012) also demonstrated $O_3$-induced increases in markers of systemic inflammation and a pro-thrombogenic environment (Devlin et al., 2012). Older controlled human

exposure studies have reported increased myocardial work (Gong et al., 1998), and increased markers of oxidative stress (Liu et al., 1999; Liu et al., 1997) following a single $O_3$ exposure and reduced serum tocopherol levels following repeated $O_3$ exposures (Foster et al., 1996) (see Section 6.3.1). These findings in humans, together with findings from animal toxicological studies, provide evidence of $O_3$-mediated cardiovascular effects that may involve changes in autonomic tone, systemic and/or vascular oxidative stress and inflammation, and activation of the fibrinolytic system.

Systemic inflammation and oxidative/nitrosative stress may similarly affect other organ systems as well as the plasma compartment. Circulating cytokines have the potential to enter the brain through diffusion and active transport and to contribute to neuroinflammation, neurotoxicity, cerebrovascular damage and a break-down of the blood brain barrier (Block and Calderón-Garcidueñas, 2009) (see Section 6.4 and Section 7.5). They can also activate neuronal afferents (Block and Calderón-Garcidueñas, 2009). Vagal afferent pathways originating in the RT may also be responsible for $O_3$-mediated activation of nucleus tractus solitarius neurons which resulted in neuronal activation in stress-responsive regions of the CNS in rats (0.5 or 2 ppm $O_3$ for 1.5-120 hours) (Gackière et al., 2011). Recent studies have demonstrated $O_3$-induced brain lipid peroxidation, cytokine production in the brain and upregulated expression of VEGF in rats (0.5 ppm $O_3$, 3 hours or 0.25-0.5 ppm $O_3$, 4 h/day, 15-60 days) (Guevara-Guzmán et al., 2009; Araneda et al., 2008; Pereyra-Muñoz et al., 2006). Further, $O_3$-induced oxidative stress resulted in increased plasma lipid peroxides (0.25 ppm, 4h/day, 15-60 days) (Santiago-López et al., 2010), which was correlated with damage to specific brain regions (Pereyra-Muñoz et al., 2006).

Oxidative stress is one mechanism by which testicular and sperm function may be disrupted (see Section 7.4.1). Studies in Leydig cells in vitro have demonstrated that steroidogenesis is blocked by oxidative stress (Diemer et al., 2003). It has been proposed that lipid peroxidation of sperm plasma membrane may lead to impaired sperm mobility and decreased sperm quality (Agarwal et al., 2003). Further, it has been proposed that oxidative stress may damage DNA in the sperm nucleus and lead to apoptosis and a decline in sperm counts (Agarwal et al., 2003). One study reported an association between $O_3$ exposure and semen quality and suggested oxidative stress as an underlying mechanism (Sokol et al., 2006). Additional evidence is required to substantiate this link.

A role for plasma antioxidants in modulating $O_3$-induced respiratory effects was suggested by a recent study (Aibo et al., 2010). In this study, pretreatment of rats with a high dose of acetaminophen resulted in increased levels of plasma cytokines and the influx of inflammatory cells into the lung following $O_3$ exposure (0.25-0.5 ppm, 6 hours) (Aibo et al., 2010). These effects were not observed in response to $O_3$ alone. Furthermore, acetaminophen-induced liver injury was exacerbated by $O_3$ exposure. A greater increase in hepatic neutrophil accumulation and greater alteration in gene expression profiles was observed in mice exposed to $O_3$ and acetaminophen compared with either exposure alone (Aibo et al., 2010).

Although not measured in this study, glutathione depletion in the liver is known to occur in acetaminophen toxicity. Since liver glutathione is the source of plasma glutathione, acetaminophen treatment may have lowered plasma glutathione levels and altered the redox balance in the vascular compartment. These findings indicate interdependence between RT, plasma and liver responses to $O_3$, possibly related to glutathione status.

### 5.3.9 Impaired Alveolar-Arterial Oxygen Transfer

Ozone may impair alveolar-arterial oxygen transfer and reduce the supply of arterial oxygen to the myocardium. This may have a greater impact in individuals with compromised cardiopulmonary systems. Gong et al. (1998) provided evidence of a small decrease in arterial oxygen saturation in human subjects exposed for 3 hours to 300 ppb $O_3$ while exercising at a light to moderate level. In addition, Delaunois et al. (1998) demonstrated pulmonary vasoconstriction in $O_3$-exposed rabbits (0.4 ppm, 4 hours). Although of interest, the contribution of this pathway to $O_3$-induced cardiovascular effects remains uncertain.

### 5.3.10 Summary

This section summarizes the modes of action and toxicity pathways resulting from $O_3$ inhalation (Figure 5-8). These pathways provide a mechanistic basis for the health effects which are described in detail in Chapters 6 and 7. However the precise sequence by which the key events lead to health effects is not entirely clear. Three distinct short-term responses have been well-characterized in humans challenged with $O_3$: decreased pulmonary function, airways inflammation, and increased bronchial reactivity. In addition, $O_3$ exposure exacerbates, and possibly also causes, asthma and allergic airways disease in humans. Animal studies have demonstrated airways remodeling and fibrotic changes in response to chronic and intermittent $O_3$ exposures and a wide range of other responses. While the RT is the primary target tissue, cardiovascular and other organ effects occur following short- and long-term exposures of animals to $O_3$.

The initial key event in the toxicity pathway of $O_3$ is the formation of secondary oxidation products in the RT. This mainly involves direct reactions with components of the ELF. The resulting secondary oxidation products transmit signals to the epithelium, nociceptive sensory nerve fibers and, if present, dendritic cells, mast cells and eosinophils. Thus, the effects of $O_3$ are mediated by components of ELF and by the multiple cell types found in the RT. Further, oxidative stress is an implicit part of this initial key event.

Another key event in the toxicity pathway of $O_3$ is the activation of neural reflexes which lead to decrements in pulmonary function (see Section 6.2.1). Evidence is accumulating that secondary oxidation products are responsible for this effect.

Eicosanoids have been implicated in humans, while both eicosanoids and aldehydes are effective in animal models. Different receptors on bronchial C-fibers have been shown to mediate separate effects of $O_3$ on pulmonary function. Nociceptive sensory nerves are involved in the involuntary truncation of inspiration which results in decreases in FVC, $FEV_1$, tidal volume and pain upon deep inspiration. Opioids block these responses while atropine has only a minimal effect. New evidence in an animal model suggests that TRPA1 receptors on bronchial C-fibers mediate this pathway. Ozone exposure also results in activation of vagal sensory nerves and a mild increase in airway obstruction measured as increased sRaw. Atropine and β-adrenergic agonists greatly inhibit this response in humans indicating that the airways obstruction is due to bronchoconstriction. Other studies in humans implicated SP release from bronchial C-fibers resulting in airway narrowing due to either neurogenic edema or bronchoconstriction. New evidence in an animal model suggests that the SP-NK receptor pathway caused bronchoconstriction following $O_3$ exposure. Activation of neural reflexes also results in extrapulmonary effects such as bradycardia.

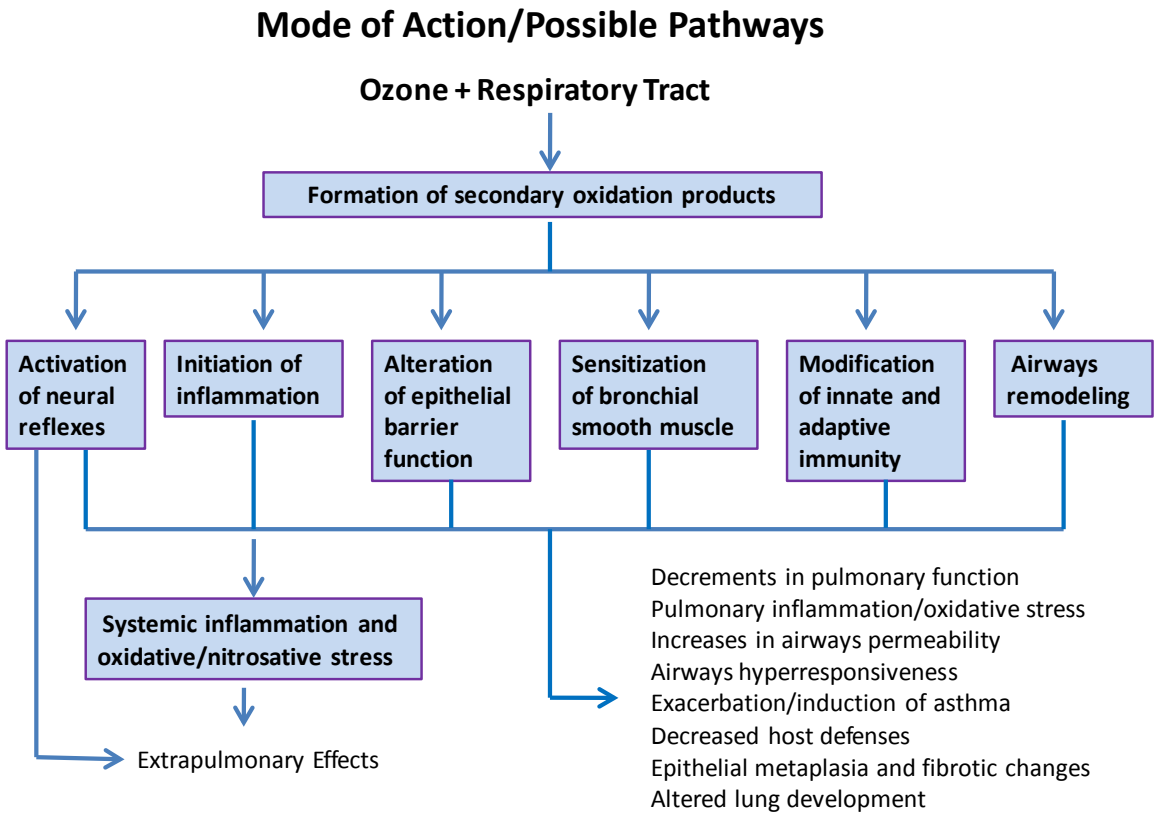



**Figure 5-8 The modes of action/possible pathways underlying the health effects resulting from inhalation exposure to $O_3$.**

Initiation of inflammation is also a key event in the toxicity pathway of $O_3$. Secondary oxidation products, as well as chemokines and cytokines elaborated by airway epithelial cells and macrophages, have been implicated in the initiation of inflammation. Vascular endothelial adhesion molecules may also play a role. Work from several laboratories using human subjects and animal models suggest that $O_3$ triggers the release of tachykinins such as SP from airway sensory nerves which could contribute to downstream effects including inflammation (see Section 6.2.3 and Section 7.2.4). Airways neutrophilia has been demonstrated in BALF, mucosal biopsy and induced sputum samples. Influx of mast cells, monocytes and macrophages also occur. Inflammation further contributes to $O_3$-mediated oxidative stress. Recent investigations show that $O_3$ exposure leads to the generation of hyaluronan fragments from high molecular weight polymers of hyaluronan normally found in the ELF in mice. Hyaluronan activates TLR4 and CD44-dependent signaling pathways in macrophages, and results in an increased number of macrophages in the BALF. Activation of these pathways occurs later than the acute neutrophilic response suggesting that they may contribute to longer-term effects of $O_3$. The mechanisms involved in clearing $O_3$-provoked inflammation remain to be clarified. It should be noted that inflammation, as measured by airways neutrophilia, is not correlated with decrements in pulmonary function as measured by spirometry.

A fourth key event in the toxicity pathway of $O_3$ is alteration of epithelial barrier function. Increased permeability occurs as a result of damage to tight junctions between epithelial cells subsequent to $O_3$-induced injury and inflammation. It may play a role in allergic sensitization and in AHR (see Section 6.2.2, Section 6.2.6, and Section 7.2.5). Tachykinins mediate this response while antioxidants may confer protection. Genetic susceptibility has been associated with functioning *Tlr4* and *Nos2* genes.

A fifth key event in the toxicity pathway of $O_3$ is the sensitization of bronchial smooth muscle. Increased bronchial reactivity can be both a rapidly occurring and a persistent response. The mechanisms responsible for early and later AHR are not well-understood (see Section 6.2.2). One proposed mechanism of sensitization, $O_3$-induced increases in epithelial permeability, would improve access of agonist to smooth muscle receptors. The evidence for this mechanism is not consistent. Another proposed mechanism, for which there is greater evidence, is neurally-mediated sensitization. In humans exposed to $O_3$, atropine blocked the early AHR response indicating the involvement of cholinergic postganglionic pathways. Animal studies demonstrated that $O_3$-induced AHR involved vagally-mediated responses and local axon reflex responses through bronchopulmonary C-fiber-mediated release of SP. Later phases of increased bronchial reactivity may involve the induction of IL-1β which in turn upregulates SP production. In guinea pigs, eosinophil-derived major basic protein contributed to the stimulation of cholinergic postganglionic pathways. A novel role for hyaluronan in mediating the later phase effects $O_3$-induced AHR has recently been demonstrated. Hyaluronan fragments stimulated AHR in a TLR4- and CD44 receptor-dependent manner. Tachykinins and secondary oxidation products of $O_3$ have been proposed as mediators of the early response and inflammation-derived products have been proposed as mediators of the later response. Inhibition of

arachidonic acid metabolism was ineffective in blocking $O_3$-induced AHR in humans while in animal models mixed results were found. Other cytokines and chemokines have been implicated in the AHR response to $O_3$ in animal models.

A sixth key event in the toxicity pathway of $O_3$ is the modification of innate/adaptive immunity. While the majority of evidence for this key event comes from animal studies, there are several studies suggesting that this pathway may also be relevant in humans. Ozone exposure of human subjects resulted in recruitment of activated innate immune cells to the airways. This included macrophages and monocytes with increased expression of cell surface markers characteristic of innate immunity and antigen presentation, the latter of which could contribute to exaggerated T-cell responses and the promotion of an allergic phenotype. Evidence of dendritic cell activation was observed in GSTM1 null human subjects exposed to $O_3$, suggesting $O_3$-mediated interaction between the innate and adaptive immune systems. Animal studies further linked $O_3$-mediated activation of the innate immune system to the development of nonspecific AHR, demonstrated an interaction between allergen and $O_3$ in the induction of nonspecific AHR, and found that $O_3$ acted as an adjuvant for allergic sensitization through the activation of both innate and adaptive immunity. Priming of the innate immune system by $O_3$ was reported in mice. This resulted in an exaggerated response to endotoxin which included enhanced TLR4 signaling in macrophages. Ozone-mediated impairment of the function of SP-A, an innate immune protein, has also been demonstrated. Taken together these studies provide evidence that $O_3$ can alter host immunologic response and lead to immune system dysfunction. These mechanisms may underlie the exacerbation and induction of asthma (see Section 6.2.6 and Section 7.2.1), as well as decreases in host defense (see Section 6.2.5 and Section 7.2.6).

Another key event in the toxicity pathway of $O_3$ is airways remodeling. Persistent inflammation and injury, which are observed in animal models of chronic and intermittent exposure to $O_3$, are associated with morphologic changes such as mucous cell metaplasia of nasal epithelium, bronchiolar metaplasia of alveolar ducts and fibrotic changes in small airways (see Section 7.2.3). Mechanisms responsible for these responses are not well-understood. However a recent study in mice demonstrated a key role for the TGF-β signaling pathway in the deposition of collagen in the airway wall following chronic intermittent exposure to $O_3$. Chronic intermittent exposure to $O_3$ has also been shown to result in effects on the developing lung and immune system.

Systemic inflammation and vascular oxidative/nitrosative stress are also key events in the toxicity pathway of $O_3$. Extrapulmonary effects of $O_3$ occur in numerous organ systems, including the cardiovascular, central nervous, reproductive, and hepatic systems (see Sections 6.3 to 6.5 and Sections 7.3 to 7.5). It has been proposed that lipid oxidation products resulting from reaction of $O_3$ with lipids and/or cellular membranes in the ELF are responsible for systemic responses; however, it is not known whether they gain access to the vascular space. Alternatively, release of diffusible mediators from the lung into the circulation may initiate or propagate inflammatory responses in the vascular or in systemic compartments.

## 5.4 Interindividual Variability in Response

Responses to $O_3$ exposure are variable within the population (Mudway and Kelly, 2000). Some studies have shown a large range of pulmonary function responses to $O_3$ among healthy young adults (i.e., 4 hours to 200 ppb $O_3$ or for 1.5 hours to 420 ppb $O_3$ while exercising at a moderate level) (Hazucha et al., 2003; Balmes et al., 1996). Since individual responses were relatively consistent across time in these studies, it was thought that responsiveness reflected an intrinsic characteristic of the subject (Mudway and Kelly, 2000). Other studies have shown that age and body mass index may influence responsiveness to $O_3$. In human subjects exercising moderately and exposed to 420 ppb $O_3$ for 1.5 hours, older adults were generally not responsive to $O_3$ (Hazucha et al., 2003), while obese young women appeared to be more responsive than lean young women (Bennett et al., 2007). In another study, the observed lack of spirometric responsiveness in one group of human subjects was not attributable to the presence of endogenous endorphins, which could vary between individuals and which could potentially block C-fiber stimulation by $O_3$ (420 ppb, 2 hours, moderate exercise (Passannante et al., 1998). Other responses to $O_3$ have also been characterized by a large degree of interindividual variability. For example, interindividual variability in the neutrophilic response has been noted in human subjects (Holz et al., 1999; Devlin et al., 1991; Schelegle et al., 1991). One study demonstrated a 3-fold difference in airways neutrophilia, measured as percent of total cells in proximal BALF, among human subjects exposed to 300 ppb $O_3$ for 1 hour while exercising at a heavy level (Schelegle et al., 1991). Another study reported a 20-fold difference in BAL neutrophils following exposure to 80-100 ppb $O_3$ for 6.6 hours in human subjects exercising at a moderate level (Devlin et al., 1991). In contrast, reproducibility of intraindividual responses to 1-hour exposure to 250 ppb $O_3$ in human subjects exercising at a light level, measured as sputum neutrophilia, was demonstrated by Holz et al. (1999). While the basis for the observed interindividual variability in responsiveness to $O_3$ is not clear, both dosimetric and mechanistic factors are likely to contribute and will be discussed below.

### 5.4.1 Dosimetric Considerations

Two studies have investigated the correlation of $O_3$ uptake with the pulmonary function responses to $O_3$ exposure (Reeser et al., 2005; Gerrity et al., 1994). These studies found that the large subject-to-subject variability in $\%\Delta FEV_1$ response to $O_3$ does not appear to have a dosimetric explanation. Reeser et al. (2005) found no significant relationship between $\%\Delta FEV_1$ and fractional absorption of $O_3$ using the bolus method. Contrary to previous findings, the percent change in dead space volume of the respiratory tract ($\%\Delta V_D$) did not correlate with $O_3$ uptake, possibly due to the contraction of dead space caused by airway closure. Gerrity et al. (1994) found that intersubject variability in $FEV_1$ and airway resistance was not related to differences in the $O_3$ dose delivered to the lower airways, whereas minute ventilation

was predictive of $FEV_1$ decrement. No study has yet demonstrated that subjects show a consistent pattern of $O_3$ retention when re-exposed over weeks of time, as has been shown to be the case for the $FEV_1$ response, or that within-subject variation in $FEV_1$ response is related to fluctuations in $O_3$ uptake. However, these studies did not control for the differences in conducting airway volume between individuals. By controlling for conducting airway volume, it may be possible to estimate how much of the intersubject variation in $FEV_1$ response at a given $O_3$ exposure is due to actual inter-individual variability in dose.

### 5.4.2 Mechanistic Considerations

This section considers mechanistic factors that may contribute to variability in responses between individuals. It has been proposed that some of the variability may be genetically determined (Yang et al., 2005a). Besides gene-environment interactions, other factors such as pre-existing diseases and conditions, nutritional status, lifestage, attenuation, and co-exposures may also contribute to inter-individual variability in responses to $O_3$ and are discussed below.

#### 5.4.2.1 Gene-environment Interactions

The pronounced interindividual variation in responses to $O_3$ infers that genetic background may play an important role in responsiveness to $O_3$ (Cho and Kleeberger, 2007; Kleeberger et al., 1997) (see also Section 8.4). Strains of mice which are prone or resistant to $O_3$-induced effects have been used to systematically identify candidate susceptibility genes. Using these recombinant inbred strains of mice and exposures to 0.3 ppm $O_3$ for up to 72 hours, genome wide linkage analyses (also known as positional cloning) demonstrated quantitative trait loci for $O_3$-induced lung inflammation and hyperpermeability on chromosome 17 (Kleeberger et al., 1997) and chromosome 4 (Kleeberger et al., 2000), respectively. More specifically, these studies found that *Tnf*, whose protein product is the inflammatory cytokine TNF-α, and *Tlr4*, whose protein product is TLR4, were candidate susceptibility genes (Kleeberger et al., 2000; Kleeberger et al., 1997). Other studies, which used targeted deletion, identified genes encoding iNOS and heat shock proteins as TLR4 effector genes (Bauer et al., 2011; Kleeberger et al., 2001) and found that IL-10 protects against $O_3$-induced pulmonary inflammation (Backus et al., 2010). Investigations in inbred mouse strains found that differences in expression of certain proteins, such as CCSP (1.8 ppm $O_3$ for 3 hours) (Broeckaert et al., 2003) and MARCO (0.3 ppm $O_3$ for up to 48 hours) (Dahl et al., 2007), were responsible for phenotypic characteristics, such as epithelial permeability and scavenging of oxidized lipids, respectively, which confer sensitivity to $O_3$.

Genetic polymorphisms have received increasing attention as modulators of $O_3$-mediated effects. Functionally relevant polymorphisms in candidate susceptibility genes have been studied at the individual and population level in humans, and also in

animal models. Genes whose protein products are involved in antioxidant defense/oxidative stress and xenobiotic metabolism, such as glutathione-S-transferase M1 (GSTM1) and NADPH:quinone oxidoreductase 1 (NQO1), have also been a major focuses of these efforts. This is because oxidative stress resulting from $O_3$ exposure is thought to contribute to the pathogenesis of asthma, and because xenobiotic metabolism detoxifies secondary oxidation products formed by $O_3$ which contribute to oxidative stress (Islam et al., 2008). TNF-α is of interest since it is linked to a candidate $O_3$ susceptibility gene and since it plays a key role in initiating airways inflammation (Li et al., 2006d). Polymorphisms of genes coding for GSTM1, NQO1 and TNF-α have been associated with altered risk of $O_3$-mediated effects (Li et al., 2006d; Yang et al., 2005a; Romieu et al., 2004b; Corradi et al., 2002; Bergamaschi et al., 2001). Additional studies have focused on functional variants in other genes involved in antioxidant defense such as catalase (*CAT*), myeloperoxidase, heme oxygenase (*HMOX-1*) and manganese superoxide dismutase (*MnSOD*) (Wenten et al., 2009; Islam et al., 2008). These studies are discussed below.

GSTM1 is a phase II antioxidant enzyme which is transcriptionally regulated by NF-e2-related factor 2-antioxidant response element (Nrf2-ARE) pathway. A large proportion (40-50%) of the general public (across ethnic populations) has the GSTM1-null genotype, which has been linked to an increased risk of health effects due to exposure to air pollutants (London, 2007). A role for GSTs in metabolizing electrophiles such as 4-hydroxynonenal, which is a secondary oxidation product resulting from $O_3$ exposure, has been demonstrated (Awasthi et al., 2004). A recent study found that the GSTM1 genotype modulated the time course of the neutrophilic inflammatory response following acute $O_3$ exposure (400 ppb for 2 hours with light to moderate exercise) in healthy adults (Alexis et al., 2009). In GSTM1-null and -sufficient subjects, $O_3$-induced sputum neutrophilia was similar at 4 hours. However, neutrophilia resolved by 24 hours in sufficient subjects but not in GSTM1-null subjects. In contrast, no differences in 24 hour sputum neutrophilia were observed between GSTM1-null and -sufficient human subjects exposed to 60 ppb $O_3$ for 6.6 hours with moderate exercise (Kim et al., 2011). It is not known whether the effect seen at the higher exposure level (Alexis et al., 2009) was due to the persistence of pro-inflammatory stimuli, impaired production of downregulators or impaired neutrophil apoptosis and clearance. However, a subsequent in vitro study by these same investigators found that GSTM1 deficiency in airway epithelial cells enhanced IL-8 production in response to 0.4 ppm $O_3$ for 4 hours (Wu et al., 2011). Furthermore, NF-κB activation was required for $O_3$-induced IL-8 production (Wu et al., 2011). Since IL-8 is a potent neutrophil activator and chemotaxin, this study provides additional evidence for the role of GSTM1 as a modulator of inflammatory responses due to $O_3$ exposure.

In addition, $O_3$ exposure increased the expression of the surface marker CD14 in airway neutrophils of GSTM1-null subjects to a greater extent than in sufficient subjects (Alexis et al., 2009). Furthermore, differences in airway macrophages were noted between the GSTM1-sufficient and -null subjects. Numbers of airway macrophages were decreased at 4 and 24 hours following $O_3$ exposure in

GSTM1-sufficient subjects (Alexis et al., 2009). Airway macrophages in GSTM1-null subjects were greater in number and found to have greater oxidative burst and phagocytic capability than those of sufficient subjects. Airway macrophages and dendritic cells from GSTM1-null subjects exposed to $O_3$ expressed higher levels of the surface marker HLA-DR, suggesting activation of the innate immune system (Alexis et al., 2009). These differences in inflammatory responses between the GSTM1-null and -sufficient subjects may provide biological plausibility for the differences in $O_3$-mediated effects reported in controlled human exposure studies (Corradi et al., 2002; Bergamaschi et al., 2001). It should also be noted that GSTM1 genotype did not affect the acute pulmonary function (i.e., spirometric) response to $O_3$ which provides additional evidence for separate mechanisms underlying the effects of $O_3$ on pulmonary function and inflammation in adults (Alexis et al., 2009). However, GSTM1-null asthmatic children were previously found to be more at risk of $O_3$-induced effects on pulmonary function than GSTM1-sufficient asthmatic children (Romieu et al., 2004b).

Another enzyme involved in the metabolism of secondary oxidation products is NQO1. NQO1 catalyzes the 2-electron reduction by NADPH of quinones to hydroquinones. Depending on the substrate, it is capable of both protective detoxification reactions and redox cycling reactions resulting in the generation of reactive oxygen species. A recent study using NQO1-null mice demonstrated that NQO1 contributes to $O_3$-induced oxidative stress, AHR and inflammation following a 3-hour exposure to 1 ppm $O_3$ (Voynow et al., 2009). These experimental results may provide biological plausibility for the increased biomarkers of oxidative stress and increased pulmonary function decrements observed in $O_3$-exposed individuals bearing both the wild-type NQO1 gene and the null GSTM1 gene (Corradi et al., 2002; Bergamaschi et al., 2001).

Besides enzymatic metabolism, other mechanisms participate in the removal of secondary oxidation products formed as a result of $O_3$ inhalation. One involves scavenging of oxidized lipids via the macrophage receptor with collagenous structure (MARCO) expressed on the cell surface of alveolar macrophages. A recent study demonstrated increased gene expression of MARCO in the lungs of an $O_3$-resistant C3H mouse strain (HeJ) but not in an $O_3$-sensitive, genetically similar strain (OuJ) (Dahl et al., 2007). Upregulation of MARCO occurred in mice exposed to 0.3 ppm $O_3$ for 24-48 hours; inhalation exposure for 6 hours at this concentration was insufficient for this response. Animals lacking the MARCO receptor exhibited greater inflammation and injury, as measured by BAL neutrophils, protein and isoprostanes, following exposure to 0.3 ppm $O_3$ (Dahl et al., 2007). MARCO also protected against the inflammatory effects of oxidized surfactant lipids (Dahl et al., 2007). Scavenging of oxidized lipids may limit $O_3$-induced injury since ozonized cholesterol species formed in the ELF (mice, 0.5-3 ppm $O_3$, 3 hours) (Pulfer et al., 2005; Pulfer and Murphy, 2004) stimulated apoptosis and cytotoxicity in vitro (Gao et al., 2009b; Sathishkumar et al., 2009; Sathishkumar et al., 2007b; Sathishkumar et al., 2007a).

Two studies reported relationships between *TNF* promoter variants and $O_3$-induced effects in humans. In one study, $O_3$-induced change in lung function was significantly lower in adult subjects with *TNF* promoter variants -308A/A and -308G/A compared with adult subjects with the variant -308G/G (Yang et al., 2005a). This response was modulated by a specific polymorphism of *LTA* (Yang et al., 2005a), a previously identified candidate susceptibility gene whose protein product is lymphotoxin-α (Kleeberger et al., 1997). In the second study, an association between the *TNF* promoter variant -308G/G and decreased risk of asthma and lifetime wheezing in children was found (Li et al., 2006d). The protective effect on wheezing was modulated by ambient $O_3$ levels and by *GSTM1* and *GSTP1* polymorphisms. The authors suggested that the *TNF*-308 G/G genotype may have a protective role in the development of childhood asthma (Li et al., 2006d).

Similarly, a promoter variant of the gene *HMOX-1*, consisting of a smaller number of (GT)n repeats, was associated with a reduced risk for new-onset asthma in non-Hispanic white children (Islam et al., 2008). The number of $(GT)_n$ repeats in this promoter has been shown to be inversely related to the inducibility of *HMOX-1*. A modulatory effect of $O_3$ was demonstrated since the beneficial effects of this polymorphism were seen only in children living in low $O_3$ communities (Islam et al., 2008). This study also identified an association between a polymorphism of the *CAT* gene and increased risk of new-onset asthma in Hispanic children; however no modulation by $O_3$ was seen (Islam et al., 2008). No association was observed in this study between a *MnSOD* polymorphism and asthma (Islam et al., 2008).

Studies to date indicate that some variability in individual responsiveness to $O_3$ may be accounted for by functional genetic polymorphisms. Further, the effects of gene-environment interactions may be different in children and adults.

#### 5.4.2.2 Pre-existing Diseases and Conditions

Pre-existing diseases and conditions can alter the response to $O_3$ exposure. For example, responsiveness to $O_3$, as measured by spirometry, is decreased in smokers and individuals with COPD (U.S. EPA, 2006b). Asthma and allergic diseases are of major importance in this discussion. In individuals with asthma, there is increased responsiveness to bronchoconstrictor challenge. This results from a combination of structural and physiological factors including increased airway inner-wall thickness, smooth muscle responsiveness and mucus secretion. Although inflammation is likely to contribute, its relationship to AHR is not clear (U.S. EPA, 2006b). However, some asthmatics have higher baseline levels of neutrophils, lymphocytes, eosinophils and mast cells in bronchial washes and bronchial biopsy tissue (Stenfors et al., 2002). It has been proposed that enhanced sensitivity to $O_3$ is conferred by the presence of greater numbers of resident airway inflammatory cells in disease states such as asthma (Mudway and Kelly, 2000).

In order to determine whether asthmatics exhibit greater responses to $O_3$, several earlier studies compared pulmonary function in asthmatic and non-asthmatic subjects

following $O_3$ exposure. Some also probed mechanisms which could account for enhanced sensitivity. While the majority focused on measurements of $FEV_1$ and FVC and found no differences between the two groups following exposures of 2-4 hours to 125-250 ppb $O_3$ or to a 30-minute exposure to 120-180 ppb $O_3$ by mouthpiece in human subjects exercising at a light to moderate level (Stenfors et al., 2002; Mudway et al., 2001; Holz et al., 1999; Scannell et al., 1996; Koenig et al., 1987; Linn et al., 1978), there were notable exceptions. In one study, greater airways obstruction in asthmatics compared with non-asthmatic subjects was observed immediately following a 2-hour exposure to 400 ppb $O_3$ while exercising at a heavy level (Kreit et al., 1989). These changes were measured as statistically significant greater decreases in $FEV_1$ and in $FEF_{25-75}$ (but not in FVC) in the absence of a bronchoconstrictor challenge (Kreit et al., 1989). These results suggest that this group of asthmatics responded to $O_3$-exposure with a greater degree of vagally-mediated bronchoconstriction compared with the non-asthmatics. A second study demonstrated a statistically significant greater decrease in $FEV_1$ and in $FEV_1$/FVC (but not in FVC) in asthmatics compared with non-asthmatics exposed to 160 ppb $O_3$ for 7.6 hours with light exercise (Horstman et al., 1995). These responses were accompanied by wheezing and inhaler use in the asthmatics (Horstman et al., 1995). Aerosol bolus dispersion measurements demonstrated a statistically significant greater change in asthmatics compared with non-asthmatics, which was suggestive of $O_3$-induced small airway dysfunction (Horstman et al., 1995). Furthermore, a statistically significant correlation was observed between the degree of baseline airway status and the $FEV_1$ response to $O_3$ in the asthmatic subjects (Horstman et al., 1995). A third study found similar decreases in FVC and $FEV_1$ in both asthmatics and non-asthmatics exposed to 400 ppb $O_3$ for 2 hours with light exercise (Alexis et al., 2000). However, a statistically significant decrease in $FEF_{75}$, a measure of small airway function, was observed in asthmatics but not in non-asthmatics (Alexis et al., 2000). Taken together, these latter studies indicate that while the magnitude of restrictive type spirometric decline was similar in asthmatics and non-asthmatics, that obstructive type changes (i.e., bronchoconstriction) were greater in asthmatics. Further, asthmatics exhibited greater sensitivity to $O_3$ in terms of small airways function.

Since asthma exacerbations occur in response to allergens and/or other triggers, some studies have focused on $O_3$-induced changes in AHR following a bronchoconstrictor challenge. No difference in sensitivity to methacholine bronchoprovocation was observed between asthmatics and non-asthmatics exposed to 400 ppb $O_3$ for 2 hours while exercising at a heavy level (Kreit et al., 1989). However, increased bronchial reactivity to inhaled allergens was demonstrated in mild allergic asthmatics exposed to 160 ppb for 7.6 hours, 250 ppb for 3 hours and 120 ppb for 1 hour while exercising at a light level or at rest (Kehrl et al., 1999; Jorres et al., 1996; Molfino et al., 1991) and in allergen-sensitized guinea pigs following $O_3$ exposure (1 ppm, 1 hour) (Sun et al., 1997). Similar, but modest, responses were reported for individuals with allergic rhinitis (Jorres et al., 1996). Further, the contractile response of isolated airways from human donor lung tissue, which were sensitized and challenged with allergen, was increased by pre-exposure to 1 ppm $O_3$ for 20 (Roux et

al., 1999). These studies provide support for $O_3$-mediated enhancement of responses to allergens in allergic subjects.

In terms of airways neutrophilia, larger responses were observed in asthmatics compared to non-asthmatics subjects, who were exercising at a light to moderate level and exposed to $O_3$, in some (Balmes et al., 1997; Scannell et al., 1996; Basha et al., 1994) but not all (Mudway et al., 2001) of the earlier studies. While each of these studies involved exposure of exercising human subjects to 200 ppb $O_3$, the duration of exposure was longer (i.e., 4-6 hours) in the former studies than in the latter study (2 hours). Further, statistically significantly increases in myeloperoxidase levels (an indicator of neutrophil activation) in bronchial washes was observed in mild asthmatics compared with non-asthmatics, despite no difference in $O_3$-stimulated neutrophil influx between the 2 groups following exposure to 200 ppb $O_3$ for 2 hours with moderate exercise (Stenfors et al., 2002). A more recent study found that atopic asthmatic subjects exhibited an enhanced inflammatory response to $O_3$ (400 ppb, 4 hours, with light to moderate exercise) (Hernandez et al., 2010). This response was characterized by greater numbers of neutrophils, higher levels of IL-6, IL-8 and IL-1β and greater macrophage cell-surface expression of TLR4 and IgE receptors in induced sputum compared with healthy subjects. This study also reported a greater increase in hyaluronan in atopic subjects and atopic asthmatics compared with healthy subjects following $O_3$ exposure. Animal studies have previously reported that hyaluronic acid activates TLR4 signaling and results in AHR (see Section 5.3.5). Furthermore, levels of IL-10, a potent anti-inflammatory cytokine, were greatly reduced in atopic asthmatics compared to healthy subjects. These results provide evidence that innate immune and adaptive responses are different in asthmatics and healthy subjects exposed to $O_3$.

Eosinophils may be an important modulator of responses to $O_3$ in asthma and allergic airways disease. Eosinophils and associated proteins are thought to affect muscarinic cholinergic receptors which are involved in vagally-mediated bronchoconstriction (Mudway and Kelly, 2000). Studies described in Section 5.3.5 which demonstrated a key role of eosinophils in $O_3$-mediated AHR may be relevant to human allergic airways disease which is characterized by airways eosinophilia (Yost et al., 2005). Furthermore, $O_3$ exposure sometimes results in airways eosinophilia in allergic subjects or animal models. For example, eosinophilia of the nasal and other airways was observed in individuals with pre-existing allergic disease following $O_3$ inhalation (160 ppb, 7.6 hours with light exercise and 270 ppb, 2 hours with moderate exercise) (Vagaggini et al., 2002; Peden et al., 1997). Further, $O_3$ exposure (0.5 ppm, 8 hours/day for 1-3 days) increased allergic responses, such as eosinophilia and augmented intraepithelial mucosubstances, in the nasal airways of ovalbumin (OVA)-sensitized rats (Wagner et al., 2002). In contrast, Stenfors et al. (2002) found no stimulation of eosinophil influx measured in bronchial washes and BALF of mild asthmatics following exposure to a lower concentration (200 ppb, 2 hours, with moderate exercise) of $O_3$.

The role of mast cells in $O_3$-mediated asthma exacerbations has been investigated. Mast cells are thought to play a key role in $O_3$-induced airways inflammation, since

airways neutrophilia was decreased in mast cell-deficient mice exposed to $O_3$ (Kleeberger et al., 1993). However, another study found that mast cells were not involved in the development of increased bronchial reactivity in $O_3$-exposed mice (Noviski et al., 1999). Nonetheless, mast cells release a wide variety of important inflammatory mediators which may lead to asthma exacerbations (Stenfors et al., 2002). A large increase in mast cell number in bronchial submucosa was observed in non-asthmatics and a significant decrease in mast cell number in bronchial epithelium was observed in mild asthmatics 6 hours following exposure to 200 ppb $O_3$ for 2 hours during mild exercise (Stenfors et al., 2002). While these results point to an $O_3$-mediated flux in bronchial mast cell populations which differed between the non-asthmatics and mild asthmatics, interpretation of these findings is difficult. Furthermore, mast cell number did not change in airway lavages in either group in response to $O_3$ (Stenfors et al., 2002)

Cytokine profiles in the airways have been investigated as an indicator of $O_3$ sensitivity. Differences in epithelial cytokine expression were observed in bronchial biopsy samples in non-asthmatic and asthmatic subjects both at baseline and 6-hours postexposure to 200 ppb $O_3$ for 2 hours with moderate exercise (Bosson et al., 2003). The asthmatic subjects had a higher baseline expression of IL-4 and IL-5 compared to non-asthmatics. In addition, expression of IL-5, IL-8, GM-CSF, and ENA-78 in asthmatics was increased significantly following $O_3$ exposure compared to non-asthmatics (Bosson et al., 2003). Some of these (IL-4, IL-5 and GM-CSF) are Th2-related cytokines or neutrophil chemoattractants, and play a role in IgE production, airways eosinophilia and suppression of Th1-cytokine production (Bosson et al., 2003). These findings suggest a link between adaptive immunity and enhanced responses of asthmatics to $O_3$.

A further consideration is the compromised status of ELF antioxidants in disease states such as asthma (Mudway and Kelly, 2000). This could possibly be due to ongoing inflammation which causes antioxidant depletion or to abnormal antioxidant transport or synthesis (Mudway and Kelly, 2000). For example, basal levels of $AH_2$ were significantly lower and basal levels of oxidized GSH and UA were significantly higher in bronchial wash fluid and BALF of mild asthmatics compared with healthy control subjects (Mudway et al., 2001). Differences in ELF antioxidant content have also been noted between species. These observations have led to the suggestion that the amount and composition of ELF antioxidants, the capacity to replenish antioxidants in the ELF or the balance between beneficial and injurious interactions between antioxidants and $O_3$ may contribute to $O_3$ sensitivity, which varies between individuals and species (Mudway et al., 2006; Mudway and Kelly, 2000; Mudway et al., 1999a). The complexity of these interactions was demonstrated by a study in which a 2-hour exposure to 200 ppb $O_3$, while exercising at a moderate level, resulted in similar increases in airway neutrophils and decreases in pulmonary function in both mild asthmatics and healthy controls, despite differences in ELF antioxidant concentrations prior to $O_3$ exposure (Mudway et al., 2001). Further, the $O_3$-induced increase in oxidized GSH and decrease in $AH_2$ observed in ELF of healthy controls was not observed in mild asthmatics (Mudway et al., 2001). While the authors concluded that basal $AH_2$ and oxidized GSH concentrations were not

predictive of responsiveness to $O_3$, they also suggested that the increased basal UA concentrations in the mild asthmatics may have played a protective role (Mudway et al., 2001). Thus compensatory mechanisms resulting in enhanced total antioxidant capacity may play a role in modulating responses to $O_3$.

Collectively these older and more recent studies provide insight into mechanisms which may contribute to enhanced responses of asthmatic and atopic individuals following $O_3$ exposure. Greater airways inflammation and/or greater bronchial reactivity have been demonstrated in asthmatics compared to non-asthmatics. This pre-existing inflammation and altered baseline bronchial reactivity may contribute to the enhanced bronchoconstriction seen in asthmatics exposed to $O_3$. Furthermore, $O_3$-induced inflammation may contribute to $O_3$-mediated AHR. An enhanced neutrophilic response to $O_3$ has been demonstrated in some asthmatics. A recent study in humans provided evidence for differences in innate immune responses related to TLR4 signaling between asthmatics and healthy subjects. Animal studies have demonstrated a role for eosinophil-derived proteins in mediating the effects of $O_3$. Since airways eosinophilia occurs in both allergic humans and allergic animal models, this pathway may underlie the exacerbation of allergic asthma by $O_3$. In addition, differences have been noted in epithelial cytokine expression in bronchial biopsy samples of healthy and asthmatic subjects. A Th2 phenotype, indicative of adaptive immune system activation and enhanced allergic responses, was observed before $O_3$ exposure and was increased by $O_3$ exposure in asthmatics. These findings support links between innate and adaptive immunity and sensitivity to $O_3$-mediated effects in asthmatics and allergic airways disease.

In addition to asthma and allergic diseases, obesity may alter responses to $O_3$. While $O_3$ is a trigger for asthma, obesity is a known risk factor for asthma (Shore, 2007). The relationship between obesity and asthma is not well understood but recent investigations have focused on alterations in endocrine function of adipose tissue in obesity. It is thought that the increases in serum levels of factors produced by adipocytes (i.e., adipokines), such as cytokines, chemokines, soluble cytokine receptors and energy regulating hormones, may contribute to the relationship between obesity and asthma. Some of these same mechanisms may be relevant to insulin resistant states such as metabolic syndrome.

In a re analysis of the data of Hazucha et al. (2003), increasing body mass index in young women was associated with increased $O_3$ responsiveness, as measured by spirometry following a 1.5-hour exposure to 420 ppb $O_3$ while exercising at a moderate level (Bennett et al., 2007). In several mouse models of obesity, airways were found to be innately more hyperresponsive and responded more vigorously to acute $O_3$ exposure than lean controls (Shore, 2007). Pulmonary inflammatory and injury in response to $O_3$ were also enhanced (Shore, 2007). It was postulated that oxidative stress resulting from obesity-related hyperglycemia could account for these effects (Shore, 2007). However, responses to $O_3$ in the different mouse models are somewhat variable and depend on whether exposures are acute or subacute. For example, diet-induced obesity augmented inflammation and injury, as measured by BALF markers, and enhanced AHR in mice exposed acutely to $O_3$ (2 ppm, 3 hours)

(Johnston et al., 2008). In contrast, the inflammatory response following sub-acute exposure to $O_3$ was dampened by obesity in a different mouse model (0.3 ppm, 72 hours) (Shore et al., 2009). It is not known whether differences in responsiveness to $O_3$ are due to differences in lung development in genetically-modified animals which result in smaller lungs and thus to differences in inhaled dose because of the altered body mass to lung size ratio.

#### 5.4.2.3 Nutritional Status

A further consideration is the compromised status of ELF antioxidants in nutritional deficiencies. Thus, many investigations have focused on antioxidant deficiency and supplementation as modulators of $O_3$-mediated effects. One study in mice found that vitamin A deficiency enhanced lung injury induced by exposure to 0.3 ppm $O_3$ for 72 hours (Paquette et al., 1996). Ascorbate deficiency was shown to increase the effects of acute (0.5-1 ppm for 4 hours), but not subacute (0.2-0.8 ppm for 7 days), $O_3$ exposure in guinea pigs (Kodavanti et al., 1995; Slade et al., 1989). Supplementation with $AH_2$ and α-TOH was protective in healthy adults who were on an $AH_2$-deficient diet and exposed to 400 ppb $O_3$ for 2 hours while exercising at a moderate level (Samet et al., 2001). In this study, the protective effect consisted of a smaller reduction in $FEV_1$ following $O_3$ exposure (Samet et al., 2001). However the inflammatory response (influx of neutrophils and levels of IL-6) measured in BALF 1 hour after $O_3$ exposure was not different between supplemented and non-supplemented subjects (Samet et al., 2001). Other investigators found that $AH_2$ and α-TOH supplementation failed to ameliorate the pulmonary function decrements or airways neutrophilia observed in humans exposed to 200 ppb $O_3$ for 2 hours while exercising at a moderate level (Mudway et al., 2006). It was suggested that supplementation may be ineffective in the absence of antioxidant deficiency (Mudway et al., 2006).

In asthmatic adults, these same dietary antioxidants reduced $O_3$-induced bronchial hyperresponsiveness (120 ppb, 45 min, light exercise) (Trenga et al., 2001). Furthermore, supplementation with $AH_2$ and α-tocopherol protected against pulmonary function decrements and nasal inflammatory responses which were associated with high levels of ambient $O_3$ in asthmatic children living in Mexico City, Mexico (Sienra-Monge et al., 2004; Romieu et al., 2002). Similarly, supplementation with ascorbate, α-tocopherol and β-carotene improved pulmonary function in Mexico City street workers (Romieu et al., 1998b).

Protective effects of supplementation with α-tocopherol alone have not been observed in humans experimentally exposed to $O_3$ (Mudway and Kelly, 2000). Alpha-TOH supplementation also failed to protect against $O_3$-induced effects in animal models of allergic rhinosinusitis and lower airways allergic inflammation (rats, 1 ppm $O_3$ for 2 days) (Wagner et al., 2007). However, protection in these same animal models was reported using γ-TOH supplementation (Wagner et al., 2009; Wagner et al., 2007). Whether or not this effect was due to enhanced antioxidant

status or to activated signaling pathways is not known. Other investigators found that α-TOH deficiency led to an increase in liver lipid peroxidation following $O_3$ exposure (rats, 0.3 ppm 3 hours/day for 7 months) (Sato et al., 1980) and a drop in liver α-TOH levels following $O_3$ exposure (mice, 0.5 ppm, 6 hours/day for 3 days) (Vasu et al., 2010). A recent study used α-TOH transfer protein null mice as a model of α-TOH deficiency and demonstrated an altered adaptive response of the lung genome to $O_3$ exposure (Vasu et al., 2010). Taken together, these studies provide evidence that the tocopherol system modulates $O_3$-induced responses.

#### 5.4.2.4 Lifestage

Responses to $O_3$ are modulated by factors associated with lifestage. On one end of the lifestage spectrum is aging. The spirometric response to $O_3$ appears to be lost in humans as they age, as was demonstrated in two studies involving exposures of human subjects exercising at levels ranging from light to heavy to 420-450 ppb $O_3$ for 1.5-2 hours (Hazucha et al., 2003; Drechsler-Parks, 1995). In mice, physiological responses to $O_3$ (600 ppb, 2 hours) were diminished with age (Hamade et al., 2010). Mechanisms accounting for this effect have not been well-studied but could include altered number and sensitivity of receptors, altered signaling pathways involved in neural reflexes or compromised status of ELF antioxidants.

On the other side of the lifestage spectrum is pre/postnatal development. Critical windows of development during the pre/postnatal period are associated with an enhanced sensitivity to environmental toxicants. Adverse birth outcomes and developmental disorders may occur as a result (Section 7.4).

Adverse birth outcomes may result from stressors which impact transplacental oxygen and nutrient transport by a variety of mechanisms including oxidative stress, placental inflammation and placental vascular dysfunction (Kannan et al., 2006). These mechanisms may be linked since oxidative/nitrosative stress is reported to cause vascular dysfunction in the placenta (Myatt et al., 2000). As described earlier in this chapter and in Section 7.4, systemic inflammation and oxidative/nitrosative stress and modification of innate and adaptive immunity are key events underlying the health effects of $O_3$ and as such they may contribute to adverse birth outcomes. An animal toxicology study showing that exposure to 2 ppm $O_3$ led to anorexia (Kavlock et al., 1979) (see Section 7.4.2) in exposed rat dams provide an additional mechanism by which $O_3$ exposure could lead to diminished transplacental nutrient transport. Disturbances of the pituitary-adrenocortico-placental system (Ritz et al., 2000) may also impact normal intrauterine growth and development. Further, restricted fetal growth may result from pro-inflammatory cytokines which limit trophoblast invasion during the early stages of pregnancy (Hansen et al., 2008). Direct effects on maternal health, such as risk of infection, and on fetal health, such as DNA damage, have also been proposed as mechanisms underlying adverse birth outcomes (Ritz et al., 2000). In addition to restricted fetal growth, preterm birth may contribute to adverse birth outcomes. Preterm birth may result from the development

of premature contractions and/or premature rupture of membranes as well as from disrupted implantation and placentation which results in suboptimal placental function (Darrow et al., 2009; Ritz et al., 2000). Genetic mutations are thought to be an important cause of placental abnormalities in the first trimester, while vascular alterations may be the main cause of placental abnormalities in later trimesters (Jalaludin et al., 2007). Ozone-mediated systemic inflammation and oxidative stress/nitrosative stress may possibly be related to these effects although there is no firm evidence.

Enhanced sensitivity to environmental toxicants during critical windows of development may also result in developmental disorders. For example, normal migration and differentiation of neural crest cells are important for heart development and are particularly sensitive to toxic insults (Ritz et al., 2002). Further, immune dysregulation and related pathologies are known to be associated with pre/postnatal environmental exposures (Dietert et al., 2010). Ozone exposure is associated with developmental effects in several organ systems. These include the lung and immune system (see below) and neurobehavioral changes which could reflect the effect of $O_3$ on CNS plasticity or the hypothalamic-pituitary axis (Auten and Foster, 2011) (see Section 7.4.9).

The majority of developmental effects due to $O_3$ have been described for the respiratory system (see Sections 7.2.3 and 7.4.8). Since its growth and development take place during both the prenatal and early postnatal periods, both prenatal and postnatal exposures to $O_3$ have been studied. Maternal exposure to 0.4-1.2 ppm $O_3$ during gestation resulted in developmental health effects in the RT of mice (Sharkhuu et al., 2011). Recent studies involving postnatal exposure to $O_3$ have focused on differences between developing and adult animals in antioxidant defenses, respiratory physiology and sensitivity to cellular injury, and on mechanisms, such as lung structural changes, antigen sensitization, interaction with nitric oxide signaling, altered airway afferent innervation and loss of alveolar repair capacity, by which early $O_3$ exposure could lead to asthma pathogenesis or exacerbations in later life (Auten and Foster, 2011).

An interesting set of studies conducted over the last 10 years in the infant rhesus monkey has identified numerous $O_3$-mediated perturbations in the developing lung and immune system (Plopper et al., 2007). These investigations were prompted by the dramatic rise in the incidence of childhood asthma and focused on the possible interaction of $O_3$ and allergens in promoting remodeling of the epithelial-mesenchymal trophic unit during postnatal development of the tracheobronchial airway wall. In humans, airways growth during the 8-12 year period of postnatal development is not well understood. Rhesus monkeys were used in these studies because the branching pattern and distribution of airways in this model are more similar to humans than those of rodents are to humans. In addition, a model of allergic airways disease, which exhibits the main features of human asthma, had already been established in the adult rhesus monkey. Studies in infant monkeys were designed to determine whether repeated exposure to $O_3$ altered postnatal growth and development, and if so, whether such effects were reversible. In addition, exposure to

$O_3$ was evaluated for its potential to increase the development of allergic airways disease. Exposures were to cyclic episodic $O_3$ over 5 months, which involved 5 biweekly cycles of alternating filtered air and $O_3$ – 9 consecutive days of filtered air and 5 consecutive days of 0.5 ppm $O_3$, 8 h/day – and to house dust mite allergen (HDMA) for 2 hours per day for 3 days on the last 3 days of $O_3$ exposure followed by 11 days of filtered air.

Key findings were numerous. First, baseline airway resistance and AHR in the infant monkeys were dramatically increased by combined exposure to both HDMA and $O_3$ (Joad et al., 2006; Schelegle et al., 2003). Secondly, $O_3$ exposure led to a large increase in BAL eosinophils (Schelegle et al., 2003) while HDMA exposure led to a large increase of eosinophils in airways tissue (Joad et al., 2006; Schelegle et al., 2003). Thirdly, the growth pattern of distal airways was changed to a large extent by exposure to $O_3$ alone and in combination with HDMA. More specifically, longer and narrower airways resulted and the number of conducting airway generations between the trachea and the gas exchange area was decreased (Fanucchi et al., 2006). This latter effect was not ameliorated by a recovery period of 6 months. Fourthly, exposure to both HDMA and $O_3$ altered the abundance and distribution of CD25+ lymphocytes in the airways (Miller et al., 2009). Lastly, several effects were seen at the level of the epithelial mesenchymal trophic unit in response to $O_3$. These include altered organization of the airways epithelium (Schelegle et al., 2003), increased abundance of mucous goblet cells (Schelegle et al., 2003), disruption of the basement membrane zone (Evans et al., 2003), reduced innervation (Larson et al., 2004), increased neuroendocrine-like cells (Joad et al., 2006), and altered orientation and abundance of smooth muscle bundles (Plopper et al., 2007; Tran et al., 2004). Six months of recovery in filtered air led to reversal of some but not all of these effects (Kajekar et al., 2007; Plopper et al., 2007; Evans et al., 2004). The authors concluded that cyclic challenge of infant rhesus monkeys to allergen and $O_3$ during the postnatal period compromised airway growth and development and resulted in changes which favor allergic airways responses and persistent effects on the immune system (Plopper et al., 2007). A more recent study in this same infant rhesus monkey model reported that early life exposure to $O_3$ resulted in decreased total peripheral blood leukocyte numbers and increased blood eosinophils as well as persistent effects on pulmonary and systemic innate immunity (Maniar-Hew et al., 2011).

Furthermore, the effect of cyclic episodic $O_3$ exposure on nasal airways was studied in the infant rhesus monkey model. The three-dimensional detail of the nasal passages was analyzed for developing predictive dosimetry models and exposure-dose-response relationships (Carey et al., 2007). The authors reported that the relative amounts of the five epithelial cell types in the nasal airways of monkeys remained consistent between infancy and adulthood [comparing to (Gross et al., 1987; Gross et al., 1982)]. Cyclic episodic $O_3$ exposure (as described in the previous paragraphs) resulted in 50-80% decreases in epithelial thickness and epithelial cell volume of the ciliated respiratory and transitional epithelium, confirming that these cell types in the nasal cavity were the most sensitive to $O_3$ exposure. The character and location of nasal lesions resulting from $O_3$ exposure were similar in the infant monkeys and adult monkeys similarly exposed. However, the nasal epithelium of

infant monkeys did not undergo nasal airway epithelial remodeling or adaptation which occurs in adult animals following $O_3$-mediated injury and which may protect against subsequent $O_3$ challenge. The authors suggested that infant monkeys may be prone to developing persistent necrotizing rhinitis following episodic longer-term exposures.

### 5.4.2.5 Attenuation of Responses

Repeated daily exposure to $O_3$ often results in a reduction in the degree of a response, i.e., an attenuation of response. This phenomenon may reflect compensatory mechanisms and is not necessarily beneficial. Furthermore, there is variability among the different $O_3$-related endpoints in terms of response attenuation, as will be described below. As a result, attenuation of some responses occurs concomitantly with the exacerbation of others.

In responsive individuals, a striking degree of attenuation of the $FEV_1$ response occurred following repeated daily exposures to $O_3$. Generally, the young $O_3$ responder was no longer responsive on the fourth or fifth day of consecutive daily $O_3$ exposure (200-500 ppb $O_3$ for 2-4 hours with light to heavy levels of exercise) and required days to weeks of nonexposure in order for the subject to regain $O_3$ responsiveness (Christian et al., 1998; Devlin et al., 1997; Linn et al., 1982b; Horvath et al., 1981; Hackney et al., 1977b). This phenomena has been reported for both lung function and symptoms such as upper airway irritation, nonproductive cough, substernal discomfort and pain upon deep inspiration (Linn et al., 1982b; Horvath et al., 1981; Hackney et al., 1977b). Repeated daily exposures also led to an attenuation of the sRaw response in moderately exercising human subjects exposed for 4 hours to 200 ppb $O_3$ (Christian et al., 1998) and to a dampened AHR response compared with a single day exposure in light exercising human subjects exposed for 2 hours to 400 ppb $O_3$ (Dimeo et al., 1981). However, one group reported persistent small airway dysfunction despite attenuation of the $FEV_1$ response on the third day of consecutive $O_3$ exposure (250 ppb, 2 hours, with moderate exercise) (Frank et al., 2001).

Studies in rodents also indicated an attenuation of the physiologic response measured by breathing patterns and tidal volume following five consecutive days of exposure to 0.35-1 ppm $O_3$ for 2.25 hours (Tepper et al., 1989). Attenuation of $O_3$-induced bradycardic responses, which also result from activation of neural reflexes, has been reported in rodents (0.5-0.6 ppm $O_3$, 2-6 h/day, 3-5 days (Hamade and Tankersley, 2009; Watkinson et al., 2001).

Multi-day exposure to $O_3$ has been found to decrease some markers of inflammation compared with a single day exposure (Christian et al., 1998; Devlin et al., 1997). For example, in human subjects exposed for 4 hours to 200 ppb $O_3$ during moderate exercise, decreased numbers of BAL neutrophils and decreased levels of BALF fibronectin and IL-6 were observed after 4 days of consecutive exposure compared with responses after 1 day (Christian et al., 1998). Results indicated an attenuation of

the inflammatory response in both proximal airways and distal lung. However markers of injury, such as lactate dehydrogenase (LDH) and protein in the BALF, were not attenuated in this study (Christian et al., 1998). Other investigators found that repeated $O_3$ exposure (200 ppb $O_3$ for 4 hours on 4 consecutive days with light exercise) resulted in increased numbers of neutrophils in bronchial mucosal biopsies despite decreased BAL neutrophilia (Jorres et al., 2000). Other markers of inflammation, including BALF protein and IL-6 remained elevated following the multi-day exposure (Jorres et al., 2000).

In rats, the increases in BALF levels of proteins, fibronectin, IL-6 and inflammatory cells observed after one day of exposure to 0.4 ppm $O_3$ for 12 hours were no longer observed after 5 consecutive days of exposure (Van Bree et al., 2002). A separate study in rats exposed to 0.35-1 ppm $O_3$ for 2.25 hours for 5 consecutive days demonstrated a lack of attenuation of the increase in BALF protein, persistence of macrophages in the centriacinar region and histological evidence of progressive tissue injury (Tepper et al., 1989). Findings that injury, measured by BALF markers or by histopathology, persist in the absence of BAL neutrophila or pulmonary function decrements suggested that repeated exposure to $O_3$ may have serious long-term consequences such as airway remodeling. In particular, the small airways were identified as a site where cumulative injury may occur (Frank et al., 2001).

Some studies examined the recovery of responses which were attenuated by repeated $O_3$ exposure. In a study of humans undergoing heavy exercise who were exposed for 2 hours to 400 ppb $O_3$ for five consecutive days (Devlin et al., 1997), recovery of the inflammatory responses which were diminished by repeated exposure required 10-20 days following the exposure (Devlin et al., 1997). In an animal study conducted in parallel (Van Bree et al., 2002), full susceptibility to $O_3$ challenge following exposure to $O_3$ for five consecutive days required 15-20 days recovery.

Several mechanisms have been postulated to explain the attenuation of some responses observed in human subjects and animal models following repeated exposure to $O_3$. First, the upregulation of antioxidant defenses (or conversely, a decrease in critical $O_3$-reactive substrates) may protect against $O_3$-mediated effects. Increases in antioxidant content of the BALF have been demonstrated in rats exposed to 0.25 and 0.5 ppm $O_3$ for several hours on consecutive days (Devlin et al., 1997; Wiester et al., 1996b; Tepper et al., 1989). Second, IL-6 was demonstrated to be an important mediator of attenuation in rats exposed to 0.5 ppm for 4 hours on two consecutive days (Mckinney et al., 1998). Third, a protective role for increases in mucus producing cells and mucus concentrations in the airways has been proposed (Devlin et al., 1997). Fourth, epithelial hyperplasia or metaplasia may decrease further effects due to subsequent $O_3$ challenge (Carey et al., 2007; Harkema et al., 1987a; Harkema et al., 1987b). These morphologic changes have been observed in nasal and lower airways in monkeys exposed chronically to 0.15-0.5 ppm $O_3$ and reflect a persistent change in epithelial architecture which may lead to other long-term sequelae. Although there is some evidence to support these possibilities, there is no consensus on mechanisms underlying response attenuation. Recent studies demonstrating that $O_3$ activates TRP receptors suggest that modulation of TRP

receptor number or sensitivity by repeated $O_3$ exposures may also contribute to the attenuation of responses.

In summary, the attenuation of pulmonary function responses by repeated exposure to $O_3$ has been linked to exacerbation of $O_3$-mediated injury. Enhanced exposure to $O_3$ due to a dampening of the $O_3$-mediated truncation of inspiration may be one factor which contributes to this relationship.

#### 5.4.2.6 Co-exposures with Particulate Matter

Numerous studies have investigated the effects of co-exposure to $O_3$ and PM because of the prevalence of these pollutants in ambient air. Results are highly variable and depend on whether exposures are simultaneous or sequential, the type of PM employed and the endpoint examined. Additive and interactive effects have been demonstrated. For example, simultaneous exposure to $O_3$ (120 ppb for 2 hours at rest) and concentrated ambient particles (CAPs) in human subjects resulted in a diminished systemic IL-6 response compared with exposure to CAPs alone (Urch et al., 2010). However, exposure to $O_3$ alone did not alter blood IL-6 levels (Urch et al., 2010). The authors provided evidence that $O_3$ mediated a switch to shallow breathing which may have accounted for the observed antagonism (Urch et al., 2010). Further, simultaneous exposure to $O_3$ (114 ppb for 2 hours at rest) and CAPs but not exposure to either alone, resulted in increased diastolic blood pressure in human subjects (Fakhri et al., 2009). Mechanisms underlying this potentiation of response were not explored. In some strains of mice, pre-exposure to $O_3$ (0.5 ppm for 2 hours) modulated the effects of carbon black PM on heart rate, HRV and breathing patterns (Hamade and Tankersley, 2009). Another recent study in mice demonstrated that treatment with carbon nanotubes followed 12 hours later by $O_3$ exposure (0.5 ppm for 3 hours) resulted in a dampening of some of the pulmonary effects of carbon nanotubes measured as markers of inflammation and injury in the BALF (Han et al., 2008). Further, Harkema and Wagner (2005) found that epithelial and inflammatory responses in the airways of rats were enhanced by co-exposure to $O_3$ (0.5 ppm for 3 days) and LPS (used as a model of biogenic PM) or to $O_3$ (1 ppm for 2 days) and OVA (used as a model of an aeroallergen). Lastly, a recent study demonstrated that maternal exposure to particulate matter (PM) resulted in augmented lung inflammation, airway epithelial mucous metaplasia and AHR in young mice exposed chronically and intermittently to 1 ppm $O_3$ (Auten et al., 2009).

In summary, many of the demonstrated responses to co-exposure were more than additive. These findings are hard to interpret but demonstrate the complexity of responses following combined exposure to PM and $O_3$.

### 5.4.2.7 Summary

Collectively, these earlier and more recent studies provide some evidence for mechanisms that may underlie the variability in responsiveness seen among individuals (Figure 5-9). Certain functional genetic polymorphisms, pre-existing conditions and diseases, nutritional status, lifestage and co-exposures contribute to altered risk of $O_3$-induced effects. Attenuation of responses may also be important, but it is incompletely understood, both in terms of the pathways involved and the resulting consequences.

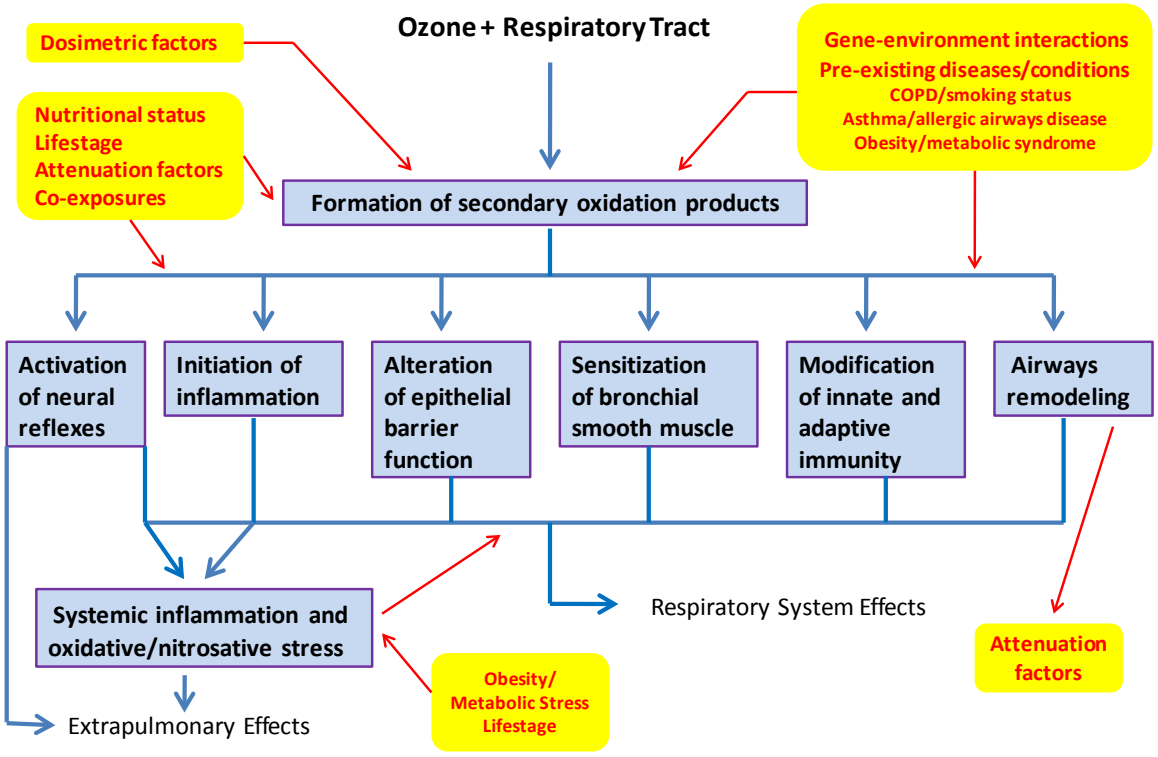



**Figure 5-9 Some factors, illustrated in yellow, that likely contribute to the interindividual variability in responses resulting from inhalation of $O_3$.**

## 5.5 Species Homology and Interspecies Sensitivity

The previous $O_3$ AQCDs discussed the homology of responses in animals and humans exposed to $O_3$ and the interspecies differences that may affect these responses and concluded that the acute and chronic functional responses of laboratory animals to $O_3$ appear qualitatively homologous to human responses. Thus, animal studies can provide important data in determining cause-effect relationships between exposure and health outcome that would be impossible to collect in human studies. Furthermore, animal studies add to a better understanding of the full range of potential $O_3$-mediated effects.

Still, care must be taken when comparing quantitative dose-response relationships in animal models to humans due to obvious interspecies differences. This section will qualitatively describe basic concepts in species homology concerning both dose and response to $O_3$ exposure. Overall, there have been few new publications examining interspecies differences in dosimetry and response to $O_3$ since the last AQCD. These studies do not overtly change the conclusions discussed in the previous document.

### 5.5.1 Interspecies Dosimetry

As discussed in Section 5.2.1, $O_3$ uptake depends on complex interactions between RT morphology, breathing route, rate, and depth, physicochemical properties of the gas, physical processes of gas transport, as well as the physical and chemical properties of the ELF and tissue layers. Understanding differences in these variables between humans and experimental animals is important to interpreting delivered doses in animal and human toxicology studies.

Physiological and anatomical differences exist between experimental species. The structure of the URT is vastly different between rodents and humans but scales according to body mass. The difference in the cross-sectional shape and size of the nasal passages affects bulk airflow patterns, particularly the shape of major airflow streams. The nasal epithelium is lined by squamous, respiratory, transitional, or olfactory cells, depending on location. The differences in airflow patterns in the URT mean that not all nasal surfaces and cell types receive the same exposure to inhaled $O_3$ leading to differences in local absorption and potential for site-specific tissue damage. The morphology of the LRT also varies within and among species. Rats and mice do not possess respiratory bronchioles; however, these structures are present in humans, dogs, ferrets, cats, and monkeys. Respiratory bronchioles are abbreviated in hamsters, guinea pigs, sheep, and pigs. The branching structure of the ciliated bronchi and bronchioles also differs between species from being a rather symmetric and dichotomous branching network of airways in humans and primates to a more monopodial branching network in other mammals. In addition, rodents have fewer terminal bronchioles due to a smaller lung size compared to humans or canines (McBride, 1992). The cellular composition in the pulmonary region is similar across mammalian species; at least 95% of the alveolar epithelial tissue is composed of

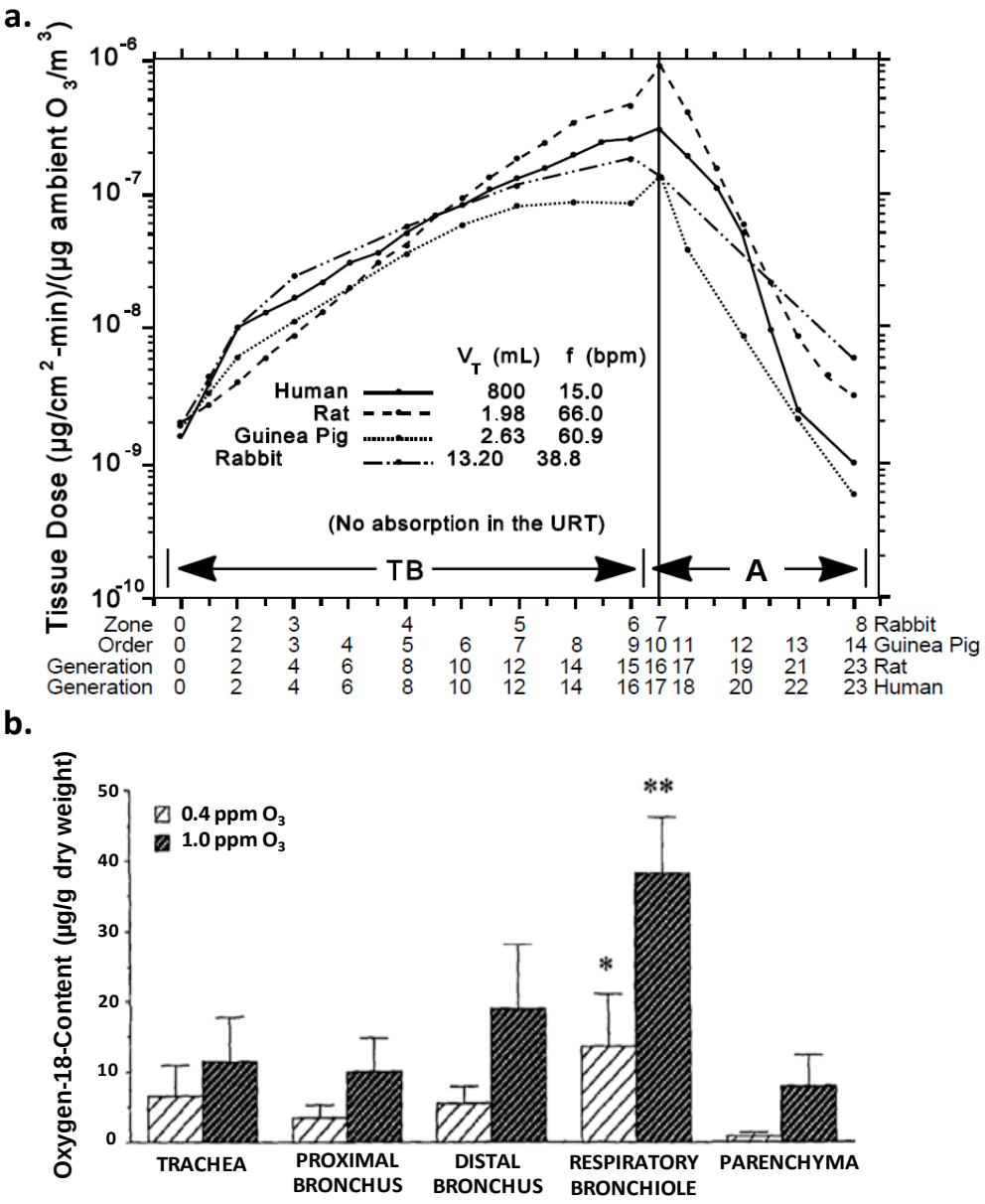
Type I cells. However, considerable differences exist between species in the number and type of cells in the TB airways. Differences also exist in breathing route and rate. Primates are oronasal breathers, while rodents are obligate nasal breathers. Past studies of the effect of body size on resting oxygen consumption also suggest that rodents inhale more volume of air per lung mass than primates. These distinctions as well as differences in nasal structure between primates and rodents affect the amount of $O_3$ uptake.

As $O_3$ absorption and reactivity relies on ELF antioxidant substances (see Section 5.2.3), variability in antioxidant concentrations and metabolism between species may affect dose and $O_3$-induced health outcomes. The thickness of the ELF in the TB airways varies among species. Mercer et al. (1992) found that the human ELF thickness in bronchi and bronchioles was 6.9 and 1.8 µm, respectively, compared to 2.6 and 1.9 µm for the same locations in the rat. Guinea pigs and mice have a lower basal activity of GSH transferase and GSH peroxidase, and lower α-TOH levels in the lung compared to rats (Ichinose et al., 1988; Sagai et al., 1987). Nasal lavage fluid analysis shows that humans have a higher proportion of their nasal antioxidants as UA and low levels of $AH_2$ whereas mice, rats, or guinea pigs have high levels of $AH_2$ and undetectable levels of UA. GSH is not detected in the nasal lavage fluid of most of these species, but is present in monkey nasal lavage fluid. Guinea pigs and rats have a higher antioxidant to protein ratio in nasal lavage fluid and BALF than humans (Hatch, 1992). The BALF profile differs from the nasal lavage fluid. Humans have a higher proportion of GSH and less $AH_2$ making up their BALF content compared to the guinea pigs and rats (Slade et al., 1993; Hatch, 1992). Similar to the nose, rats have the highest antioxidant to protein mass ratio found in BALF (Slade et al., 1993). Antioxidant defenses also vary with age (Servais et al., 2005) and exposure history (Duan et al., 1996). Duan et al. (1996); Duan et al. (1993) reported that differences in antioxidant levels between species and lung regions did not appear to be the primary factor in $O_3$ induced tissue injury. However, a close correlation between site-specific $O_3$ dose, the degree of epithelial injury, and reduced glutathione depletion was observed in monkeys (Plopper et al., 1998).

Even with these differences, humans and animals are similar in the pattern of regional $O_3$ dose distribution. As discussed for humans in Section 5.2.2, $O_3$ flux to the air-liquid interface of the ELF slowly decreases distally in the TB region and then rapidly decreases distally in the alveolar region (Miller et al., 1985). Modeled tissue dose in the human RT, representing $O_3$ flux to the liquid-tissue interface, is very low in the trachea, increases to a maximum in the CAR, and then rapidly decreases distally in the alveolar region (Figure 5-10a). Similar patterns of $O_3$ tissue dose profiles normalized to inhaled $O_3$ concentration were predicted for rat, guinea pig, and rabbit [(Miller et al., 1988) (Figure 5-10a)]. Overton et al. (1987) modeled rat and guinea pig $O_3$ dose distribution and found that after comparing two different morphometrically based anatomical models for each species, considerable difference in predicted percent RT and alveolar region uptakes were observed. This was due to the variability between the two anatomical models in airway path distance to the first alveolated duct. As a result, the overall dose profile was similar between species however the $O_3$ uptake efficiency varied due to RT size and path length

(Section 5.2.2). A similar pattern of $O_3$ dose distribution was measured in monkeys exposed to 0.4 and 1.0 ppm $^{18}O_3$ (Plopper et al., 1998) (Figure 5-10b). Less $^{18}O$ was measured in the trachea, proximal bronchus, and distal bronchus than was observed in the respiratory bronchioles. Again indicating the highest concentration of $O_3$ tissue dose is localized to the CAR, which are the respiratory bronchioles in nonhuman primates. In addition, the lowest $^{18}O$ detected in the RT was in the parenchyma (i.e., alveolar region), reflecting the rapid decrease in tissue $O_3$ dose predicted by models for the alveolar regions of humans and other animals.

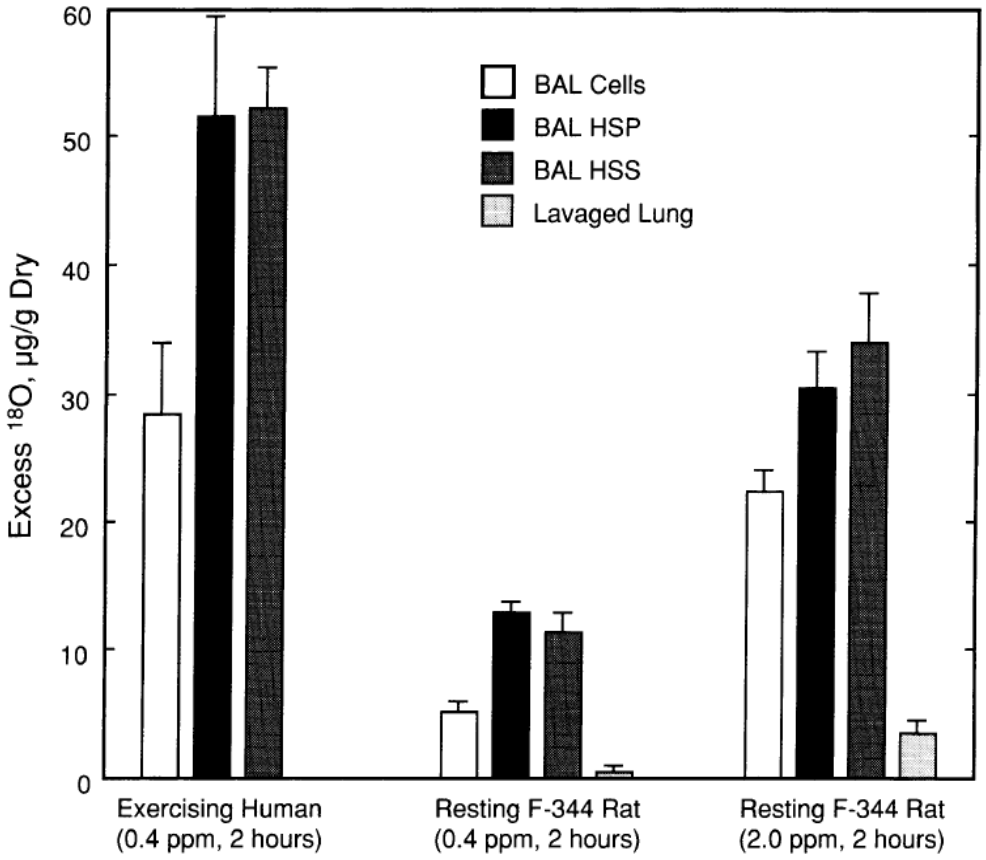
Humans and animal models are similar in the pattern of regional $O_3$ dose, but absolute values differ. Hatch et al. (1994) reported that exercising humans exposed to oxygen-18 labeled $O_3$ (400 ppb) accumulated 4-5 times higher concentrations of $O_3$ reaction product in BAL cells, surfactant and protein fractions compared to resting rats similarly exposed (400 ppb) (Figure 5-11). The use of $^{18}O$ was specifically employed in an attempt to accurately measure $O_3$ dose to BALF fractions and lung tissue and was normalized to the dried mass of lavaged constituents. It was necessary to expose resting rats to 2 ppm $O_3$ to achieve the same BALF accumulation of $^{18}O$ reaction product that was observed in humans exposed to 400 ppb with intermittent heavy exercise ($\dot{V}_E$ ~60 L/min). The concentration of $^{18}O$ reaction product in BALF paralleled the accumulation of BALF protein and cellular effects of the $O_3$ exposure observed such that these responses to 2.0 ppm $O_3$ were similar to those of the 400 ppb $O_3$ in exercising humans. This suggests that animal data obtained in resting conditions would underestimate the reaction of $O_3$ with cells in the RT and presumably the resultant risk of effect for humans. However these results should be interpreted with caution given an important limitation in the $^{18}O$ labeling technique when used for interspecies comparisons. The reaction between $O_3$ and some reactants such as ascorbate produce $^{18}O$-labeled products that are lost during sample processing. When levels of ascorbate or other such reactants vary between species, this lost portion of the total $^{18}O$-reaction products will also vary, leading to uncertainty in interspecies comparisons.




Note: Panel (a) presents the predicted tissue dose of $O_3$ (as µg of $O_3$ per $cm^2$ of segment surface area per min, standardized to a tracheal $O_3$ value of 1 µg/$m^3$) for various regions of the rabbit, guinea pig, rat, and human RT. TB = tracheobronchial region, A = alveolar region. Panel (b) presents a comparison of excess $^{18}O$ in the five regions of the TB airways of rhesus monkeys exposed to $O_3$ for 2h. *p <0.05 comparing the same $O_3$ concentration across regions. **p <0.05 comparing different $O_3$ concentrations in the same region.

Source: Panel (a) Miller et al. (1988),Springer-Verlag (b) Plopper et al. (1998)

**Figure 5-10 Humans and animals are similar in the regional pattern of $O_3$ tissue dose distribution.**




Note: The excess $^{18}O$ in each fraction is expressed relative to the dry weight of that fraction. Fractions assayed include cells, high speed pellet (HSP), high speed supernatant (HSS), and lavaged lung homogenates.

Source: Hatch et al. (1994)

**Figure 5-11 Oxygen-18 incorporation into different fractions of BALF from humans and rats exposed to 0.4 and 2.0 ppm $^{18}O_3$.**

Recently, a quantitative comparison of $O_3$ transport in the airways of rats, dogs, and humans was conducted using a three-compartment airways model, based on upper and lower airway casts and mathematical calculation for alveolar parameters (Tsujino et al., 2005). This one-dimensional gas transport model examined how interspecies anatomical and physiological differences affect intra-airway $O_3$ concentrations and the amount of gas absorbed. The morphological model consisted of cylindrical tubes with constant volume and no airway branching patterns. Peak, real-time, and mean $O_3$ concentrations were higher in the upper and lower airways of humans compared to rats and dogs, but lowest in the alveoli of humans. The amount of $O_3$ absorbed was lowest in humans when normalized by body weight. The intra-airway concentration decreased distally in all species. Sensitivity analysis demonstrated that

$V_T$, $f_B$, and upper and lower airways surface area had a statistically significant impact on model results. The model is limited in that it did not account for chemical reactions in the ELF or consider gas diffusion as a driving force for $O_3$ transport. Also, the model was run at a respiratory rate of 16/min simulating a resting individual, however exercise may cause a further deviation from animal models as was seen in Hatch et al. (1994).

Overall, animal models exhibit qualitatively similar patterns of $O_3$ net and tissue dose distribution with the largest tissue dose delivered to the CAR. However, due to anatomical and biochemical RT differences the absolute values of $O_3$ dose delivered differs. Past results suggest that animal data obtained in resting conditions would underestimate the $O_3$ reactions with cells in the BALF and presumably the resultant risk of effect for humans, especially for humans during exercise.

### 5.5.2 Interspecies Homology of Response

Biological response to $O_3$ exposure broadly shows commonalities in many species. Among rodents, non-human primates, and humans, for example, ample data suggest that $O_3$ induces oxidative stress, cell injury, upregulation of cytokines/chemokines, inflammation, alterations in lung function, and disruption of normal lung growth and development (see Chapters 6 and 7).

The effects related to early life exposures can differ appreciably across species due to the maturation stage of the lung and immune systems at birth. Evidence from non-human primate studies shows that early life $O_3$ exposure disrupts lung development producing physiologic perturbations that are similar to those observed in children exposed to urban air pollution (Fanucchi et al., 2006; Joad et al., 2006). Studies of $O_3$ effects on lung surface chemistry also show some degree of homology. Lipid oxidation products specific to $O_3$ reactions with unsaturated fatty acids have been reported, for example, in lavage fluids from both rodents and humans (Frampton et al., 1999; Pryor et al., 1996). In humans, the extent to which systemic effects occur is less well studied; plasma indices of lipid oxidation such as isoprostanes unfortunately do not pinpoint the compartment(s) where oxidative stress has transpired. That oxidative stress occurs systemically in both rodents and non-human primates (Chuang et al., 2009), nevertheless, suggests that it likely also occurs in humans.

Despite the overall similarities in responses to $O_3$ among species, studies have reported variability in the responsiveness to $O_3$ between and within species, as well as between endpoints. Rodents appear to have a slightly higher tachypneic response to $O_3$ and are less sensitive to changes in pulmonary function responses than humans (U.S. EPA, 1996a). However, rats experience attenuation of pulmonary function and tachypneic ventilatory responses, similar to humans (Wiester et al., 1996b). Hatch et al. (1986) reported that guinea pigs were the most responsive to $O_3$-induced inflammatory cell and protein influx. Rabbits were the least responsive and rats, hamsters, and mice were intermediate responders. Further analysis of this study by Miller et al. (1988) found that the protein levels in BALF from guinea pigs increased

more rapidly with predicted pulmonary tissue dose than in rats and rabbits. Alveolar macrophages isolated from guinea pigs and humans mounted similar qualitative and quantitative cytokine responses to in vitro $O_3$ (0.1-1.0 ppm for 60 minutes) exposure (Arsalane et al., 1995).

Also, because of their higher body surface to volume ratio, rodents can rapidly lower body temperature during exposure leading to lowered $O_3$ dose and toxicity (Watkinson et al., 2003; Iwasaki et al., 1998; Slade et al., 1997). In addition to lowering the $O_3$ dose to the lungs, this hypothermic response may cause: (1) lower metabolic rate, (2) altered enzyme kinetics, and (3) altered membrane function. The thermoregulatory mechanisms also may disrupt heart rate that may lead to: (1) decreased cardiac output, (2) lowered blood pressure, and (3) decreased tissue perfusion (Watkinson et al., 2003). These responses have not been observed in humans except at very high exposures, thus further complicating extrapolation of effects from animals to humans.

The degree to which $O_3$ induces injury and inflammation responses appears to be variable between species. However, the majority of those studies did not normalize the response to the dose received to account for species differences in $O_3$ absorption. For example, Dormans et al. (1999) found that rats, mice, and guinea pigs all exhibited $O_3$-induced (0.2 - 0.4 ppm for 3-56 days) inflammation; however, guinea pigs were the most responsive with respect to alveolar macrophage elicitation and pulmonary cell density in the centriacinar region. Mice were the most responsive in terms of bronchiolar epithelial hypertrophy and biochemical changes (e.g., LDH, glutathione reductase, glucose-6-phosphate dehydrogenase activity), and had the slowest recovery from $O_3$ exposure. All species displayed increased collagen in the ductal septa and large lamellar bodies in Type II pneumocytes at the longest exposure and highest concentration; whereas this response occurred in the rat and guinea pig at lower $O_3$ levels (0.2 ppm) as well. Overall, the authors rated mice as most responsive, followed by guinea pigs, then rats (Dormans et al., 1999). Rats were also less responsive in terms of epithelial necrosis and inflammatory responses as a result of $O_3$ exposure (1.0 ppm for 8 hours) compared with monkeys and ferrets, which manifested a similar response (Sterner-Kock et al., 2000). Results of this study should be interpreted with caution since no dose metric was used to normalize the total inhaled dose or local organ dose between species.

To further understand the genetic basis for age-dependent differential response to $O_3$, adult (15 week old) and neonatal (15-16 day old) mice from 8 genetically diverse strains were examined for $O_3$-induced (0.8 ppm for 5 hours) pulmonary injury and lung inflammation (Vancza et al., 2009). Ozone exposure increased polymorphonuclear leukocytes (PMN) influx in all strains of neonatal mice tested, but significantly greater PMNs occurred in neonatal compared to adult mice for only some sensitive strains, suggesting a genetic background effect. This strain difference was not due to differences in delivered dose of $O_3$ to the lung, evidenced by $^{18}O$ lung enrichment. The sensitivity of strains for $O_3$-induced increases in BALF protein and PMNs was different for different strains of mice suggesting that genetic factors contributed to heightened responses. Interestingly, adult mice accumulated more than

twice the levels of $^{18}O$ reaction product of $O_3$ than corresponding strain neonates. Thus, it appeared that the infant mice showed a 2-fold- to 3-fold higher response than the adults when expressed relative to the accumulated $O_3$ reaction product in their lungs. The apparent decrease in delivered $O_3$ dose in neonates could be a result of a more rapid loss of body temperature in infant rodents incident to maternal separation and chamber air flow.

In animal studies, inhaled $O_3$ concentration and exposure history rarely reflect actual human environmental exposures. Generally, very high exposure concentrations are used to induce murine AHR, which in some human subjects is observed at far more relevant concentrations. This calls into question whether the differences in airway reactivity are simply a function of differential nasopharyngeal scrubbing or whether the complexities encompassing a variety of contributory biological pathways show species divergence. Furthermore, in non-human primates exposed during early life, eosinophil trafficking occurs, which has not been observed in rodents (unless sensitized) (Maniar-Hew et al., 2011). This response has been shown to be persistent when $O_3$ challenges are administered after a recovery period of ≥9 months during which no exposure transpired.

Quantitative extrapolation is challenging due to a number of uncertainties. Unfortunately, many input parameters needed to conduct quantitative extrapolations across species have not been obtained or currently remain undefined. It is not clear whether characterization of the ELF provides the information needed to compute a profile of reaction products or whether environmentally relevant exposure has altered the physicochemical interactions that occur within the RT surface compartment (e.g., $O_3$ diffusion through regions where the ELF is thin). That systemic effects have been documented in both rodents and non-human primates leads to the question of whether reaction products, cytokines/chemokines, or both enter the nasopharyngeal or bronchial circulation, both of which show species-dependent differences (Chuang et al., 2009; Cole and Freeman, 2009).

In addition, the response to $O_3$ insult across species and more recent health effects such as immune system development are uncertain. Non-human primate studies have shown hypo-responsiveness to endotoxin challenge as a consequence of exposure; whether this occurs in rodents and humans is largely unknown (Maniar-Hew et al., 2011). In addition, structural changes (e.g., airways remodeling, fibrogenesis) might differ appreciably across species. Moreover, whether the upper airways differentially contribute to either distal lung or systemic impacts has not been explored.

Some outcomes (e.g., inflammation) support the conclusion of homologous responses across species. However, factors such as age, exposure history, diet, endogenous substrate generation and homeostatic regulation, the cellular machinery that regulates inflammatory cell trafficking, responses to other environmental challenges, and the precise chemical species (whether ELF or cell membrane-derived) that account for exposure-related initiation of pathophysiologic sequelae might differ across species, but the extent of species-specific contributing factors remains unknown. Consequently, some level of uncertainty cannot be dismissed. Nonetheless, if experimental animals show pathophysiological consequences of

exposure, the overall weight of the toxicological evidence supports the likelihood that qualitatively similar effects occur in humans given appropriate exposure scenarios.

### 5.5.3 Summary

In summary, biological response to $O_3$ exposure broadly shows commonalities in many species and thus supports the use of animal studies in determining mechanistic and cause-effect relationships and as supporting evidence that similar effects could occur in humans if $O_3$ exposure is sufficient. However, there is uncertainty regarding the similarity of response to $O_3$ across species for some recently described endpoints. Differences exist between species in a number of factors that influence $O_3$ dosimetry and responses, such as RT anatomy, breathing patterns, and ELF antioxidant concentrations and chemical species. While humans and animals are similar in the pattern of regional $O_3$ dose distribution, these differences will likely result in differences in the absolute values of $O_3$ dose delivered throughout the RT. Thus, these considerations can impact quantitative comparison between species.

## 5.6 Chapter Summary

Ozone is a highly reactive gas and a powerful oxidant with a short half-life. Both $O_3$ uptake and responses are dependent upon the formation of secondary reaction products in the ELF; however more complex interactions occur. Total RT uptake in humans at rest is 80-95% efficient and it is influenced by a number of factors including RT morphology, breathing route, frequency, and volume, physicochemical properties of the gas, physical processes of gas transport, as well as the physical and chemical properties of the ELF and tissue layers. In fact, even though the average RT dose may be at a level where health effects would not be predicted, local regions of the RT may receive considerably higher than average doses due to RT inhomogeneity and differences in the pathlengths, and therefore be at greater risk of effects. About half of the $O_3$ that will be absorbed from the airstream is removed in the URT, which provides a defense against $O_3$ entering the lungs. However, the local dose to the URT tissue is site-specific and dependent on the nasal anatomy, nasal fluid composition, and ventilation and airflow patterns of the nasal passageways. The primary uptake site of $O_3$ delivery to the LRT epithelium is believed to be the CAR, however changes in a number of factors (e.g., physical activity) can alter the distribution of $O_3$ uptake in the RT. Ozone uptake is chemical reaction-dependent and the substances present in the ELF appear in most cases to limit interaction of $O_3$ with underlying tissues and to prevent penetration of $O_3$ distally into the RT. Still, reactions of $O_3$ with soluble ELF components or possibly plasma membranes result in distinct products, some of which are highly reactive and can injure and/or transmit signals to RT cells.

Thus, in addition to contributing to the driving force for $O_3$ uptake, formation of secondary oxidation products initiates pathways that provide the mechanistic basis for health effects that are described in detail in Chapters 6 and 7 and that involve the RT as well as extrapulmonary systems. These pathways include activation of neural reflexes, initiation of inflammation, alteration of epithelial barrier function, sensitization of bronchial smooth muscle, modification of innate and adaptive immunity, airways remodeling, and systemic inflammation and oxidative/nitrosative stress. With the exception of airways remodeling, these pathways have been demonstrated in both animals and human subjects in response to the inhalation of $O_3$.

Both dosimetric and mechanistic factors contribute to the understanding of interindividual variability in responses to $O_3$. This variability is influenced by differences in RT volume and surface area, certain genetic polymorphisms, pre-existing conditions and disease, nutritional status, lifestages, attenuation, and co-exposures. Some of these factors also underlie differences in species homology and sensitivity. Qualitatively, animal models exhibit similar patterns of $O_3$ net and tissue dose distribution with the largest tissue dose of $O_3$ delivered to the CAR. However, due to anatomical and biochemical RT differences, the absolute value of delivered $O_3$ dose differs, with animal data obtained in resting conditions underestimating the dose to the RT and presumably the resultant risk of effect for humans, especially humans during exercise. Even though interspecies differences can complicate quantitative comparison between species, many short-term responses of laboratory animals to $O_3$ appear qualitatively homologous to those of the human. Furthermore, animal studies add to a better understanding of the full range of potential $O_3$-mediated effects. Given the commonalities in many responses across species, animal studies that observe $O_3$-induced effects may be used as supporting evidence that similar effects could occur in humans or in determining mechanistic and cause-effect relationships if $O_3$ exposure is sufficient.

## References


Abraham, WM; Delehunt, JC; Yerger, L; Marchette, B; Oliver, W, Jr. (1984). Changes in airway permeability and responsiveness after exposure to ozone. Environ Res 34: 110-119.

Agarwal, A; Saleh, RA; Bedaiwy, MA. (2003). Role of reactive oxygen species in the pathophysiology of human reproduction [Review]. Fertil Steril 79: 829-843.

Ahmad, S; Ahmad, A; McConville, G; Schneider, BK; Allen, CB; Manzer, R; Mason, RJ; White, CW. (2005). Lung epithelial cells release ATP during ozone exposure: Signaling for cell survival. Free Radic Biol Med 39: 213-226. http://dx.doi.org/10.1016/j.freeradbiomed.2005.03.009

Aibo, DI; Birmingham, NP; Lewandowski, R; Maddox, JF; Roth, RA; Ganey, PE; Wagner, JG; Harkema, JR. (2010). Acute exposure to ozone exacerbates acetaminophen-induced liver injury in mice. Toxicol Sci 115: 267-285. http://dx.doi.org/10.1093/toxsci/kfq034

Al-Hegelan, M; Tighe, RM; Castillo, C; Hollingsworth, JW. (2011). Ambient ozone and pulmonary innate immunity [Review]. Immunol Res 49: 173-191. http://dx.doi.org/10.1007/s12026-010-8180-z

Alexis, N; Soukup, J; Nierkens, S; Becker, S. (2001b). Association between airway hyperreactivity and bronchial macrophage dysfunction in individuals with mild asthma. Am J Physiol Lung Cell Mol Physiol 280: L369-L375.

Alexis, N; Urch, B; Tarlo, S; Corey, P; Pengelly, D; O'Byrne, P; Silverman, F. (2000). Cyclooxygenase metabolites play a different role in ozone-induced pulmonary function decline in asthmatics compared to normals. Inhal Toxicol 12: 1205-1224.

Alexis, NE; Lay, JC; Hazucha, M; Harris, B; Hernandez, ML; Bromberg, PA; Kehrl, H; Diaz-Sanchez, D; Kim, C; Devlin, RB; Peden, DB. (2010). Low-level ozone exposure induces airways inflammation and modifies cell surface phenotypes in healthy humans. Inhal Toxicol 22: 593-600. http://dx.doi.org/10.3109/08958371003596587

Alexis, NE; Zhou, H; Lay, JC; Harris, B; Hernandez, ML; Lu, TS; Bromberg, PA; Diaz-Sanchez, D; Devlin, RB; Kleeberger, SR; Peden, DB. (2009). The glutathione-S-transferase Mu 1 null genotype modulates ozone-induced airway inflammation in human subjects. J Allergy Clin Immunol 124: 1222-1228. http://dx.doi.org/10.1016/j.jaci.2009.07.036

Alfaro, MF; Putney, L; Tarkington, BK; Hatch, GE; Hyde, DM; Schelegle, ES. (2004). Effect of rapid shallow breathing on the distribution of 18O-labeled ozone reaction product in the respiratory tract of the rat. Inhal Toxicol 16: 77-85.

Alfaro, MF; Walby, WF; Adams, WC; Schelegle, ES. (2007). Breath condensate levels of 8-isoprostane and leukotriene B4 after ozone inhalation are greater in sensitive versus nonsensitive subjects. Exp Lung Res 33: 115-133.

Araneda, S; Commin, L; Atlagich, M; Kitahama, K; Parraguez, VH; Pequignot, JM; Dalmaz, Y. (2008). VEGF overexpression in the astroglial cells of rat brainstem following ozone exposure. Neurotoxicology 29: 920-927. http://dx.doi.org/10.1016/j.neuro.2008.09.006

Aris, RM; Christian, D; Hearne, PQ; Kerr, K; Finkbeiner, WE; Balmes, JR. (1993). Ozone-induced airway inflammation in human subjects as determined by airway lavage and biopsy. Am J Respir Crit Care Med 148: 1363-1372.

Arito, H; Uchiyama, I; Arakawa, H; Yokoyama, E. (1990). Ozone-induced bradycardia and arrhythmia and their relation to sleep-wakefulness in rats. Toxicol Lett 52: 169-178. http://dx.doi.org/10.1016/0378-4274(90)90151-B

Arsalane, K; Gosset, P; Vanhee, D; Voisin, C; Hamid, Q; Tonnel, AB; Wallaert, B. (1995). Ozone stimulates synthesis of inflammatory cytokines by alveolar macrophages in vitro. Am J Respir Cell Mol Biol 13: 60-68.

Asplund, PT; Ben-Jebria, A; Rigas, ML; Ultman, JS. (1996). Longitudinal distribution of ozone absorption in the lung: Effect of continuous inhalation exposure. Arch Environ Occup Health 51: 431-438.

Auten, RL; Foster, WM. (2011). Biochemical effects of ozone on asthma during postnatal development [Review]. Biochim Biophys Acta 1810: 1114-1119. http://dx.doi.org/10.1016/j.bbagen.2011.01.008

Auten, RL; Potts, EN; Mason, SN; Fischer, B; Huang, Y; Foster, WM. (2009). Maternal exposure to particulate matter increases postnatal ozone-induced airway hyperreactivity in juvenile mice. Am J Respir Crit Care Med 180: 1218-1226. http://dx.doi.org/10.1164/rccm.200901-0116OC

Awasthi, YC; Yang, Y; Tiwari, NK; Patrick, B; Sharma, A; Li, J; Awasthi, S. (2004). Regulation of 4-hydroxynonenal-mediated signaling by gluathione S-transferases. Free Radic Biol Med 37: 607-619. http://dx.doi.org/10.1016/j.freeradbiomed.2004.05.033

Backus, GS; Howden, R; Fostel, J; Bauer, AK; Cho, HY; Marzec, J; Peden, DB; Kleeberger, SR. (2010). Protective role of interleukin-10 in ozone-induced pulmonary inflammation. Environ Health Perspect 118: 1721-1727. http://dx.doi.org/10.1289/ehp.1002182

Ballinger, CA; Cueto, R; Squadrito, G; Coffin, JF; Velsor, LW; Pryor, WA; Postlethwait, EM. (2005). Antioxidant-mediated augmentation of ozone-induced membrane oxidation. Free Radic Biol Med 38: 515-526. http://dx.doi.org/10.1016/j.freeradbiomed.2004.11.009

Balmes, JR; Aris, RM; Chen, LL; Scannell, C; Tager, IB; Finkbeiner, W; Christian, D; Kelly, T; Hearne, PQ; Ferrando, R; Welch, B. (1997). Effects of ozone on normal and potentially sensitive human subjects. Part I: Airway inflammation and responsiveness to ozone in normal and asthmatic subjects (pp. 1-37; discussion 81-99). (ISSN 1041-5505). Boston, MA: Health Effects Institute.

Balmes, JR; Chen, LL; Scannell, C; Tager, I; Christian, D; Hearne, PQ; Kelly, T; Aris, RM. (1996). Ozone-induced decrements in FEV1 and FVC do not correlate with measures of inflammation. Am J Respir Crit Care Med 153: 904-909.

Bang, S; Kim, KY; Yoo, S; Kim, YG; Hwang, SW. (2007). Transient receptor potential A1 mediates acetaldehyde-evoked pain sensation. Eur J Neurosci 26: 2516-2523. http://dx.doi.org/10.1111/j.1460-9568.2007.05882.x

Basha, MA; Gross, KB; Gwizdala, CJ; Haidar, AH; Popovich, J, Jr. (1994). Bronchoalveolar lavage neutrophilia in asthmatic and healthy volunteers after controlled exposure to ozone and filtered purified air. Chest 106: 1757-1765.

Bastacky, J; Lee, CY; Goerke, J; Koushafar, H; Yager, D; Kenaga, L; Speed, TP; Chen, Y; Clements, JA. (1995). Alveolar lining layer is thin and continuous: Low-temperature scanning electron microscopy of rat lung. J Appl Physiol 79: 1615-1628.

Bates, ML; Brenza, TM; Ben-Jebria, A; Bascom, R; Ultman, JS. (2009). Longitudinal distribution of ozone absorption in the lung: Comparison of cigarette smokers and nonsmokers. Toxicol Appl Pharmacol 236: 270-275.

Bauer, AK; Rondini, EA; Hummel, KA; Degraff, LM; Walker, C; Jedlicka, AE; Kleeberger, SR. (2011). Identification of candidate genes downstream of TLR4 signaling after ozone exposure in mice: A role for heat shock protein 70. Environ Health Perspect 119: 1091-1097. http://dx.doi.org/10.1289/ehp.1003326

Beckett, WS; McDonnell, WF; Horstman, DH; House, DE. (1985). Role of the parasympathetic nervous system in acute lung response to ozone. J Appl Physiol 59: 1879-1885.

Becquemin, MM; Bertholon, JF; Bouchikhi, A; Malarbet, JL; Roy, M. (1999). Oronasal ventilation partitioning in adults and children: Effect on aerosol deposition in airways. Radiat Prot Dosimetry 81: 221-228.

Belvisi, MG; Stretton, CD; Verleden, GM; Ledingham, SJ; Yacoub, MH; Barnes, PJ. (1992). Inhibition of cholinergic neurotransmission in human airways by opioids. J Appl Physiol 72: 1096-1100.

Bennett, W; Zeman, K; Jarabek, A. (2003). Nasal contribution to breathing with exercise: effect of race and gender. J Appl Physiol 95: 497-503. http://dx.doi.org/10.1152/japplphysiol.00718.2002

Bennett, WD; Hazucha, MJ; Folinsbee, LJ; Bromberg, PA; Kissling, GE; London, SJ. (2007). Acute pulmonary function response to ozone in young adults as a function of body mass index. Inhal Toxicol 19: 1147-1154. http://dx.doi.org/10.1080/08958370701665475

Bennett, WD; Zeman, KL; Jarabek, AM. (2008). Nasal contribution to breathing and fine particle deposition in children versus adults. J Toxicol Environ Health A 71: 227-237. http://dx.doi.org/10.1080/15287390701598200

Bergamaschi, E; De Palma, G; Mozzoni, P; Vanni, S; Vettori, MV; Broeckaert, F; Bernard, A; Mutti, A. (2001). Polymorphism of quinone-metabolizing enzymes and susceptibility to ozone-induced acute effects. Am J Respir Crit Care Med 163: 1426-1431.

Bhalla, DK; Gupta, SK. (2000). Lung injury, inflammation, and inflammatory stimuli in rats exposed to ozone. J Toxicol Environ Health 59: 211-228. http://dx.doi.org/10.1080/009841000156899

Block, ML; Calderón-Garcidueñas, L. (2009). Air pollution: Mechanisms of neuroinflammation and CNS disease [Review]. Trends Neurosci 32: 506-516. http://dx.doi.org/10.1016/j.tins.2009.05.009

Blomberg, A; Mudway, IS; Nordenhall, C; Hedenstrom, H; Kelly, FJ; Frew, AJ; Holgate, ST; Sandstrom, T. (1999). Ozone-induced lung function decrements do not correlate with early airway inflammatory or antioxidant responses. Eur Respir J 13: 1418-1428.

Bosson, J; Blomberg, A; Pourazar, J; Mudway, IS; Frew, AJ; Kelly, FJ; Sandström, T. (2009). Early suppression of NFkappaB and IL-8 in bronchial epithelium after ozone exposure in healthy human subjects. Inhal Toxicol 21: 913-919. http://dx.doi.org/10.1080/08958370802657389

Bosson, J; Stenfors, N; Bucht, A; Helleday, R; Pourazar, J; Holgate, ST; Kelly, FJ; Sandstrom, T; Wilson, S; Frew, AJ; Blomberg, A. (2003). Ozone-induced bronchial epithelial cytokine expression differs between healthy and asthmatic subjects. Clin Exp Allergy 33: 777-782.

Broeckaert, F; Clippe, A; Wattiez, R; Falmagne, P; Bernard, A. (2003). Lung hyperpermeability, Clara-cell secretory potein (CC16), and susceptibility to ozone of five inbred strains of mice. Inhal Toxicol 15: 1209-1230.

Bush, ML; Asplund, PT; Miles, KA; Ben-Jebria, A; Ultman, JS. (1996). Longitudinal distribution of O3 absorption in the lung: gender differences and intersubject variability. J Appl Physiol 81: 1651-1657.

Bush, ML; Zhang, W; Ben-Jebria, A; Ultman, JS. (2001). Longitudinal distribution of ozone and chlorine in the human respiratory tract: Simulation of nasal and oral breathing with the single-path diffusion model. Toxicol Appl Pharmacol 173: 137-145. http://dx.doi.org/10.1006/taap.2001.9182

Caceres, AI; Brackmann, M; Elia, MD; Bessac, BF; del Camino, D; D'Amours, M; Witek, JS; Fanger, CM; Chong, JA; Hayward, NJ; Homer, RJ; Cohn, L; Huang, X; Moran, MM; Jordt, SE. (2009). A sensory neuronal ion channel essential for airway inflammation and hyperreactivity in asthma. PNAS 106: 9099-9104. http://dx.doi.org/10.1073/pnas.0900591106

Carey, SA; Minard, KR; Trease, LL; Wagner, JG; Garcia, GJ; Ballinger, CA; Kimbell, JS; Plopper, CG; Corley, RA; Postlethwait, EM; Harkema, JR. (2007). Three-dimensional mapping of ozone-induced injury in the nasal airways of monkeys using magnetic resonance imaging and morphometric techniques. Toxicol Pathol 35: 27-40. http://dx.doi.org/10.1080/01926230601072343

Chang, LY; Huang, Y; Stockstill, BL; Graham, JA; Grose, EC; Menache, MG; Miller, FJ; Costa, DL; Crapo, JD. (1992). Epithelial injury and interstitial fibrosis in the proximal alveolar regions of rats chronically exposed to a simulated pattern of urban ambient ozone. Toxicol Appl Pharmacol 115: 241-252. http://dx.doi.org/10.1016/0041-008X(92)90329-Q

Chen, C; Arjomandi, M; Balmes, J; Tager, I; N, H. (2007a). Effects of chronic and acute ozone exposure on lipid peroxidation and antioxidant capacity in healthy young adults. Environ Health Perspect 115: 1732-1737. http://dx.doi.org/10.1289/ehp.10294

Chen, C; Arjomandi, M; Qin, H; Balmes, J; Tager, I; Holland, N. (2006a). Cytogenetic damage in buccal epithelia and peripheral lymphocytes of young healthy individuals exposed to ozone. Mutagenesis 21: 131-137. http://dx.doi.org/10.1093/mutage/gel007

Cho, HY; Hotchkiss, JA; Harkema, JR. (1999). Inflammatory and epithelial responses during the development of ozone-induced mucous cell metaplasia in the nasal epithelium of rats. Toxicol Sci 51: 135-145.

Cho, HY; Kleeberger, SR. (2007). Genetic mechanisms of susceptibility to oxidative lung injury in mice. Free Radic Biol Med 42: 433-445. http://dx.doi.org/10.1016/j.freeradbiomed.2006.11.021

Cho, HY; Morgan, DL; Bauer, AK; Kleeberger, SR. (2007). Signal transduction pathways of tumor necrosis factor--mediated lung injury induced by ozone in mice. Am J Respir Crit Care Med 175: 829-839. http://dx.doi.org/10.1164/rccm.200509-1527OC

Cho, HY; Zhang, LY; Kleeberger, SR. (2001). Ozone-induced lung inflammation and hyperreactivity are mediated via tumor necrosis factor-alpha receptors. Am J Physiol 280: L537-L546.

Christian, DL; Chen, LL; Scannell, CH; Ferrando, RE; Welch, BS; Balmes, JR. (1998). Ozone-induced inflammation is attenuated with multiday exposure. Am J Respir Crit Care Med 158: 532-537.

Chuang, GC; Yang, Z; Westbrook, DG; Pompilius, M; Ballinger, CA; White, RC; Krzywanski, DM; Postlethwait, EM; Ballinger, SW. (2009). Pulmonary ozone exposure induces vascular dysfunction, mitochondrial damage, and atherogenesis. Am J Physiol Lung Cell Mol Physiol 297: L209-L216. http://dx.doi.org/10.1152/ajplung.00102.2009

Cohen-Hubal, EA; Kimbell, JS; Fedkiw, PS. (1996). Incorporation of nasal-lining mass-transfer resistance into a CFD model for prediction of ozone dosimetry in the upper respiratory tract. Inhal Toxicol 8: 831-857.

Cole, MP; Freeman, BA. (2009). Promotion of cardiovascular disease by exposure to the air pollutant ozone [Review]. Am J Physiol Lung Cell Mol Physiol 297: L209-L216. http://dx.doi.org/10.1152/ajplung.00187.2009

Coleridge, HM; Coleridge, JCG; Ginzel, KH; Baker, DG; Banzett, RB; Morrison, MA. (1976). Stimulation of `irritant' receptors and afferent C-fibers in the lungs by prostaglandins. Nature 264: 451-453.

Coleridge, JCG; Coleridge, HM; Schelegle, ES; Green, JF. (1993). Acute inhalation of ozone stimulates bronchial C-fibers and rapidly adapting receptors in dogs. J Appl Physiol 74: 2345-2352.

Connor, LM; Ballinger, CA; Albrecht, TB; Postlethwait, EM. (2004). Interfacial phospholipids inhibit ozone reactive absorption-mediated cytotoxicity in vitro. Am J Physiol 286: L1169-L1178. http://dx.doi.org/10.1152/ajplung.00397.2003

Corradi, M; Alinovi, R; Goldoni, M; Vettori, M; Folesani, G; Mozzoni, P; Cavazzini, S; Bergamaschi, E; Rossi, L; Mutti, A. (2002). Biomarkers of oxidative stress after controlled human exposure to ozone. Toxicol Lett 134: 219-225.

Costa, DL; Schafrank, SN; Wehner, RW; Jellett, E. (1985). Alveolar permeability to protein in rats differentially susceptible to ozone. J Appl Toxicol 5: 182-186. http://dx.doi.org/10.1002/jat.2550050309

Cross, CE; Motchnik, PA; Bruener, BA; Jones, DA; Kaur, H; Ames, BN; Halliwell, B. (1992). Oxidative damage to plasma constituents by ozone. FEBS Lett 298: 269-272. http://dx.doi.org/10.1016/0014-5793(92)80074-Q

Dahl, M; Bauer, AK; Arredouani, M; Soininen, R; Tryggvason, K; Kleeberger, SR; Kobzik, L. (2007). Protection against inhaled oxidants through scavenging of oxidized lipids by macrophage receptors MARCO and SR-AI/II. J Clin Invest 117: 757-764. http://dx.doi.org/10.1172/JCI29968

Darrow, LA; Klein, M; Flanders, WD; Waller, LA; Correa, A; Marcus, M; Mulholland, JA; Russell, AG; Tolbert, PE. (2009). Ambient air pollution and preterm birth: A time-series analysis. Epidemiology 20: 689-698. http://dx.doi.org/10.1097/EDE.0b013e3181a7128f

Delaunois, A; Segura, P; Montano, LM; Vargas, MH; Ansay, M; Gustin, P. (1998). Comparison of ozone-induced effects on lung mechanics and hemodynamics in the rabbit. Toxicol Appl Pharmacol 150: 58-67.

Dempsey, JA; Johnson, BD; Saupe, KW. (1990). Adaptations and limitations in the pulmonary system during exercise [Review]. Chest 97: 81S-87S.

Dempsey, JA; McKenzie, DC; Haverkamp, HC; Eldridge, MW. (2008). Update in the understanding of respiratory limitations to exercise performance in fit, active adults [Review]. Chest 134: 613-622. http://dx.doi.org/10.1378/chest.07-2730

Devlin, RB; Duncan, KE; Jardim, M; Schmitt, MT; Rappold, AG; Diaz-Sanchez, D. (2012). Controlled exposure of healthy young volunteers to ozone causes cardiovascular effects. Circulation 126: 104-111. http://dx.doi.org/10.1161/CIRCULATIONAHA.112.094359

Devlin, RB; Folinsbee, LJ; Biscardi, F; Hatch, G; Becker, S; Madden, MC; Robbins, M; Koren, HS. (1997). Inflammation and cell damage induced by repeated exposure of humans to ozone. Inhal Toxicol 9: 211-235.

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

Diemer, T; Allen, JA; Hales, KH; Hales, DB. (2003). Reactive oxygen disrupts mitochondria in MA-10 tumor Leydig cells and inhibits steroidogenic acute regulatory (StAR) protein and steroidogenesis. Endocrinology 144: 2882-2891. http://dx.doi.org/10.1210/en.2002-0090

Dietert, RR; DeWitt, JC; Germolec, DR; Zelikoff, JT. (2010). Breaking patterns of environmentally influenced disease for health risk reduction: Immune perspectives [Review]. Environ Health Perspect 118: 1091-1099. http://dx.doi.org/10.1289/ehp.1001971

Dimeo, MJ; Glenn, MG; Holtzman, MJ; Sheller, JR; Nadel, JA; Boushey, HA. (1981). Threshold concentration of ozone causing an increase in bronchial reactivity in humans and adaptation with repeated exposures. Am Rev Respir Dis 124: 245-248.

Dormans, JAM, A; Van Bree, L; Boere, AJF; Marra, M; Rombout, PJA. (1999). Interspecies differences in time course of pulmonary toxicity following repeated exposure to ozone. Inhal Toxicol 11: 309-329. http://dx.doi.org/10.1080/089583799197113

Dostert, C; Petrilli, V; Van Bruggen, R; Steele, C; Mossman, BT; Tschopp, J. (2008). Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. Science 320: 674-677. http://dx.doi.org/10.1126/science.1156995

Drechsler-Parks, DM. (1995). The dose-response relationship in older men exposed to ozone. Exp Gerontol 30: 65-75.

Duan, X; Buckpitt, AR; Pinkerton, KE; Ji, C; Plopper, CG. (1996). Ozone-induced alterations in glutathione in lung subcompartments of rats and monkeys. Am J Respir Cell Mol Biol 14: 70-75.

Duan, X; Buckpitt, AR; Plopper, CG. (1993). Variation in antioxidant enzyme activities in anatomic subcompartments within rat and rhesus monkey lung. Toxicol Appl Pharmacol 123: 73-82.

Emmons, K; Foster, WM. (1991). Smoking cessation and acute airway response to ozone. Arch Environ Occup Health 46: 288-295. http://dx.doi.org/10.1080/00039896.1991.9934389

Enami, S; Hoffmann, MR; Colussi, AJ. (2008a). Acidity enhances the formation of a persistent ozonide at aqueous ascorbate/ozone gas interfaces. PNAS 105: 7365-7369. http://dx.doi.org/10.1073/pnas.0710791105

Enami, S; Hoffmann, MR; Colussi, AJ. (2008b). Ozonolysis of uric acid at the air/water interface. J Phys Chem B 112: 41534156. http://dx.doi.org/10.1021/jp712010k

Enami, S; Hoffmann, MR; Colussi, AJ. (2009a). How phenol and alpha-tocopherol react with ambient ozone at gas/liquid interfaces. J Phys Chem A 113: 7002-7010. http://dx.doi.org/10.1021/jp901712k

Enami, S; Hoffmann, MR; Colussi, AJ. (2009b). Ozone oxidizes glutathione to a sulfonic acid. Chem Res Toxicol 22: 35-40. http://dx.doi.org/10.1021/tx800298j10.1021/tx800298j

Enami, S; Hoffmann, MR; Colussi, AJ. (2009c). Simultaneous detection of cysteine sulfenate, sulfinate, and sulfonate during cysteine interfacial ozonolysis [Letter]. J Phys Chem B 113: 9356-9358. http://dx.doi.org/10.1021/jp904316n

Engel, LA. (1985). Intraregional gas mixing and distribution. In Gas mixing and distribution in the lung. New York: Marcel Dekker.

Esther, CR; Peden, DB; Alexis, NE; Hernandez, ML. (2011). Airway purinergic responses in healthy, atopic nonasthmatic, and atopic asthmatic subjects exposed to ozone. Inhal Toxicol 23: 324-330. http://dx.doi.org/10.3109/08958378.2011.572096

Evans, MJ; Fanucchi, MV; Baker, GL; Van Winkle, LS; Pantle, LM; Nishio, SJ; Schelegle, ES; Gershwhin, LJ; Miller, LA; Hyde, DM; Sannes, PL; Plopper, CG. (2003). Atypical development of the tracheal basement membrane zone of infant rhesus monkeys exposed to ozone and allergen. Am J Physiol 285: L931-L939. http://dx.doi.org/10.1152/ajplung.00175.2003

Evans, MJ; Fanucchi, MV; Baker, GL; Van Winkle, LS; Pantle, LM; Nishio, SJ; Schelegle, ES; Gershwin, LJ; Miller, LA; Hyde, DM; Plopper, CG. (2004). The remodelled tracheal basement membrane zone of infant rhesus monkeys after 6 months of recovery. Clin Exp Allergy 34: 1131-1136. http://dx.doi.org/10.1111/j.1365-2222.2004.02004.x CEA2004

Fabbri, LM; Aizawa, H; O'Byrne, PM; Bethel, RA; Walters, EH; Holtzman, MJ; Nadel, JA. (1985). An anti-inflammatory drug (BW755C) inhibits airway hyperresponsiveness induced by ozone in dogs. J Allergy Clin Immunol 76: 162-166. http://dx.doi.org/10.1016/0091-6749(85)90695-5

Fakhri, AA; Ilic, LM; Wellenius, GA; Urch, B; Silverman, F; Gold, DR; Mittleman, MA. (2009). Autonomic effects of controlled fine particulate exposure in young healthy adults: Effect modification by ozone. Environ Health Perspect 117: 1287-1292. http://dx.doi.org/10.1289/ehp.0900541

Fanucchi, MV; Plopper, CG; Evans, MJ; Hyde, DM; Van Winkle, LS; Gershwin, LJ; Schelegle, ES. (2006). Cyclic exposure to ozone alters distal airway development in infant rhesus monkeys. Am J Physiol Lung Cell Mol Physiol 291: L644-L650. http://dx.doi.org/10.1152/ajplung.00027.2006

Feng, R; He, W; Ochi, H; Castranova, V. (2006). Ozone exposure impairs antigen-specific immunity but activates IL-7-induced proliferation of CD4-CD8- thymocytes in BALB/c mice. J Toxicol Environ Health A 69: 1511-1526. http://dx.doi.org/10.1080/15287390500468696

Foster, WM; Brown, RH; Macri, K; Mitchell, CS. (2000). Bronchial reactivity of healthy subjects: 18-20 h postexposure to ozone. J Appl Physiol 89: 1804-1810.

Foster, WM; Freed, AN. (1999). Regional clearance of solute from peripheral airway epithelia: Recovery after sublobar exposure to ozone. J Appl Physiol 86: 641-646.

Foster, WM; Stetkiewicz, PT. (1996). Regional clearance of solute from the respiratory epithelia: 18-20 h postexposure to ozone. J Appl Physiol 81: 1143-1149.

Foster, WM; Wills-Karp, M; Tankersley, CG; Chen, X; Paquette, NC. (1996). Bloodborne markers in humans during multiday exposure to ozone. J Appl Physiol 81: 794-800.

Frampton, MW; Morrow, PE; Torres, A; Cox, C; Voter, KZ; Utell, MJ; Gibb, FR; Speers, DM. (1997a). Ozone responsiveness in smokers and nonsmokers. Am J Respir Crit Care Med 155: 116-121.

Frampton, MW; Morrow, PE; Torres, A; Voter, KZ; Whitin, JC; Cox, C; Speers, DM; Tsai, Y; Utell, MJ. (1997b). Effects of ozone on normal and potentially sensitive human subjects: Part II. Airway inflammation and responsiveness to ozone in nonsmokers and smokers. Boston, MA: Health Effects Institute.

Frampton, MW; Pryor, WA; Cueto, R; Cox, C; Morrow, PE; Utell, MJ. (1999). Ozone exposure increases aldehydes in epithelial lining fluid in human lung. Am J Respir Crit Care Med 159: 1134-1137.

Frank, R; Liu, MC; Spannhake, EW; Mlynarek, S; Macri, K; Weinmann, GG. (2001). Repetitive ozone exposure of young adults: Evidence of persistent small airway dysfunction. Am J Respir Crit Care Med 164: 1253-1260.

Freed, AN; Chou, CL; Fuller, SD; Croxton, TL. (1996). Ozone-induced vagal reflex modulates airways reactivity in rabbits. Respir Physiol Neurobiol 105: 95-102.

Freed, AN; Cueto, R; Pryor, WA. (1999). Antioxidant transport modulates peripheral airway reactivity and inflammation during ozone exposure. J Appl Physiol 87: 1595-1603.

Fujita, M; Sasayama, S; Ohno, A; Nakajima, H; Asanoi, H. (1987). Importance of angina for development of collateral circulation. Heart 57: 139-143.

Gackière, F; Saliba, L; Baude, A; Bosler, O; Strube, C. (2011). Ozone inhalation activates stress-responsive regions of the CNS. J Neurochem 117: 961-972. http://dx.doi.org/10.1111/j.1471-4159.2011.07267.x

Gao, X; Raghavamenon, AC; D'Auvergne, O; Uppu, RM. (2009b). Cholesterol secoaldehyde induces apoptosis in J774 macrophages via mitochondrial pathway but not involving reactive oxygen species as mediators. Biochem Biophys Res Commun 389: 382-387. http://dx.doi.org/10.1016/j.bbrc.2009.09.005

Garantziotis, S; Li, Z; Potts, EN; Kimata, K; Zhuo, L; Morgan, DL; Savani, RC; Noble, PW; Foster, WM; Schwartz, DA; Hollingsworth, JW. (2009). Hyaluronan mediates ozone-induced airway hyperresponsiveness in mice. J Biol Chem 284: 11309-11317. http://dx.doi.org/10.1074/jbc.M802400200

Garantziotis, S; Li, Z; Potts, EN; Lindsey, JY; Stober, VP; Polosukhin, VV; Blackwell, TS; Schwartz, DA; Foster, WM; Hollingsworth, JW. (2010). TLR4 is necessary for hyaluronan-mediated airway hyperresponsiveness after ozone inhalation. Am J Respir Crit Care Med 181: 666-675. http://dx.doi.org/10.1164/rccm.200903-0381OC

Garcia, GJ; Schroeter, JD; Segal, RA; Stanek, J; Foureman, GL; Kimbell, JS. (2009a). Dosimetry of nasal uptake of water-soluble and reactive gases: A first study of interhuman variability. Inhal Toxicol 21: 607-618. http://dx.doi.org/10.1080/08958370802320186

Gerrity, TR; Biscardi, F; Strong, A; Garlington, AR; Brown, JS; Bromberg, PA. (1995). Bronchoscopic determination of ozone uptake in humans. J Appl Physiol 79: 852-860.

Gerrity, TR; McDonnell, WF; House, DE. (1994). The relationship between delivered ozone dose and functional responses in humans. Toxicol Appl Pharmacol 124: 275-283.

Gerrity, TR; Weaver, RA; Berntsen, J; House, DE; O'Neil, JJ. (1988). Extrathoracic and intrathoracic removal of O3 in tidal-breathing humans. J Appl Physiol 65: 393-400.

Giamalva, D; Church, DF; Pryor, WA. (1985). A comparison of the rates of ozonation of biological antioxidants and oleate and linoleate esters. Biochem Biophys Res Commun 133: 773-779.

Gong, H, Jr; Wong, R; Sarma, RJ; Linn, WS; Sullivan, ED; Shamoo, DA; Anderson, KR; Prasad, SB. (1998). Cardiovascular effects of ozone exposure in human volunteers. Am J Respir Crit Care Med 158: 538-546.

Graham, DE; Koren, HS. (1990). Biomarkers of inflammation in ozone-exposed humans: Comparison of the nasal and bronchoalveolar lavage. Am J Respir Crit Care Med 142: 152-156.

Gross, EA; Starr, TB; Randall, HW; Morgan, KT. (1987). Morphometric analysis of the primate nasal cavity [Abstract]. Toxicologist 7: 193.

Gross, EA; Swenberg, JA; Fields, S; Popp, JA. (1982). Comparative morphometry of the nasal cavity in rats and mice. J Anat 135: 83-88.

Guevara-Guzmán, R; Arriaga, V; Kendrick, KM; Bernal, C; Vega, X; Mercado-Gómez, OF; Rivas-Arancibia, S. (2009). Estradiol prevents ozone-induced increases in brain lipid peroxidation and impaired social recognition memory in female rats. Neuroscience 159: 940-950. http://dx.doi.org/10.1016/j.neuroscience.2009.01.047

Gunnison, AF; Hatch, GE. (1999). O3-induced inflammation in prepregnant, pregnant, and lactating rats correlates with O3 dose estimated by 18O. Am J Physiol 276: L332-L340.

Gunnison, AF; Hatch, GE; Crissman, K; Bowers, A. (1996). Comparative sensitivity of lactating and virgin female rats to ozone-induced pulmonary inflammation. Inhal Toxicol 8: 607-623.

Hackney, JD; Linn, WS; Mohler, JG; Collier, CR. (1977b). Adaptation to short-term respiratory effects of ozone in men exposed repeatedly. J Appl Physiol 43: 82-85.

Hamade, AK; Misra, V; Rabold, R; Tankersley, CG. (2010). Age-related changes in cardiac and respiratory adaptation to acute ozone and carbon black exposures: Interstrain variation in mice. Inhal Toxicol 22: 84-94. http://dx.doi.org/10.3109/08958378.2010.503974

Hamade, AK; Tankersley, CG. (2009). Interstrain variation in cardiac and respiratory adaptation to repeated ozone and particulate matter exposures. Am J Physiol Regul Integr Comp Physiol 296: R1202-R1215. http://dx.doi.org/10.1152/ajpregu.90808.2008

Hamilton, RF; Li, L; Eschenbacher, WL; Szweda, L; Holian, A. (1998). Potential involvement of 4-hydroxynonenal in the response of human lung cells to ozone. Am J Physiol 274: L8-L16.

Han, SG; Andrews, R; Gairola, CG; Bhalla, DK. (2008). Acute pulmonary effects of combined exposure to carbon nanotubes and ozone in mice. Inhal Toxicol 20: 391-398. http://dx.doi.org/10.1080/08958370801904014

Hansen, CA; Barnett, AG; Pritchard, G. (2008). The effect of ambient air pollution during early pregnancy on fetal ultrasonic measurements during mid-pregnancy. Environ Health Perspect 116: 362-369. http://dx.doi.org/10.1289/ehp.10720

Haque, R; Umstead, TM; Freeman, WM; Floros, J; Phelps, DS. (2009). The impact of surfactant protein-A on ozone-induced changes in the mouse bronchoalveolar lavage proteome. Proteome Science 7: 12. http://dx.doi.org/10.1186/1477-5956-7-12

Haque, R; Umstead, TM; Ponnuru, P; Guo, X; Hawgood, S; Phelps, DS; Floros, J. (2007). Role of surfactant protein-A (SP-A) in lung injury in response to acute ozone exposure of SP-A deficient mice. Toxicol Appl Pharmacol 220: 72-82. http://dx.doi.org/10.1016/j.taap.2006.12.017

Harkema, JR; Hotchkiss, JA; Barr, EB; Bennett, CB; Gallup, M; Lee, JK; Basbaum, C. (1999). Long-lasting effects of chronic ozone exposure on rat nasal epithelium. Am J Respir Cell Mol Biol 20: 517-529.

Harkema, JR; Plopper, CG; Hyde, DM; St George, JA; Dungworth, DL. (1987a). Effects of an ambient level of ozone on primate nasal epithelial mucosubstances: quantitative histochemistry. Am J Pathol 127: 90-96.

Harkema, JR; Plopper, CG; Hyde, DM; St George, JA; Wilson, DW; Dungworth, DL. (1987b). Response of the macaque nasal epithelium to ambient levels of ozone: A morphologic and morphometric study of the transitional and respiratory epithelium. Am J Pathol 128: 29-44.

Harkema, JR; Plopper, CG; Hyde, DM; St George, JA; Wilson, DW; Dungworth, DL. (1993). Response of macaque bronchiolar epithelium to ambient concentrations of ozone. Am J Pathol 143: 857-866.

Harkema, JR; Wagner, JG. (2005). Epithelial and inflammatory responses in the airways of laboratory rats coexposed to ozone and biogenic substances: Enhancement of toxicant-induced airway injury. Exp Toxicol Pathol 57: 129-141. http://dx.doi.org/10.1016/j.etp.2005.05.013

Hatch, GE. (1992). Comparative biochemistry of airway lining fluid. In RA Parent (Ed.), Comparative biology of the normal lung, vol 1: Treatise on pulmonary toxicology (pp. 617-632). Boca Raton, FL: CRC Press, Inc.

Hatch, GE; Slade, R; Harris, LP; Mcdonnell, WF; Devlin, RB; Koren, HS; Costa, DL; Mckee, J. (1994). Ozone dose and effect in humans and rats: A comparison using oxygen-18 labeling and bronchoalveolar lavage. Am J Respir Crit Care Med 150: 676-683.

Hatch, GE; Slade, R; Stead, AG; Graham, JA. (1986). Species comparison of acute inhalation toxicity of ozone and phosgene. J Toxicol Environ Health 19: 43-53. http://dx.doi.org/10.1080/15287398609530905

Hatch, GE; Wiester, MJ; Overton, JH, Jr; Aissa, M. (1989). Respiratory tract dosimetry of [18]O-labeled ozone in rats: Implications for a rat-human extrapolation of ozone dose. In T Schneider; SD Lee; GJR Wolters; LD Grant (Eds.), Atmospheric ozone research and its policy implications (pp. 553-560). Amsterdam, The Netherlands: Elsevier Science Publishers B.V. http://www.elsevier.com/wps/find/bookdescription.cws_home/502592/description#description

Hazbun, ME; Hamilton, R; Holian, A; Eschenbacher, WL. (1993). Ozone-induced increases in substance P and 8-epi-prostaglandin F2 alpha in the airways of human subjects. Am J Respir Cell Mol Biol 9: 568-572. http://dx.doi.org/10.1165/ajrcmb/9.5.568

Hazucha, MJ; Bates, DV; Bromberg, PA. (1989). Mechanism of action of ozone on the human lung. J Appl Physiol 67: 1535-1541.

Hazucha, MJ; Folinsbee, LJ; Bromberg, PA. (2003). Distribution and reproducibility of spirometric response to ozone by gender and age. J Appl Physiol 95: 1917-1925.

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

Hernandez, ML; Lay, JC; Harris, B; Esther, CR; Brickey, WJ; Bromberg, PA; Diaz-Sanchez, D; Devlin, RB; Kleeberger, SR; Alexis, NE; Peden, DB. (2010). Atopic asthmatic subjects but not atopic subjects without asthma have enhanced inflammatory response to ozone. J Allergy Clin Immunol 126: 537-544. http://dx.doi.org/10.1016/j.jaci.2010.06.043

Hoigné, J; Bader, H. (1983). Rate constants of reactions of ozone with organic and inorganic compounds in water - II: Dissociating organic compounds. Water Res 17: 185-194. http://dx.doi.org/10.1016/0043-1354(83)90099-4

Hollingsworth, JW; Cook, DN; Brass, DM; Walker, JKL; Morgan, DL; Foster, WM; Schwartz, DA. (2004). The role of Toll-like receptor 4 in environmental airway injury in mice. Am J Respir Crit Care Med 170: 126-132. http://dx.doi.org/10.1164/rccm.200311-1499OC

Hollingsworth, JW; Free, ME; Li, Z; Andrews, LN; Nakano, H; Cook, DN. (2010). Ozone activates pulmonary dendritic cells and promotes allergic sensitization through a Toll-like receptor 4-dependent mechanism [Letter]. J Allergy Clin Immunol 125: 1167-1170. http://dx.doi.org/10.1016/j.jaci.2010.03.001

Hollingsworth, JW; Maruoka, S; Li, Z; Potts, EN; Brass, DM; Garantziotis, S; Fong, A; Foster, WM; Schwartz, DA. (2007). Ambient ozone primes pulmonary innate immunity in mice. J Immunol 179: 4367-4375.

Holtzman, MJ; Cunningham, JH; Sheller, JR; Irsigler, GB; Nadel, JA; Boushey, HA. (1979). Effect of ozone on bronchial reactivity in atopic and nonatopic subjects. Am Rev Respir Dis 120: 1059-1067.

Holtzman, MJ; Fabbri, LM; O'Byrne, PM; Gold, BD; Aizawa, H; Walters, EH; Alpert, SE; Nadel, JA. (1983). Importance of airway inflammation for hyperresponsiveness induced by ozone. Am Rev Respir Dis 127: 686-690.

Holz, O; Jorres, RA; Timm, P; Mucke, M; Richter, K; Koschyk, S; Magnussen, H. (1999). Ozone-induced airway inflammatory changes differ between individuals and are reproducible. Am J Respir Crit Care Med 159: 776-784.

Horstman, DH; Ball, BA; Brown, J; Gerrity, T; Folinsbee, LJ. (1995). Comparison of pulmonary responses of asthmatic and nonasthmatic subjects performing light exercise while exposed to a low level of ozone. Toxicol Ind Health 11: 369-385.

Horvath, SM; Gliner, JA; Folinsbee, LJ. (1981). Adaptation to ozone: Duration of effect. Am Rev Respir Dis 123: 496-499.

Hotchkiss, JA; Harkema, JR; Henderson, RF. (1991). Effect of cumulative ozone exposure on ozone-induced nasal epithelial hyperplasia and secretory metaplasia in rats. Exp Lung Res 15: 589-600.

Hu, PC; Miller, FJ; Daniels, MJ; Hatch, G. (1982). Protein accumulation in lung lavage fluid following ozone exposure. Environ Res 29: 377-388. http://dx.doi.org/10.1016/0013-9351(82)90039-1

Hu, SC; Ben-Jebria, A; Ultman, JS. (1992). Longitudinal distribution of ozone absorption in the lung: Quiet respiration in healthy subjects. J Appl Physiol 73: 1655-1667.

Hu, SC; Ben-Jebria, A; Ultman, JS. (1994). Longitudinal distribution of ozone absorption in the lung: Effects of respiratory flow. J Appl Physiol 77: 574-583.

Ichinose, T; Arakawa, K; Shimojo, N; Sagai, M. (1988). Biochemical effects of combined gases of nitrogen dioxide and ozone: II Species differences in lipid peroxides and antioxidative protective enzymes in the lungs. Toxicol Lett 42: 167-176.

ICRP (International Commission on Radiological Protection). (1994). Human respiratory tract model for radiological protection: A report of a task group of the International Commission on Radiological Protection. ICRP Publication 66 [Review]. Ann ICRP 24: 1-482.

Ignatenko, AV; Cherenkevich, SN. (1985). Reactivity of amino-acids and proteins in reactions with ozone. Kinet Catal 26: 1145-1148.

Islam, T; Mcconnell, R; Gauderman, WJ; Avol, E; Peters, JM; Gilliland, FD. (2008). Ozone, oxidant defense genes and risk of asthma during adolescence. Am J Respir Crit Care Med 177: 388-395. http://dx.doi.org/10.1164/rccm.200706-863OC

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

Jalaludin, B; Mannes, T; Morgan, G; Lincoln, D; Sheppeard, V; Corbett, S. (2007). Impact of ambient air pollution on gestational age is modified by season in Sydney, Australia. Environ Health 6: 16. http://dx.doi.org/10.1186/1476-069X-6-16

James, DS; Stidley, CA; Lambert, WE; Chick, TW; Mermier, CM; Samet, JM. (1997). Oronasal distribution of ventilation at different ages. Arch Environ Occup Health 52: 118-123.

Jiang, D; Liang, J; Fan, J; Yu, S; Chen, S; Luo, Y; Prestwich, GD; Mascarenhas, MM; Garg, HG; Quinn, DA; Homer, RJ; Goldstein, DR; Bucala, R; Lee, PJ; Medzhitov, R; Noble, PW. (2005). Regulation of lung injury and repair by Toll-like receptors and hyaluronan. Nat Med 11: 1173-1179. http://dx.doi.org/10.1038/nm1315

Joad, JP; Kott, KS; Bric, JM. (1996). The local C-fiber contribution to ozone-induced effects on the isolated guinea pig lung. Toxicol Appl Pharmacol 141: 561-567.

Joad, JP; Kott, KS; Bric, JM; Peake, JL; Plopper, CG; Schelegle, ES; Gershwin, LJ; Pinkerton, KE. (2006). Structural and functional localization of airway effects from episodic exposure of infant monkeys to allergen and/or ozone. Toxicol Appl Pharmacol 214: 237-243. http://dx.doi.org/10.1016/j.taap.2005.12.012

Johansson, E; Wesselkamper, SC; Shertzer, HG; Leikauf, GD; Dalton, TP; Chen, Y. (2010). Glutathione deficient C57BL/6J mice are not sensitized to ozone-induced lung injury. Biochem Biophys Res Commun 396: 407-412. http://dx.doi.org/10.1016/j.bbrc.2010.04.105

Johnston, RA; Mizgerd, JP; Shore, SA. (2005a). CXCR2 is essential for maximal neutrophil recruitment and methacholine responsiveness after ozone exposure. Am J Physiol Lung Cell Mol Physiol 288: L61-L67. http://dx.doi.org/10.1152/ajplung.00101.2004 00101.2004

Johnston, RA; Schwartzman, IN; Flynt, L; Shore, SA. (2005b). Role of interleukin-6 in murine airway responses to ozone. Am J Physiol Lung Cell Mol Physiol 288: L390-L397. http://dx.doi.org/10.1152/ajplung.00007.2004

Johnston, RA; Theman, TA; Lu, FL; Terry, RD; Williams, ES; Shore, SA. (2008). Diet-induced obesity causes innate airway hyperresponsiveness to methacholine and enhances ozone-induced pulmonary inflammation. J Appl Psychol 104: 1727-1735. http://dx.doi.org/10.1152/japplphysiol.00075.2008

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

Jorres, RA; Holz, O; Zachgo, W; Timm, P; Koschyk, S; Muller, B; Grimminger, F; Seeger, W; Kelly, FJ; Dunster, C; Frischer, T; Lubec, G; Waschewski, M; Niendorf, A; Magnussen, H. (2000). The effect of repeated ozone exposures on inflammatory markers in bronchoalveolar lavage fluid and mucosal biopsies. Am J Respir Crit Care Med 161: 1855-1861.

Kabel, JR; Ben-Jebria, A; Ultman, JS. (1994). Longitudinal distribution of ozone absorption in the lung: Comparison of nasal and oral quiet breathing. J Appl Physiol 77: 2584-2592.

Kafoury, RM; Pryor, WA; Squadrito, GL; Salgo, MG; Zou, X; Friedman, M. (1998). Lipid ozonation products activate phospholipases A2, C, and D. Toxicol Appl Pharmacol 150: 338-349.

Kajekar, R; Pieczarka, EM; Smiley-Jewell, SM; Schelegle, ES; Fanucchi, MV; Plopper, CG. (2007). Early postnatal exposure to allergen and ozone leads to hyperinnervation of the pulmonary epithelium. Respir Physiol Neurobiol 155: 55-63. http://dx.doi.org/10.1016/j.resp.2006.03.002

Kannan, S; Misra, DP; Dvonch, T; Krishnakumar, A. (2006). Exposures to airborne particulate matter and adverse perinatal outcomes: A biologically plausible mechanistic framework for exploring potential effect modification by nutrition. Environ Health Perspect 114: 1636-1642.

Kanofsky, JR; Sima, PD. (1995). Reactive absorption of ozone by aqueous biomolecule solutions: Implications for the role of sulfhydryl compounds as targets for ozone. Arch Biochem Biophys 316: 52-62.

Kari, F; Hatch, G; Slade, R; Crissman, K; Simeonova, PP; Luster, M. (1997). Dietary restriction mitigates ozone-induced lung inflammation in rats: A role for endogenous antioxidants. Am J Respir Cell Mol Biol 17: 740-747.

Katre, A; Ballinger, C; Akhter, H; Fanucchi, M; Kim, DK; Postlethwait, E; Liu, RM. (2011). Increased transforming growth factor beta 1 expression mediates ozone-induced airway fibrosis in mice. Inhal Toxicol 23: 486-494. http://dx.doi.org/10.3109/08958378.2011.584919

Kavlock, R; Daston, G; Grabowski, CT. (1979). Studies on the developmental toxicity of ozone. I. Prenatal effects. Toxicol Appl Pharmacol 48: 19-28. http://dx.doi.org/10.1016/S0041-008X(79)80004-6

Kehrl, HR; Peden, DB; Ball, BA; Folinsbee, LJ; Horstman, DH. (1999). Increased specific airway reactivity of persons with mild allergic asthma after 7.6 hours of exposure to 0.16 ppm ozone. J Allergy Clin Immunol 104: 1198-1204.

Kehrl, HR; Vincent, LM; Kowalsky, RJ; Horstman, DH; O'Neil, JJ; McCartney, WH; Bromberg, PA. (1987). Ozone exposure increases respiratory epithelial permeability in humans. Am Rev Respir Dis 135: 1124-1128.

Kermani, S; Ben-Jebria, A; Ultman, JS. (2006). Kinetics of ozone reaction with uric acid, ascorbic acid, and glutathione at physiologically relevant conditions. Arch Biochem Biophys 451: 8-16. http://dx.doi.org/10.1016/j.abb.2006.04.015

Kim, CS; Alexis, NE; Rappold, AG; Kehrl, H; Hazucha, MJ; Lay, JC; Schmitt, MT; Case, M; Devlin, RB; Peden, DB; Diaz-Sanchez, D. (2011). Lung function and inflammatory responses in healthy young adults exposed to 0.06 ppm ozone for 6.6 hours. Am J Respir Crit Care Med 183: 1215-1221. http://dx.doi.org/10.1164/rccm.201011-1813OC

Kleeberger, SR; Levitt, RC; Zhang, LY; Longphre, M; Harkema, J; Jedlicka, A; Eleff, SM; DiSilvestre, D; Holroyd, KJ. (1997). Linkage analysis of susceptibility to ozone-induced lung inflammation in inbred mice. Nat Genet 17: 475-478.

Kleeberger, SR; Reddy, S; Zhang, LY; Jedlicka, AE. (2000). Genetic susceptibility to ozone-induced lung hyperpermeability: Role of toll-like receptor 4. Am J Respir Cell Mol Biol 22: 620-627.

Kleeberger, SR; Reddy, SP; Zhang, LY; Cho, HY; Jedlicka, AE. (2001). Toll-like receptor 4 mediates ozone-induced murine lung hyperpermeability via inducible nitric oxide synthase. Am J Physiol 280: L326-L333.

Kleeberger, SR; Seiden, JE; Levitt, RC; Zhang, LY. (1993). Mast cells modulate acute ozone-induced inflammation of the murine lung. Am J Respir Crit Care Med 148: 1284-1291.

Kodavanti, UP; Costa, DL; Dreher, KL; Crissman, K; Hatch, GE. (1995). Ozone-induced tissue injury and changes in antioxidant homeostasis in normal and ascorbate-deficient guinea pigs. Biochem Pharmacol 50: 243-251. http://dx.doi.org/10.1016/0006-2952(95)00122-G

Kodavanti, UP; Thomas, R; Ledbetter, AD; Schladweiler, MC; Shannahan, JH; Wallenborn, JG; Lund, AK; Campen, MJ; Butler, EO; Gottipolu, RR; Nyska, A; Richards, JE; Andrews, D; Jaskot, RH; Mckee, J; Kotha, S. R.; Patel, RB; Parianandi, NL. (2011). Vascular and cardiac impairments in rats Inhaling ozone and diesel exhaust particles. Environ Health Perspect 119: 312-318. http://dx.doi.org/10.1289/ehp.1002386

Koenig, JQ; Covert, DS; Marshall, SG; Van Belle, G; Pierson, WE. (1987). The effects of ozone and nitrogen dioxide on pulmonary function in healthy and in asthmatic adolescents. Am J Respir Crit Care Med 136: 1152-1157.

Koren, HS; Devlin, RB; Graham, DE; Mann, R; Mcgee, MP; Horstman, DH; Kozumbo, WJ; Becker, S; House, DE; Mcdonnell, WF; Bromberg, PA. (1989). Ozone-induced inflammation in the lower airways of human subjects. Am J Respir Crit Care Med 139: 407-415.

Kreit, JW; Gross, KB; Moore, TB; Lorenzen, TJ; D'Arcy, J; Eschenbacher, WL. (1989). Ozone-induced changes in pulmonary function and bronchial responsiveness in asthmatics. J Appl Physiol 66: 217-222.

Krishna, MT; Springall, D; Meng, QH; Withers, N; Macleod, D; Biscione, G; Frew, A; Polak, J; Holgate, S. (1997). Effects of ozone on epithelium and sensory nerves in the bronchial mucosa of healthy humans. Am J Respir Crit Care Med 156: 943-950.

Larsen, ST; Matsubara, S; Mcconville, G; Poulsen, SS; Gelfand, EW. (2010). Ozone increases airway hyperreactivity and mucus hyperproduction in mice previously exposed to allergen. J Toxicol Environ Health A 73: 738-747. http://dx.doi.org/10.1080/15287391003614034

Larson, SD; Schelegle, ES; Walby, WF; Gershwin, LJ; Fanuccihi, MV; Evans, MJ; Joad, JP; Tarkington, BK; Hyde, DM; Plopper, CG. (2004). Postnatal remodeling of the neural components of the epithelial-mesenchymal trophic unit in the proximal airways of infant rhesus monkeys exposed to ozone and allergen. Toxicol Appl Pharmacol 194: 211-220. http://dx.doi.org/10.1016/j.taap.2003.09.025

Lay, JC; Alexis, NE; Kleeberger, SR; Roubey, RA; Harris, BD; Bromberg, PA; Hazucha, MJ; Devlin, RB; Peden, DB. (2007). Ozone enhances markers of innate immunity and antigen presentation on airway monocytes in healthy individuals [Letter]. J Allergy Clin Immunol 120: 719-722. http://dx.doi.org/10.1016/j.jaci.2007.05.005

Li, YF; Gauderman, WJ; Avol, E; Dubeau, L; Gilliland, FD. (2006d). Associations of tumor necrosis factor G-308A with childhood asthma and wheezing. Am J Respir Crit Care Med 173: 970-976. http://dx.doi.org/10.1164/rccm.200508-1256OC

Li, Z; Potts-Kant, EN; Garantziotis, S; Foster, WM; Hollingsworth, JW. (2011). Hyaluronan signaling during ozone-induced lung injury requires TLR4, MyD88, and TIRAP. PLoS ONE 6: e27137. http://dx.doi.org/10.1371/journal.pone.0027137

Li, Z; Potts, EN; Piantadosi, CA; Foster, WM; Hollingsworth, JW. (2010). Hyaluronan fragments contribute to the ozone-primed immune response to lipopolysaccharide. J Immunol 185: 6891-6898. http://dx.doi.org/10.4049/jimmunol.1000283

Linn, WS; Buckley, RD; Spier, CE; Blessey, RL; Jones, MP; Fischer, DA; Hackney, JD. (1978). Health effects of ozone exposure in asthmatics. Am Rev Respir Dis 117: 835-843.

Linn, WS; Medway, DA; Anzar, UT; Valencia, LM; Spier, CE; FS-D, T; Fischer, DA; Hackney, JD. (1982b). Persistence of adaptation to ozone in volunteers exposed repeatedly for six weeks. Am Rev Respir Dis 125: 491-495.

Liu, L; Leech, JA; Urch, RB; Poon, R; Zimmerman, B; Kubay, JM; Silverman, FS. (1999). A comparison of biomarkers of ozone exposure in human plasma, nasal lavage, and sputum. Inhal Toxicol 11: 657-674. http://dx.doi.org/10.1080/089583799196790

Liu, L; Leech, JA; Urch, RB; Silverman, FS. (1997). In vivo salicylate hyroxylation: A potential biomarker for assessing acute ozone exposure and effects in humans. Am J Respir Crit Care Med 156: 1405-1412.

London, SJ. (2007). Gene-air pollution interactions in asthma [Review]. Proc Am Thorac Soc 4: 217-220. http://dx.doi.org/10.1513/pats.200701-031AW

Long, NC; Suh, J; Morrow, JD; Schiestl, RH; Krishna Murthy, GG; Brain, JD; Frei, B. (2001). Ozone causes lipid peroxidation but little antioxidant depletion in exercising and nonexercising hamsters. J Appl Physiol 91: 1694-1700.

Longphre, M; Zhang, LY; Harkema, JR; Kleeberger, SR. (1999). Ozone-induced pulmonary inflammation and epithelial proliferation are partially mediated by PAF. J Appl Physiol 86: 341-349.

Maniar-Hew, K; Postlethwait, EM; Fanucchi, MV; Ballinger, CA; Evans, MJ; Harkema, JR; Carey, SA; McDonald, RJ; Bartolucci, AA; Miller, LA. (2011). Postnatal episodic ozone results in persistent attenuation of pulmonary and peripheral blood responses to LPS challenge. Am J Physiol Lung Cell Mol Physiol 300: L462-L471. http://dx.doi.org/10.1152/ajplung.00254.2010

Matsumura, Y. (1970). The effects of ozone, nitrogen dioxide, and sulfur dioxide on the experimentally induced allergic respiratory disorder in guinea pigs: II. The effects of ozone on the absorption and the retention of antigen in the lung. Am Rev Respir Dis 102: 438-443.

McBride, JT. (1992). Architecture of the tracheobronchial tree. In RA Parent (Ed.), Comparative biology of the normal lung, v 1: Treatise on pulmonary toxicology (pp. 49-61). Boca Raton, FL: CRC Press.

McDonnell, WF; Horstman, DH; Hazucha, MJ; Seal, E, Jr; Haak, ED; Salaam, SA; House, DE. (1983). Pulmonary effects of ozone exposure during exercise: Dose-response characteristics. J Appl Physiol 54: 1345-1352.

Mckinney, WJ; Jaskot, RH; Richards, JH; Costa, DL; Dreher, KL. (1998). Cytokine mediation of ozone-induced pulmonary adaptation. Am J Respir Cell Mol Biol 18: 696-705.

Mercer, RR; Anjilvel, S; Miller, FJ; Crapo, JD. (1991). Inhomogeneity of ventilatory unit volume and its effects on reactive gas uptake. J Appl Physiol 70: 2193-2205.

Mercer, RR; Russell, ML; Crapo, JD. (1992). Mucous lining layers in human and rat airways [Abstract]. Am Rev Respir Dis 145: A355.

Mikerov, AN; Gan, X; Umstead, TM; Miller, L; Chinchilli, VM; Phelps, DS; Floros, J. (2008a). Sex differences in the impact of ozone on survival and alveolar macrophage function of mice after Klebsiella pneumoniae infection. Respir Res 9: 24. http://dx.doi.org/10.1186/1465-9921-9-24

Mikerov, AN; Haque, R; Gan, X; Guo, X; Phelps, DS; Floros, J. (2008b). Ablation of SP-A has a negative impact on the susceptibility of mice to Klebsiella pneumoniae infection after ozone exposure: Sex differences. Respir Res 9: 77. http://dx.doi.org/10.1186/1465-9921-9-77

Mikerov, AN; Umstead, TM; Gan, X; Huang, W; Guo, X; Wang, G; Phelps, DS; Floros, J. (2008c). Impact of ozone exposure on the phagocytic activity of human surfactant protein A (SP-A) and SP-A variants. Am J Physiol Lung Cell Mol Physiol 294: L121-L130. http://dx.doi.org/10.1152/ajplung.00288.2007

Miller, FJ. (1995). Uptake and fate of ozone in the respiratory tract [Review]. Toxicol Lett 82-83: 277-285.

Miller, FJ; Kimbell, JS. (1995). Regional dosimetry of inhaled reactive gases. In RO McClellan; RF Henderson (Eds.), Concepts in inhalation toxicology (2nd ed., pp. 257-287). Washington, DC: Taylor & Francis.

Miller, FJ; McNeal, CA; Kirtz, JM; Gardner, DE; Coffin, DL; Menzel, DB. (1979). Nasopharyngeal removal of ozone in rabbits and guinea pigs. Toxicology 14: 273-281. http://dx.doi.org/10.1016/0300-483X(79)90009-X

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

Miller, FJ; Overton, JH, Jr; Jaskot, RH; Menzel, DB. (1985). A model of the regional uptake of gaseous pollutants in the lung: I. The sensitivity of the uptake of ozone in the human lung to lower respiratory tract secretions and exercise. Toxicol Appl Pharmacol 79: 11-27. http://dx.doi.org/10.1016/0041-008X(85)90364-3

Miller, LA; Gerriets, JE; Tyler, NK; Abel, K; Schelegle, ES; Plopper, CG; Hyde, DM. (2009). Ozone and allergen exposure during postnatal development alters the frequency and airway distribution of CD25+ cells in infant rhesus monkeys. Toxicol Appl Pharmacol 236: 39-48. http://dx.doi.org/10.1016/j.taap.2008.12.031

Molfino, NA; Wright, SC; Katz, I; Tarlo, S; Silverman, F; Mcclean, PA; Szalai, JP; Raizenne, M; Slutsky, AS; Zamel, N. (1991). Effect of low concentrations of ozone on inhaled allergen responses in asthmatic subjects. Lancet 338: 199-203.

Morgan, KT; Monticello, TM. (1990). Airflow, gas deposition, and lesion distribution in the nasal passages. Environ Health Perspect 85: 209-218.

Mudway, IS; Behndig, AF; Helleday, R; Pourazar, J; Frew, AJ; Kelly, FJ; Blomberg, A. (2006). Vitamin supplementation does not protect against symptoms in ozone-responsive subjects. Free Radic Biol Med 40: 1702-1712.

Mudway, IS; Blomberg, A; Frew, AJ; Holgate, ST; Sandstrom, T; Kelly, FJ. (1999a). Antioxidant consumption and repletion kinetics in nasal lavage fluid following exposure of healthy human volunteers to ozone. Eur Respir J 13: 1429-1438.

Mudway, IS; Housley, D; Eccles, R; Richards, RJ; Datta, AK; Tetley, TD; Kelly, FJ. (1996). Differential depletion of human respiratory tract antioxidants in response to ozone challenge. Free Radic Res 25: 499-513.

Mudway, IS; Kelly, FJ. (1998). Modeling the interactions of ozone with pulmonary epithelial lining fluid antioxidants. Toxicol Appl Pharmacol 148: 91-100.

Mudway, IS; Kelly, FJ. (2000). Ozone and the lung: A sensitive issue. Mol Aspects Med 21: 1-48.

Mudway, IS; Kelly, FJ. (2004b). An investigation of inhaled ozone dose and the magnitude of airway inflammation in healthy adults: Online data supplement. Am J Respir Crit Care Med 169: 1089-1095. http://dx.doi.org/10.1164/rccm.200309-1325PP

Mudway, IS; Krishna, MT; Frew, AJ; MacLeod, D; Sandstrom, T; Holgate, ST; Kelly, FJ. (1999b). Compromised concentrations of ascorbate in fluid lining the respiratory tract in human subjects after exposure to ozone. Occup Environ Med 56: 473-481.

Mudway, IS; Stenfors, N; Blomberg, A; Helleday, R; Dunster, C; Marklund, SL; Frew, AJ; Sandstrom, T; Kelly, FJ. (2001). Differences in basal airway antioxidant concentrations are not predictive of individual responsiveness to ozone: A comparison of healthy and mild asthmatic subjects. Free Radic Biol Med 31: 962-974.

Murphy, RC; Johnson, KM. (2008). Cholesterol, reactive oxygen species, and the formation of biologically active mediators. J Biol Chem 283: 15521-15525. http://dx.doi.org/10.1074/jbc.R700049200

Myatt, L; Kossenjans, W; Sahay, R; Eis, A; Brockman, D. (2000). Oxidative stress causes vascular dysfunction in the placenta [Review]. J Matern Fetal Med 9: 79-82. http://dx.doi.org/10.1002/(SICI)1520-6661(200001/02)9:1<79::AID-MFM16>3.0.CO;2-O

Nishiyama, H; Ikeda, H; Kaneko, T; Fu, L; Kudo, M; Ito, T; Okubo, T. (1998). Neuropeptides mediate the ozone-induced increase in the permeability of the tracheal mucosa in guinea pigs. Am J Physiol 275: L231-L238.

Nodelman, V; Ultman, JS. (1999). Longitudinal distribution of chlorine absorption in human airways: A comparison to ozone absorption. J Appl Physiol 87: 2073-2080.

Noviski, N; Brewer, JP; Skornik, WA; Galli, SJ; Drazen, JM; Martin, TR. (1999). Mast cell activation is not required for induction of airway hyperresponsiveness by ozone in mice. J Appl Physiol 86: 202-210.

O'Byrne, P; Walters, E; Gold, B; Aizawa, H; Fabbri, L; Alpert, S; Nadel, J; Holtzman, M. (1983). Neutrophil depletion inhibits airway hyperresponsiveness induced by ozone exposure. Am Rev Respir Dis 130: 214-219.

O'Byrne, PM; Walters, EH; Aizawa, H; Fabbri, LM; Holtzman, MJ; Nadel, JA. (1984). Indomethacin inhibits the airway hyperresponsiveness but not the neutrophil influx induced by ozone in dogs. Am Rev Respir Dis 130: 220-224.

Oslund, KL; Hyde, DM; Putney, LF; Alfaro, MF; Walby, WF; Tyler, NK; Schelegle, ES. (2008). Activation of neurokinin-1 receptors during ozone inhalation contributes to epithelial injury and repair. Am J Respir Cell Mol Biol 39: 279-288. http://dx.doi.org/10.1165/rcmb.2008-0009OC

Oslund, KL; Hyde, DM; Putney, LF; Alfaro, MF; Walby, WF; Tyler, NK; Schelegle, ES. (2009). Activation of calcitonin gene-related peptide receptor during ozone inhalation contributes to airway epithelial injury and repair. Toxicol Pathol 37: 805-813. http://dx.doi.org/10.1177/0192623309345691

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

Overton, JH; Graham, RC; Menache, MG; Mercer, RR; Miller, FJ. (1996). Influence of tracheobronchial region expansion and volume on reactive gas uptake and interspecies dose extrapolations. Inhal Toxicol 8: 723-745.

Overton, JH; Graham, RC; Miller, FJ. (1987). A model of the regional uptake of gaseous pollutants in the lung: II. The sensitivity of ozone uptake in laboratory animal lungs to anatomical and ventilatory parameters. Toxicol Appl Pharmacol 88: 418-432. http://dx.doi.org/10.1016/0041-008X(87)90216-X

Paquette, NC; Zhang, LY; Ellis, WA; Scott, AL; Kleeberger, SR. (1996). Vitamin A deficiency enhances ozone-induced lung injury. Am J Physiol 270: L475-L482.

Park, JW; Taube, C; Swasey, C; Kodama, T; Joetham, A; Balhorn, A; Takeda, K; Miyahara, N; Allen, CB; Dakhama, A; Kim, SH; Dinarello, CA; Gelfand, EW. (2004). Interleukin-1 receptor antagonist attenuates airway hyperresponsiveness following exposure to ozone. Am J Respir Cell Mol Biol 30: 830-836. http://dx.doi.org/10.1165/rcmb.2003-0373OC

Passannante, AN; Hazucha, MJ; Bromberg, PA; Seal, E; Folinsbee, L; Koch, G. (1998). Nociceptive mechanisms modulate ozone-induced human lung function decrements. J Appl Physiol 85: 1863-1870.

Pearson, AC; Bhalla, DK. (1997). Effects of ozone on macrophage adhesion in vitro and epithelial and inflammatory responses in vivo: The role of cytokines. J Toxicol Environ Health 50: 143-157.

Peden, DB. (2011). The role of oxidative stress and innate immunity in O3 and endotoxin-induced human allergic airway disease [Review]. Immunol Rev 242: 91-105. http://dx.doi.org/10.1111/j.1600-065X.2011.01035.x

Peden, DB; Boehlecke, B; Horstman, D; Devlin, R. (1997). Prolonged acute exposure to 0.16 ppm ozone induces eosinophilic airway inflammation in asthmatic subjects with allergies. J Allergy Clin Immunol 100: 802-808.

Peden, DB; Setzer, RW, Jr; Devlin, RB. (1995). Ozone exposure has both a priming effect on allergen-induced responses and an intrinsic inflammatory action in the nasal airways of perennially allergic asthmatics. Am J Respir Crit Care Med 151: 1336-1345.

Peden, DB; Swiersz, M; Ohkubo, K; Hahn, B; Emery, B; Kaliner, MA. (1993). Nasal secretion of the ozone scavenger uric acid. Am Rev Respir Dis 148: 455-461.

Perepu, RS; Garcia, C; Dostal, D; Sethi, R. (2010). Enhanced death signaling in ozone-exposed ischemic-reperfused hearts. Mol Cell Biochem 336: 55-64. http://dx.doi.org/10.1007/s11010-009-0265-4

Pereyra-Muñoz, N; Rugerio-Vargas, C; Angoa-Pérez, M; Borgonio-Pérez, G; Rivas-Arancibia, S. (2006). Oxidative damage in substantia nigra and striatum of rats chronically exposed to ozone. J Chem Neuroanat 31: 114-123. http://dx.doi.org/10.1016/j.jchemneu.2005.09.006

Perez-Gil, J. (2008). Structure of pulmonary surfactant membranes and films: The role of proteins and lipid-protein interactions [Review]. Biochim Biophys Acta 1778: 1676-1695. http://dx.doi.org/10.1016/j.bbamem.2008.05.003

Pichavant, M; Goya, S; Meyer, EH; Johnston, RA; Kim, HY; Matangkasombut, P; Zhu, M; Iwakura, Y; Savage, PB; Dekruyff, RH; Shore, SA; Umetsu, DT. (2008). Ozone exposure in a mouse model induces airway hyperreactivity that requires the presence of natural killer T cells and IL-17. J Exp Med 205: 385-393. http://dx.doi.org/10.1084/jem.20071507

Picher, M; Burch, LH; Boucher, RC. (2004). Metabolism of P2 receptor agonists in human airways: Implications for mucociliary clearance and cystic fibrosis. J Biol Chem 279: 20234-20241. http://dx.doi.org/10.1074/jbc.M400305200

Plopper, CG; Hatch, GE; Wong, V; Duan, X; Weir, AJ; Tarkington, BK; Devlin, RB; Becker, S; Buckpitt, AR. (1998). Relationship of inhaled ozone concentration to acute tracheobronchial epithelial injury, site-specific ozone dose and glutathione depletion in rhesus monkeys. Am J Respir Cell Mol Biol 19: 387-399.

Plopper, CG; Mango, GW; Hatch, GE; Wong, VJ; Toskala, E; Reynolds, SD; Tarkington, BK; Stripp, BR. (2006). Elevation of susceptibility to ozone-induced acute tracheobronchial injury in transgenic mice deficient in Clara cell secretory protein. Toxicol Appl Pharmacol 213: 74-85. http://dx.doi.org/10.1016/j.taap.2005.09.003

Plopper, CG; Smiley-Jewell, SM; Miller, LA; Fanucchi, MV; Evans, MJ; Buckpitt, AR; Avdalovic, M; Gershwin, LJ; Joad, JP; Kajekar, R; Larson, S; Pinkerton, KE; Van Winkle, LS; Schelegle, ES; Pieczarka, EM; Wu, R; Hyde, DM. (2007). Asthma/allergic airways disease: Does postnatal exposure to environmental toxicants promote airway pathobiology? Toxicol Pathol 35: 97-110. http://dx.doi.org/10.1080/01926230601132030

Postlethwait, EM; Cueto, R; Velsor, LW; Pryor, WA. (1998). O3-induced formation of bioactive lipids: Estimated surface concentrations and lining layer effects. Am J Physiol 274: L1006-L1016.

Postlethwait, EM; Joad, JP; Hyde, DM; Schelegle, ES; Bric, JM; Weir, AJ; Putney, LF; Wong, VJ; Velsor, LW; Plopper, CG. (2000). Three-dimensional mapping of ozone-induced acute cytotoxicity in tracheobronchial airways of isolated perfused rat lung. Am J Respir Cell Mol Biol 22: 191-199.

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

Postlethwait, EM; Ultman, JS. (2001). Airspace surface chemistry mediates O3-induced lung injury. Hum Ecol Risk Assess 7: 1145-1159. http://dx.doi.org/10.1080/20018091094907

Pryor, WA. (1976). Free radical reactions in biology: Initiation of lipid autoxidation by ozone and nitrogen dioxide. Environ Health Perspect 16: 180-181.

Pryor, WA. (1992). How far does ozone penetrate into the pulmonary air/tissue boundary before it reacts? Free Radic Biol Med 12: 83-88. http://dx.doi.org/10.1016/0891-5849(92)90060-T

Pryor, WA. (1994). Mechanisms of radical formation from reactions of ozone with target molecules in the lung. Free Radic Biol Med 17: 451-465.

Pryor, WA; Bermudez, E; Cueto, R; Squadrito, GL. (1996). Detection of aldehydes in bronchoalveolar lavage of rats exposed to ozone. Toxicol Sci 34: 148-156.

Pryor, WA; Das, B; Church, DF. (1991). The ozonation of unsaturated fatty acids: Aldehydes and hydrogen peroxide as products and possible mediators of ozone toxicity. Chem Res Toxicol 4: 341-348.

Pryor, WA; Giamalva, DH; Church, DF. (1984). Kinetics of ozonation. 2. Amino acids and model compounds in water and comparisons to rates in nonpolar solvents. J Am Chem Soc 106: 7094-7100.

Pulfer, MK; Murphy, RC. (2004). Formation of biologically active oxysterols during ozonolysis of cholesterol present in lung surfactant. J Biol Chem 279: 26331-26338. http://dx.doi.org/10.1074/jbc.M403581200

Pulfer, MK; Taube, C; Gelfand, E; Murphy, RC. (2005). Ozone exposure in vivo and formation of biologically active oxysterols in the lung. J Pharmacol Exp Ther 312: 256-264. http://dx.doi.org/10.1124/jpet.104.073437

Que, LG; Stiles, JV; Sundy, JS; Foster, WM. (2011). Pulmonary function, bronchial reactivity, and epithelial permeability are response phenotypes to ozone and develop differentially in healthy humans. J Appl Physiol 111: 679-687. http://dx.doi.org/10.1152/japplphysiol.00337.2011

Rashba-Step, J; Tatoyan, A; Duncan, R; Ann, D; Pushpa-Rehka, TR; Sevanian, A. (1997). Phospholipid peroxidation induces cytosolic phospholipase A2 activity: Membrane effects versus enzyme phosphorylation. Arch Biochem Biophys 343: 44-54. http://dx.doi.org/10.1006/abbi.1997.0134

Reeser, WH; Lee, GM; Taylor, A; Wang, L; Arnold, SF; Ultman, JS; Ben-Jebria, A. (2005). Uptake of ozone in human lungs and its relationship to local physiological response. Inhal Toxicol 17: 699-707. http://dx.doi.org/10.1080/08958370500224433

Rigas, ML; Ben-Jebria, A; Ultman, JS. (1997). Longitudinal distribution of ozone absorption in the lung: Effects of nitrogen dioxide, sulfur dioxide, and ozone exposures. Arch Environ Occup Health 52: 173-178.

Rigas, ML; Catlin, SN; Ben-Jebria, A; Ultman, JS. (2000). Ozone uptake in the intact human respiratory tract: Relationship between inhaled dose and actual dose. J Appl Physiol 88: 2015-2022.

Ritz, B; Yu, F; Chapa, G; Fruin, S. (2000). Effect of air pollution on preterm birth among children born in Southern California between 1989 and 1993. Epidemiology 11: 502-511.

Ritz, B; Yu, F; Fruin, S; Chapa, G; Shaw, GM; Harris, JA. (2002). Ambient air pollution and risk of birth defects in Southern California. Am J Epidemiol 155: 17-25.

Romieu, I; Meneses, F; Ramirez, M; Ruiz, S; Padilla, RP; Sienra, JJ; Gerber, M; Grievink, L; Dekker, R; Walda, I; Brunekreef, B. (1998b). Antioxidant supplementation and respiratory functions among workers exposed to high levels of ozone. Am J Respir Crit Care Med 158: 226-232.

Romieu, I; Sienra-Monge, JJ; Ramirez-Aguilar, M; Moreno-Macias, H; Reyes-Ruiz, NI; Estela del Rio-Navarro, B; Hernandez-Avila, M; London, SJ. (2004b). Genetic polymorphism of GSTM1 and antioxidant supplementation influence lung function in relation to ozone exposure in asthmatic children in Mexico City. Thorax 59: 8-10.

Romieu, I; Sienra-Monge, JJ; Ramirez-Aguilar, M; Tellez-Rojo, MM; Moreno-Macias, H; Reyes-Ruiz, NI; Del Rio-Navarro, BE; Ruiz-Navarro, MX; Hatch, G; Slade, R; Hernandez-Avila, M. (2002). Antioxidant supplementation and lung functions among children with asthma exposed to high levels of air pollutants. Am J Respir Crit Care Med 166: 703-709.

Roux, E; Hyvelin, JM; Savineau, JP; Marthan, R. (1999). Human isolated airway contraction: Interaction between air pollutants and passive sensitization. Am J Respir Crit Care Med 160: 439-445.

Rutkowski, JM; Santiag, LY; Ben-Jebria, A; Ultman, JS. (2011). Comparison of ozone-specific (OZAC) and oxygen radical (ORAC) antioxidant capacity assays for use with nasal lavage fluid. Toxicol In Vitro 25: 1406-1413. http://dx.doi.org/10.1016/j.tiv.2011.04.008

Sagai, M; Arakawa, K; Ichinose, T; Shimojo, N. (1987). Biochemical effects on combined gases of nitrogen dioxide and ozone: I. Species differences of lipid peroxides and phospholipids in lungs. Toxicology 46: 251-265. http://dx.doi.org/10.1016/0300-483X(87)90207-1

Samet, JM; Hatch, GE; Horstman, D; Steck-Scott, S; Arab, L; Bromberg, PA; Levine, M; McDonnell, WF; Devlin, RB. (2001). Effect of antioxidant supplementation on ozone-induced lung injury in human subjects. Am J Respir Crit Care Med 164: 819-825.

Santiago-López, D; Bautista-Martínez, JA; Reyes-Hernandez, CI; Aguilar-Martínez, M; Rivas-Arancibia, S. (2010). Oxidative stress, progressive damage in the substantia nigra and plasma dopamine oxidation, in rats chronically exposed to ozone. Toxicol Lett 197: 193-200. http://dx.doi.org/10.1016/j.toxlet.2010.05.020

Santiago, LY; Hann, MC; Ben-Jebria, A; Ultman, JS. (2001). Ozone absorption in the human nose during unidirectional airflow. J Appl Physiol 91: 725-732.

Sarangapani, R; Gentry, PR; Covington, TR; Teeguarden, JG; Clewell HJ, III. (2003). Evaluation of the potential impact of age- and gender-specific lung morphology and ventilation rate on the dosimetry of vapors. Inhal Toxicol 15: 987-1016. http://dx.doi.org/10.1080/08958370390226350

Sathishkumar, K; Gao, X; Raghavamenon, AC; Parinandi, N; Pryor, WA; Uppu, RM. (2009). Cholesterol secoaldehyde induces apoptosis in H9c2 cardiomyoblasts through reactive oxygen species involving mitochondrial and death receptor pathways. Free Radic Biol Med 47: 548-558. http://dx.doi.org/10.1016/j.freeradbiomed.2009.05.020

Sathishkumar, K; Murthy, SN; Uppu, RM. (2007a). Cytotoxic effects of oxysterols produced during ozonolysis of cholesterol in murine GT1-7 hypothalamic neurons. Free Radic Res 41: 82-88. http://dx.doi.org/10.1080/10715760600950566

Sathishkumar, K; Xi, X; Martin, R; Uppu, RM. (2007b). Cholesterol secoaldehyde, an ozonation product of cholesterol, induces amyloid aggregation and apoptosis in murine GT1-7 hypothalamic neurons. J Alzheimers Dis 11: 261-274.

Sato, S; Shimura, S; Hirose, T; Maeda, S; Kawakami, M; Takishima, T; Kimura, S. (1980). Effects of long-term ozone exposure and dietary vitamin E in rats. Tohoku J Exp Med 130: 117-128.

Sawyer, K; Brown, J; HazuchaM; Bennett, WD. (2007). The effect of exercise on nasal uptake of ozone in healthy human adults. J Appl Physiol 102: 1380-1386. http://dx.doi.org/10.1152/japplphysiol.00269.2006

Scannell, C; Chen, L; Aris, RM; Tager, I; Christian, D; Ferrando, R; Welch, B; Kelly, T; Balmes, JR. (1996). Greater ozone-induced inflammatory responses in subjects with asthma. Am J Respir Crit Care Med 154: 24-29.

Schelegle, ES; Adams, WC; Siefkin, AD. (1987). Indomethacin pretreatment reduces ozone-induced pulmonary function decrements in human subjects. Am Rev Respir Dis 136: 1350-1354.

Schelegle, ES; Carl, ML; Coleridge, HM; Coleridge, JCG; Green, JF. (1993). Contribution of vagal afferents to respiratory reflexes evoked by acute inhalation of ozone in dogs. J Appl Physiol 74: 2338-2344.

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

Schelegle, ES; Siefkin, AD; McDonald, RJ. (1991). Time course of ozone-induced neutrophilia in normal humans. Am J Respir Crit Care Med 143: 1353-1358.

Schelegle, ES; Walby, WF; Adams, WC. (2007). Time course of ozone-induced changes in breathing pattern in healthy exercising humans. J Appl Physiol 102: 688-697. http://dx.doi.org/10.1152/japplphysiol.00141.2006

Schlesinger, RB; Ben-Jebria, A; Dahl, AR; Snipes, MB; Ultman, J. (1997). Disposition of inhaled toxicants. In EJ Massaro (Ed.), Handbook of human toxicology (pp. 493-550). Boca Raton, FL: CRC Press.

Schroter, RC; Sudlow, MF. (1969). Flow patterns in models of the human bronchial airways. Respir Physiol Neurobiol 7: 341-355.

Seltzer, J; Bigby, BG; Stulbarg, M; Holtzman, MJ; Nadel, JA; Ueki, IF; Leikauf, GD; Goetzl, EJ; Boushey, HA. (1986). O3-induced change in bronchial reactivity to methacholine and airway inflammation in humans. J Appl Physiol 60: 1321-1326.

Servais, S; Boussouar, A; Molnar, A; Douki, T; Pequignot, JM; Favier, R. (2005). Age-related sensitivity to lung oxidative stress during ozone exposure. Free Radic Res 39: 305-316. http://dx.doi.org/10.1080/10715760400011098

Sharkhuu, T; Doerfler, DL; Copeland, C; Luebke, RW; Gilmour, MI. (2011). Effect of maternal exposure to ozone on reproductive outcome and immune, inflammatory, and allergic responses in the offspring. J Immunotoxicol 8: 183-194. http://dx.doi.org/10.3109/1547691X.2011.568978

Shore, SA. (2007). Obesity and asthma: Lessons from animal models [Review]. J Appl Physiol 102: 516-528. http://dx.doi.org/10.1152/japplphysiol.00847.2006

Shore, SA; Lang, JE; Kasahara, DI; Lu, FL; Verbout, NG; Si, H; Williams, ES; Terry, RD; Lee, A; Johnston, RA. (2009). Pulmonary responses to subacute ozone exposure in obese vs. lean mice. J Appl Physiol 107: 1445-1452. http://dx.doi.org/10.1152/japplphysiol.00456.2009

Shore, SA; Schwartzman, IN; Le Blanc, B; Krishna Murthy, GG; Doerschuk, CM. (2001). Tumor necrosis factor receptor 2 contributes to ozone-induced airway hyperresponsiveness in mice. Am J Respir Crit Care Med 164: 602-607.

Sienra-Monge, JJ; Ramirez-Aguilar, M; Moreno-Macias, H; Reyes-Ruiz, NI; Del Rio-Navarro, BE; Ruiz-Navarro, MX; Hatch, G; Crissman, K; Slade, R; Devlin, RB; Romieu, I. (2004). Antioxidant supplementation and nasal inflammatory responses among young asthmatics exposed to high levels of ozone. Clin Exp Immunol 138: 317-322. http://dx.doi.org/10.1111/j.1365-2249.2004.02606.x

Slade, R; Crissman, K; Norwood, J; Hatch, G. (1993). Comparison of antioxidant substances in bronchoalveolar lavage cells and fluid from humans, guinea pigs, and rats. Exp Lung Res 19: 469-484.

Slade, R; Highfill, JW; Hatch, GE. (1989). Effects of depletion of ascorbic acid or nonprotein sulfhydryls on the acute inhalation toxicity of nitrogen dioxide, ozone, and phosgene. Inhal Toxicol 1: 261-271.

Slade, R; Watkinson, WP; Hatch, GE. (1997). Mouse strain differences in ozone dosimetry and body temperature changes. Am J Physiol 272: L73-L77.

Sokol, RZ; Kraft, P; Fowler, IM; Mamet, R; Kim, E; Berhane, KT. (2006). Exposure to environmental ozone alters semen quality. Environ Health Perspect 114: 360-365. http://dx.doi.org/10.1289/ehp.8232

Stenfors, N; Pourazar, J; Blomberg, A; Krishna, MT; Mudway, I; Helleday, R; Kelly, FJ; Frew, AJ; Sandstrom, T. (2002). Effect of ozone on bronchial mucosal inflammation in asthmatic and healthy subjects. Respir Med 96: 352-358.

Sterner-Kock, A; Kock, M; Braun, R; Hyde, DM. (2000). Ozone-induced epithelial injury in the ferret is similar to nonhuman primates. Am J Respir Crit Care Med 162: 1152-1156.

Sun, J; Koto, H; Chung, KF. (1997). Interaction of ozone and allergen challenges on bronchial responsiveness and inflammation in sensitised guinea pigs. Int Arch Allergy Immunol 112: 191-195.

Taylor-Clark, TE; McAlexander, MA; Nassenstein, C; Sheardown, SA; Wilson, S; Thornton, J; Carr, MJ; Undem, BJ. (2008). Relative contributions of TRPA1 and TRPV1 channels in the activation of vagal bronchopulmonary C-fibres by the endogenous autacoid 4-oxononenal. J Physiol 586: 3447-3459. http://dx.doi.org/10.1113/jphysiol.2008.153585

Taylor-Clark, TE; Undem, BJ. (2010). Ozone activates airway nerves via the selective stimulation of TRPA1 ion channels. J Physiol 588: 423-433. http://dx.doi.org/10.1113/jphysiol.2009.183301

Taylor, AB; Borhan, A; Ultman, JS. (2007). Three-dimensional simulations of reactive gas uptake in single airway bifurcations. Ann Biomed Eng 35: 235-249. http://dx.doi.org/10.1007/s10439-006-9195-4

Taylor, AB; Lee, GM; Nellore, K; Ben-Jebria, A; Ultman, JS. (2006). Changes in the carbon dioxide expirogram in response to ozone exposure. Toxicol Appl Pharmacol 213: 1-9. http://dx.doi.org/10.1016/j.taap.2005.09.009

Tepper, JS; Costa, DL; Fitzgerald, S; Doerfler, DL; Bromberg, PA. (1993). Role of tachykinins in ozone-induced acute lung injury in guinea pigs. J Appl Physiol 75: 1404-1411.

Tepper, JS; Costa, DL; Lehmann, JR; Weber, MF; Hatch, GE. (1989). Unattenuated structural and biochemical alterations in the rat lung during functional adaptation to ozone. Am J Respir Crit Care Med 140: 493-501.

Thomson, E; Kumarathasan, P; Goegan, P; Aubin, RA; Vincent, R. (2005). Differential regulation of the lung endothelin system by urban particulate matter and ozone. Toxicol Sci 88: 103-113. http://dx.doi.org/10.1093/toxsci/kfi272

Thomson, E; Kumarathasan, P; Vincent, R. (2006). Pulmonary expression of preproET-1 and preproET-3 mRNAs is altered reciprocally in rats after inhalation of air pollutants. Exp Biol Med 231: 979-984.

Tran, MU; Weir, AJ; Fanucchi, MV; Rodriguez, AE; Pantle, LM; Smiley-Jewell, SM; Van Winkle, LS; Evans, MJ; Miller, LA; Schelegle, ES; Gershwin, LJ; Hyde, DM; Plopper, CG. (2004). Smooth muscle hypertrophy in distal airways of sensitized infant rhesus monkeys exposed to house dust mite allergen. Clin Exp Allergy 34: 1627-1633. http://dx.doi.org/10.1111/j.1365-2222.2004.02057.x

Trenga, CA; Koenig, JQ; Williams, PV. (2001). Dietary antioxidants and ozone-induced bronchial hyperresponsiveness in adults with asthma. Arch Environ Occup Health 56: 242-249.

Trevisani, M; Siemens, J; Materazzi, S; Bautista, DM; Nassini, R; Campi, B; Imamachi, N; Andrè, E; Patacchini, R; Cottrell, GS; Gatti, R; Basbaum, AI; Bunnett, NW; Julius, D; Geppetti, P. (2007). 4-Hydroxynonenal, an endogenous aldehyde, causes pain and neurogenic inflammation through activation of the irritant receptor TRPA1. PNAS 104: 13519-13524. http://dx.doi.org/10.1073/pnas.0705923104

Tsujino, I; Kawakami, Y; Kaneko, A. (2005). Comparative simulation of gas transport in airway models of rat, dog, and human. Inhal Toxicol 17: 475-485. http://dx.doi.org/10.1080/08958370590964476

U.S. EPA (U.S. Environmental Protection Agency). (1986). Air quality criteria for ozone and other photochemical oxidants [EPA Report]. (EPA-600/8-84-020aF - EPA-600/8-84-020eF). Research Triangle Park, NC. http://www.ntis.gov/search/product.aspx?ABBR=PB87142949

U.S. EPA (U.S. Environmental Protection Agency). (1996a). Air quality criteria for ozone and related photochemical oxidants [EPA Report]. (EPA/600/P-93/004AF). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (2005). Guidelines for carcinogen risk assessment [EPA Report]. (EPA/630/P-03/001F). Washington, DC. http://www.epa.gov/cancerguidelines/

U.S. EPA (U.S. Environmental Protection Agency). (2006b). Air quality criteria for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-05/004AF). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=149923

U.S. EPA (U.S. Environmental Protection Agency). (2009f). The U.S. Environmental Protection Agencys strategic plan for evaluating the toxicity of chemicals [EPA Report]. (EPA/100/K-09/001). Washington, DC. http://www.epa.gov/osa/spc/toxicitytesting/docs/toxtest_strategy_032309.pdf

Ultman, JS; Anjilvel, S. (1990). Monte Carlo simulation of ozone uptake in an asymmetric lung model. In DJ Schneck; CL Lucas (Eds.), Biofluid mechanics 3: Proceedings of the third Mid-Atlantic Conference on Biofluid Mechanics; October; Blacksburg, VA (pp. 45-52). New York, NY: New York University Press.

Ultman, JS; Ben-Jebria, A; Arnold, SF. (2004). Uptake distribution of ozone in human lungs: Intersubject variability in physiologic response (pp. 1-23; discussion 25-30). (ISSN 1041-5505

Ultman, JS; Ben-Jebria, A; Hu, SC. (1994). Noninvasive determination of respiratory ozone absorption: The bolus-response method. (HEI Research Report 69). Cambridge, MA: Health Effects Institute.

Uppu, RM; Cueto, R; Squadrito, GL; Pryor, WA. (1995). What does ozone react with at the air/lung interface? Model studies using human red blood cell membranes. Arch Biochem Biophys 319: 257-266.

Urch, B; Speck, M; Corey, P; Wasserstein, D; Manno, M; Lukic, KZ; Brook, JR; Liu, L; Coull, B; Schwartz, J; Gold, DR; Silverman, F. (2010). Concentrated ambient fine particles and not ozone induce a systemic interleukin-6 response in humans. Inhal Toxicol 22: 210-218. http://dx.doi.org/10.3109/08958370903173666

Vagaggini, B; Taccola, M; Clanchetti, S; Carnevali, S; Bartoli, ML; Bacci, E; Dente, FL; Di Franco, A; Giannini, D; Paggiaro, PL. (2002). Ozone exposure increases eosinophilic airway response induced by previous allergen challenge. Am J Respir Crit Care Med 166: 1073-1077.

Van Bree, L; Dormans, JAM, A; Koren, HS; Devlin, RB; Rombout, PJA. (2002). Attenuation and recovery of pulmonary injury in rats following short-term, repeated daily exposure to ozone. Inhal Toxicol 14: 883-900. http://dx.doi.org/10.1080/08958370290084674

Vancza, EM; Galdanes, K; Gunnison, A; Hatch, G; Gordon, T. (2009). Age, strain, and gender as factors for increased sensitivity of the mouse lung to inhaled ozone. Toxicol Sci 107: 535-543. http://dx.doi.org/10.1093/toxsci/kfn253

Vasu, VT; Oommen, S; Lim, Y; Valacchi, G; Hobson, B; Eiserich, JP; Leonard, SW; Traber, MG; Cross, CE; Gohil, K. (2010). Modulation of ozone-sensitive genes in alpha-tocopherol transfer protein null mice. Inhal Toxicol 22: 1-16. http://dx.doi.org/10.3109/08958370902838145

Vaughan, TR, Jr; Jennelle, LF; Lewis, TR. (1969). Long-term exposure to low levels of air pollutants: Effects on pulmonary function in the beagle. Arch Environ Health 19: 45-50.

Verhein, KC; Hazari, MS; Moulton, BC; Jacoby, IW; Jacoby, DB; Fryer, AD. (2011). Three days after a single exposure to ozone the mechanism of airway hyperreactivity is dependent upon substance P and nerve growth factor. Am J Physiol Lung Cell Mol Physiol 300: L176-L184. http://dx.doi.org/10.1152/ajplung.00060.2010

Vesely, KR; Schelegle, ES; Stovall, MY; Harkema, JR; Green, JF; Hyde, DM. (1999). Breathing pattern response and epithelial labeling in ozone-induced airway injury in neutrophil-depleted rats. Am J Respir Cell Mol Biol 20: 699-709.

Vincent, R; Vu, D; Hatch, G; Poon, R; Dreher, K; Guenette, J; Bjarnason, S; Potvin, M; Norwood, J; Mcmullen, E. (1996b). Sensitivity of lungs of aging Fischer 344 rats to ozone: Assessment by bronchoalveolar lavage. Am J Physiol 271: L555-L565.

Vivier, E; Raulet, DH; Moretta, A; Caligiuri, MA; Zitvogel, L; Lanier, LL; Yokoyama, WM; Ugolini, S. (2011). Innate or adaptive immunity? The example of natural killer cells [Review]. Science 331: 44-49. http://dx.doi.org/10.1126/science.1198687

Voynow, JA; Fischer, BM; Zheng, S; Potts, EN; Grover, AR; Jaiswal, AK; Ghio, AJ; Foster, WM. (2009). NAD(P)H quinone oxidoreductase 1 is essential for ozone-induced oxidative stress in mice and humans. Am J Respir Cell Mol Biol 41: 107-113. http://dx.doi.org/10.1165/rcmb.2008-0381OC

Wagner, JG; Harkema, JR; Jiang, Q; Illek, B; Ames, BN; Peden, DB. (2009). Gamma-tocopherol attenuates ozone-induced exacerbation of allergic rhinosinusitis in rats. Toxicol Pathol 37: 481-491. http://dx.doi.org/10.1177/0192623309335630

Wagner, JG; Hotchkiss, JA; Harkema, JR. (2002). Enhancement of nasal inflammatory and epithelial responses after ozone and allergen coexposure in brown Norway rats. Toxicol Sci 67: 284-294.

Wagner, JG; Jiang, Q; Harkema, JR; Illek, B; Patel, DD; Ames, BN; Peden, DB. (2007). Ozone enhancement of lower airway allergic inflammation is prevented by gamma-tocopherol. Free Radic Biol Med 43: 1176-1188. http://dx.doi.org/10.1016/j.freeradbiomed.2007.07.013

Watkinson, WP; Campen, MJ; Nolan, JP; Costa, DL. (2001). Cardiovascular and systemic responses to inhaled pollutants in rodents: Effects of ozone and particulate matter. Environ Health Perspect 109: 539-546.

Watkinson, WP; Campen, MJ; Wichers, LB; Nolan, JP; Costa, DL. (2003). Cardiac and thermoregulatory responses to inhaled pollutants in healthy and compromised rodents: Modulation via interaction with environmental factors. Environ Res 92: 35-47.

Weibel, ER. (1980). Design and structure of the human lung. In AP Fishman (Ed.), Assessment of pulmonary function (pp. 18-65). New York, NY: McGraw-Hill.

Weinmann, GG; Liu, MC; Proud, D; Weidenbach-Gerbase, M; Hubbard, W; Frank, R. (1995b). Ozone exposure in humans: Inflammatory, small and peripheral airway responses. Am J Respir Crit Care Med 152: 1175-1182.

Weinmann, GG; Weidenbach-Gerbase, M; Foster, WM; Zacur, H; Frank, R. (1995c). Evidence for ozone-induced small-airway dysfunction: Lack of menstrual-cycle and gender effects. Am J Respir Crit Care Med 152: 988-996.

Welch, RW; Wang, Y; Crossman, A, Jr; Park, JB; Kirk, KL; Levine, M. (1995). Accumulation of vitamin C (ascorbate) and its oxidized metabolite dehydroascorbic acid occurs by separate mechanisms. J Biol Chem 270: 12584-12592. http://dx.doi.org/10.1074/jbc.270.21.12584

Wenten, M; Gauderman, WJ; Berhane, K; Lin, PC; Peters, J; Gilliland, FD. (2009). Functional variants in the catalase and myeloperoxidase genes, ambient air pollution, and respiratory-related school absences: An example of epistasis in gene-environment interactions. Am J Epidemiol 170: 1494-1501. http://dx.doi.org/10.1093/aje/kwp310

Wiester, MJ; Stevens, MA; Menache, MG; McKee, JL, Jr; Gerrity, TR. (1996a). Ozone uptake in healthy adult males during quiet breathing. Toxicol Sci 29: 102-109.

Wiester, MJ; Tepper, JS; King, ME; Menache, MG; Costa, DL. (1988). Comparative study of ozone (O3) uptake in three strains of rats and in the guinea pig. Toxicol Appl Pharmacol 96: 140-146.

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

Wiester, MJ; Williams, TB; King, ME; Menache, MG; Miller, FJ. (1987). Ozone uptake in awake Sprague-Dawley rats. Toxicol Appl Pharmacol 89: 429-437. http://dx.doi.org/10.1016/0041-008X(87)90162-1

Williams, AS; Eynott, PR; Leung, SY; Nath, P; Jupp, R; De Sanctis, GT; Resnick, R; Adcock, IM; Chung, KF. (2009a). Role of cathepsin S in ozone-induced airway hyperresponsiveness and inflammation. Pulm Pharmacol Ther 22: 27-32. http://dx.doi.org/10.1016/j.pupt.2008.11.002

Williams, AS; Issa, R; Durham, A; Leung, SY; Kapoun, A; Medicherla, S; Higgins, LS; Adcock, IM; Chung, KF. (2008a). Role of p38 mitogen-activated protein kinase in ozone-induced airway hyperresponsiveness and inflammation. Eur J Pharmacol 600: 117-122. http://dx.doi.org/10.1016/j.ejphar.2008.09.031

Williams, AS; Issa, R; Leung, SY; Puneeta, N; Gregory, D; Ferguson, D; Brydon, L; Bennett, I; Adcock, M; Chung, KF. (2007a). Attenuation of ozone-induced airway inflammation and hyper-responsiveness by c-Jun NH2 terminal kinase inhibitor SP600125. J Pharmacol Exp Ther 322: 351-359. http://dx.doi.org/10.1124/jpet.107.121624

Williams, AS; Leung, SY; Nath, P; Khorasani, NM; Bhavsar, P; Issa, R; Mitchell, JA; Adcock, IM; Chung, KF. (2007b). Role of TLR2, TLR4, and MyD88 in murine ozone-induced airway hyperresponsiveness and neutrophilia. J Appl Physiol 103: 1189-1195. http://dx.doi.org/10.1152/japplphysiol.00172.2007

Williams, AS; Nath, P; Leung, SY; Khorasani, N; Mckenzie, ANJ; Adcock, IM; Chung, KF. (2008b). Modulation of ozone-induced airway hyperresponsiveness and inflammation by interleukin-13. Eur Respir J 32: 571-578. http://dx.doi.org/10.1183/09031936.00121607

Wu, W; Doreswamy, V; Diaz-Sanchez, D; Samet, JM; Kesic, M; Dailey, L; Zhang, W; Jaspers, I; Peden, DB. (2011). GSTM1 modulation of IL-8 expression in human bronchial epithelial cells exposed to ozone. Free Radic Biol Med 51: 522-529. http://dx.doi.org/10.1016/j.freeradbiomed.2011.05.006

Wu, ZX; Barker, JS; Batchelor, TP; Dey, RD. (2008c). Interleukin (IL)-1 regulates ozone-enhanced tracheal smooth muscle responsiveness by increasing substance P (SP) production in intrinsic airway neurons of ferret. Respir Physiol Neurobiol 164: 300-311. http://dx.doi.org/10.1016/j.resp.2008.07.019

Wu, ZX; Satterfield, BE; Dey, RD. (2003). Substance P released from intrinsic airway neurons contributes to ozone-enhanced airway hyperresponsiveness in ferret trachea. J Appl Physiol 95: 742-750.

Yang, IA; Holz, O; Jorres, RA; Magnussen, H; Barton, SJ; Rodriguez, S; Cakebread, JA; Holloway, JW; Holgate, ST. (2005a). Association of tumor necrosis factor alpha polymorphisms and ozone-induced change in lung function. Am J Respir Crit Care Med 171: 171-176.

Yokoyama, E; Frank, R. (1972). Respiratory uptake of ozone in dogs. Arch Environ Occup Health 25: 132-138.

Yoon, HK; Cho, HY; Kleeberger, SR. (2007). Protective role of matrix metalloproteinase-9 in ozone-induced airway inflammation. Environ Health Perspect 115: 1557-1563. http://dx.doi.org/10.1289/ehp.10289

Yost, BL; Gleich, GJ; Jacoby, DB; Fryer, AD. (2005). The changing role of eosinophils in long-term hyperreactivity following a single ozone exposure. Am J Physiol Lung Cell Mol Physiol 289: L627-L635. http://dx.doi.org/10.1152/ajplung.00377.2004

# 6 INTEGRATED HEALTH EFFECTS OF SHORT-TERM OZONE EXPOSURE

## 6.1 Introduction

This chapter reviews, summarizes, and integrates the evidence for various health outcomes associated with short-term (i.e., hours, days, or weeks) exposures to $O_3$. Numerous controlled human exposure, epidemiologic, and toxicological studies have permitted evaluation of the relationships between short-term $O_3$ exposure and a range of endpoints related to respiratory effects (Section 6.2), cardiovascular effects (Section 6.3), and mortality (Section 6.2, Section 6.3, and Section 6.6). A smaller number of studies were available to assess the effects of $O_3$ exposure on other physiological systems such as the central nervous system (Section 6.4), liver and metabolism (Section 6.5.1), and cutaneous and ocular tissues (Section 6.5.2). This chapter evaluates the majority of recent [i.e., published since the completion of the 2006 $O_3$ AQCD (U.S. EPA, 2006b)] short-term exposure studies; however, those for birth outcomes and infant mortality are evaluated in Chapter 7 (Section 7.4), because they compare associations among overlapping short- and long-term exposure windows that are difficult to distinguish.

Within each individual section of this chapter, a brief summary of conclusions from the 2006 $O_3$ AQCD is included along with an evaluation of recent evidence that is intended to build upon the body of evidence from previous reviews. The studies evaluated are organized by health endpoint (e.g., lung function, pulmonary inflammation) then by scientific discipline (e.g., controlled human exposure, epidemiology, and toxicology). Each major section (e.g., respiratory, cardiovascular, mortality) concludes with an integrated summary of the findings and a conclusion regarding causality based upon the framework described in the Preamble to this ISA. The causal determinations are presented for a broad health effect category, such as respiratory effects, with coherence and plausibility based on the total evidence available across disciplines and across the suite of related health endpoints, including cause-specific mortality.

## 6.2 Respiratory Effects

Based on evidence integrated across controlled human exposure, epidemiologic, and toxicological studies, the 2006 $O_3$ AQCD concluded "that acute $O_3$ exposure is causally associated with respiratory system effects" (U.S. EPA, 2006b). Contributing to this conclusion were the consistency and coherence across scientific disciplines for the effects of short-term $O_3$ exposure on a variety of respiratory outcomes including "pulmonary function decrements, respiratory symptoms, lung inflammation, and increased lung permeability, airway hyperresponsiveness." Collectively, these findings provided biological plausibility for associations in epidemiologic studies

observed between short-term increases in ambient $O_3$ concentration and increases in respiratory symptoms and respiratory-related hospitalizations and emergency department (ED) visits.

Controlled human exposure studies have provided strong and quantifiable exposure-response data on the human health effects of $O_3$. The most salient observations from studies reviewed in the 1996 and 2006 $O_3$ AQCDs (U.S. EPA, 2006b, 1996a) included: (1) young healthy adults exposed to $O_3$ concentrations $\geq$ 80 ppb develop significant reversible, transient decrements in pulmonary function and symptoms of breathing discomfort if minute ventilation ($\dot{V}_E$) or duration of exposure is increased sufficiently; (2) relative to young adults, children experience similar spirometric responses but lower incidence of symptoms from $O_3$ exposure; (3) relative to young adults, $O_3$-induced spirometric responses are decreased in older individuals; (4) there is a large degree of intersubject variability in physiologic and symptomatic responses to $O_3$, but responses tend to be reproducible within a given individual over a period of several months; (5) subjects exposed repeatedly to $O_3$ for several days experience an attenuation of spirometric and symptomatic responses on successive exposures, which is lost after about a week without exposure; and (6) acute $O_3$ exposure initiates an inflammatory response that may persist for at least 18 to 24 hours postexposure.

Substantial evidence for biologically plausible $O_3$-induced respiratory morbidity has been derived from the coherence between toxicological and controlled human exposure study findings for parallel endpoints. For example, $O_3$-induced lung function decrements and increased airway hyperresponsiveness have been observed in both animals and humans. Airway hyperresponsiveness could be an important consequence of exposure to ambient $O_3$ because the airways are then predisposed to narrowing upon inhalation of a variety of ambient stimuli. Additionally, airway hyperresponsiveness tends to resolve more slowly and appears less subject to attenuation with repeated $O_3$ exposures than lung function decrements. Increased permeability and inflammation have been observed in the airways of humans and animals alike after $O_3$ exposure, although these processes are not necessarily associated with immediate changes in lung function or hyperresponsiveness. Furthermore, the potential relationship between repetitive bouts of acute inflammation and the development of chronic respiratory disease is unknown. Another feature of $O_3$-related respiratory morbidity is impaired host defense and reduced resistance to lung infection, which has been strongly supported by toxicological evidence and, to a limited extent, by evidence from controlled human exposure studies. Recurrent respiratory infection in early life is associated with increased incidence of asthma in humans.

In concordance with experimental studies, epidemiologic studies have provided clear evidence for decrements in lung function related to short-term ambient $O_3$ exposure. These effects have been demonstrated in healthy children attending camps, adults exercising or working outdoors, and children with and without asthma (U.S. EPA, 2006b, 1996a). In addition to lung function decrements, short-term increases in ambient $O_3$ concentration have been associated with increases in respiratory symptoms (e.g., cough, wheeze, shortness of breath), notably in large U.S. panel

studies of children with asthma (Gent et al., 2003; Mortimer et al., 2000).
The evidence across disciplines for $O_3$ effects on a range of respiratory endpoints collectively provides support for epidemiologic studies that have demonstrated consistent associations between short-term increases in ambient $O_3$ concentration and increases in respiratory hospital admissions and ED visits, specifically during the summer or warm months. In contrast with other respiratory health endpoints, previous epidemiologic evidence has not clearly supported a relationship between short-term $O_3$ exposure and respiratory mortality. Although $O_3$ has been consistently associated with nonaccidental and cardiopulmonary mortality, the contribution of respiratory causes to these findings was uncertain as the few studies that have examined mortality specifically from respiratory causes reported inconsistent associations with ambient $O_3$ concentrations.

As will be discussed throughout this section, consistent with the strong body of evidence presented in the 2006 $O_3$ AQCD, recent studies continue to support associations between short-term $O_3$ exposure and respiratory effects, in particular, lung function decrements in controlled human exposure studies, airway inflammatory responses in toxicological studies, and respiratory-related hospitalizations and ED visits. Recent epidemiologic studies contribute new evidence for potentially at-risk populations and associations linking ambient $O_3$ concentrations with biological markers of airway inflammation and oxidative stress, which is consistent with the extensive evidence from controlled human exposure and toxicological studies. Furthermore, extending the potential range of well-established $O_3$-associated respiratory effects, recent multicity studies and a multicontinent study demonstrate associations between short-term increases in ambient $O_3$ concentration and respiratory-related mortality.

## 6.2.1 Lung Function

### 6.2.1.1 Controlled Human Exposure

This section focuses on studies examining $O_3$ effects on lung function and respiratory symptoms in volunteers exposed, for periods of up to 8 hours, to $O_3$ concentrations ranging from 40 to 500 ppb, while at rest or during exercise of varying intensity. Responses to acute $O_3$ exposures in the range of ambient concentrations include decreased inspiratory capacity; mild bronchoconstriction; rapid, shallow breathing patterns during exercise; and symptoms of cough and pain on deep inspiration (PDI). Reflex inhibition of inspiration results in a decrease in forced vital capacity (FVC) and total lung capacity (TLC) and, in combination with mild bronchoconstriction, contributes to a decrease in the forced expiratory volume in 1 second ($FEV_1$).

In studies that have exposed subjects during exercise, the majority of shorter duration ($\leq$ 4-hour exposures) studies utilized an intermittent exercise protocol in which

subjects rotated between 15-minute periods of exercise and rest. A limited number of 1- to 2-hour studies, mainly focusing on exercise performance, have utilized a continuous exercise regime. A quasi continuous exercise protocol is common to prolonged exposure studies where subjects complete 50-minute periods of exercise followed by 10-minute rest periods.

The majority of controlled human exposure studies have been conducted within exposure chambers, although a smaller number of studies used a facemask to expose subjects to $O_3$. Little effort has been made herein to differentiate between facemask and chamber exposures since $FEV_1$ and respiratory symptom responses appear minimally differentially affected by these exposure modalities. Similar responses between facemask and chamber exposures have been reported for exposures to 80 and 120 ppb $O_3$ (6.6-hour, moderate quasi continuous exercise, 40 L/min) and 300 ppb $O_3$ (2 hours, heavy intermittent exercise, 70 L/min) (Adams, 2003a, b, 2002).

The majority of controlled human exposure studies investigating the effects $O_3$ are of a randomized, controlled, crossover design in which subjects were exposed, without knowledge of the exposure condition and in random order to clean filtered air (FA; the control) and, depending on the study, to one or more $O_3$ concentrations. The FA control exposure provides an unbiased estimate of the effects of the experimental procedures on the outcome(s) of interest. Comparison of responses following this FA exposure to those following an $O_3$ exposure allows for estimation of the effects of $O_3$ itself on an outcome measurement while controlling for independent effects of the experimental procedures. As individuals may experience small changes in various health endpoints from exercise, diurnal variation, or other effects in addition to those of $O_3$ during the course of an exposure, the term "$O_3$-induced" is used herein to designate effects that have been corrected or adjusted for such extraneous responses as measured during FA exposures.

Spirometry, viz., $FEV_1$, is a common health endpoint used to assess effects of $O_3$ on respiratory health in controlled human exposure studies. In considering 6.6-hour exposures to FA, group mean $FEV_1$ changes have ranged from -0.7% (McDonnell et al., 1991) to 2.7% (Adams, 2006a). On average, across ten 6.6-hour exposure studies, there has been a 1.0% (n = 279) increase in $FEV_1$ (Kim et al., 2011; Schelegle et al., 2009; Adams, 2006a, 2003a, 2002; Adams and Ollison, 1997; Folinsbee et al., 1994; McDonnell et al., 1991; Horstman et al., 1990; Folinsbee et al., 1988). Regardless of the reason for small changes in $FEV_1$ over the course of FA exposures, whether biologically based or a systematic effect of the experimental procedures, the use of FA responses as a control for the assessment of responses following $O_3$ exposure in randomized exposure studies serves to eliminate alternative explanations other than those of $O_3$ itself in causing the measured responses.

With respect to $FEV_1$ responses in young healthy adults, an $O_3$-induced change in $FEV_1$ is typically the difference between the decrement observed with $O_3$ exposure and the improvement observed with FA exposure. Noting that some healthy individuals experience small improvements while others have small decrements in $FEV_1$ following FA exposure, investigators have used the randomized, crossover

design with each subject serving as their own control (exposure to FA) to discern relatively small effects with certainty since alternative explanations for these effects are controlled for by the nature of the experimental design. The utility of intraindividual FA control exposures becomes more apparent when considering individuals with respiratory disease. The occurrence of exercise-induced bronchospasm is well recognized in patients with asthma and chronic obstructive pulmonary disease (COPD) and may be experienced during both FA and $O_3$ exposures. Absent correction for FA responses, exercise-induced changes in $FEV_1$ could be mistaken for responses due to $O_3$. This biological phenomenon serves as an example to emphasize the need for a proper control exposure in assessing the effects of $O_3$ as well as the role of this control in eliminating the influence of other factors on the outcomes of interest.

## Pulmonary Function Effects of Ozone Exposure in Healthy Subjects

### Acute Exposure of Healthy Subjects

The majority of controlled human exposure studies have investigated the effects of exposure to $O_3$ in young healthy nonsmoking adults (18-35 years of age). These studies typically use fixed concentrations of $O_3$ under carefully regulated environmental conditions and subject activity levels. The magnitude of respiratory effects (decrements in spirometry measurements and increases in symptomatic responses) in these individuals is a function of $O_3$ concentration (C), minute ventilation ($\dot{V}_E$), and exposure duration (time). Any physical activity will increase minute ventilation and therefore the dose of inhaled $O_3$. Dose of inhaled $O_3$ to the lower airways is also increased due to a shift from nasal to oronasal breathing with a consequential decrease in $O_3$ scrubbing by the upper airways. Thus, the intensity of physiological response following an acute exposure will be strongly associated with minute ventilation.

The product of $C \times \dot{V}_E \times$ time is commonly used as a surrogate for $O_3$ dose to the respiratory tract in controlled human exposure studies. A large body of data regarding the interdependent effects of C, $\dot{V}_E$, and time on pulmonary responses was assessed in the 1986 and 1996 $O_3$ AQCDs (U.S. EPA, 1996a, 1986). Acute responses were modeled as a function of total inhaled dose ($C \times \dot{V}_E \times$ time) which was found to be a better predictor of response to $O_3$ than C, $\dot{V}_E$, or time of exposure, alone, or as a combination of any two of these factors. However, intake dose ($C \times \dot{V}_E \times$ time) did not adequately capture the temporal dynamics of pulmonary responses in a comparison between a constant (square-wave) and a variable (triangular) $O_3$ exposure (average 120 ppb $O_3$; moderate exercise, $\dot{V}_E$ = 40 L/min; 8 hour duration) conducted by Hazucha et al. (1992). Recent nonlinear statistical models clearly describe the temporal dynamics of $FEV_1$ responses as a function of C, $\dot{V}_E$, time, and age of the exposed subject (McDonnell et al., 2010, 2007).

For healthy young adults exposed at rest for 2 hours, 500 ppb is the lowest $O_3$ concentration reported to produce a statistically significant $O_3$-induced group mean

$FEV_1$ decrement of 6.4% (n = 10) (Folinsbee et al., 1978) to 6.7% (n = 13) (Horvath et al., 1979). Airway resistance was not clearly affected during at-rest exposure to these $O_3$ concentrations. For exposures of 1-2 hours to ≥ 120 ppb $O_3$, statistically significant symptomatic responses and effects on $FEV_1$ are observed when $\dot{V}_E$ is sufficiently increased by exercise (McDonnell et al., 1999b). For instance, 5% of young healthy adults exposed to 400 ppb $O_3$ for 2 hours during rest experienced pain on deep inspiration. Respiratory symptoms were not observed at lower exposure concentrations (120-300 ppb) or with only 1 hour of exposure even at 400 ppb. However, when exposed to 120 ppb $O_3$ for 2 hours during light-to-moderate intermittent exercise ($\dot{V}_E$ of 22 - 35 L/min), 9% of individuals experienced pain on deep inspiration, 5% experienced cough, and 4% experienced shortness of breath. With very heavy continuous exercise ($\dot{V}_E$ = 89 L/min), an $O_3$-induced group mean decrement of 9.7% in $FEV_1$ has been reported for healthy young adults exposed for 1 hour to 120 ppb $O_3$ (Gong et al., 1986). Symptoms are present and decrements in forced expiratory volumes and flows occur at 160-240 ppb $O_3$ following 1 hour of continuous heavy exercise ($\dot{V}_E$ ≈ 55 to 90 L/min (Gong et al., 1986; Avol et al., 1984; Folinsbee et al., 1984; Adams and Schelegle, 1983) and following 2 hours of intermittent heavy exercise ($\dot{V}_E$ ≈ 65-68 L/min) (Linn et al., 1986; Kulle et al., 1985; McDonnell et al., 1983). With heavy intermittent exercise (15-min intervals of rest and exercise [$\dot{V}_E$ = 68 L/min]), symptoms of breathing discomfort and a group mean $O_3$-induced decrement of 3.4% in $FEV_1$ occurred in young healthy adults exposed for 2 hours to 120 ppb $O_3$ (McDonnell et al., 1983).[1] Table 6-1 provides examples of typical exercise protocols utilized in controlled human exposures to $O_3$. The $\dot{V}_E$ rates in this table are per body surface area (BSA) which is, on average, about 1.7 $m^2$ and 2.0 $m^2$ for young healthy adult females and males, respectively, who participated in controlled $O_3$ exposure studies.

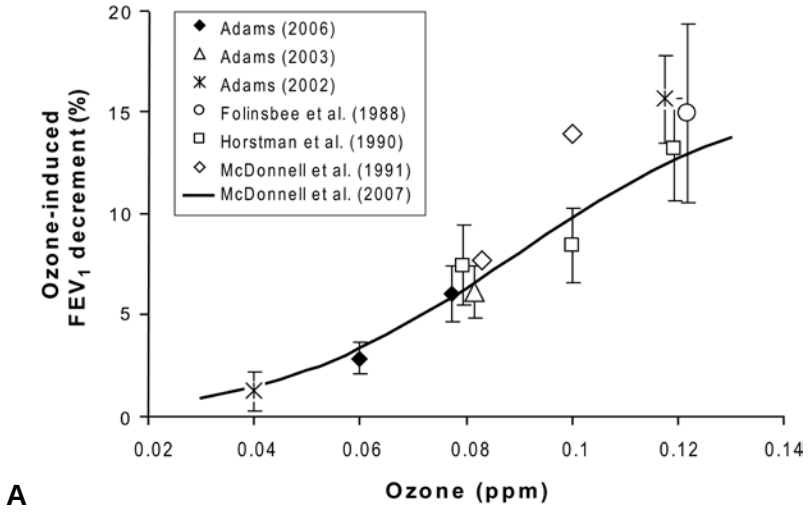
[1] In total, subjects were exposed to $O_3$ for 2.5 hours. Intermittent exercise periods, however, were only conducted for the first 2 hours of exposure and $FEV_1$ was determined 5 minutes after the exercise was completed.

## Table 6-1 Activity levels used in controlled exposures of healthy young adults to $O_3$.

| Activity[a,b] | Study Duration (hours) | $\dot{V}_E$ (L/min per $m^2$ BSA) | Heart Rate (bpm) | Treadmill Speed (mph) | Treadmill Grade (%) | Cycle (watts) |
|---|---|---|---|---|---|---|
| Rest | 2 | 4 | 70 | n.a. | n.a. | n.a. |
| Light quasi-continuous exercise | 6.6-7.6 | 15 | 110 | 3.5-4.4 | 0 | 42 |
| Moderate quasi-continuous exercise | 6.6 | 17-23 | 115-130 | 3.3-3.5 | 4-5 | 72 |
| Heavy intermittent exercise | 1-2 | 27-33 | 160 | 3.5-5 | 10-12 | 100 |
| Very heavy continuous exercise | 1 | 45 | 160 | n.a. | n.a. | 260 |

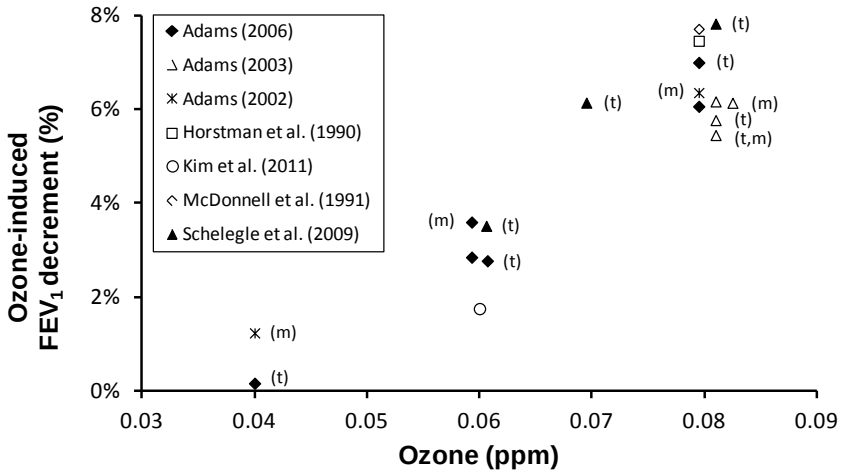
[a]Based on group mean exercise specific data provided in the individual studies. On average, subjects were 23 years of age. For exercise protocols, the minute ventilation and heart rate are for the exercise periods. Quasi-continuous exercise consists of 50 minutes of exercise periods followed by 10 minutes of rest. Intermittent exercise consists of alternating periods of 15 minutes of exercise and 15 minutes of rest.

[b]References: Horvath et al. (1979) for rest; Adams (2000) and Horstman et al. (1995) for light quasi-continuous exercise; Adams (2006a, 2002, 2000), Folinsbee et al. (1988), Horstman et al. (1990), and McDonnell et al. (1991) for moderate quasi-continuous exercise; Kehrl et al. (1987), Kreit et al. (1989), and McDonnell et al. (1983) for heavy intermittent exercise, and Gong et al. (1986) for very heavy continuous exercise.




**A**

Source: Brown et al. (2008).




**B**

Top, panel A: All studies exposed subjects to a constant (square-wave) concentration in a chamber, except Adams (2002) where a facemask was used. All responses at and above 0.06 ppm were statistically significant. The McDonnell et al. (2007) curve illustrates the predicted $FEV_1$ decrement at 6.6 hours as a function of $O_3$ concentration for a 23 year-old (the average age of subjects that participated in the illustrated studies). Note that this curve was not "fitted" to the plotted data. Error bars (where available) are the standard error of responses.

Bottom, panel B: All studies used constant (square-wave) exposures in a chamber unless designated as triangular (t) and/or facemask (m) exposures. All responses at and above 0.07 ppm were statistically significant. At 0.06 ppm, Adams (2006a) and Kim et al. (2011) responses to square-wave chamber exposures were statistically significant. During each hour of the exposures, subjects were engaged in moderate quasi continuous exercise (40 L/min) for 50 minutes and rest for 10 minutes. Following the third hour, subjects had an additional 35-minute rest period for lunch. The data at 0.06, 0.08 and 0.12 ppm have been offset for illustrative purposes.

Studies appearing in the figure legends: Adams (2006a, 2003a, 2002), Folinsbee et al. (1988), Horstman et al. (1990), Kim et al. (2011), McDonnell et al. (2007); McDonnell et al. (1991), and Schelegle et al. (2009).

## Figure 6-1 Cross-study comparison of mean $O_3$-induced $FEV_1$ decrements following 6.6 hours of exposure to $O_3$.

For prolonged (6.6 hours) exposures relative to shorter exposures, significant pulmonary function responses and symptoms have been observed at lower $O_3$ concentrations and at a moderate level of exercise ($\dot{V}_E$ = 40 L/min). The 6.6-hour experimental protocol was intended to simulate the performance of heavy physical labor for a full workday (Folinsbee et al., 1988). The results from studies using 6.6 hours of constant or square-wave exposures to between 40 and 120 ppb $O_3$ are illustrated in Figure 6-1(A). Figure 6-1(B) focuses on the range from 40 to 80 ppb and includes triangular exposure protocols as well as facemask exposures. Exposure to 40 ppb $O_3$ for 6.6 hours produces small, statistically nonsignificant changes in $FEV_1$ that are relatively similar to responses from FA exposure (Adams, 2002). Volunteers exposed to 60 ppb $O_3$ experience group mean $O_3$-induced $FEV_1$ decrements of about 3% (Kim et al., 2011; Brown et al., 2008; Adams, 2006a)[1]; those exposed to 80 ppb have group mean decrements that range from 6 to 8% (Adams, 2006a, 2003a; McDonnell et al., 1991; Horstman et al., 1990); at 100 ppb, group mean decrements range from 8 to 14% (McDonnell et al., 1991; Horstman et al., 1990); and at 120 ppb, group mean decrements of 13 to 16% are observed (Adams, 2002; Horstman et al., 1990; Folinsbee et al., 1988). As illustrated in Figure 6-1, there is a smooth intake dose-response curve without evidence of a threshold for exposures between 40 and 120 ppb $O_3$. This is consistent with Hazucha and Lefohn (2007), who suggested that a randomly selected group of healthy individuals of sufficient size would include hypo-, normo-, and hyper-responsive individuals such that the average response would show no threshold for any spirometric endpoint. Taken together, these data indicate that mean $FEV_1$ is clearly decreased by 6.6-hour exposures to 60 ppb $O_3$ and higher concentrations in subjects performing moderate exercise. Discussed later in this subsection, the recent McDonnell et al. (2012) and Schelegle et al. (2012) studies analyzed large datasets and fit compartmental models, which included the concept of a response threshold.

The time course of responses during prolonged (6.6 hours) square-wave $O_3$ exposures with moderate exercise ($\dot{V}_E$ = 40 L/min) depends on $O_3$ concentration. At 120 ppb $O_3$, Folinsbee et al. (1988) observed that somewhat small $FEV_1$ decrements and symptoms of breathing discomfort become apparent in healthy subjects following the second hour of exposure with a more rapid change in responses between the 3rd and 5th hour of exposure and a diminishing response or plateau in responses over the last hour of exposure. Relative to FA, the change in $FEV_1$ at 120 ppb $O_3$ became statistically significant after 4.6 hours. Following the same exposure protocol, Horstman et al. (1990) observed a linear increase in $FEV_1$ responses with time following 2 hours of exposure to 120 ppb $O_3$ that was statistically different from FA responses after 3 hours. At 100 ppb $O_3$, $FEV_1$ responses diverged from FA after 3 hours and were statistically different at 4.6 hours (Horstman et al., 1990). At 80 ppb $O_3$, $FEV_1$ responses diverged from FA after 4.6 hours and were statistically different from FA at 5.6 hours (Horstman et al., 1990). Subsequently, Adams (2006a) observed $FEV_1$ decrements and total respiratory

[1] Adams (2006a) did not find effects on $FEV_1$ at 60 ppb to be statistically significant. In an analysis of the Adams (2006a) data, even after removal of potential outliers, Brown et al. (2008) found the average effect on $FEV_1$ at 60 ppb to be small, but highly statistically significant ($p < 0.002$) using several common statistical tests.

symptoms at 80 ppb $O_3$ to diverge from FA responses after 3 hours, but did not become statistically different until 6.6 hours. At 60 ppb $O_3$, $FEV_1$ responses generally tracked responses in FA for the first 4.6 hours of exposure and diverged after 5.6 hours (Adams, 2006a). $FEV_1$ responses, but not symptomatic responses, become statistically different between 60 ppb $O_3$ and FA at 6.6 hours (Kim et al., 2011; Brown et al., 2008). At 40 ppb, $FEV_1$ and symptomatic responses track FA for 5.6 hours of exposure and may begin to diverge after 6.6 hours (Adams, 2002). In prolonged (6.6 hours) square-wave $O_3$ exposures between 40 and 120 ppb with moderate exercise ($\dot{V}_E$ = 40 L/min), the time required for group mean responses to differ between $O_3$ and FA exposures increases with decreasing $O_3$ concentration.

As opposed to constant (i.e., square-wave) concentration patterns used in the studies described above, many studies conducted at the levels of 40-80 ppb have used variable (i.e., triangular) $O_3$ concentration patterns. It has been suggested that a triangular exposure profile can potentially lead to higher $FEV_1$ responses than square-wave profiles despite having the same average $O_3$ concentration over the exposure period. Hazucha et al. (1992) were the first to investigate the effects of variable versus constant concentration exposures on responsiveness to $O_3$. In their study, volunteers were randomly exposed to a triangular concentration profile (averaging 120 ppb over the 8-hour exposure) that increased linearly from 0-240 ppb for the first 4 hours of the 8-hour exposure, then decreased linearly from 240 to 0 ppb over the next 4 hours of the 8-hour exposure, and to an square-wave exposure of 120 ppb $O_3$ for 8 hours. While the total inhaled $O_3$ doses at 4 hours and 8 hours for the square-wave and the triangular concentration profile were almost identical, the $FEV_1$ responses were dissimilar. For the square-wave exposure, $FEV_1$ declined ~5% by the fifth hour and then remained at that level. With the triangular $O_3$ profile, there was minimal $FEV_1$ response over the first 3 hours followed by a rapid decrease in $FEV_1$ to a decrement of 10.3% over the next 3 hours. During the seventh and eighth hours, mean $FEV_1$ decrement improved to 6.3% as the $O_3$ concentration decreased from 120 to 0 ppb (mean = 60 ppb). These findings illustrate that the severity of symptoms and the magnitude of spirometric responses are time-dependent functions of $O_3$ delivery rate with periods of both effect development and recovery during the course of an exposure.

Subsequently, others have also demonstrated that variable concentration exposures can elicit greater $FEV_1$ and symptomatic responses than do square-wave exposures to $O_3$ (Adams, 2006a, b, 2003a). Adams (2006b) reproduced the findings of Hazucha et al. (1992) at 120 ppb. However, Adams (2006a, 2003a) found that responses from an 80 ppb $O_3$ (average) triangular exposure did not differ significantly from those observed with the 80 ppb $O_3$ square-wave exposure at 6.6 hours. Nevertheless, $FEV_1$ and symptoms were significantly different from pre-exposure at 4.6 hours (when the $O_3$ concentration was 150 ppb) in the triangular exposure, but not until 6.6 hours in the square-wave exposure. At the lower $O_3$ concentration of 60 ppb, no temporal pattern differences in $FEV_1$ responses could be discerned between square-wave and triangular exposure profiles (Adams, 2006a). However, both total symptom scores and pain on deep inspiration tended to be greater following the 60 ppb triangular than the 60 ppb square-wave exposure. At 80 ppb $O_3$, respiratory symptoms tended to

increase more rapidly during the triangular than square-wave exposure protocol, but then decreased during the last hour of exposure to be less than that observed with the square-wave exposure at 6.6 hours. Both total symptom scores and pain on deep inspiration were significantly increased following exposures to 80 ppb $O_3$ relative to all other exposure protocols, i.e., FA, 40, and 60 ppb exposures. Following the 6.6-hour exposures, respiratory symptoms at 80 ppb were roughly 2-3 times greater than those observed at 60 ppb. At 40 ppb, triangular and square-wave patterns produced spirometric and subjective symptom responses similar to FA exposure (Adams, 2006a, 2002).

For $O_3$ exposures of 60 ppb and greater, studies (Adams, 2006a, b, 2003a; Hazucha et al., 1992) demonstrate that during triangular exposure protocols, volunteers exposed during moderate exercise ($\dot{V}_E$ = 40 L/min) may develop greater spirometric and/or symptomatic responses during and following peak $O_3$ concentrations as compared to responses over the same time interval of square-wave exposures. This observation is not unexpected since the inhaled dose rate during peaks of the triangular protocols approached twice that of the square-wave protocols, e.g., 150 ppb versus 80 ppb peak concentration. At time intervals toward the end of an exposure, $O_3$ delivery rates for the triangular protocols were less than those of square-wave. At these later time intervals, there is some recovery of responses during triangular exposure protocols, whereas there is a continued development of or a plateau of responses in the square-wave exposure protocols. Thus, responses during triangular protocols relative to square-wave protocols may be expected to diverge and be greater following peak exposures and then converge toward the end of an exposure. Subsequent discussion will focus on exposures between 40 and 80 ppb where $FEV_1$ pre-to-post responses are similar (although not identical) between triangular and square-wave protocols having equivalent average exposure concentrations.

Schelegle et al. (2009) recently investigated the effects of 6.6-hour variable $O_3$ exposure protocols at mean concentrations of 60, 70, 80, and 87 ppb on respiratory symptoms and pulmonary function in young healthy adults (16 F, 15 M; 21.4 ± 0.6 years) exposed during moderate quasi continuous exercise ($\dot{V}_E$ = 40 L/min). The mean $FEV_1$ (± standard error) decrements at 6.6 hours (end of exposure relative to pre-exposure) were: −0.80 ± 0.90%, 2.72 ± 1.48%, 5.34 ± 1.42%, 7.02 ± 1.60%, and 11.42 ± 2.20% for exposure to FA, 60, 70, 80, and 87 ppb $O_3$, respectively. Statistically significant decrements in $FEV_1$ and increases in total subjective symptom scores ($p < 0.05$) were found following exposure to mean concentrations of 70, 80, and 87 ppb $O_3$ relative to FA. Statistically significant effects were not found at 60 ppb. One of the expressed purposes of the Schelegle et al. (2009) study was to determine the minimal mean $O_3$ concentration that produces a statistically significant decrement in $FEV_1$ and respiratory symptoms in healthy individuals completing 6.6-hour exposure protocols. At 70 ppb, Schelegle et al. (2009) observed a statistically significant $O_3$-induced $FEV_1$ decrement of 6.1% at 6.6 hours and a significant increase in total subjective symptoms at 5.6 and 6.6 hours. A re-analysis found the $FEV_1$ responses at 70 ppb to be significantly different from FA responses beginning at 4.6 hours of exposure (Lefohn et al., 2010a). At 60 ppb,

an $O_3$-induced 3.5% $FEV_1$ decrement was not found to be statistically significant. However, this effect is similar in magnitude to the 2.9% $FEV_1$ decrement at 60 ppb observed by Adams (2006a), which was found to be statistically significant by Brown et al. (2008).

More recently, Kim et al. (2011) investigated the effects of a 6.6-hour exposure to 60 ppb $O_3$ during moderate quasi continuous exercise ($\dot{V}_E$ = 40 L/min) on pulmonary function and respiratory symptoms in young healthy adults (32 F, 27 M; 25.0 ± 0.5 years) who were roughly half glutathione S-transferase μ-1 (GSTM1)-null genetically and half GSTM1-positive. Sputum neutrophil levels were also measured in a subset of the subjects (13 F, 11 M). The mean $FEV_1$ (± standard error) decrements at 6.6 hours (end of exposure relative to pre-exposure) were significantly different ($p = 0.008$) between the FA (0.002 ± 0.46%) and $O_3$ (1.76 ± 0.50%) exposures. The inflammatory response following $O_3$ exposure was also significantly ($p < 0.001$) increased relative to the FA exposure. Respiratory symptoms were not affected by $O_3$ exposure. There was also no significant effect of GSTM1 genotype on $FEV_1$ or inflammatory responses to $O_3$.

Consideration of the minimal $O_3$ concentration producing statistically significant effects on $FEV_1$ and respiratory symptoms (e.g., cough and pain on deep inspiration) following 6.6-hour exposures warrants additional discussion. As discussed above, numerous studies have demonstrated statistically significant $O_3$-induced group mean $FEV_1$ decrements of 6-8% and an increase in respiratory symptoms at 80 ppb. Schelegle et al. (2009) have now reported a statistically significant $O_3$-induced group mean $FEV_1$ decrement of 6%, as well as increased respiratory symptoms, at 70 ppb. At 60 ppb, there is information available from 4 separate studies (Kim et al., 2011; Schelegle et al., 2009; Adams, 2006a, 2002).[1] The group mean $O_3$-induced $FEV_1$ decrements observed in these studies were 3.6% (facemask, square-wave) by Adams (2006a, 2002)[2], 2.8% (triangular exposure) and 2.9% (square-wave exposure) by Adams (2006a), 3.5% (triangular exposure) by Schelegle et al. (2009), and 1.8% (square-wave exposure) by Kim et al. (2011). Based on data from these studies, at 60 ppb, the weighted-average group mean $O_3$-induced $FEV_1$ decrement (i.e., adjusted for FA responses) is 2.7% (n = 150). Although not consistently statistically significant, these group mean changes in $FEV_1$ at 60 ppb are consistent among studies, i.e., none observed an average improvement in lung function following a 6.6-hour exposure to 60 ppb $O_3$. Indeed, as was illustrated in Figure 6-1, the group mean $FEV_1$ responses at 60 ppb fall on a smooth intake dose-response curve for exposures between 40 and 120 ppb $O_3$. Furthermore, in a re-analysis of the 60 ppb square-wave data from Adams (2006a), Brown et al. (2008) found the mean effects on $FEV_1$ to be highly statistically significant ($p < 0.002$) using several common statistical tests even after removal of 3 potential outliers. A statistically

[1] Adams (2006a); (2002) both provide data for an additional group of 30 healthy subjects that were exposed via facemask to 60 ppb (square-wave) $O_3$ for 6.6 hours with moderate exercise ($\dot{V}_E$ = 23 L/min per $m^2$ BSA). These subjects are described on page 133 of Adams (2006a) and pages 747 and 761 of Adams (2002). The $FEV_1$ decrement may be somewhat increased due to a target $\dot{V}_E$ of 23 L/min per $m^2$ BSA relative to other studies with which it is listed having the target $\dot{V}_E$ of 20 L/min per $m^2$ BSA. Based on Adams (2003a, b, 2002) the facemask exposure is not expect to affect the $FEV_1$ responses relative to a chamber exposure.

[2] This group average $FEV_1$ response is for a set of subjects exposed via facemask to 60 ppb $O_3$, see page 133 of Adams (2006a).

significant increase in total respiratory symptoms at 60 ppb has only been reported by Adams (2006a) for a triangular exposure protocol at 5.6 hours and 6.6 hours relative to baseline (not FA). Although not statistically significant, there was a tendency for an increase in total symptoms and pain on deep inspiration following the 60 ppb exposures (triangular and square-wave) relative to those following both FA and 40 ppb exposures. The time-course and magnitude of $FEV_1$ responses at 40 ppb resemble those occurring during FA exposures (Adams, 2006a, 2002). In both of these studies, there was a tendency (not statistically significant) for a small increase in total symptoms and pain on deep inspiration following the 40 ppb exposures relative to those following FA. Taken together, the available evidence shows that detectable effects of $O_3$ on group mean $FEV_1$ persist down to 60 ppb, but not 40 ppb in young healthy adults exposed for 6.6 hours during moderate exercise. Although group mean $FEV_1$ responses at 60 ppb are relatively small (2-3% mean $FEV_1$ decrement), it should be emphasized that there is considerable intersubject variability, with some responsive individuals consistently experiencing larger than average $FEV_1$ responses.

In addition to overt effects of $O_3$ exposure on the large airways indicated by spirometric responses, $O_3$ exposure also affects the function of the small airways and parenchymal lung. Foster et al. (1997); (1993) examined the effect of $O_3$ on ventilation distribution. In healthy adult males (n = 6; and 26.7 ± 7 years old) exposed to $O_3$ (330 ppb with light intermittent exercise for 2 hours), there was a significant reduction in ventilation to the lower lung (31% of lung volume) and significant increases in ventilation to the upper- and middle-lung regions (Foster et al., 1993). In a subsequent study of healthy males (n = 15; and 25.4 ± 2 years old) exposed to $O_3$ (350 ppb with moderate intermittent exercise for 2.2 hours), $O_3$ exposure caused a delayed gas washout in addition to a 14% $FEV_1$ decrement (Foster et al., 1997). The pronounced slow phase of gas washout following $O_3$ exposure represented a 24% decrease in the washout rate. A day following $O_3$ exposure, 50% of the subjects still had (or developed) a delayed washout relative to the pre-$O_3$ maneuver. These studies suggest a prolonged $O_3$ effect on the small airways and ventilation distribution in healthy young individuals.

There is a rapid recovery of $O_3$-induced spirometric responses and symptoms; 40 to 65% recovery appears to occur within about 2 hours following exposure (Folinsbee and Hazucha, 1989). For example, following a 2-hour exposure to 400 ppb $O_3$ with intermittent exercise, Nightingale et al. (2000) observed a 13.5% mean decrement in $FEV_1$. By 3 hours postexposure, however, only a 2.7% $FEV_1$ decrement persisted. Partial recovery also occurs following cessation of exercise despite continued exposure to $O_3$ (Folinsbee et al., 1977) and at low $O_3$ concentrations during exposure (Hazucha et al., 1992). A slower recovery phase, especially after exposure to higher $O_3$ concentrations, may take at least 24 hours to complete (Folinsbee and Hazucha, 2000; Folinsbee et al., 1993). Repeated daily exposure studies at higher concentrations typically show that $FEV_1$ response to $O_3$ is enhanced on the second day of exposure. This enhanced response suggests a residual effect of the previous exposure, about 22 hours earlier, even though the pre-exposure 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 (Folinsbee et al., 1994).

### Predicted Responses in Healthy Subjects

Studies analyzing large data sets (hundreds of subjects) provide better predictive ability of acute changes in $FEV_1$ at low levels of $O_3$ and $\dot{V}_E$ than is possible via comparisons between smaller studies. A few such studies described in the 2006 $O_3$ AQCD (U.S. EPA, 2006b) analyzed $FEV_1$ responses in healthy young adults (18-35 years of age) recruited from the area around Chapel Hill, NC and exposed for 2 hours to $O_3$ concentrations of up to 400 ppb at rest or with intermittent exercise (McDonnell et al., 1997; Seal et al., 1996; Seal et al., 1993). McDonnell et al. (1999b) examined changes in respiratory symptoms with $O_3$ exposure in a subset of the Chapel Hill data. In general, these studies showed that $FEV_1$ and respiratory symptom responses increase with increasing $O_3$ concentration and $\dot{V}_E$ and decrease with increasing subject age. More recent studies expand upon these analyses of $FEV_1$ responses to also include longer duration (up to 8 hours) studies and periods of recovery following exposure.

McDonnell et al. (2007) provided a nonlinear empirical model for predicting group average $FEV_1$ responses as a function of $O_3$ concentration, exposure time, $\dot{V}_E$, and age of the exposed individual. The model predicts temporal dynamics of $FEV_1$ change in response to any set of $O_3$ exposure conditions that might reasonably be experienced in the ambient environment. The model substantially differs from earlier statistical models in that it effectively considers the concurrent processes of damage and repair, i.e., the model allows effects on $FEV_1$ to accumulate during exposure at the same time they are reduced due to the reversible nature of the effects. The model was based on response data of healthy, nonsmoking, white males (n = 541), 18-35 years old, from 15 studies conducted at the U.S. EPA Human Studies Facility in Chapel Hill, NC.

McDonnell et al. (2010) tested the predictive ability of the model (Mcdonnell et al., 2007) against independent data (i.e., data that were not used to fit the model) of Adams (2006a, b, 2003a, 2002, 2000), Hazucha et al. (1992), and Schelegle et al. (2009). The model generally captured the dynamics of group average $FEV_1$ responses within about a one percentage point of the experimental data. Consistent with Bennett et al. (2007), an increased body mass index (BMI) was found to be associated with enhanced $FEV_1$ responses to $O_3$ by McDonnell et al. (2010). The BMI effect is of the same order of magnitude but in the opposite direction of the age effect whereby $FEV_1$ responses diminish with increasing age. Although the effects of age and BMI are relatively strong, these characteristics account for only a small amount of the observed variability in individual responses.

Alternatively, Lefohn et al. (2010a) proposed that $FEV_1$ responses to $O_3$ exposure might be described by a cumulative integrated exposure index with a sigmoidal weighting function similar to the W126 used for predicting vegetation effects (see Section 9.5). The integrated exposure index is the sum of the hourly average $O_3$

concentrations times their respective weighing factors. Based on a limited number of studies, the authors assumed weighting factors ranged from near zero at 50 ppb up to approximately 1.0 for concentrations at ≥ 125 ppb. The concentrations of 60, 70 and 80 ppb correspond to the assumed weights of 0.14, 0.28, and 0.50, respectively, and apply only to the case of exposure during moderate exercise ($\dot{V}_E$ = 20 L/min per $m^2$ BSA). Lefohn et al. (2010a) calculated the cumulative exposure index for the protocols used by Adams (2006a, 2003a) and Schelegle et al. (2009). They found statistically significant $O_3$ effects after 4 hours on $FEV_1$ at 105 ppb-hour based on Schelegle et al. (2009) and at 235 ppb-hour based on Adams (2006a, 2003a). Based on this analysis, the authors recommended a 5-hour accumulation period to protect against $O_3$ effects on lung function.

More recently the McDonnell et al. (2007) model, as well as a variant containing a response threshold (described in more detail below), was fit to a larger dataset consisting of the $FEV_1$ responses of 741 young healthy adults (104 F, 637 M; mean age 23.8 yrs) from 23 individual controlled exposure studies conducted in either Chapel Hill, NC or Davis, CA (McDonnell et al., 2012). Concentrations across individual studies ranged from 40 ppb to 400 ppb, activity level ranged from rest to heavy exercise, duration of exposure was from 2 to 7.6 hours, and some studies provided data during periods of recovery following exposure. The resulting empirical models can estimate the frequency distribution of individual responses for any exposure scenario as well as summary measures of the distribution such the mean or median response and the proportions of individuals with $FEV_1$ decrements > 10%, 15%, and 20%. Predictions were found to be close agreement with the experimental data. The responses of males and females were, on average, approximately equal when activity level was controlled by normalizing $\dot{V}_E$ to BSA. Thus, any effects of sex upon $FEV_1$ responses to $O_3$ exposure can be accounted for by utilizing $\dot{V}_E$/BSA. In this large dataset, the coefficient of BMI was not statistically significantly different from zero, although its magnitude was similar to that estimated by of the earlier study (McDonnell et al., 2010). The threshold model fit the experimental data better than the non-threshold model, particularly at the earliest time points of low concentration exposures.

Schelegle et al. (2012) also analyzed a large dataset with substantial overlap to that used by McDonnell et al. (2012). From an initial dataset consisting of the $FEV_1$ responses of 704 young healthy adults (76 F, 628 M; mean age 23.8 yrs) from 21 individual controlled exposure studies conducted in either Chapel Hill, NC or Davis, CA, their model was fit to the $FEV_1$ responses of 220 young healthy adults (51 F, mean age 22 yrs; 169 M, mean age 24 yrs). Eighty-one of the excluded individuals appeared to be the result of inherent variability of repeated $FEV_1$ measurements in certain individuals and were present in both FA and $O_3$ exposure protocols. The resulting model unrealistically overestimated the $FEV_1$ responses of 11 individuals that participated in short-duration exposures (2.5 hours) with heavy exercise ($\dot{V}_E$ = 35 L/min per BSA) and high $O_3$ concentrations (240, 300, and 400 ppb). However, in general, for most exposure scenarios, the authors concluded that their model coefficients based on 220 individuals reliably predicted the mean $FEV_1$ decrements for the full dataset of 704 individuals.

Both McDonnell et al. (2012) and Schelegle et al. (2012) developed two compartment models that considered a dose of onset in response or a threshold of response. The first compartment in the McDonnell et al. (2012) model considers the level of oxidant stress in response to $O_3$ exposure to increase over time as a function of dose rate ($C \times \dot{V}_E$) and decrease by clearance or metabolization over time according to first order reaction kinetics. In the second compartment of the threshold model, once oxidant stress reaches some threshold level, the decrement in $FEV_1$ increases as a sigmoid-shaped function of the oxidant stress with age. In the Schelegle et al. (2012) model, a first compartment acts as a reservoir in which oxidant stress builds up until the dose of onset at which time it spills over into a second compartment. The second compartment is identical to the first compartment in McDonnell et al. (2012) model. The oxidant levels in the second compartment were multiplied by a responsiveness coefficient to predict $FEV_1$ responses for the Schelegle et al. (2012) model.

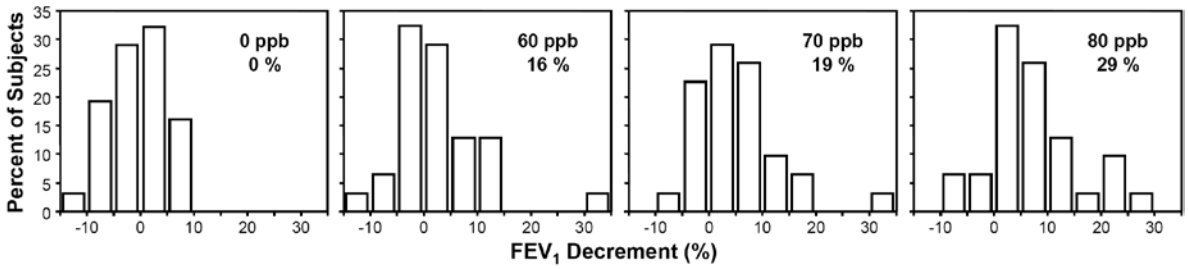
Exposures predicted to not reach threshold in the McDonnell et al. (2012) dataset were those with moderate, near continuous exercise for 1 hour to 60 and 80 ppb $O_3$, and for 2 hours to 40 ppb; and those at rest for 1 hour to 180 and 240 ppb $O_3$, and for 2 hours to 120 ppb $O_3$. However, there were also exposures above the threshold having small predicted responses due to the sigmoid shape of the exposure-response function. Schelegle et al. (2012) reported an average predicted dose of onset in response was 1,080 μg $O_3$. For a prolonged (6.6 hours) $O_3$ exposure with moderate quasi continuous exercise ($\dot{V}_E$ = 20 L/min per BSA), this dose of onset would not be reached until between 4-5 hrs of exposure to 60 ppb or 3-4 hrs of exposure to 80 ppb. However, 14% of the individuals in the Schelegle et al. (2012) study had a dose of onset of less than 400 μg $O_3$. More consistent with the threshold in response reported by McDonnell et al. (2012), this dose of onset (i.e., 400 μg $O_3$) would be reached in 1-2 hrs of exposure to 50-80 ppb $O_3$ with moderate quasi continuous exercise.

### Intersubject Variability in Response of Healthy Subjects

Consideration of group mean changes is important in discerning if observed effects are due to $O_3$ exposure rather than chance alone. Inter-individual variability in responses is, however, considerable and pertinent to assessing the fraction of the population that might actually be affected during an $O_3$ exposure. Hackney et al. (1975) first recognized a wide range in the sensitivity of subjects to $O_3$. The range in the subjects' ages (29 to 49 years) and smoking status (0 to 50 pack years) in the Hackney et al. (1975) study are now understood to affect the spirometric and symptomatic responses to $O_3$. Subsequently, DeLucia and Adams (1977) examined responses to $O_3$ in six healthy non-smokers and found that two exhibited notably greater sensitivity to $O_3$. Since that time, numerous studies have documented considerable variability in responsiveness to $O_3$ even in subjects recruited to assure homogeneity in factors recognized or presumed to affect responses.

An individual's $FEV_1$ response to a 2 hour $O_3$ exposure is generally reproducible over several months and presumably reflects the intrinsic responsiveness of the individual to $O_3$ (Hazucha et al., 2003; McDonnell et al., 1985c). The frequency

distribution of individual $FEV_1$ responses following these relatively short exposures becomes skewed as the group mean response increases, with some individuals experiencing large reductions in $FEV_1$ (Weinmann et al., 1995a; Kulle et al., 1985). For 2-hour exposures with intermittent exercise causing a predicted average $FEV_1$ decrement of 10%, individual decrements ranged from approximately 0 to 40% in white males aged 18-36 years (McDonnell et al., 1997). For an average $FEV_1$ decrement of 13%, Ultman et al. (2004) reported $FEV_1$ responses ranging from a 4% improvement to a 56% decrement in young healthy adults (32 M, 28 F) exposed for 1 hour to 250 ppb $O_3$. One-third of the subjects had $FEV_1$ decrements of >15%, and 7% of the subjects had decrements of >40%. The differences in $FEV_1$ responses did not appear to be explained by intersubject differences in the fraction of inhaled $O_3$ retained in the lung (Ultman et al., 2004).




Note: During each hour of the exposures, subjects were engaged in moderate quasi continuous exercise (40 L/min) for 50 minutes and rest for 10 minutes. Following the third hour, subjects had an additional 35 minute rest period for lunch. Subjects were exposed to a triangular $O_3$ concentration profile having the average $O_3$ concentration provided in each panel. As average $O_3$ concentration increased, the distribution of responses became asymmetric with a few individuals exhibiting large $FEV_1$ decrements. The percentage indicated in each panel is the portion of subjects having a $FEV_1$ decrement in excess of 10%.

Source: Adapted with permission of American Thoracic Society (Schelegle et al., 2009).

**Figure 6-2 Frequency distributions of $FEV_1$ decrements observed by Schelegle et al. (2009) in young healthy adults (16 F, 15 M) following 6.6-hour exposures to $O_3$ or filtered air.**

Consistent with the 1- to 2-hour studies, the distribution of individual responses following 6.6-hour exposures becomes skewed with increasing $O_3$ exposure concentration and magnitude of the group mean $FEV_1$ response (McDonnell, 1996). Figure 6-2 illustrates frequency distributions of individual $FEV_1$ responses observed in 31 young healthy adults following 6.6-hour exposures between 0 and 80 ppb $O_3$. Schelegle et al. (2009) found >10% $FEV_1$ decrements in 16, 19, 29, and 42% of individuals exposed for 6.6 hours to 60, 70, 80, and 87 ppb $O_3$, respectively. Just as there are differences in mean decrements between studies having similar exposure scenarios (Figure 6-1 at 80 and 120 ppb), there are differences in the proportion of individuals affected with >10% $FEV_1$ decrements. At 80 ppb $O_3$, the proportion affected with >10% $FEV_1$ decrements was 17% (n = 30) by Adams (2006a)[1], 26%

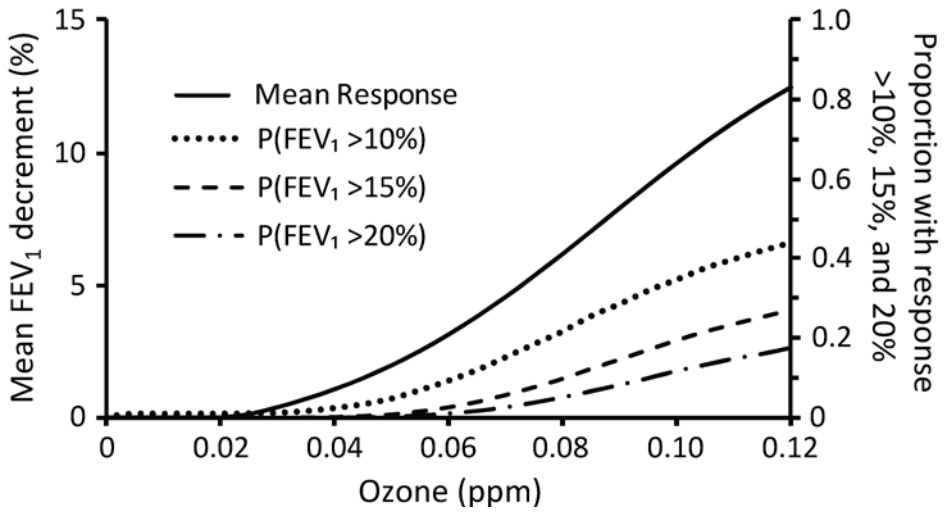
[1] Not assessed by Adams (2006a), the proportion was provided in Figure 8-1B of the 2006 $O_3$ AQCD (U.S. EPA, 2006b).

(n = 60) by McDonnell (1996), and 29% (n = 31) by Schelegle et al. (2009). At 60 ppb $O_3$, the proportion with >10% $FEV_1$ decrements was 20% (n = 30) by Adams (2002)[1], 3% (n = 30) by Adams (2006a)[1], 16% (n = 31) by Schelegle et al. (2009), and 5% (n = 59) by Kim et al. (2011). Based on these studies, the weighted average proportion of individuals with >10% $FEV_1$ decrements is 10% following exposure to 60 ppb $O_3$ and 25% following exposure to 80 ppb $O_3$. Due to limited data within the published papers, these proportions were not corrected for responses to FA exposure during which lung function typically improves in healthy adults. For example, uncorrected versus $O_3$-induced (i.e., adjusted for response during FA exposure) proportions of individuals having >10% $FEV_1$ decrements in the Adams (2006a)[2] study were, respectively, 3% versus 7% at 60 ppb and 17% versus 23% at 80 ppb. Thus, uncorrected proportions may underestimate the actual fraction of healthy individuals affected in some studies.

In addition to examining individual responses on a study-by-study basis, the recently published McDonnell et al. (2012) model can also be utilized to directly calculate the proportion of individuals expected to experience $O_3$-induced (i.e., adjusted for response during FA exposure) $FEV_1$ decrements of a given magnitude under a variety of exposure conditions and demographic characteristics. This model was fit to the data of young healthy adults (104 F, 637 M; 18-36 yrs of age) that participated in controlled $O_3$ exposure studies conducted in Chapel Hill, NC and Davis, CA. Figure 6-3 illustrates the proportions of individuals predicted to have greater than 10%, 15%, and 20% $O_3$-induced $FEV_1$ decrements a following a 6.6 hour exposure to $O_3$ with moderate exercise. Consistent with the observed responses of individual studies cited above, the model predicts that >10% $FEV_1$ decrements occur in 9% of the individuals exposed to 60 ppb and 22% of those exposed to 80 ppb $O_3$.

[1] This information is from page 761 of Adams (2002). Adams (2006a, 2002) both provide data for a group of 30 healthy subjects that were exposed via facemask to 60 ppb (square-wave) $O_3$ for 6.6 hours with moderate exercise ($\dot{V}_E$ = 23 L/min per $m^2$ BSA). These subjects are described on page 133 of Adams (2006a) and pages 747 and 761 of Adams (2002). The $FEV_1$ decrement may be somewhat increased due to a target $\dot{V}_E$ of 23 L/min per $m^2$ BSA relative to other studies with which it is listed having the target $\dot{V}_E$ of 20 L/min per $m^2$ BSA. Based on Adams (2003a, b, 2002), similar $FEV_1$ responses are expected between facemask and chamber exposures.

[2] Not assessed by Adams (2006a), uncorrected and $O_3$-induced proportions are from Figures 8-1B and 8-2, respectively, of the 2006 $O_3$ AQCD (U.S. EPA, 2006b).




Note: Predictions based threshold model of McDonnell et al. (2012) for healthy 23.8 year old adults exposed to a constant concentration of $O_3$ for 6.6 hrs. During each hour of the exposures, subjects were presumed engaged in moderate quasi continuous exercise (20 L/min/BSA) for 50 minutes and rest for 10 minutes. Following the third hour, subjects had an additional 35 minute rest period for lunch.

Source: Adapted from McDonnell et al. (2012).

**Figure 6-3 Proportion of individuals predicted to have greater than 10%, 15%, and 20% $O_3$-induced $FEV_1$ decrements a following 6.6-hour exposure to $O_3$ with moderate exercise.**

Given considerable inter-individual variability in responses, the interpretation of biologically small group mean decrements requires careful consideration. Following prolonged 6.6-hour exposures to an average level of 60 ppb $O_3$, data available from four studies yield a weighted-average group mean $O_3$-induced $FEV_1$ decrement (i.e., adjusted for FA responses) of 2.7% (n = 150) (Kim et al., 2011; Schelegle et al., 2009; Adams, 2006a, 1998). The data from these studies also yield a weighted-average proportion (uncorrected for FA responses) of subjects with >10% $FEV_1$ decrements of 10% (n = 150) (Kim et al., 2011; Schelegle et al., 2009; Adams, 2006a, 1998). In an individual with relatively "normal" lung function, with recognition of the technical and biological variability in measurements, confidence can be given that within-day changes in $FEV_1$ of $\geq$ 5% are clinically meaningful (Pellegrino et al., 2005; ATS, 1991). Here focus is given to individuals with >10% decrements in $FEV_1$ since some individuals in the Schelegle et al. (2009) study experienced 5-10% $FEV_1$ decrements following exposure to FA. A 10% $FEV_1$ decrement is also generally accepted as an abnormal response and a reasonable criterion for assessing exercise-induced bronchoconstriction (Dryden et al., 2010; ATS, 2000a). The data are not available in the published papers to determine the $O_3$-induced proportion for either the Adams (1998) or Schelegle et al. (2009) studies. As already stated, however, this uncorrected proportion likely underestimates the actual proportion of healthy individuals experiencing $O_3$-induced $FEV_1$ decrements

in excess of 10%. Therefore, by considering uncorrected responses and those individuals having >10% decrements, 10% is an underestimate of the proportion of healthy individuals that are likely to experience clinically meaningful changes in lung function following exposure for 6.6 hours to 60 ppb $O_3$ during moderate exercise. Of the studies conducted at 60 ppb, only Kim et al. (2011) reported $FEV_1$ decrements at 60 ppb to be statistically significant. However, Brown et al. (2008) found those from Adams (2006a) to be highly statistically significant. Though group mean decrements are biologically small and generally do not attain statistical significance, a considerable fraction of exposed individuals experience clinically meaningful decrements in lung function.

### Factors Modifying Responsiveness to Ozone

Physical activity increases $\dot{V}_E$ and therefore the dose of inhaled $O_3$. Consequently, the intensity of physiological response during and following an acute $O_3$ exposure will be strongly associated with $\dot{V}_E$. Apart from inhaled $O_3$ dose and related environmental factors (e.g., repeated daily exposures), individual-level factors, such as health status, age, sex, race/ethnicity, race, smoking habit, diet, and socioeconomic status (SES) have been considered as potential modifiers of a physiologic response to such exposures.

#### Responses in Individuals with Pre-existing Disease

Individuals with respiratory disease are of primary concern in evaluating the health effects of $O_3$ because a given change in function is likely to have more impact on a person with pre-existing function impairment and reduced reserve.

Possibly due to the age of subjects studied, patients with COPD performing light to moderate exercise do not generally experience statistically significant pulmonary function decrements following 1- and 2-hour exposures to ≤ 300 ppb $O_3$ (Kehrl et al., 1985; Linn et al., 1983; Linn et al., 1982a; Solic et al., 1982). Following a 4-hour exposure to 240 ppb $O_3$ during exercise, Gong et al. (1997b) found an $O_3$-induced $FEV_1$ decrement of 8% in COPD patients which was not statistically different from the decrement of 3% in healthy subjects. Demonstrating the need for control exposures and the presumed effect of exercise, four of the patients in the Gong et al. (1997b) study had $FEV_1$ decrements of >14% following both the FA and $O_3$ exposures. Although the clinical significance is uncertain, small transient decreases in arterial blood oxygen saturation have also been observed in some of these studies.

Based on studies reviewed in the 1996 and 2006 $O_3$ AQCDs, subjects with asthma appear to be at least as sensitive to acute effects of $O_3$ as healthy subjects. Horstman et al. (1995) found the $O_3$-induced $FEV_1$ decrement in 17 subjects with mild-to-moderate asthma to be significantly larger than that in 13 healthy subjects (19% versus 10%, respectively) exposed to 160 ppb $O_3$ during light exercise ($\dot{V}_E$ of 15 L/min per $m^2$ BSA) for a 7.6-hour exposure. In subjects with asthma, a significant positive correlation between $O_3$-induced spirometric responses and baseline lung

function was observed, i.e., responses increased with severity of disease. In the shorter duration study by Kreit et al. (1989), 9 subjects with asthma also showed a considerable larger average $O_3$-induced $FEV_1$ decrement than 9 healthy controls (25% versus 16%, respectively) following exposure to 400 ppb $O_3$ for 2 hours with moderate-heavy exercise ($\dot{V}_E$ = 54 L/min). Alexis et al. (2000) [400 ppb; 2 hours; exercise, $\dot{V}_E$ = 30 L/min] and Jorres et al. (1996) [250 ppb; 3 hours; exercise, $\dot{V}_E$ = 30 L/min] reported a tendency for slightly greater $FEV_1$ decrements in subjects with asthma than healthy subjects. Several studies reported similar responses between individuals with asthma and healthy individuals (Scannell et al., 1996; Hiltermann et al., 1995; Basha et al., 1994). The lack of differences in the Hiltermann et al. (1995) [400 ppb; 2 hours; exercise, $\dot{V}_E$ = 20 L/min] and Basha et al. (1994) [200 ppb; 6 hours; exercise, $\dot{V}_E$ = 25 L/min] studies was not surprising, however, given extremely small sample sizes (5-6 subjects per group) and corresponding lack of statistical power. Power was not likely problematic for Scannell et al. (1996) [200 ppb; 4 hours; exercise, $\dot{V}_E \approx$ 44 L/min] with 18 subjects with mild asthma and 81 age-matched healthy controls from companion studies (Balmes et al., 1996; Aris et al., 1995). Of note, Mudway et al. (2001) reported a tendency for subjects with asthma to have smaller $O_3$-induced $FEV_1$ decrements than healthy subjects (3% versus 8%, respectively) when exposed to 200 ppb $O_3$ for 2 hours during exercise. However, the subjects with asthma in Mudway et al. (2001) also tended to be older than the healthy subjects, which could partially explain their smaller response since $FEV_1$ responses to $O_3$ diminish with age.

In a study published since the 2006 $O_3$ AQCD, Stenfors et al. (2010) exposed subjects with persistent asthma (n = 13; aged 33 years) receiving chronic inhaled corticosteroid therapy to 200 ppb $O_3$ for 2 hours with moderate exercise. An average $O_3$-induced $FEV_1$ decrement of 8.4% was observed, whereas, only a 3.0% $FEV_1$ decrement is predicted for similarly exposed age-matched healthy controls (Mcdonnell et al., 2007). Vagaggini et al. (2010) exposed subjects with mild-to-moderate asthma (n = 23; 33 ± 11 years) to 300 ppb $O_3$ for 2 hours with moderate exercise. Although the group mean $O_3$-induced $FEV_1$ decrement was only 4%, eight subjects were categorized as “responders” with >10% $FEV_1$ decrements. Baseline lung function did not differ between the responders and nonresponders suggesting that, in contrast to Horstman et al. (1995), $O_3$-induced $FEV_1$ responses were not associated with disease severity.

### Lifestage

Children, adolescents, and young adults (<18 years of age) appear, on average, to have nearly equivalent spirometric responses to $O_3$, but have greater responses than middle-aged and older adults when similarly exposed to $O_3$ (U.S. EPA, 1996a). Symptomatic responses to $O_3$ exposure, however, appear to increase with age until early adulthood and then gradually decrease with increasing age (U.S. EPA, 1996a). For example, healthy children (n=22; mean age 10 yrs) exposed to FA and 120 ppb $O_3$ (2.5 hours; heavy intermittent exercise, $\dot{V}_E$=32-35 L/min per $m^2$ BSA) experienced similar spirometric responses, but lesser symptoms than similarly exposed young healthy adults (n=21-22; mean age 22 yrs)(McDonnell et al., 1985a).

For subjects aged 18-36 years, McDonnell et al. (1999b) reported that symptom responses from $O_3$ exposure also decrease with increasing age. Diminished symptomatic responses in children and the elderly might put these groups at increased risk for continued $O_3$ exposure, i.e., a lack of symptoms may result in their not avoiding or ceasing exposure. Once lung growth and development reaches the peak (18-20 years of age in females and early twenties in males), pulmonary function, which is at its maximum as well, begins to decline progressively with age as does $O_3$ sensitivity.

A couple analyses of large datasets have assessed the effects of age on responsiveness to $O_3$ exposure. McDonnell et al. (2007) found $O_3$-induced $FEV_1$ responses to decrease significantly with increasing age in an analysis of data from studies of healthy, nonsmoking, white males (n = 541), 18-35 years old (mean age 24.1), conducted in Chapel Hill, NC. Using this same dataset, McDonnell et al. (2010) reported that $O_3$-induced $FEV_1$ responses, while decreasing significantly with age, also increased significantly with BMI. In a larger dataset of 741 young healthy adults (104 F, 637 M; mean age 23.8 yrs) from studies conducted in either Chapel Hill, NC or Davis, CA, McDonnell et al. (2012) did not find a statistically significant effect of either age or BMI on the $FEV_1$ responses. Analysis of the Davis data alone showed a tendency for increases in $O_3$-induced $FEV_1$ responses with increases in both age and BMI, whereas $FEV_1$ responses in the Chapel Hill data decreased with age and increased with BMI. The authors speculated that the lack of a significant age effect may be, in part, due to a significant correlation (r = 0.23) between age and BMI in the 142 subjects from studies conducted at Davis. No correlation (r = 0.03) between age and BMI was observed in the Chapel Hill data.

In healthy individuals, the fastest rate of decline in $O_3$ responsiveness appears between the ages of 18 and 35 years (Passannante et al., 1998; Seal et al., 1996), more so for females then males (Hazucha et al., 2003). During the middle age period (35-55 years), $O_3$ sensitivity continues to decline, but at a much lower rate. Beyond this age (>55 years), acute $O_3$ exposure elicits minimal spirometric changes. Whether the same age-dependent pattern of $O_3$ sensitivity decline also holds for nonspirometric pulmonary function, airway reactivity or inflammatory endpoints has not been determined. Although there is considerable evidence that spirometric and symptomatic responses to $O_3$ exposure decrease with age beyond young adulthood, this evidence comes from cross-sectional analyses and has not been confirmed by longitudinal studies of the same individuals.

### Sex

Several studies have suggested that physiological differences between sexes may predispose females to greater $O_3$-induced health effects. In females, lower plasma and nasal lavage fluid (NLF) levels of uric acid (the most prevalent antioxidant), the initial defense mechanism of $O_3$ neutralization in airway surface liquid, may be a contributing factor (Housley et al., 1996). Consequently, reduced absorption of $O_3$ in the upper airways may promote its deeper penetration. Dosimetric measurements have shown that the absorption distribution of $O_3$ is independent of sex when

absorption is normalized to anatomical dead space (Bush et al., 1996). Thus, a sex-related differential removal of $O_3$ by uric acid seems to be minimal. In general, the physiologic response of young healthy females to $O_3$ exposure appears comparable to the response of young males (Hazucha et al., 2003). Based on analysis of a large dataset (104 F, 637 M; 18-36 yrs of age), $FEV_1$ responses of males and females to $O_3$ exposure were, on average, approximately equal when activity level was controlled by normalizing $\dot{V}_E$ to BSA (McDonnell et al., 2012). Additionally, with activity level assessed as $\dot{V}_E$/BSA, Schelegle et al. (2012) observed no significant difference in the average dose to onset of responses between males and females.

Several studies have investigated the effects of the menstrual cycle on responses to $O_3$ in healthy young women. In a study of 9 women exposed during exercise to 300 ppb $O_3$ for an hour, Fox et al. (1993) found lung function responses to $O_3$ significantly enhanced during the follicular phase relative to the luteal phase. However, Weinmann et al. (1995c) found no difference in responses between the follicular and luteal phases as well as no significant differences between 12 males and 12 females exposed during exercise to 350 ppb $O_3$ for 2.15 hours. Seal et al. (1996) also reported no effect of menstrual cycle phase in their analysis of responses of 150 women (n = 25 per exposure group; 0, 120, 240, 300, and 400 ppb $O_3$). Seal et al. (1996) conceded that the methods used by Fox et al. (1993) more precisely defined menstrual cycle phase.

### Race/Ethnicity

Only two controlled human exposure studies have assessed differences in lung function responses between races. Seal et al. (1993) compared lung function responses of whites (93 M, 94 F) and blacks (undefined ancestry; 92 M, 93 F) exposed to a range of $O_3$ concentrations (0-400 ppb). The main effects of the sex-race group and $O_3$ concentration were statistically significant (both at $p < 0.001$), although the interaction between sex-race group and $O_3$ concentration was not significant ($p = 0.13$). These findings indicate some overall difference between the sex-race groups that is independent of $O_3$ concentration, i.e., the concentration-response (C-R) curves for the four sex-race groups are parallel. In a multiple comparison procedure on data collapsed across all $O_3$ concentrations for each sex-race group, both black men and black women had significantly larger decrements in $FEV_1$ than did white men. The authors noted that the $O_3$ dose per unit of lung tissue would be greater in blacks and females than whites and males, respectively. It cannot be ruled out that this difference in tissue dose might have affected responses to $O_3$. The college students recruited for the Seal et al. (1993) study were noted by the authors as probably being from better educated and SES advantaged families, thus reducing the potential influence of these variables on results. In a follow-up analysis, Seal et al. (1996) reported that, of three SES categories, individuals in the middle SES category showed greater concentration-dependent decline in percent-predicted $FEV_1$ (4-5% at 400 ppb $O_3$) than low and high SES groups. The authors did not have an “immediately clear” explanation for this finding.

More recently, Que et al. (2011) assessed pulmonary responses in blacks of African American ancestry (22 M, 24 F) and Caucasians (55 M, 28 F) exposed to 220 ppb $O_3$ for 2.25 hours (alternating 15 min periods of rest and brisk treadmill walking). On average, the black males experienced a 16.8% decrement in $FEV_1$ following $O_3$ exposure which was significantly larger than mean $FEV_1$ decrements of 6.2, 7.9, and 8.3% in black females and Caucasian males and Caucasian females, respectively. In the study by Seal et al. (1993), there was potential that the increased $FEV_1$ decrements in blacks relative to whites were due to increased $O_3$ tissue doses since exercise rates were normalized to BSA. Differences in $O_3$ tissue doses between the races should not have occurred in the Que et al. (2011) study because exercise rates were normalized to lung volume (viz., 6-8 times FVC). Thus, the increased mean $FEV_1$ decrement in black males is not likely attributable to systematically larger $O_3$ tissue doses in blacks relative to whites.

### Smoking

Smokers are less responsive to $O_3$ for some (but not all) health endpoints than nonsmokers. Spirometric and plethysmographic pulmonary function decline, respiratory symptoms, and nonspecific airway hyperreactivity of smokers to $O_3$ were all weaker than data reported for nonsmokers. However, the time course of development and recovery of these effects as well their reproducibility in smokers were not different from nonsmokers (Frampton et al., 1997a). Another similarity between smokers and nonsmokers is that, the inflammatory response to $O_3$ does not appear to depend on smoking status or the responsiveness of individuals to changes in lung function (Torres et al., 1997). Chronic airway inflammation with desensitization of bronchial nerve endings and an increased production of mucus may plausibly explain the reduced responses to $O_3$ in smokers relative to nonsmokers (Frampton et al., 1997a; Torres et al., 1997).

### Antioxidant supplementation

The first line of defense against oxidative stress is antioxidants-rich ELF which scavenges free radicals and limits lipid peroxidation. Exposure to $O_3$ depletes the antioxidant level in nasal ELF probably due to scrubbing of $O_3$ (Mudway et al., 1999a); however, the concentration and the activity of antioxidant enzymes either in ELF or plasma do not appear to be related to $O_3$ responsiveness (Samet et al., 2001; Avissar et al., 2000; Blomberg et al., 1999). Carefully controlled studies of dietary antioxidant supplementation have demonstrated some protective effects of α-tocopherol and ascorbate on spirometric lung function from $O_3$ but not on the intensity of subjective symptoms or inflammatory response including cell recruitment, activation and a release of mediators (Samet et al., 2001; Trenga et al., 2001). Dietary antioxidants have also been reported to attenuate $O_3$-induced bronchial hyperresponsiveness in asthmatics (Trenga et al., 2001).

### Genetic polymorphisms

Some studies (e.g., Corradi et al., 2002; Bergamaschi et al., 2001) reviewed in the 2006 $O_3$ AQCD reported that genetic polymorphisms of antioxidant enzymes may

modulate pulmonary function and inflammatory response to $O_3$ challenge. It was suggested that healthy carriers of NAD(P)H:quinone oxidoreductase wild type (NQO1wt) in combination with GSTM1 null were more responsive to $O_3$. Bergamaschi et al. (2001) reported that subjects having NQO1wt and GSTM1 null genotypes had increased $O_3$ responsiveness ($FEV_1$ decrements and epithelial permeability), whereas subjects with other combinations of these genotypes were less affected. A subsequent study from the same laboratory reported a positive association between $O_3$ responsiveness, as characterized by the level of oxidative stress and inflammatory mediators (8-isoprostane, $LTB_4$ and TBARS) in exhaled breath condensate and the NQO1wt and GSTM1null genotypes (Corradi et al., 2002). However, none of the spirometric endpoints (e.g., $FEV_1$) were affected by $O_3$ exposure.

In a controlled exposure of subjects with mild-to-moderate asthma (n = 23; 33 ± 11 years) to 300 ppb $O_3$ for 2 hours with moderate exercise, Vagaggini et al. (2010) found that six of the subjects had a NQO1*wt* and GSTM1 null, but this genotype was not associated with the changes in lung function or inflammatory responses to $O_3$. Kim et al. (2011) also recently reported that GSTM1 genotype was not predictive of $FEV_1$ responses to $O_3$ in young healthy adults (32 F, 27 M; 25.0 ± 0.5 year) who were roughly half GSTM1-null and half GSTM1-sufficient. Sputum neutrophil levels, measured in a subset of the subjects (13 F, 11 M), were also not significantly associated with GSTM1 genotype.

In a study of healthy volunteers with GSTM1 sufficient (n = 19; 24 ± 3) and GSTM1 null (n = 16; 25 ± 5) genotypes exposed to 400 ppb $O_3$ for 2 hours with exercise, Alexis et al. (2009) found that inflammatory responses but not lung function responses to $O_3$ were dependent on genotype. At 4 hours post-$O_3$ exposure, both GSTM1 genotype groups had significant increases in sputum neutrophils with a tendency for a greater increase in GSTM1 sufficient than null subjects. At 24 hours postexposure, sputum neutrophils had returned to baseline levels in the GSTM1 sufficient individuals. In the GSTM1 null subjects, however, sputum neutrophil levels increased from 4 hours to 24 hours and were significantly greater than both baseline levels and levels at 24 hours in the GSTM1 sufficient individuals. Since there was no FA control in the Alexis et al. (2009) study, effects of the exposure other than $O_3$ itself cannot be ruled out. In general, the findings between studies are inconsistent.

### Body Mass Index

In a retrospective analysis of data from 541 healthy, nonsmoking, white males between the ages of 18-35 years from 15 studies conducted at the U.S. EPA Human Studies Facility in Chapel Hill, NC, McDonnell et al. (2010) found that increased BMI was associated with enhanced $FEV_1$ responses to $O_3$. The BMI effect was of the same order of magnitude but in the opposite direction of the age effect whereby $FEV_1$ responses diminish with increasing age. In a similar retrospective analysis, Bennett et al. (2007) found enhanced $FEV_1$ decrements following $O_3$ exposure with increasing BMI in a group of 75 healthy, nonsmoking, women (age 24 ± 4 years;

BMI range 15.7 to 33.4), but not 122 healthy, nonsmoking, men (age 25 ± 4 years; BMI range 19.1 to 32.9). In the women, greater $O_3$-induced $FEV_1$ decrements were seen in overweight (BMI >25) than in normal weight (BMI from 18.5 to 25), and in normal weight than in underweight (BMI <18.5) (P trend ≤ 0.022). Together, these results indicate that higher BMI may be a risk factor for pulmonary effects associated with $O_3$ exposure.

### Repeated Ozone Exposure Effects

The attenuation of responses observed after repeated consecutive $O_3$ exposures in controlled human exposure studies has also been referred to in the literature as "adaptation" or "tolerance" (e.g., Linn et al., 1988). In animal toxicology studies, however, the term tolerance has more classically been used to describe the phenomenon wherein a prior exposure to a low, nonlethal concentration of $O_3$ provides some protection against death and lung edema at a higher, normally lethal exposure concentration (see Section 9.3.5 of U.S. EPA, 1986). The term "attenuation" will be used herein to refer to the reduction in responses to $O_3$ observed with repeated $O_3$ exposures in controlled human exposure studies. Neither tolerance nor attenuation should be presumed to imply complete protection from the biological effects of inhaled $O_3$, because continuing injury still occurs despite the desensitization to some responses.

The attenuation of responses due to ambient $O_3$ exposure was first investigated by Hackney et al. (1977a); (1976). Experiencing frequent ambient $O_3$ exposures, Los Angeles residents were compared to groups having less ambient $O_3$ exposure. Following a controlled laboratory exposure to 370-400 ppb $O_3$ for 2 hours with light intermittent exercise (2-2.5 times resting $\dot{V}_E$), the Los Angeles residents exhibited minimal $FEV_1$ responses relative to groups having less ambient $O_3$ exposure. Subsequently, Linn et al. (1988) examined the seasonal variation in Los Angeles residents' responses to $O_3$ exposure. A group of 8 responders (3M, 5F) and 9 nonresponders (4M, 5F) were exposed to 180 ppb $O_3$ for 2 hours with heavy intermittent exercise ($\dot{V}_E$ = 35 L/min per $m^2$ BSA) on four occasions (spring, fall, winter, and the following spring). In responders, relative to the first spring exposures, $FEV_1$ responses were attenuated in the fall and winter, but returned to similar decrements the following spring. By comparison, the nonresponders, on average, showed no $FEV_1$ decrements on any of the four occasions. In subjects recruited regardless of $FEV_1$ responsiveness to $O_3$ from the area around Chapel Hill, NC, no seasonal effect of ambient $O_3$ exposure on $FEV_1$ responses following chamber exposures to $O_3$ has been observed (Hazucha et al., 2003; McDonnell et al., 1985c).

Based on studies reviewed in previous $O_3$ AQCDs, several conclusions can be drawn about repeated 1- to 2-hour $O_3$ exposures. Repeated exposures to $O_3$ causes enhanced (i.e., greater decrements) FVC and $FEV_1$ responses on the second day of exposure. The enhanced response appears to depend to some extent on the magnitude of the initial response (Horvath et al., 1981). 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

(Folinsbee et al., 1994). With continued daily exposures (i.e., beyond the second day) there is a substantial (or even total) attenuation of pulmonary function responses, typically on the third to fifth days of repeated $O_3$ exposure. This attenuation of responses is lost in 1 week (Kulle et al., 1982; Linn et al., 1982b) or perhaps 2 weeks (Horvath et al., 1981) without $O_3$ exposure. In temporal conjunction with pulmonary function changes, symptoms induced by $O_3$ (e.g., cough, pain on deep inspiration, and chest discomfort), are also increased on the second exposure day but are attenuated with repeated $O_3$ exposure thereafter (Folnsbee et al., 1995; Foxcroft and Adams, 1986; Linn et al., 1982b; Folinsbee et al., 1980). In longer-duration (4-6.6 hours), lower-concentration studies that do not cause an enhanced second-day response, the attenuation of response to $O_3$ appears to proceed more rapidly (Folinsbee et al., 1994).

Consistent with other investigators, Frank et al. (2001) found FVC and $FEV_1$ decrements to be significantly attenuated following four consecutive days of exposure to $O_3$ (250 ppb, 2 hours). However, the effects of $O_3$ on the small airways (assessed by a combined index of isovolumetric forced expiratory flow between 25 and 75% of vital capacity [$FEF_{25-75}$] and flows at 50% and 75% of FVC) showed a persistent functional reduction from Day 2 through Day 4. Notably, in contrast to FVC and $FEV_1$ which exhibited a recovery of function between days, there was a persistent effect of $O_3$ on small airways function such that the baseline function on Day 2 through Day 4 was depressed relative to Day 1. Frank et al. (2001) also found neutrophil (PMN) numbers in BAL remained significantly higher following $O_3$ (24 hours after last $O_3$ exposure) compared to FA. Markers from bronchioalveolar lavage fluid (BALF) following 4 consecutive days of both 2-hour (Devlin et al., 1997) and 4-hour (Jorres et al., 2000; Christian et al., 1998) exposures have indicated ongoing cellular damage irrespective of the attenuation of some cellular inflammatory responses of the airways, lung function and symptoms response. These data suggest that the persistent small airways dysfunction assessed by Frank et al. (2001) is likely induced by both neurogenic and inflammatory mediators, since the density of bronchial C-fibers is much lower in the small than large airways.

### Summary of Controlled Human Exposure Studies on Lung Function

Responses in humans exposed to $O_3$ concentrations found in the ambient environment include: decreased inspiratory capacity; mild bronchoconstriction; rapid, shallow breathing pattern during exercise; and symptoms of cough and pain on deep inspiration (U.S. EPA, 2006b, 1996a). Discussed in subsequent Section 6.2.2.1 and Section 6.2.3.1, controlled exposure to $O_3$ also results in airway hyperresponsiveness, pulmonary inflammation, immune system activation, and epithelial injury (Que et al., 2011; Mudway and Kelly, 2004a). Reflex inhibition of inspiration results in a decrease in forced vital capacity and, in combination with mild bronchoconstriction, contributes to a decrease in the $FEV_1$. Healthy young adults exposed to $O_3$ concentrations $\geq$ 60 ppb develop statistically significant reversible, transient decrements in lung function and symptoms of breathing discomfort if minute ventilation or duration of exposure is increased sufficiently

(Kim et al., 2011; McDonnell et al., 2010; Schelegle et al., 2009; Brown et al., 2008; Adams, 2006a). With repeated $O_3$ exposures over several days, $FEV_1$ and symptom responses become attenuated in both healthy individuals and asthmatics, but this attenuation of responses is lost after about a week without exposure (Gong et al., 1997a; Folinsbee et al., 1994; Kulle et al., 1982). In contrast to the attenuation of $FEV_1$ responses, there appear to be persistent $O_3$ effects on small airways function as well as ongoing cellular damage during repeated exposures.

There is a large degree of intersubject variability in lung function decrements (McDonnell, 1996). However, these lung function responses tend to be reproducible within a given individual over a period of several months indicating differences in the intrinsic responsiveness of individuals (Hazucha et al., 2003; McDonnell et al., 1985c). In healthy young adults, $O_3$-induced decrements in $FEV_1$ do not appear to depend on sex (Hazucha et al., 2003), body surface area or height (McDonnell et al., 1997), lung size or baseline FVC (Messineo and Adams, 1990). There is limited evidence that blacks may experience greater $O_3$-induced decrements in $FEV_1$ than age-matched whites (Que et al., 2011; Seal et al., 1993). Healthy children experience similar spirometric responses but lesser symptoms from $O_3$ exposure relative to young adults (McDonnell et al., 1985b). On average, spirometric and symptom responses to $O_3$ exposure appear to decline with increasing age beyond about 18 years of age (McDonnell et al., 1999b; Seal et al., 1996). There is a tendency for slightly increased spirometric responses in individuals with mild asthma and allergic rhinitis relative to healthy young adults (Jorres et al., 1996). Spirometric responses in subjects with asthma appear to be affected by baseline lung function, i.e., responses increase with disease severity (Horstman et al., 1995).

Available information on recovery of lung function following $O_3$ exposure indicates that an initial phase of recovery in healthy individuals proceeds relatively rapidly, with acute spirometric and symptom responses resolving within about 2 to 4 hours (Folinsbee and Hazucha, 1989). Small residual lung function effects are almost completely resolved within 24 hours. One day following $O_3$ exposure, persistent effects on the small airways assessed by decrements in $FEF_{25-75}$ and altered ventilation distribution have been reported (Frank et al., 2001; Foster et al., 1997).

### 6.2.1.2 Epidemiology

The $O_3$-induced lung function decrements consistently demonstrated in controlled human exposure studies (Section 6.2.1.1) provide biological plausibility for the epidemiologic evidence consistently linking short-term increases in ambient $O_3$ concentration with lung function decrements in diverse populations. In the 1996 and 2006 $O_3$ AQCDs, coherence with controlled human exposure study results was found not only for epidemiologic associations observed in groups with expected higher ambient $O_3$ exposures and higher exertion levels, including children attending summer camps and adults exercising or working outdoors, but also for associations observed in children and individuals with asthma (U.S. EPA, 2006b, 1996a). Recent

epidemiologic studies focused more on children with asthma rather than groups with increased outdoor exposures or other healthy populations. Whereas recent studies contributed less consistent evidence, the cumulative body of evidenc e indicates decreases in lung function in association with increases in ambient $O_3$ concentration in children with asthma. Collectively, studies in adults with asthma and individuals without asthma found both $O_3$-associated decreases and increases in lung function. Recent studies did provide additional data to assess whether particular lags of $O_3$ exposure were more strongly associated with decrements in lung function; whether $O_3$ associations were confounded by copollutant exposures; and whether associations were modified by factors such as corticosteroid (CS) use, genetic polymorphisms, and elevated BMI.

### Populations with Increased Outdoor Exposures

Epidemiologic studies primarily use ambient $O_3$ concentrations to represent exposure; however, few studies have accounted for time spent outdoors, which has been shown to influence the relationship between ambient concentrations and individual exposures to $O_3$ (Section 4.3.3). Epidemiologic studies of individuals engaged in outdoor recreation, exercise, or work are noteworthy for the likely greater extent to which ambient $O_3$ concentrations represent ambient $O_3$ exposures. Ambient $O_3$ concentrations, locations, and time periods for epidemiologic studies of populations with increased outdoor exposures are presented in Table 6-2. Most of these studies measured ambient $O_3$ at the site of subjects' outdoor activity and related lung function changes to the $O_3$ concentrations measured during outdoor activity, which have contributed to higher $O_3$ personal exposure-ambient concentration correlations and ratios (Section 4.3.3). Because of improved $O_3$ exposure estimates, measurement of lung function before and after discrete periods of activity, and examination of $O_3$ effects during exertion when the dose of $O_3$ reaching the lungs may be higher due to higher ventilation and inhalation of larger volumes of air, epidemiologic studies of populations with increased outdoor exposures are more comparable to controlled human exposure studies. The collective body of epidemiologic evidence clearly demonstrates decrements in lung function in association with increases in ambient $O_3$ exposure during outdoor activity (Figure 6-4 [and Table 6-3], Figure 6-5 [and Table 6-4], Figure 6-6 [and Table 6-5]. Expanding upon findings from controlled human exposure studies, these epidemiologic studies provide strong evidence for respiratory effects in children and adults related to ambient $O_3$ exposure.

## Table 6-2 Mean and upper percentile $O_3$ concentrations in epidemiologic studies of lung function in populations with increased outdoor exposures.

| Study* | Location | Study Period | $O_3$ Averaging Time | Mean/Median Concentration (ppb) | Upper Percentile Concentrations (ppb) |
|---|---|---|---|---|---|
| Thurston et al. (1997) | Connecticut River Valley, CT | June 1991-1993 | 1-h max | 83.6 | Max: 160 |
| Berry et al. (1991) | Mercer County, NJ | July 1988 | 1-h max[a] | NR | Max: 204 |
| Spektor and Lippmann (1991) | Fairview Lake, NJ | July-August 1988 | 1-h avg[b] | 69 | Max: 137 |
| Avol et al. (1990) | Idyllwild, CA | June-August 1988 | 1-h avg[b] | 94 | Max: 161 |
| Burnett et al. (1990) | Lake Couchiching, Ontario, Canada | June-July 1983 | 1-h avg[b] | 59 | Max: 95 |
| Higgins et al. (1990) | San Bernardino, CA | June-July 1987 | 1-h avg[b] | 123 | Max: 245 |
| Raizenne et al. (1989) | Lake Erie, Ontario, Canada | June-August 1986 | 1-h avg[b] | 71 | Max: 143 |
| Spektor et al. (1988a) | Fairview Lake, NJ | July-August 1984 | 1-h avg[b] | 53 | Max: 113 |
| Neas et al. (1999) | Philadelphia, PA | July-September 1993 | 12-h avg[a] (9 a.m. - 9 p.m.) | 57.5 (near Camp 1)<br>55.9 (near Camp 2) | Max (near Camp 1): 106 |
| Nickmilder et al. (2007) | Southern Belgium | July-August 2002 | 1-h max | NR | Max (across 6 camps): 24.6-112.8[c] |
| | | | 8-h max | NR | Max (across 6 camps): 19.0-81.1[c] |
| Girardot et al. (2006) | Great Smoky Mountain NP, TN | August-October 2002 June-August 2003 | Hike-time avg (2-9 h)[d] | 48.1 | Max: 74.2 |
| Korrick et al. (1998) | Mt. Washington, NH | Summers 1991, 1992 | Hike-time avg (2-12 h)[d] | 40 | Max: 74 |
| Hoppe et al. (2003) | Munich, Germany | Summers 1992-1995 | 30-min max (1-4 p.m.) | High $O_3$ days: 62.1<br>Control $O_3$ days: 26.6 | Max (overall): 82 |
| Spektor et al. (1988b) | Tuxedo, NY | June-August 1985 | Exercise-time avg (15 - 55 min) | NR | Max: 124 |
| Selwyn et al. (1985) | Houston, TX | May-October 1981 | Exercise-time 15-min max (4-7 p.m.) | 47 | Max: 135 |
| Brunekreef et al. (1994) | Eastern Netherlands | June-August 1991 | Exercise-time avg[a] (10-145 min) | 44.4[c] | Max: 99.5[c] |
| Braun-Fahrlander et al. (1994) | Southern Switzerland | May-October 1989 | Exercise-time 30-min avg (1-4 p.m.) | NR | Max: 80[c] |
| Castillejos et al. (1995) | Mexico City, Mexico | June 1990-October 1991 | 1-h max[a] | 112.3 | Max: 365 |

| Study* | Location | Study Period | $O_3$ Averaging Time | Mean/Median Concentration (ppb) | Upper Percentile Concentrations (ppb) |
|---|---|---|---|---|---|
| Hoek et al. (1993) | Wageningen, Netherlands | May-July 1989 | 1-h max[a] | NR | Max: 105[c] |
| Hoppe et al. (1995) | Munich, Germany | April-September 1993 | 30-min max (1-4 p.m.) | High $O_3$ days: 64<br>Control $O_3$ days: 32 | Max (overall): 77 |
| Chan and Wu (2005) | Taichung City, Taiwan | November-December 2001 | 8-h avg (9 a.m.-5 p.m.)<br>1-h max | 35.6<br>52.6 | Max: 65.1<br>95.5 |
| Brauer et al. (1996) | British Columbia, Canada | June-August 1993 | 1-h max[a] | 40 | Max: 84 |
| Romieu et al. (1998b) | Mexico City, Mexico | March-August 1996 | Work-shift avg (mean 9 h)[a] | 67.3 | 95th: 105.8 |
| Thaller et al. (2008) | Galveston, TX | Summers 2002-2004 | 1-h max[a] | 35 (median) | Max: 118 |

* Note: Studies presented in order of first appearance in the text of this section.

NR = not reported.

[a]Some or all measurements obtained from monitors located off site of outdoor activity.

[b]1-h avg preceding lung function measurement, as reported in the pooled analysis by Kinney et al. (1996).

[c]Concentrations converted from μg/$m^3$ to ppb using the conversion factor of 0.51 assuming standard temperature (25°C) and pressure (1 atm).

[d]Individual-level estimates calculated from concentrations measured in different segments of hiking trail.

### Children Attending Summer Camps

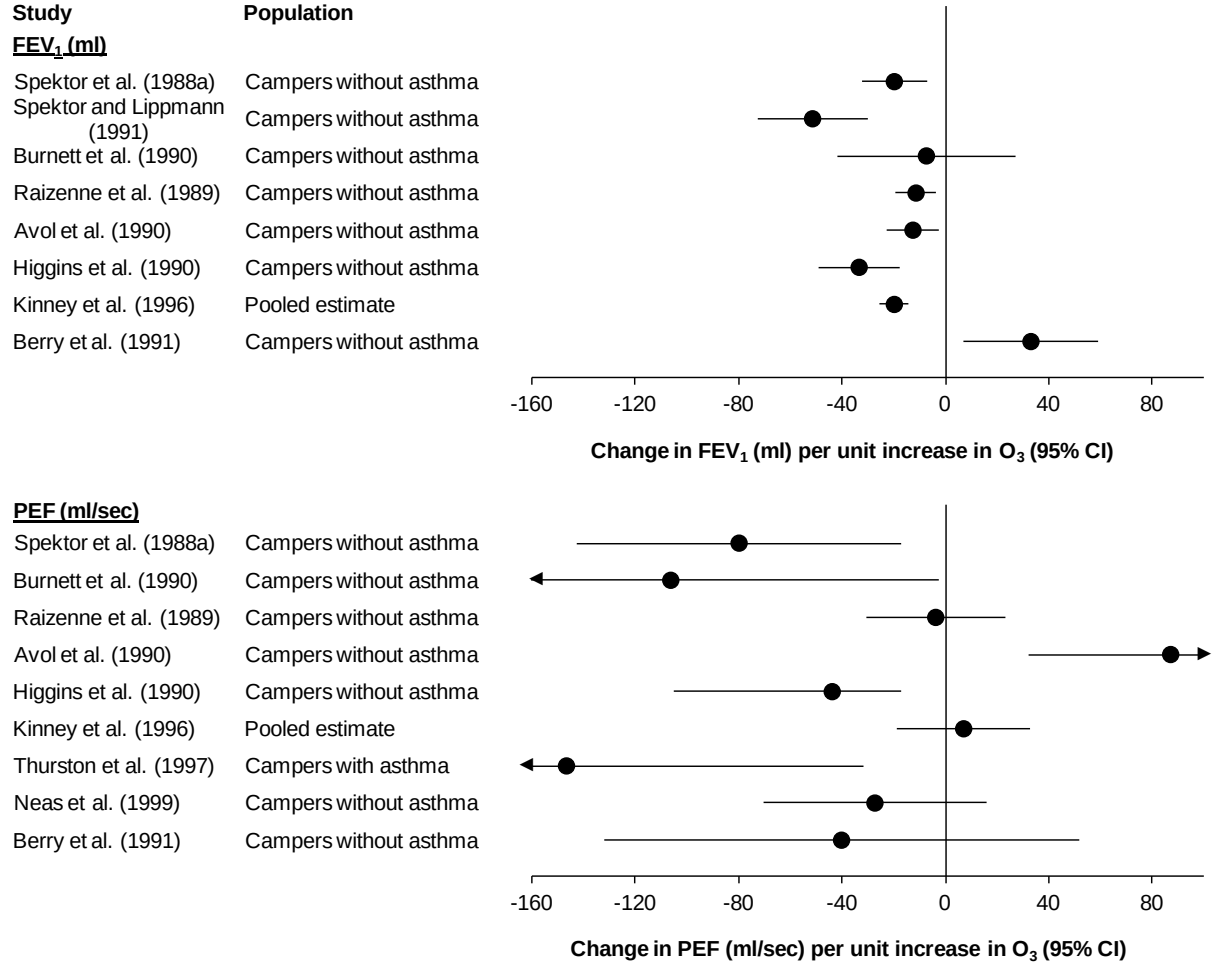
Studies of children attending summer camps, most of which were discussed in the 1996 $O_3$ AQCD, have provided important evidence of the effect of ambient $O_3$ exposure on respiratory effects in young, healthy children. In addition to the improved exposure assessment as described above, these studies were noted for their daily assessment of lung function by trained staff over 1- to 2-week periods in the mornings and late afternoons before and after hours of outdoor activity (Thurston et al., 1997; Berry et al., 1991; Spektor and Lippmann, 1991; Avol et al., 1990; Burnett et al., 1990; Higgins et al., 1990; Raizenne et al., 1989; Spektor et al., 1988a).

In groups mostly comprising healthy children (ages 7-17 years), decrements in $FEV_1$ were associated consistently with increases in ambient $O_3$ concentration averaged over the 1-12 hours preceding lung function measurement (Figure 6-4 [and Table 6-3]). Kinney et al. (1996) corroborated this association in a re-analysis combining 5,367 lung function measurements collected from 616 healthy children from six studies (Spektor and Lippmann, 1991; Avol et al., 1990; Burnett et al., 1990; Higgins et al., 1990; Raizenne et al., 1989; Spektor et al., 1988a). Based on uniform statistical methods, a -20 ml (95% CI: -25, -14) change in afternoon $FEV_1$ was estimated for a 40-ppb increase in $O_3$ concentration averaged over the 1 hour before lung function measurement (Kinney et al., 1996) (all effect estimates are standardized to increments specific to the $O_3$ averaging time as detailed in Section 2.5). All of the studies in the pooled analysis were conducted during summer months but were diverse in locations examined (i.e., Northeast U.S., Canada,

California), range in ambient concentrations of $O_3$ (presented within Table 6-2) and other pollutants measured, and magnitudes of association observed. Study-specific effect estimates ranged between a 0.76 and 48 mL decrease or between a 0.3% and 2.2% decrease in study mean $FEV_1$ per 40-ppb increase in 1-h avg $O_3$.

Among camp studies (including the pooled analysis, plus others), associations for peak expiratory flow (PEF) were more variable than were those for $FEV_1$, as indicated by the wider range in effect estimates and wider 95% CIs (Figure 6-4 [and Table 6-3]). Nonetheless, in most cases, increases in ambient $O_3$ concentration were associated with decreases in PEF. The largest $O_3$-associated decrease in PEF (mean 2.8% decline per 40-ppb increase in 1-h max $O_3$) was found in a group of campers with asthma, in whom an increase in ambient $O_3$ concentration also was associated with increases in chest symptoms and bronchodilator use (Thurston et al., 1997).

For both $FEV_1$ and PEF, the magnitude of association was not related to the study mean ambient 1-h avg or max $O_3$ concentration. With exclusion of results from Spektor and Lippmann (1991), larger $O_3$-associated $FEV_1$ decrements were found in populations with lower mean $FEV_1$. No such trend was found with mean PEF. Sufficient data were not available to assess whether the temporal variability in $O_3$ concentrations, activity levels of subjects, or associations with other pollutants contributed to between-study heterogeneity in $O_3$ effect estimates.




Note: Results generally are presented in order of increasing mean ambient $O_3$ concentration. Effect estimates are from single-pollutant models and are standardized to a 40-ppb increase for 1-h avg or 1-h max $O_3$ concentration and a 30-ppb increase for 12-h avg $O_3$ concentration.

**Figure 6-4 Changes in $FEV_1$ (mL) or PEF (mL/sec) in association with ambient $O_3$ concentrations among children attending summer camp.**

## Table 6-3 Changes in $FEV_1$ or PEF in association with ambient $O_3$ concentrations among children attending summer camp for studies presented in Figure 6-4.

| Study* | Location | Population, Mean $FEV_1$ (mL) or PEF (mL/sec) | Standardized Percent Change (95% CI)[a] | Standardized Effect Estimate (95% CI)[a] |
|---|---|---|---|---|
| **$FEV_1$** | | | | **(mL)** |
| Spektor et al. (1988a) | Fairview Lake, NJ | 91 campers without asthma ages 8-15 yr, 2,140 | -0.93 (-1.5, -0.35)[b] | -20.0 (-32.5, -7.5)[b] |
| Spektor and Lippmann (1991) | Fairview Lake, NJ | 46 campers without asthma ages 8-14 yr, 2,390 | -2.2 (-3.0, -1.3)[b] | -51.6 (-72.8, -30.4)[b] |
| Burnett et al. (1990) | Lake Couchiching, Ontario, Canada | 29 campers without asthma ages 7-15 yr, 2,410 | -0.32 (-1.7, 1.1)[b] | -7.6 (-42.1, 26.9)[b] |
| Raizenne et al. (1989) | Lake Erie, Ontario, Canada | 112 campers without asthma mean age 11.6 yr, 2,340 | -0.50 (-0.83, -0.16)[b] | -11.6 (-19.4, -3.8)[b] |
| Avol et al. (1990) | Pine Springs, CA | 295 campers without asthma ages 8-17 yr, 2,190 | -0.58 (-1.0, -0.12)[b] | -12.8 (-23.0, -2.6)[b] |
| Higgins et al. (1990) | San Bernardino, CA | 43 campers without asthma ages 7-13 yr, 2,060 | -1.6 (-2.4, -0.87)[b] | -33.6 (-49.3, -17.9)[b] |
| Kinney et al. (1996) | Pooled analysis of preceding 6 studies | 616 campers without asthma ages 7-17 yr, 2,300 | -0.87 (-1.1, -0.63) | -20.0 (-25.5, -14.5)[b] |
| Berry et al. (1991) | Hamilton, NJ | 14 campers without asthma age <14 yr, NA | NA | 32.8 (6.9, 58.7) |
| **PEF** | | | | **(mL/sec)** |
| Spektor et al. (1988a) | Fairview Lake, NJ | 91 campers without asthma ages 8-15 yr, 4,360 | -1.8 (-3.3, -0.40)[b] | -80.0 (-142.7, -17.3)[b] |
| Burnett et al. (1990) | Lake Couchiching, Ontario, Canada | 29 campers without asthma ages 7-15 yr, 5,480 | -1.9 (-3.8, -0.05[b] | -106.4 (-209.9, -2.9)[b] |
| Raizenne et al. (1989) | Lake Erie, Ontario, Canada | 112 campers without asthma mean age 11.6 yr, 5,510 | -0.07 (-0.56, 0.41)[b] | -4.0 (-30.7, 22.7)[b] |
| Avol et al. (1990) | Pine Springs, CA | 295 campers without asthma ages 8-17 yr, 4,520 | 1.9 (0.71, 3.1)[b] | 86.8 (31.9, 142)[b] |
| Higgins et al. (1990) | San Bernardino, CA | 43 campers without asthma ages 7-13 yr, 5,070 | -0.87 (-2.1, 0.34)[b] | -44.0 (-105, 17.2)[b] |
| Kinney et al. (1996) | Pooled analysis of preceding 6 studies | 616 campers without asthma ages 7-17 yr, 4,222 | 0.16 (-0.45, 0.77)[b] | 6.8 (-19.1, 32.7)[b] |
| Thurston et al. (1997) | CT River Valley, CT | 166 campers with asthma ages 7-13 yr, 5,333 | -2.8 (-4.9, -0.59) | -146.7 (-261.7, -31.7) |
| Neas et al. (1999) | Philadelphia, PA | 156 campers without asthma ages 6-11 yr, 4,717 | -0.58 (-1.5, 0.33) | -27.5 (-70.8, 15.8) |
| Berry et al. (1991) | Hamilton, NJ | 14 campers without asthma age <14 yr, NA | NA | -40.4 (-132.1, 51.3) |

*Includes studies from Figure 6-4.

NA = Data not available.

[a]All results are standardized to a 40-ppb increase in 1-h avg or 1-h max $O_3$, except that from Neas et al. (1999), which is standardized to a 30-ppb increase in 12-h avg (9 a.m.-9 p.m.) $O_3$.

[b]Effect estimates based on results reported in the pooled analysis by Kinney et al. (1996).

Similar to controlled human exposure studies, some camp studies found interindividual variability in the magnitude of $O_3$-associated changes in lung function. Based on separate regression analyses of serial measurements from individual subjects, increases in ambient $O_3$ concentration were associated with a wide range of changes in lung function across subjects (Berry et al., 1991; Higgins et al., 1990; Spektor et al., 1988a). For example, among children attending camp in Fairview Lake, NJ, 36% of subjects had statistically significant $O_3$-associated decreases in $FEV_1$, and the 90th percentile of response was a 6.3% decrease in $FEV_1$ per a 40-pbb increase in 1-h avg $O_3$ (Spektor et al., 1988a).

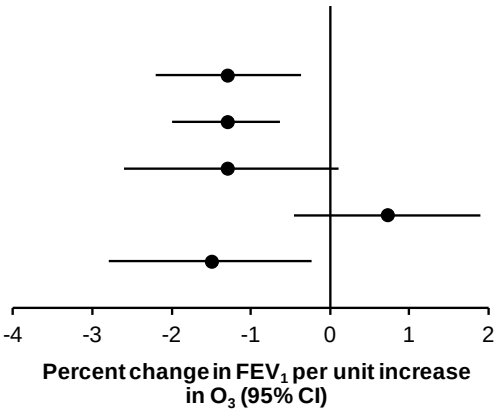
In contrast with previous studies, a recent study of children attending six different summer camps in Belgium did not find an association between ambient $O_3$ concentration and lung function (Nickmilder et al., 2007). This study examined similar ambient $O_3$ concentrations as did previous studies (Table 6-2) but used a less rigorous methodology. Lung function was measured only once in each subject, and mean lung function was compared among camps. Children at camps with higher daily 1-h max or 8-h max $O_3$ concentrations did not consistently have larger decreases in mean intraday $FEV_1$ or $FEV_1$/FVC (Nickmilder et al., 2007).

### Populations Exercising Outdoors

As discussed in the 1996 and 2006 $O_3$ AQCDs, epidemiologic studies of adults exercising outdoors have provided evidence for lung function decrements in healthy adults associated with increases in ambient $O_3$ exposure during exercise with durations (10 min to 12 hours) and intensities (heart rates 121-190 beats per min) in the range of those examined in controlled human exposure studies (Table 6-1). Associations were found consistently in studies of adults exercising outdoor for up to 2 hours, which similar to the camp studies, measured lung function before and after exercise by trained staff on multiple occasions. Collectively, studies of exercising adults found $FEV_1$ decrements of 1.3 to 1.5% per unit increase in $O_3$[1] (Figure 6-5 [and Table 6-4]). The magnitude of association did not appear to be related to study mean ambient $O_3$ concentrations (Table 6-2), exercise duration, or the mean heart rate measured during exercise (Figure 6-5 [and Table 6-4]). Increases in ambient $O_3$ concentration generally were associated with decreases in lung function in the smaller body of studies of children exercising outdoors (Table 6-4).

[1]Effect estimates were standardized to a 40-ppb increase in $O_3$ averaged over 15 min to 1 h and a 30-ppb increase for $O_3$ averaged over 2 to 12 hours.

| Study | Population | Exercise duration | Mean heart rate (bpm)[a] |
|---|---|---|---|
| Brunekreef et al. (1994) | Adults exercising | 10 min-1 h | 161, 176 |
| Spektor et al. (1988b) | Adults exercising | 15-55 min | 162, 145 |
| Hoppe et al. (2003) | Adults exercising | 2 h | NR |
| Girardot et al. (2006) | Adults hiking | 2-9 h | max: 121 |
| Korrick et al. (1998) | Adults hiking | 2-12 h | max: 122 |




Note: Studies generally are presented in order of increasing duration of outdoor exercise. Data for mean heart rate refer to the maximum or mean measured during exercise or in different groups or conditions as described in Table 6-4.

[a]bpm = beats per minute. NR = Not reported. Effect estimates are from single-pollutant models and are standardized to a 40-ppb increase for $O_3$ concentrations averaged over 15 minutes to 1 hour and a 30-ppb increase for $O_3$ concentrations averaged over 2 to 12 hours.

**Figure 6-5 Percent change in $FEV_1$ in association with ambient $O_3$ concentrations among adults exercising outdoors.**

## Table 6-4 Percent change in $FEV_1$ in association with ambient $O_3$ concentrations among adults exercising outdoors for studies presented in Figure 6-5, and among children exercising outdoors.

| Study* | Location | Population | Exercise Duration, Mean Heart Rate | $O_3$ Averaging Time | Parameter | Standardized Percent Change (95% CI)[a] |
|---|---|---|---|---|---|---|
| **Studies of adults** | | | | | | |
| Brunekreef et al. (1994) | Eastern Netherlands | 29 adults exercising, ages 18-37 yr | 10 min - 2.4 h, HR: 161 bpm (training), 176 bpm (races) | Exercise duration | $FEV_1$<br>PEF | -1.3 (-2.2, -0.37)<br>-2.5 (-3.8, -1.2) |
| Spektor et al. (1988b) | Tuxedo, NY | 30 adults exercising, ages 21-44 yr | 15 - 55 min, HR:162 bpm if $\dot{V}_E$ >100 L, 145 bpm if $\dot{V}_E$ 60-100 L | 30-min avg | $FEV_1$ | -1.3 (-2.0, -0.64) |
| Hoppe et al. (2003) | Munich, Germany | 43 adults and children exercising, ages 13-38 yr | 2 h, HR: NR | 30-min max (1-4 p.m.) | $FEV_1$<br>PEF | -1.3 (-2.6, 0.10)<br>-2.8 (-5.9, 0.31) |
| Girardot et al. (2006) | Great Smoky Mt, TN | 354 adult day hikers, ages 18-82 yr | 1.8-9 h, max HR:121 bpm | Hike duration | $FEV_1$<br>PEF | 0.72 (-0.46, 1.90)<br>3.5 (-0.11, 7.2) |
| Korrick et al. (1998) | Mt. Washington, NH | 530 adult day hikers, ages 18-64 yr | 2-12 h, max HR: 122 bpm | Hike duration | $FEV_1$<br>PEF | -1.5 (-2.8, -0.24)<br>-0.54 (-4.0, 2.9) |
| Selwyn et al. (1985) | Houston, TX | 24 adults exercising, ages 29-47 yr | Duration: NR, max HR: 179 bpm in males, 183 bpm in females | 15-min max | $FEV_1$ | -16 mL (-28.8, -3.2)[b] |
| **Studies of children not included in Figure 6-4.** | | | | | | |
| Braun-Fahrlander et al. (1994) | Southern Switzerland | 128 children exercising, ages 9-11 yr | 10 min, max HR: 180 bpm | 30-min avg | PEF | -3.8 (-6.7, -0.96) |
| Castillejos et al. (1995) | Mexico City, Mexico | 40 children exercising, ages 7-11 yr | 2 periods, each with 15 min exercise and 15 min rest, max HR: <190 bpm | 1-h avg over combined exercise-rest period | $FEV_1$ | -0.48 (-0.72, -0.24) |
| Hoek et al. (1993) | Wageningen, Netherlands | 65 children exercising, ages 7-12 yr | 25 min-1.5 h, HR: NR | 1-h avg during exercise | PEF | 1.9 (0.83, 3.0) |

*Includes studies from Figure 6-5, plus others.

HR = heart rate, bpm = beats per minute, $\dot{V}_E$ = minute ventilation, NR = Not reported.

[a]Effect estimates are standardized to a 40-ppb increase for $O_3$ concentrations averaged over 15 minutes to 1 hour and a 30-ppb increase for $O_3$ concentrations averaged over 2 to 12 hours.

[b]Results not included in the figure because data were not available to calculate percent change in lung function.

Compared with the studies of individuals exercising outdoors described above, studies of day-hikers assessed lung function only on one day per subject but examined longer periods of outdoor activity and included much larger sample sizes. Studies of adult day-hikers had similar design but produced contrasting results (Girardot et al., 2006; Korrick et al., 1998). Among 530 hikers on Mt. Washington, NH, Korrick et al. (1998) reported posthike declines in $FEV_1$ and FVC of 1.5% and 1.3%, respectively, per a 30-ppb increase in 2- to 12-h avg $O_3$. Associations with $FEV_1$/FVC, $FEF_{25-75\%}$, and PEF were weaker. In contrast, among 354 hikers on Great Smoky Mt, TN, Girardot et al. (2006) found that higher $O_3$ concentrations were associated with posthike increases in many of the same lung function indices (Figure 6-5 [and Table 6-4]). These studies were similar in the examination of a mostly white, healthy population and of changes in lung function associated with ambient $O_3$ concentrations measured on site during multihour (2-12 hours) periods of outdoor exercise. Mean $O_3$ concentrations were similar as were the population mean and variability in lung function. However, Girardot et al. (2006) differed from Korrick et al. (1998) in several aspects, including a shorter hike time (mean: 5 versus 8 hours), older age of subjects (mean: 43 versus 35 yr), and measurement of lung function by a larger number of less well-trained technicians. The impact of these differences on the heterogeneity in results between the studies was not examined.

Similar to the camp studies, some studies of outdoor exercise examined and found interindividual variability in the magnitude of $O_3$-associated decreases in lung function. In Korrick et al. (1998), although a 30-ppb increase in 2- to 12-h avg ambient $O_3$ concentration was associated with a group mean change in $FEF_{25-75\%}$ of -0.81% (95% CI: -4.9, 3.3), some individuals experienced a >10% decline. The odds of >10% decline in $FEF_{25-75\%}$ increased with increasing ambient $O_3$ concentration (OR: 2.3 [95% CI: 1.2, 6.7] per 30-ppb increase in 2- to 12-h avg $O_3$). Likewise, Hoppe et al. (2003) found that compared with days with 30-min max (1-4 p.m.) ambient $O_3$ concentrations <40 ppb, on days with $O_3$ >50 ppb, 14% of athletes had at least a 10% decrease in lung function or 20% increase in airway resistance.

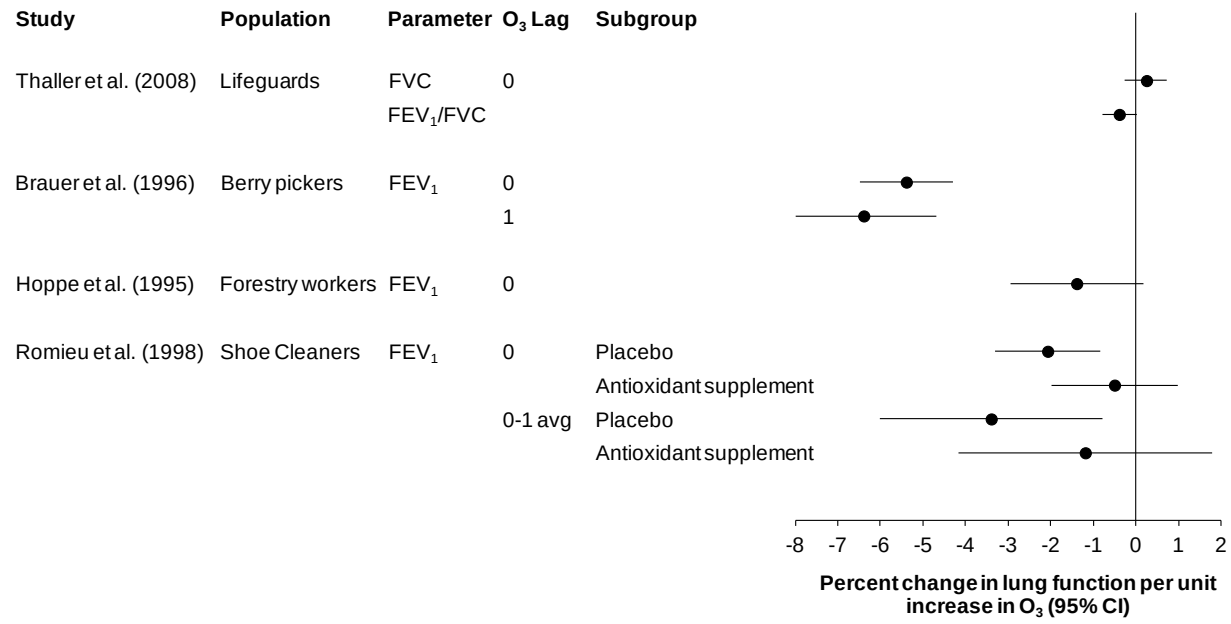
### Outdoor Workers

Consistent findings in outdoor workers add to the evidence that short-term increases in ambient $O_3$ exposure can decrease lung function in healthy adults (Figure 6-6 [and Table 6-5]). Except for Hoppe et al. (1995), studies used central site ambient $O_3$ concentrations. However, in outdoor workers, ambient concentrations have been more highly correlated with and similar in magnitude to personal exposures (Section 4.3.3) likely because workers spend long periods of time outdoors (6-14 hours across studies) and the $O_3$ averaging times examined correspond to periods of outdoor work. For example, in a subset of berry pickers, the correlation and ratio of personal to ambient 24-h avg $O_3$ concentrations (15 km from work site) were 0.64 and 0.96, respectively (Brauer and Brook, 1997). The 6-h avg personal-ambient ratio in a population of shoe cleaners in Mexico City, Mexico, was 0.56 (O'Neill et al., 2003). Many studies of outdoor workers found that in addition to same-day concentrations, $O_3$ concentrations lagged 1 or 2 days (Chan and Wu, 2005; Brauer et

al., 1996) or averaged over 2 days (Romieu et al., 1998b) were associated with equal or larger decrements in lung function (Figure 6-6 [and Table 6-5]).

Similar to other populations with increased outdoor exposure, most of the magnitudes of $O_3$-associated lung function decrements in outdoor workers were small, i.e., <1% to 3.4% per unit increase in $O_3$ concentration[1]. The magnitude of decrease was not found to depend strongly on duration of outdoor work or ambient $O_3$ concentration. The largest decrease (6.4% per 40-ppb increase in 1-h max $O_3$) was observed in berry pickers in British Columbia who were examined during a period of relatively low ambient $O_3$ concentrations (work shift mean: 26.0 ppb [SD: 11.8]) but had long daily periods of outdoor work (8-14 hours) (Brauer et al., 1996) (Figure 6-6 [and Table 6-5]). However, a much smaller $O_3$-associated decrease in $FEV_1$ was found in shoe cleaners in Mexico City who were examined during a period of higher $O_3$ concentrations (work shift mean: 67.3 ppb [SD: 24]) but had a period of outdoor work that was as long as that of the berry pickers. The smallest magnitude of decrease (-0.4% [95% CI: -0.8, 0] in afternoon $FEV_1$/FVC per 40-ppb increase in 1-h max $O_3$) was observed in lifeguards in Galveston, TX (Thaller et al., 2008) whose outdoor work periods were shorter than those of the berry pickers but characterized by a similar range of ambient $O_3$ concentrations. Not all studies provided information on ventilation rate or pulse rate, thus it was not possible to ascertain whether differences in the magnitude of $O_3$-associated lung function decrement across studies were related to differences in the level of exertion of among the various groups of workers.

[1]Effect estimates were standardized to a 40-ppb increase for $O_3$ averaged over 30 minutes to 1 hour and a 30-ppb increase for $O_3$ averaged over 8 hours or 12 hours.




Note: Studies generally are presented in order of increasing mean ambient $O_3$ concentration. Effect estimates are from single-pollutant models and are standardized to a 40-ppb increase for 30-min, 1-h avg, or 1-h max $O_3$ concentrations.

**Figure 6-6 Percent change in lung function in association with ambient $O_3$ concentrations among outdoor workers.**

**Table 6-5 Percent change in $FEV_1$ or $FEV_1$/FVC in association with ambient $O_3$ concentrations among outdoor workers for studies presented in Figure 6-6.**

| Study* | Location | Population | Parameter | Outdoor Work Duration | $O_3$ Averaging Time | $O_3$ Lag | Subgroup | Standardized Percent Change (95% CI)[a] |
|---|---|---|---|---|---|---|---|---|
| Thaller et al. (2008) | Galveston, TX | 142 lifeguards, ages 16-27 yr | FVC | 6-8 h | 1-h max | 0 | | 0.24 (-0.28, 0.72) |
| | | | | | 12-h avg (7 a.m.-7 p.m.) | | | 0.15 (-0.60, 0.90) |
| | | | $FEV_1$/FVC | | 1-h max | | | -0.40 (-0.80, 0) |
| | | | | | 12-h avg (7 a.m.-7 p.m.) | | | -0.60 (-1.2, 0) |
| Brauer et al. (1996) | British Columbia, Canada | 58 berry pickers, ages 10-69 yr | $FEV_1$ | 8-14 h | 1-h max | 0 | | -5.4 (-6.5, -4.3) |
| | | | | | | 1 | | -6.4 (-8.0, -4.7) |
| Hoppe et al. (1995) | Munich, Germany | 41 forestry workers, ages 20-60 yr | $FEV_1$ | Not reported | 30-min max (1 - 4 p.m.) | 0 | | -1.4 (-3.0, 0.16) |
| Romieu et al. (1998b) | Mexico City, Mexico | 47 male shoe cleaners, mean (SD) age: 38.9 (10) yr | $FEV_1$ | Mean (SD): 9 (1) h | 1-h avg before lung function measurement | 0 | Placebo | -2.1 (-3.3, -0.85) |
| | | | | | | | Antioxidant | -0.52 (-2.0, 0.97) |
| | | | | | | 0-1 avg | Placebo | -3.4 (-6.0, -0.78) |
| | | | | | | | Antioxidant | -1.2 (-4.2, 1.8) |
| Chan and Wu (2005)[b] | Taichung City, Taiwan | 43 mail carriers. Mean (SD) age: 39 (8) yr | Nighttime PEF | 8 h | 1-h max | 0 | | -1.3 (-1.7, -0.92) |
| | | | | | | 1 | | -1.4 (-1.7, -1.2) |
| | | | | | 8-h avg (9 a.m. - 5 p.m.) | 0 | | -1.6 (-2.2, -1.1) |
| | | | | | | 1 | | -1.9 (-2.5, -1.3) |

*Includes results from Figure 6-6, plus others.

[a]Effect estimates are standardized to a 40-ppb increase for 30-min, 1-h avg, or 1-h max $O_3$ and a 30-ppb increase for 8-h avg or 12-h avg $O_3$.

[b]PEF results not included in figure.

### Associations at Lower Ozone Concentrations

In some studies of populations with increased outdoor exposures, $O_3$-associated lung function decrements were observed when maximum or average ambient $O_3$ concentrations over 30 minutes to 12 hours did not exceed 80 ppb (Chan and Wu, 2005; Korrick et al., 1998; Hoppe et al., 1995; Braun-Fahrlander et al., 1994) (presented within Table 6-2). Korrick et al. (1998) found lung function decrements in association with higher hike-time average (2-12 hours) $O_3$ concentrations in the range 40-74 ppb but not <40 ppb. Several other studies that included higher maximum ambient $O_3$ concentrations restricted analyses to observations with 10-min to 1-h avg $O_3$ concentrations <80 ppb (Table 6-6). Higgins et al. (1990) found that $O_3$-associated lung function decrements in children attending camp were limited largely to 1-h avg ambient concentrations >120 ppb; however, many other studies found associations in the lower range of $O_3$ concentrations (Table 6-6). Among adults exercising outdoors, Spektor et al. (1988b) found that for most lung function

parameters, effect estimates in analyses restricted to 30-min max ambient $O_3$ concentrations <80 ppb were similar to those obtained for the full range of $O_3$ concentrations (Table 6-6). In a study of children attending summer camp, similar effects were estimated for the full range of 1-h avg $O_3$ concentrations and those <60 ppb (Spektor et al., 1988a). Brunekreef et al. (1994) found increases in ambient $O_3$ concentration (10-min to 1-hour) during outdoor exercise to be associated with decreases in $FEV_1$ in analyses restricted to concentrations <61 (Table 6-6) and <51 ppb (quantitative results not reported). Whereas Brunekreef et al. (1994) found that effect estimates were near zero with $O_3$ concentrations <41 ppb, Brauer et al. (1996) found that associations persisted with 1-h max $O_3$ concentrations <40 ppb (quantitative results not provided).

**Table 6-6 Associations between ambient $O_3$ concentration and $FEV_1$ decrements in different ranges of ambient $O_3$ concentrations.**

| Study | Location | Population | $O_3$ Averaging Time | $O_3$ Concentration Range | Standardized Percent Change (95% CI)[a] |
|---|---|---|---|---|---|
| Brunekreef et al. (1994) | Eastern Netherlands | 29 adults exercising, ages 18-37 yr | 10-min to 2.4-h avg during exercise | Full range<br>$O_3$ <61 ppb | -1.3 (-2.2, -0.37)<br>-2.1 (-4.5, 0.32) |
| Spektor et al. (1988a) | Fairview Lake, NJ | 91 children without asthma at camp, ages 8-15 yr | 1-h avg before afternoon $FEV_1$ measurement | Full range<br>$O_3$ <80 ppb<br>$O_3$ <60 ppb | -2.7 (-3.3, -2.0)<br>-1.4 (-2.5, -0.34)<br>-2.2 (-3.7, -0.80) |
| Spektor et al. (1988b) | Tuxedo, NY | 30 adults exercising, ages 21-44 yr | 30-min avg during exercise | Full range<br>$O_3$ <80 ppb | -1.3 (-2.0, -0.64)<br>-1.3 (-2.4, -0.08) |
| Korrick et al. (1998) | Mt. Washington, NH | 530 adult day hikers, ages 18-64 yr | 2-12 h avg during hike | Full range<br>$O_3$ 40-74 ppb | -1.5 (-2.8, -0.24)<br>-2.6 (-4.9, -0.32) |
| Higgins et al. (1990) | San Bernardino, CA | 43 children without asthma at camp, ages 7-13 yr | 1-h avg around time of $FEV_1$ measurement | >120 ppb<br><120 ppb | -1.4 (-2.8, 0.03)<br>0.35 (-1.3, 2.0) |

[a]Results are presented in order of maximum $O_3$ concentration included in models. Effect estimates are standardized to a 40-ppb increase for $O_3$ concentrations averaged over 10 min to 1 h and a 30-ppb increase for $O_3$ concentrations averaged over 2 to 12 h.

### Children with Asthma

Increases in ambient $O_3$ concentration have been associated with lung function decrements in children with asthma in epidemiologic studies conducted across diverse geographical locations and a range of ambient $O_3$ concentrations (Table 6-7). Whereas most studies of populations with increased outdoor exposures monitored $O_3$ concentrations at the site of subjects' outdoor activities and used trained staff to measure lung function, studies of children with asthma relied more on $O_3$ measured at central monitoring sites and lung function measured by subjects. However, these methods for exposure and outcome assessment in studies of children with asthma likely are sources of nondifferential measurement error. Further, compared with the

camp studies, studies of children with asthma have provided an understanding of the changes in lung function associated with patterns of outdoor activity and ambient $O_3$ exposure that likely better represent those of children in the general population. These studies also have provided more information on potential at-risk populations for $O_3$-associated respiratory effects and on potential confounding by copollutant exposure or meteorology.

**Table 6-7 Mean and upper percentile concentrations of $O_3$ in epidemiologic studies of lung function in children with asthma.**

| Study* | Location | Study Period | $O_3$ Averaging Time | Mean/Median Concentration (ppb) | Upper Percentile Concentrations (ppb) |
|---|---|---|---|---|---|
| Jalaludin et al. (2000) | Sydney, Australia | Feb-Dec 1994 | 15-h avg (6 a.m.-9 p.m.) | 12 | Max: 43 |
| | | | 1-h max | 26 | 91 |
| Lewis et al. (2005) | Detroit, MI | Feb 2001-May 2002 | 24-h avg | 27.6, 26.5[a] | Overall max: 66.3[a] |
| | | | 8-h max | 40.4, 41.4[a] | Overall max: 92.0[a] |
| Just et al. (2002) | Paris, France | April-June 1996 | 24-h avg | 30.0[b] | Max: 61.7[b] |
| Hoppe et al. (2003) | Munich, Germany | Summers 1992-1995 | 30-min max (1 p.m.-4 p.m.) | High $O_3$ days: 66.9[c] | Max: 91 high $O_3$ days[c] |
| | | | | Control $O_3$ days: 32.5[c] | Max: 39 Control $O_3$ days[c] |
| Thurston et al. (1997) | CT River Valley, CT | June 1991-1993 | 1-h max | 83.6[c] | Max: 160[c] |
| Romieu et al. (2006); (2004b; 2002) | Mexico City, Mexico | Oct 1998-Apr 2000 | 8-h max | 69 | Max: 184 |
| | | | 1-h max | 102 | Max: 309 |
| Romieu et al. (1997) | Southern Mexico City, Mexico | Apr-July 1991; Nov 1991-Feb 1992 | 1-h max | 196 | Max: 390 |
| Romieu et al. (1996) | Northern Mexico City, Mexico | Apr-July 1991; Nov 1991-Feb 1992 | 1-h max | 190 | Max: 370 |
| O'Connor et al. (2008) | Boston, MA; Bronx, Manhattan NY; Chicago, IL; Dallas, TX, Seattle, WA; Tucson, AZ (ICAS) | Aug 1998-July 2001 | 24-h avg | NR | NR |
| Mortimer et al. (2002) Mortimer et al. (2000) | Bronx, East Harlem, NY; Baltimore, MD; Washington, DC; Detroit, MI, Cleveland, OH; Chicago, IL; St. Louis, MO (NCICAS) | June-Aug 1993 | 8-h avg (10 a.m.-6 p.m.) | 48 | NR |
| Gielen et al. (1997) | Amsterdam, Netherlands | Apr-July 1995 | 8-h max | 34.2[b] | Max: 56.5[b] |
| Liu et al. (2009a),Dales et al. (2009) | Windsor, ON, Canada | Oct-Dec2005 | 24-h avg | 13.0 | 95th: 26.5 |
| | | | 1-h max | 27.2 | 75th: 32.8 |

| Study* | Location | Study Period | $O_3$ Averaging Time | Mean/Median Concentration (ppb) | Upper Percentile Concentrations (ppb) |
|---|---|---|---|---|---|
| Rabinovitch et al. (2004) | Denver, CO | Nov-Mar 1999-2002 | 1-h max | 28.2 | 75th: 36.0, Max 70.0 |
| Barraza-Villarreal et al. (2008) | Mexico City, Mexico | June 2003-June 2005 | 8-h moving avg | 31.6 | Max: 86.3 |
| Wiwatanadate and Trakultivakorn (2010) | Chiang Mai, Thailand | August 2005-June 2006 | 24-h avg | 17.5 | 90th: 26.8, Max: 34.7 |
| Delfino et al. (2004) | Alpine, CA | September-October 1999; April-June 2000 | 8-h max | 62.9 | 90th: 83.9, Max: 105.9 |
| Hernández-Cadena et al. (2009) | Mexico City, Mexico | May-September 2005 | 24-h avg | 26.3 | 75th: 35.3; Max: 62.8 |
| | | | 1-h max | 74.5 | 75th: 92.5; Max: 165 |

*Note: Studies presented in order of first appearance in the text of this section.

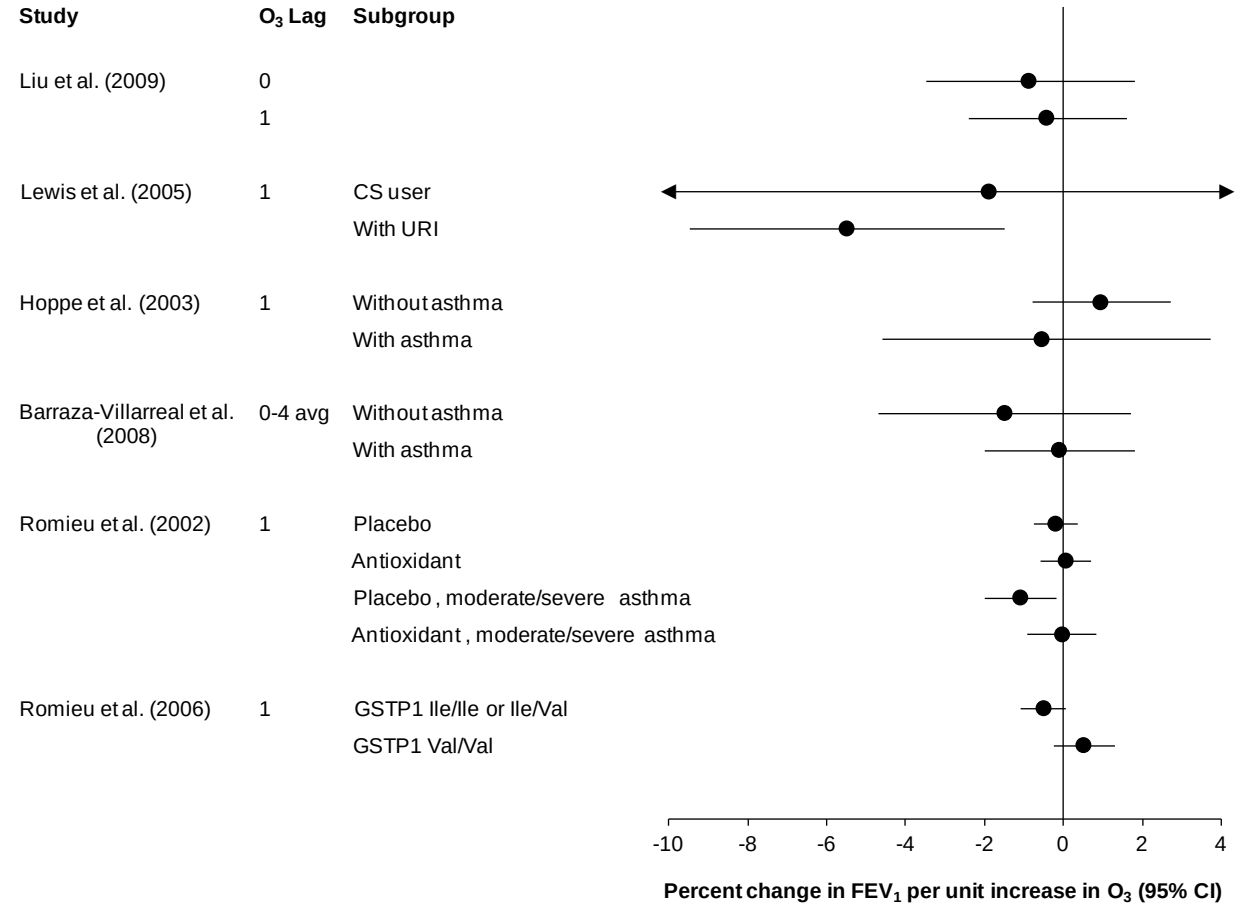
ICAS = Inner City Asthma Study, NR = Not Reported, NCICAS = National Cooperative Inner-City Asthma Study.

[a]Measurements at two sites established by investigators and located within 5 km of most subjects' residences.

[b]Concentrations converted from μg/m$^3$ to ppb using the conversion factor of 0.51 assuming standard temperature (25°C) and pressure (1 atm).

[c]Measured where subjects spent daytime hours.

In a majority of studies, including a large U.S. multicity study and several smaller studies conducted in the United States, Mexico City, Mexico, and Europe, an increase in ambient $O_3$ concentration (various averaging times and lags) was associated with a decrement in $FEV_1$ (Figure 6-7 [and Table 6-8]) or PEF (Figure 6-8 [and Table 6-9]) in children with asthma. Results were more variable for $FEV_1$ than for PEF. In most studies, $FEV_1$ was measured by technicians whereas PEF was measured by study subjects or their parents. However, associations with $O_3$ also were found with PEF measured by trained technicians (Romieu et al., 2004b; Thurston et al., 1997), which are subject to less measurement error. Further, in some studies, associations with $FEV_1$ were limited to specific subgroups. Some studies found that increases in ambient $O_3$ concentration were associated with greater lung function variability, i.e., a deviation from a baseline level. These results pointed to associations of $O_3$ with poorer lung function, as indicated by a decrease from the individual's mean lung function over the study period (Jalaludin et al., 2000), a decrease in lung function over the course of the day (Lewis et al., 2005), or a decrease in the lowest daily measurement (Just et al., 2002). Within many studies, increases in $O_3$ concentration were associated with decreases in lung function and increases in respiratory symptoms at the same or similar lag (Just et al., 2002; Mortimer et al., 2002; Gielen et al., 1997; Romieu et al., 1997; Thurston et al., 1997; Romieu et al., 1996) (see Figure 6-12 [and Table 6-20]) for symptom results).




Note: Results generally are presented in order of increasing mean ambient $O_3$ concentration. CS = Corticosteroid, URI = Upper respiratory infection. Effect estimates are from single-pollutant models and are standardized to a 40-ppb increase for 30-min or 1-h max $O_3$ concentrations, a 30-ppb increase for 8-h max or 8-h avg $O_3$ concentrations, and a 20-ppb increase for 24-h avg $O_3$ concentrations.

**Figure 6-7 Percent change in $FEV_1$ in association with ambient $O_3$ concentrations among children with asthma.**

## Table 6-8 Percent change in $FEV_1$ in association with ambient $O_3$ concentrations among children with asthma for studies presented in Figure 6-7 plus others.

| Study* | Location/ Population | $O_3$ Averaging Time | $O_3$ Lag | Parameter | Subgroup | Standardized Percent Change (95% CI)[a] |
|---|---|---|---|---|---|---|
| Liu et al. (2009a) | Windsor, ON, Canada<br>182 children with asthma,<br>ages 9-14 yr | 24-h avg | 0 | $FEV_1$ | | -0.89 (-3.5, 1.8) |
| | | | 1 | | | -0.44 (-2.4, 1.6) |
| Lewis et al. (2005) | Detroit, MI<br>86 children with asthma,<br>mean (SD)<br>age 9.1 (1.4) yr | 8-h max | 1 | Lowest daily $FEV_1$ | CS user | -1.9 (-10.4, 7.5) |
| | | | | | With URI | -5.5 (-9.5, -1.5) |
| | | | 2 | | CS user | -7.3 (-12.3, -1.9) |
| | | | | | With URI | -4.9 (-10.0, 0.48) |
| Hoppe et al. (2003) | Munich, Germany<br>43 people with asthma,<br>ages 12-23 yr<br>44 children without asthma,<br>ages 6-8 yr | 30-min max (1-4 p.m.) | 1 | Afternoon $FEV_1$ | Without asthma | 0.93 (-0.80, 2.7) |
| | | | | | With asthma | -0.56 (-4.6, 3.7) |
| | | | | Afternoon FVC | Without asthma | -0.09 (-1.7, 1.6) |
| | | | | | With asthma | -3.5 (-5.9, -1.0) |
| Barraza-Villarreal et al. (2008) | Mexico City, Mexico<br>208 children,<br>ages 6-14 yr | 8-h avg | 0-4 avg | $FEV_1$ | 50 without asthma | -1.5 (-4.7, 1.7) |
| | | | | | 158 with asthma | -0.12 (-2.0, 1.8) |
| Romieu et al. (2002) | Mexico City, Mexico<br>158 children with asthma,<br>ages 6-17 yr | 1-h max | 1 | $FEV_1$ | Placebo | -0.21 (-0.77, 0.36) |
| | | | | | Antioxidant supplement | 0.05 (-0.60, 0.69) |
| | | | | | Placebo, moderate/severe asthma | -1.1 (-2.0, -0.19) |
| | | | | | Antioxidant supplement, moderate/severe asthma | -0.04 (-0.92, 0.83) |
| Romieu et al. (2006) | Mexico City, Mexico<br>151 children with asthma,<br>mean age 9 yr | 1-h max | 1 | $FEV_1$ | GSTP1 Ile/Ile or Ile/Val | -0.51 (-1.1, 0.05) |
| | | | | | GSTP1 Val/Val | 0.50 (-0.25, 1.3) |

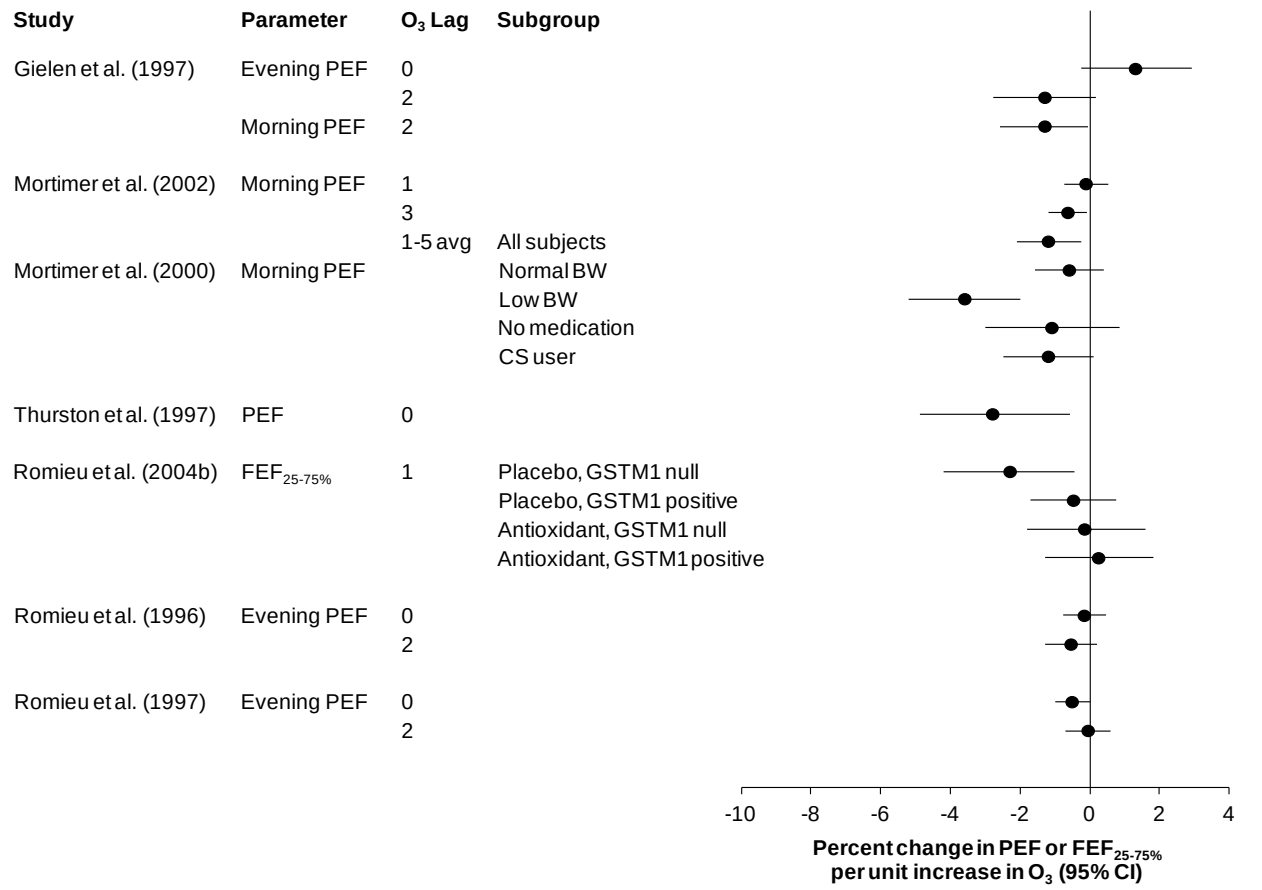
| Study* | Location/ Population | $O_3$ Averaging Time | $O_3$ Lag | Parameter | Subgroup | Standardized Percent Change (95% CI)[a] |
|---|---|---|---|---|---|---|
| **Studies not included in Figure 6-7[b]** | | | | | | |
| Dales et al. (2009) | Windsor, ON, Canada<br>182 children with asthma, ages 9-14 yr | 1-h max | 0 | Evening percent predicted $FEV_1$ | | -0.47 (-1.9, 0.95) |
| Rabinovitch et al. (2004) | Denver, CO<br>86 children with asthma, ages 6-12 yr | 1-h max | 0-2 avg | Morning $FEV_1$ (mL) | | 55 (-2.4, 108) |
| O'Connor et al. (2008) | Boston, MA; Bronx, Manhattan NY; Chicago, IL; Dallas, TX, Seattle, WA; Tucson, AZ<br>861 children with asthma, mean (SD) age 7.7 (2.0) yr | 24-h avg | 1-5 avg | Percent predicted $FEV_1$ | | -0.41 (-1.0, 0.21) |

*Includes studies in Figure 6-7, plus others

CS = corticosteroid, URI = Upper respiratory infection.

[a]Effect estimates are standardized to a 40-ppb increase for 30-min or 1-h max $O_3$, a 30-ppb increase for 8-h max or 8-h avg $O_3$, and a 20-ppb increase for 24-h avg $O_3$.

[b]Results not presented in Figure 6-7 because a different form of $FEV_1$ with a different scale was examined or because sufficient data were not available to calculate percent change in $FEV_1$.




Note: Results generally are presented in order of increasing mean ambient $O_3$ concentration. BW = birth weight, CS = Corticosteroid. Effect estimates are from single pollutant models and are standardized to a 40-ppb increase for 1-h max $O_3$ concentrations and a 30-ppb increase for 8-h max or 8-h avg $O_3$ concentrations.

**Figure 6-8 Percent change in PEF or $FEF_{25-75\%}$ in association with ambient $O_3$ concentrations among children with asthma.**

## Table 6-9 Percent change in PEF or $FEF_{25-75\%}$ in association with ambient $O_3$ concentrations among children with asthma for studies presented in Figure 6-8 plus others.

| Study* | Location/Population | $O_3$ Averaging Time | $O_3$ Lag | Parameter | Subgroup | Standardized Percent Change (95% CI)[a] |
|---|---|---|---|---|---|---|
| Gielen et al. (1997) | Amsterdam, Netherlands<br>61 children with asthma, ages 7-13 yr | 8-h max | 0<br>2<br>2 | Evening PEF<br>Evening PEF<br>Morning PEF | | 1.3 (-0.25, 2.9)<br>-1.3 (-2.8, 0.16)<br>-1.3 (-2.6, -0.08) |
| Mortimer et al. (2002) | Bronx, East Harlem, NY; Baltimore, MD; Washington, DC; Detroit, MI, Cleveland, OH; Chicago, IL; St. Louis, MO<br>846 children with asthma, ages 4-9 yr | 8-h avg (10 a.m.-6 p.m.) | 1<br>3<br>1-5 avg | Morning PEF | All subjects | -0.12 (-0.76, 0.52)<br>-0.64 (-1.2, -0.10)<br>-1.2 (-2.1, -0.26) |
| Mortimer et al. (2000) | Bronx, East Harlem, NY; Baltimore, MD; Washington, DC; Detroit, MI, Cleveland, OH; Chicago, IL; St. Louis, MO<br>846 children with asthma, ages 4-9 yr | 8-h avg (10 a.m.-6 p.m.) | 1-5 avg | Morning PEF | Normal BW<br>Low BW (<5.5 lbs.)<br>No medication<br>CS user | -0.60 (-1.6, 0.39)<br>-3.6 (-5.2, -2.0)<br>-1.1 (-3.0, 0.84)<br>-1.2 (-2.5, 0.11) |
| Thurston et al. (1997) | CT River Valley, CT<br>166 children with asthma, ages 7-13 yr | 1-h max | 0 | Intraday change PEF | | -2.8 (-4.9, -0.59) |
| Romieu et al. (2004b) | Mexico City, Mexico<br>158 children with asthma, mean age 9 yr | 1-h max | 1 | $FEF_{25-75\%}$ | Placebo, GSTM1 null<br>Placebo, GSTM1 positive<br>Antioxidant, GSTM1 null<br>Antioxidant, GST M1 positive | -2.3 (-4.2, -0.44)<br>-0.48 (-1.7, 0.74)<br>-0.16 (-1.8, 1.6)<br>0.24 (-1.3, 1.8) |
| Romieu et al. (1996) | Northern Mexico City, Mexico<br>71 children with asthma, ages 5-7 yr | 1-h max | 0<br>2 | Evening PEF | | -0.17 (-0.79, 0.46)<br>-0.55 (-1.3, 0.19) |
| Romieu et al. (1997) | Southern Mexico City, Mexico<br>65 children with asthma, ages 5-13 yr | 1-h max | 0<br>2 | Evening PEF | | -0.52 (-1.0, -0.01)<br>-0.06 (-0.70, 0.58) |

| Study* | Location/Population | $O_3$ Averaging Time | $O_3$ Lag | Parameter | Subgroup | Standardized Percent Change (95% CI)[a] |
|---|---|---|---|---|---|---|
| **Studies not included in Figure 6-8**[b] | | | | | | |
| Jalaludin et al. (2000) | Sydney, Australia<br>45 children with asthma and AHR,<br>mean (SD)<br>age 9.6 (1) yr | 24-h avg<br>1-h max | 0 | Daily deviation from mean PEF | | -5.2 (-8.3, -2.2)[c]<br>-1.1 (-2.4, 0.18)[c] |
| Wiwatanadate and Trakultivakorn (2010) | Chiang Mai, Thailand<br>31 children with asthma, ages 4-11 yr | 24-h avg | 0<br>5 | Daily avg PEF (L/min) | | 1.0 (-1.6, 3.6)<br>-2.6 (-5.2, 0) |
| O'Connor et al. (2008) | Boston, MA; Bronx, Manhattan NY; Chicago, IL; Dallas, TX, Seattle, WA; Tucson, AZ<br>861 Children with asthma, mean (SD)<br>age 7.7 (2.0) yr | 24-h avg | 1-5 avg | Change in percent predicted PEF | | -0.22 (-0.86, 0.43) |
| Just et al. (2002) | Paris, France<br>82 children with asthma, mean (SD)<br>age 10.9 (2.5) yr | 8-h avg | 0-2 avg | Percent variability PEF | | 15.3 (0, 30.6) |

*Includes studies in Figure 6-8, plus others

BW = birth weight, CS = corticosteroid, AHR = Airway hyperresponsiveness.

[a]Effect estimates are standardized to a 40-ppb increase for 1-h max $O_3$, a 30-ppb increase for 8-h max or avg $O_3$, and a 20-ppb increase for 24-h avg $O_3$.

[b]Results are not presented in Figure 6-8 because a different form of PEF with a different scale was examined or because sufficient data were not available to calculate percent change in PEF.

[c]Outcome defined as the normalized percent deviation from individual mean PEF during the study period. Quantitative results from generalized estimating equations were provided only for models that included $PM_{10}$ and $NO_2$.

The most geographically representative data were provided by the large, multi-U.S. city National Cooperative Inner City Asthma Study (NCICAS) (Mortimer et al., 2002; Mortimer et al., 2000) and Inner-City Asthma Study (ICAS) (O'Connor et al., 2008). Although the two studies differed in the cities, seasons, racial distribution of subjects, and lung function indices examined, results were fairly similar. In ICAS, which included children with asthma and atopy (i.e., allergic sensitization) and year-round examinations of lung function, a 20-ppb increase in the lag 1-5 average of 24-h avg $O_3$ was associated with a 0.41-point decrease in percent predicted $FEV_1$ (95% CI: -1.0, 0.21) and a 0.22-point decrease in percent predicted PEF (95% CI: -0.86, 0.43) (O'Connor et al., 2008).

Increases in lag 1-5 avg $O_3$ (8-h avg, 10 a.m.-6 p.m.) also were associated with declines in PEF in NCICAS, which included different U.S. cities, summer-only measurements, larger proportions of Black and Hispanic children, and fewer subjects with atopy (79%) (Mortimer et al., 2002). Ozone concentrations lagged 3 to 5 days were associated with larger PEF decrements than were $O_3$ concentrations lagged 1 to 2 days (Figure 6-8 [and Table 6-9]). NCICAS additionally identified groups potentially at increased risk of $O_3$-associated PEF decrements, namely, males,

children of Hispanic ethnicity, children living in crowded housing, and as indicated in Figure 6-8 (and Table 6-9), children with birth weight <5.5 lbs (Mortimer et al., 2000). Somewhat paradoxically, $O_3$ was associated with a larger decrease in PEF among subjects taking cromolyn, medication typically used to treat asthma due to allergy, but a smaller decrease among subjects with allergic sensitization (as determined by skin prick test). NCICAS also indicated robust associations with consideration of other sources of heterogeneity. Except for Baltimore, MD, effect estimates were similar across the study cities (1.1 to 1.7% decrease in PEF per 30-ppb increase in lag 1-5 avg of 8-h avg $O_3$). Results were similar with $O_3$ averaged from all available city monitors and concentrations averaged from the three monitors closest to subjects' ZIP code centroid (1.2% and 1.0% decrease in PEF, respectively, per 30-ppb increase in $O_3$). At concentrations <80 ppb, a 30-ppb increase in lag 1-5 of 8-h avg $O_3$ was associated with a 1.4% decrease (95% CI: -2.6, -0.21) in PEF, (Mortimer et al., 2002) which was similar to the effect estimated for the full range of $O_3$ concentrations (Figure 6-8 [and Table 6-9]). In a study of children with asthma in the Netherlands, Gielen et al. (1997) estimated similar effects on PEF for the full range of 8-h max $O_3$ concentrations and concentrations <51 ppb.

Several but not all controlled human exposure studies have reported slightly larger $O_3$-induced $FEV_1$ decrements in adults with asthma than adults without asthma (Section 6.2.1.1). However, in the few epidemiologic studies that compared children with and without asthma, evidence did not conclusively indicate that children with asthma were at increased risk of $O_3$-associated lung function decrements. Hoppe et al. (2003) and Jalaludin et al. (2000) generally found larger $O_3$-associated decrements in FVC and PEF, respectively, in children with asthma; whereas Raizenne et al. (1989) did not consistently demonstrate differences between campers with and without asthma. In their study of children in Mexico City, Mexico, Barraza-Villarreal et al. (2008) estimated larger $O_3$-associated decreases in children without asthma; however, 72% of these children had atopy. These findings indicate that children with atopy, who also have airway inflammation and similar respiratory symptoms, may experience respiratory effects from short-term ambient $O_3$ exposure.

As shown in Figure 6-7 (and Table 6-8]) and Figure 6-8 (and Table 6-9), lung function decrements in children with asthma mostly ranged from <1% to 2% per unit increase in ambient $O_3$ concentration[1]. Larger magnitudes of decrease, were found in children with asthma who were using CS, had a concurrent upper respiratory infection (URI), were GSTM1 null, had airway hyperresponsiveness, or had increased outdoor exposure (Romieu et al., 2006; Lewis et al., 2005; Romieu et al., 2004b; Jalaludin et al., 2000) than among children with asthma overall (Barraza-Villarreal et al., 2008; Lewis et al., 2005; Delfino et al., 2004; Romieu et al., 2002). For example, Jalaludin et al. (2000) estimated a -5.2% deviation from mean $FEV_1$ per 20-ppb increase in 24-h avg $O_3$ concentration among children with asthma and airway hyperresponsiveness and a much smaller -0.71% deviation among children with asthma without airway hyperresponsiveness. In a group of 86 children with asthma in Detroit, MI, Lewis et al. (2005) reported that associations between ambient

[1]Effect estimates were standardized to a 40-, 30-, and 20-ppb increase for 1-h max, 8-h max or 8-h avg, and 24-h avg $O_3$.

$O_3$ concentration and $FEV_1$ were confined largely to children with asthma who used CS or had a concurrent URI, 7.3% and 4.9% decreases, respectively, in the mean of lowest daily $FEV_1$ per 30-ppb increase in 8-h max ambient $O_3$ concentration.

Heterogeneity in response to ambient $O_3$ exposure also was demonstrated by observations that some children with asthma experienced larger $O_3$-associated lung function decrements than the population mean effect estimate. Similar observations were made in controlled human exposure studies (Section 6.2.1.1). Mortimer et al. (2002) found that for a 30-ppb increase in lag 1-5 avg of 8-h avg $O_3$, there was a 30% ([95% CI: 4%, 61%]) higher incidence of >10% decline in PEF. Likewise, Hoppe et al. (2003) found that while the percentages of $O_3$-associated lung function decrements were variable and small, 47% of children with asthma experienced a >10% decline in $FEV_1$, FVC, or PEF or 20% increase in airway resistance on days with 30-min (1-4 p.m.) max ambient $O_3$ concentrations >50 ppb relative to days with <40 ppb $O_3$.

## Effect Modification

### Effect modification by corticosteroid use

In controlled human exposure studies, CS treatment of subjects with asthma generally has not prevented $O_3$-induced $FEV_1$ decrements (Section 6.2.1.1). Epidemiologic evidence is equivocal, with findings that use of inhaled CS attenuated (Hernández-Cadena et al., 2009), increased (Lewis et al., 2005), and did not affect (Mortimer et al., 2000) ambient $O_3$-associated lung function decrements. In winter-only studies, consideration of CS use largely did not influence associations between ambient $O_3$ and various lung function indices (Liu et al., 2009a; Rabinovitch et al., 2004). Similarly equivocal epidemiologic evidence was found for modification of associations with respiratory symptoms (Section 6.2.4.1). The assessment of effect modification by CS use has been hampered by differences in the severity of asthma among CS users and the definition of CS use. Additionally, investigators did not assess adherence to reported CS regimen, and misclassification of CS use may bias findings. For example, Mortimer et al. (2000) classified children by no or any CS use at baseline but did not measure daily use during the study period. Lewis et al. (2005) defined CS use as use for at least 50% of study days and estimated larger $O_3$-associated $FEV_1$ decrements among CS users (Figure 6-7 [and Table 6-8]) than among CS nonusers (quantitative results not reported). In this study, most children with moderate to severe asthma (91%) were classified as CS users. However, CS users had a higher percent predicted $FEV_1$. In contrast, Hernández-Cadena et al. (2009) observed larger $O_3$-related decrements in $FEV_1$ among the 60 CS nonusers than among the 25 CS users. A definition for CS use was not provided; however, children with persistent asthma were included among the group of CS nonusers. Thus, across studies, both CS use and nonuse have been used to indicate more severe, uncontrolled asthma.

### Effect modification by antioxidant capacity

Ozone is a powerful oxidant whose secondary oxidation products have been described to initiate the key modes of action that mediate decreases in lung function, including the activation of neural reflexes (Section 5.3.2). Additionally, $O_3$ exposure of humans and animals has induced changes in the levels of antioxidants in the ELF (Section 5.3.3). These observations provide biological plausibility for diminished antioxidant capacity to increase the risk of $O_3$-associated respiratory effects and for augmented antioxidant capacity to decrease risk.

### Antioxidant supplementation

Controlled human exposure studies have demonstrated the protective effects of α-tocopherol (vitamin E) and ascorbate (vitamin C) supplementation on $O_3$-induced lung function decrements (Section 6.2.1.1), and an epidemiologic study of children with asthma conducted in Mexico City, Mexico, produced similar findings. Particularly among children with moderate to severe asthma, an increase in ambient $O_3$ concentration was associated with a smaller decrease in $FEV_1$ in the group supplemented with vitamin C and E as compared with the placebo group (Romieu et al., 2002) (Figure 6-7 [and Table 6-8]). Romieu et al. (2009) also demonstrated an interaction between dietary antioxidant intake and ambient $O_3$ concentrations by finding that the main effect of diet was modified by ambient $O_3$ concentrations. Diets high in antioxidant vitamins and/or omega-3 fatty acids protected against $FEV_1$ decrements at 8-h max $O_3$ concentrations $\geq$ 38 ppb. Results for the main effect of $O_3$ on $FEV_1$ or effect modification by diet were not presented.

### Genetic polymorphisms

Antioxidant capacity also can be characterized by variants in genes encoding oxidant metabolizing enzymes with altered enzymatic activity. A potential role for such genetic variants in modifying $O_3$-associated health effects is biologically plausible given the well-characterized evidence for the secondary oxidation products of $O_3$ mediating downstream effects and has been indicated in some epidemiologic studies. Specifically, ambient $O_3$-associated $FEF_{25-75\%}$ decrements were larger among children with asthma with the GSTM1 null genotype, which is associated with lack of oxidant metabolizing activity (Romieu et al., 2004b). The difference in association between GSTM1 null and positive subjects was minimal in children supplemented with antioxidant vitamins (Figure 6-8 [and Table 6-9]). Controlled human exposure studies have not consistently found larger $O_3$-induced lung function decrements in GSTM1 null subjects (Section 6.2.1.1). Effect modification by GSTP1 variants is less clear. Romieu et al. (2006) observed larger $O_3$-associated decreases in $FEV_1$ in children with asthma with the GSTP1 Ile/Ile or Ile/Val variant, which are associated with relatively higher oxidative metabolism activity (Figure 6-7 [and Table 6-8]). An increase in ambient $O_3$ concentration was associated with an increase in $FEV_1$ among children with the GSTP1 Val/Val variant, which is associated with reduced oxidative metabolism. Rather than reflecting effect modification by the GSTP1 variant, these results may reflect effect modification by asthma severity, as 77% of

subjects with the GSTP1 Ile/Ile genotype had moderate to severe asthma. In support of this alternate hypothesis, another analysis of the same cohort indicated a larger $O_3$-associated decrement in $FEV_1$ among children with moderate to severe asthma than among all children with asthma (Romieu et al., 2002).

### Exposure Measurement Error

Across the studies of children with asthma, lung function decrements were associated with ambient $O_3$ concentrations assigned to subjects using various exposure assessment methods. As described in Section 4.3.3, exposure measurement error due to use of ambient concentrations measured at central sites has varied, depending on the population and season examined. Because there are a limited number of studies of each method, it is difficult to conclude that a particular method of exposure assessment produced stronger results.

Seasonal differences have been observed in the personal-ambient $O_3$ relationship (Section 4.3.3); however, in children with asthma, $O_3$-associated lung function decrements were found in studies conducted in summer months and over multiple seasons. Lung function was associated with $O_3$ measured on site of subjects' daytime hours in summer months (Hoppe et al., 2003; Thurston et al., 1997), factors that have contributed to higher personal-ambient $O_3$ ratios and correlations. Many year-round studies in Mexico City, Mexico (Romieu et al., 2006; 2004b; 2002; 1997; 1996), and a study in Detroit, MI (Lewis et al., 2005) found associations with $O_3$ measured at sites within 5 km of children's home or school. Children with asthma examined by Romieu et al. (2006); (2004b; 2002) had a personal-ambient ratio and correlation for 48- to 72-h avg $O_3$ concentrations of 0.17 and 0.35, respectively (Ramírez-Aguilar et al., 2008). These findings indicate that the effects of personal $O_3$ exposure on lung function decrements may have been underestimated in the children in Mexico City. Associations were found with $O_3$ concentrations averaged across multiple community monitoring sites (O'Connor et al., 2008; Just et al., 2002; Mortimer et al., 2002; Jalaludin et al., 2000) and measured at a single site (Gielen et al., 1997), which may be attributable to observations of high temporal correlation among.$O_3$ concentrations measured at multiple sites within a region (Darrow et al., 2011a; Gent et al., 2003).

Studies of children with asthma restricted to winter months provided little evidence of an association between various single- and multi-day lags of ambient $O_3$ concentration and lung function decrements with several observations of $O_3$-associated increases in lung function (Dales et al., 2009; Liu et al., 2009a; Rabinovitch et al., 2004). One explanation for these results may be lower indoor than outdoor $O_3$ concentrations, variable indoor to outdoor ratios, and lower correlations between personal and ambient $O_3$ concentrations in non-summer months (Sections 4.3.2 and 4.3.3). As noted for other respiratory endpoints such as respiratory hospital admissions, ED visits, and mortality, associations with $O_3$ generally are lower in colder seasons.

### Adults with Respiratory Disease

Relative to studies in children with asthma, studies of adults with asthma or COPD have been limited in number. Details from these studies regarding location, time period, and ambient $O_3$ concentrations are presented in Table 6-10. Increases in ambient $O_3$ concentration were not consistently associated with lung function decrements in adults with respiratory disease. Several different exposure assessment methods were used, including monitoring personal exposures (Delfino et al., 1997), monitoring on site of outdoor activity (Girardot et al., 2006; Korrick et al., 1998), and using measurements from one (Peacock et al., 2011; Wiwatanadate and Liwsrisakun, 2011; Thaller et al., 2008; Ross et al., 2002) to several central monitors (Khatri et al., 2009; Lagorio et al., 2006; Park et al., 2005a). There was not a clear indication that differences in exposure assessment methodology contributed to inconsistencies in findings.

**Table 6-10 Mean and upper percentile concentrations of $O_3$ in epidemiologic studies of lung function in adults with respiratory disease.**

| Study* | Location | Study Period | $O_3$ Averaging Time | Mean/Median Concentration (ppb) | Upper Percentile Concentrations (ppb) |
|---|---|---|---|---|---|
| Delfino et al. (1997) | Alpine, CA | May-July 1994 | 12-h avg personal (8 a.m.-8 p.m.) | 18 | 90th: 38<br>Max: 80 |
| Girardot et al. (2006) | Great Smoky Mountain NP, TN | August-October 2002 June-August 2003 | Hike-time avg (2-9 h) | 48.1[a] | Max: 74.2[a] |
| Korrick et al. (1998) | Mt. Washington, NH | Summer 1991, 1992 | Hike-time avg (2-12 h) | 40[a] | Max: 74[a] |
| Peacock et al. (2011) | London, England | All-year 1995-1997 | 8-h max | 15.5 | Autumn/Winter Max: 32<br>Spring/Summer Max: 74 |
| Wiwatanadate and Liwsrisakun (2011) | Chiang Mai, Thailand | August 2005-June 2006 | 24-h avg | 17.5 | 90th: 26.8<br>Max: 34.7 |
| Thaller et al. (2008); Brooks (2010) | Galveston, TX | Summer 2002-2004 | 1-h max | 35 (median) | Max: 118 |
| Ross et al. (2002) | East Moline, IL | April-October 1994 | 8-h avg | 41.5 | Max: 78.3 |
| Khatri et al. (2009) | Atlanta, GA | May-September 2003, 2005, 2006 | 8-h max | With asthma: 61 (median)[b]<br>No asthma: 56 (median)[b] | 75th (with asthma): 74[b]<br>75th (no asthma): 64[b] |
| Lagorio et al. (2006) | Rome, Italy | May-June, November-December 1999 | 24-h avg | Spring: 36.2[c]<br>Winter: 8.2[c] | Overall max: 48.6[c] |
| Park et al. (2005a) | Incheon, Korea | March-June 2002 | 24-h avg | Dust event days: 23.6<br>Control days: 25.1 | NR |

*Note: Studies presented in order of first appearance in the text of this section.

NR = Not reported.

[a]Individual-level estimates calculated from concentrations measured in different segments of hiking trail.

[b]Individual-level estimates calculated based on time spent in the vicinity of various $O_3$ monitors.

[c]Concentrations converted from µg/m$^3$ to ppb using the conversion factor of 0.51 assuming standard temperature (25°C) and pressure (1 atm).

Comparisons of adults with asthma (8-18% of study population) and without asthma did not conclusively demonstrate that adults with asthma had larger ambient $O_3$-associated lung function decrements. Several studies examined on-site or central-site ambient $O_3$ concentrations measured while subjects were outdoors. Ambient $O_3$ measured during time spent outdoors has been closer in magnitude and more correlated with personal exposures (Section 4.3.3). In a panel study of lifeguards (ages 16-27 years) in Galveston, TX, a larger $O_3$-associated decrement in $FEV_1$/FVC was found among the 16 lifeguards with asthma (-1.6% [95% CI: -2.8, -0.4] per 40 ppb increase in 1-h max $O_3$) than among the 126 lifeguards without asthma (-0.40% [95% CI: -0.80, 0] per 40-ppb increase in 1-h max $O_3$) (Brooks, 2010). In Korrick et al. (1998), hikers with a history of asthma or wheeze had larger

$O_3$-associated lung function decrements (e.g., -4.4% [95% CI: -7.5, -1.2] in $FEV_1$ per 30-ppb increase in 2-12 h avg $O_3$). In contrast, Girardot et al. (2006) generally did not find $O_3$-associated lung function decrements in hikers with or without respiratory disease history. In a cross-sectional study of 38 adults with asthma and 13 adults without asthma, Khatri et al. (2009) used central site $O_3$ measurements but aimed to account for spatial variability by calculating an average of concentrations measured at sites closest to each subject's location during each hour. Investigators reported a larger $O_3$-associated decrease in percent predicted $FEV_1$/FVC in the 38 subjects with atopy (with or without asthma) (-12 points [95% CI: -21, -3] per 30-ppb increase in lag 2 of 8-h max $O_3$) than in subjects with asthma (-4.7 points [95% CI: -12, 2.3]). Among adults with asthma, $O_3$ was associated with an increase in $FEV_1$.

In panel studies that exclusively examined adults with asthma, increases in ambient $O_3$ concentrations, across the multiple lags examined, generally were associated with increases in lung function (Wiwatanadate and Liwsrisakun, 2011; Lagorio et al., 2006; Park et al., 2005a). These studies were conducted in Europe and Asia during periods of low ambient $O_3$ concentrations, including one conducted in Korea during a period of dust storms (Park et al., 2005a).

Some studies included children and adults with asthma. Among subjects ages 9-46 years (41% adults) in Alpine, CA with low personal 12-h avg $O_3$ exposures (55% samples below limit of detection) and a majority of sampling hours spent indoors (mean 71%), Delfino et al. (1997) reported that neither increases in 12-h avg personal exposure nor increases in ambient $O_3$ concentration were associated with decreases in PEF. Ross et al. (2002) examined subjects ages 5-49 years (proportion of adults not reported) in East Moline, IL and found that a 20-ppb increase in lag 0 (of 24-h avg $O_3$) was associated with a 2.6 L/min decrease (95% CI: -4.3, -0.90) in evening PEF. In this population with asthma, an increase in lag 0 ozone also was associated with an increase in symptom score.

Controlled human exposure studies have found diminished, statistically nonsignificant $O_3$-induced lung function responses in older adults with COPD (Section 6.2.1.1). Similarly, epidemiologic studies do not provide strong evidence that short-term increases in ambient $O_3$ exposure result in lung function decrements in adults with COPD. Inconsistent associations were reported for PEF, $FEV_1$, and FVC in a study that followed 94 adults with COPD (ages 40-83 years) in London, England daily over two years (Peacock et al., 2011). For example, an increase in lag 1 of 8-h max $O_3$ was associated with a decrease in PEF in an analysis of summer 1996 (-1.7 L/min [95% CI: -3.1, -0.39] per 30-ppb increase $O_3$), but the association was near null and imprecise in summer 1997 (-0.21 L/min [95% CI: -2.4, 2.0]). Further, in this study, an increase in ambient $O_3$ concentration was associated with lower odds of a large PEF decrement (OR for a >20% drop from an individual's median value: 0.89 [95% CI: 0.72, 1.10] per 30-ppb increase in lag 1 of 8-h max $O_3$) and was not consistently associated with increases in respiratory symptoms (Peacock et al., 2011). Inconsistent associations also were reported in a small panel study of 11 adults with COPD (mean age 67 years) in Rome, Italy (Lagorio et al., 2006).

### Populations Not Restricted to Individuals with Asthma

Several studies have examined associations between ambient $O_3$ concentrations and lung function in groups that included children with and without asthma; however, a limited number of studies have examined groups of children or adults restricted to healthy individuals. Details from studies not restricted to individuals with asthma regarding location, time period, and ambient $O_3$ concentrations are presented in Table 6-11.

**Table 6-11 Mean and upper percentile concentrations of $O_3$ in epidemiologic studies of lung function in populations not restricted to individuals with asthma.**

| Study* | Location | Study Period | $O_3$ Averaging Time | Mean/Median Concentration (ppb) | Upper Percentile Concentrations (ppb) |
|---|---|---|---|---|---|
| Avol et al. (1998b) | 6 southern CA communities | Spring and summer 1994 | 24-h avg personal | NR | NR |
| Hoppe et al. (2003) | Munich, Germany | Summers 1992-1995 | 30-min max (1-4 p.m.) | High $O_3$ days: 70.4[a]<br>Control $O_3$ days: 29.8[a] | Max (high $O_3$ days): 99[a]<br>Max (control $O_3$ days): 39[a] |
| Chen et al. (1999) | 3 Taiwan communities | May 1995-January 1996 | 1-h max (8 a.m.-6 p.m.) | NR | Max: 110.3[a] |
| Gold et al. (1999) | Mexico City, Mexico | January-November 1991 | 24-h avg | 52.0[a] | Max: 103[a] |
| Ward et al. (2002) | Birmingham and Sandwell, England | January-March, May-July 1997 | 24-h avg | Winter median: 13.0<br>Summer median: 22.0 | Winter Max: 33<br>Summer Max: 41 |
| Ulmer et al. (1997) | Freudenstadt and Villingen, Germany | March-October 1994 | 30-min avg | Freudenstadt median: 50.6<br>Villingen median: 32.1 | Freudenstadt 95th: 89.8<br>Villingen 95th: 70.1 |
| Linn et al. (1996) | Rubidoux, Upland, Torrance, CA | September-June 1992-1994 | 24-h avg personal<br>24-h avg central site | 5<br>23 | Max: 16<br>Max: 53 |
| Scarlett et al. (1996) | Surrey, England | June-July 1994 | 8-h max | 50.7[a] | Max: 128[a] |
| Neuberger et al. (2004) | Vienna, Austria | June-October 1999, January-April 2000 | NR | NR | NR |
| Alexeeff et al. (2008); (2007) | Greater Boston, MA; NAS | January 1995-June 2005 | 48-h avg | 24.4[b] | NR |
| Steinvil et al. (2009) | Tel Aviv, Israel | September 2002-November 2007 | 8-h avg (10 a.m.-6 p.m.) | 41.1 | 75th: 48.7<br>Max: 72.8 |
| Naeher et al. (1999) | Multiple communities, VA | May-September 1995-1996 | 24-h avg | 34.9 | Max: 56.6 |
| Son et al. (2010) | Ulsan, Korea | All-year, 2003-2007 | 8-h max | 35.9 (avg of 13 monitors) | Max: 59.5 |

*Note: Studies presented in order of first appearance in the text of this section.

NR = Not Reported, NAS = Normative Aging Study.

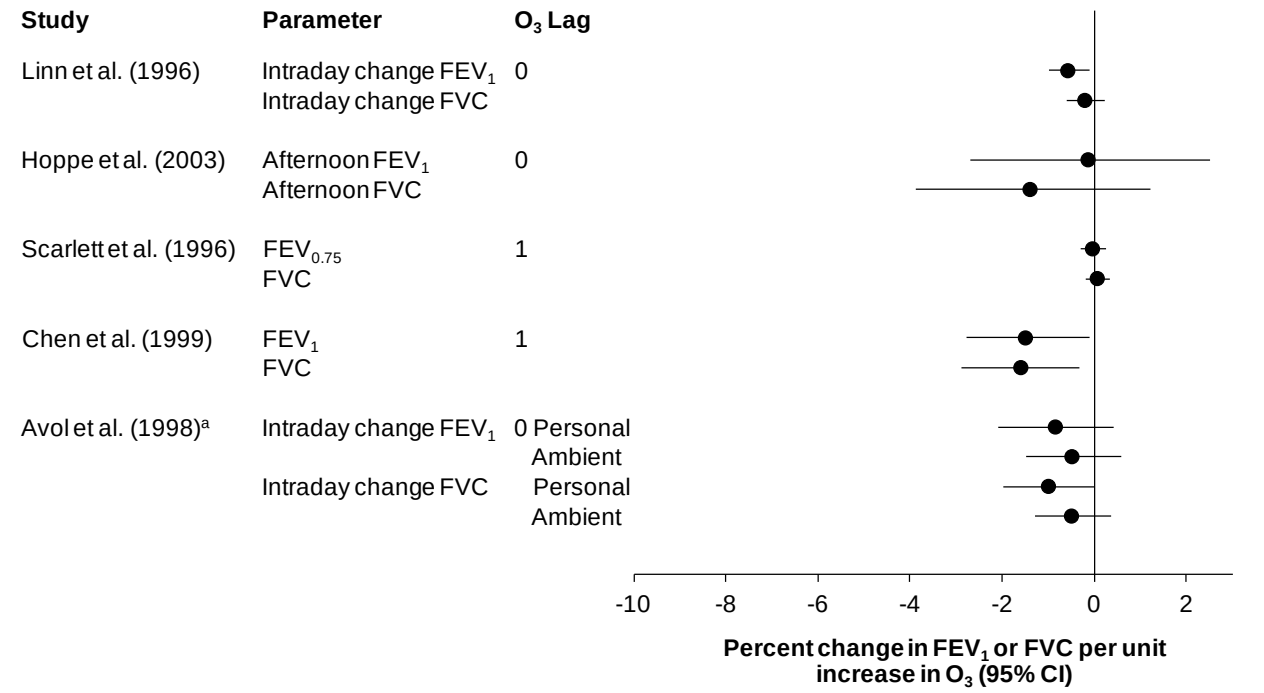
[a]Measured at subjects' schools where lung function was measured.

[b]Measured at central monitoring sites established by investigators. Concentrations were averaged across four monitors.

### Children

Based on studies available at the time of the 2006 $O_3$ AQCD, evidence consistently links increases in ambient $O_3$ concentration with decrements in $FEV_1$ and PEF in children (U.S. EPA, 2006b) (Figure 6-9 [and Table 6-12]). These associations were

found with personal $O_3$ exposures (Avol et al., 1998b), ambient $O_3$ measured at children's schools where lung function was measured (Hoppe et al., 2003; Chen et al., 1999; Gold et al., 1999), and ambient $O_3$ measured at sites within the community (Ward et al., 2002; Ulmer et al., 1997; Linn et al., 1996). Among children in California who spent a mean 2-3 hours per day outdoors and whose personal-ambient $O_3$ correlation was 0.28 across multiple seasons, Avol et al. (1998b) found slightly larger $O_3$-associated decrements in $FEV_1$ and FVC for 24-h avg personal exposures than for 1-h max ambient measurements (Figure 6-9 [and Table 6-12]). The effect estimates for personal exposures were similar in magnitude to those found in other studies of children for ambient $O_3$ measured at schools (Hoppe et al., 2003; Chen et al., 1999). In another study of children in California, Linn et al. (1996) did not present results for personal $O_3$ exposures but found $FEV_1$ decrements in association with increases in ambient $O_3$ concentration in children who spent 1-2 hours per day outdoors and whose personal-ambient correlation was 0.61. Because of between-study heterogeneity in populations and ambient $O_3$ concentrations examined, it is difficult to assess how the method of exposure assessment may have influenced findings.




Note: Results generally are presented in order of increasing mean ambient $O_3$ concentration. Effect estimates are from single-pollutant models and are standardized to a 40-, 30-, and 20-ppb increase for 1-h (or 30-min) max, 8-h max, and 24-h avg $O_3$ concentrations, respectively.

[a]The 95% CI was constructed using a standard error that was estimated from the p-value.

**Figure 6-9 Percent change in $FEV_1$ or FVC in association with ambient $O_3$ concentrations in studies of children in the general population.**

## Table 6-12 Percent change in $FEV_1$ or FVC in association with ambient $O_3$ concentrations in studies of children in the general population presented in Figure 6-9 plus others.

| Study* | Location/ Population | $O_3$ Averaging Time | $O_3$ Lag | Parameter | Standardized Percent Change (95% CI)[a] |
|---|---|---|---|---|---|
| Linn et al. (1996) | 3 southern CA communities<br>269 children, 4th and 5th grades | 24-h avg | 0 | Intraday change $FEV_1$<br>Intraday change FVC | -0.58 (-1.0, -0.13)<br>-0.21 (-0.62, 0.20) |
| Hoppe et al. (2003) | Munich, Germany<br>44 children, ages 6-8 yr | 30-min max (1 - 4 p.m.) | 0 | Afternoon $FEV_1$<br>Afternoon FVC | -0.14 (-2.7, 2.5)<br>-1.4 (-3.9, 1.2) |
| Scarlett et al. (1996) | Surrey, England<br>154 children, ages 7-11 yr | 8-h max | 1 | $FEV_{0.75}$<br>FVC | -0.04 (-0.32, 0.23)<br>0.06 (-0.21, 0.33) |
| Chen et al. (1999) | 3 Taiwan communities<br>941 children, ages 8-13 yr | 1-h max (8 a.m.-6 p.m.) | 1 | $FEV_1$<br>FVC | -1.5 (-2.8, -0.12)<br>-1.6 (-2.9, -0.33) |
| Avol et al. (1998b) | 3 southern CA communities<br>195 children, ages 10-12 yr | 24-h avg personal<br>1-h max ambient<br>24-h avg personal<br>1-h max ambient | 0 | Intraday change $FEV_1$<br>Intraday change $FEV_1$<br>Intraday change FVC<br>Intraday change FVC | -0.85 (-2.1, 0.42)[b]<br>-0.49 (-1.5, 0.57)[b]<br>-1.0 (-2.0, 0)[b]<br>-0.50 (-1.3, 0.35)[b] |
| **Studies of children not included in Figure 6-9[c]** | | | | | |
| Ulmer et al. (1997) | Freudenstadt and Villingen, Germany<br>135 children, ages 8-10 yr | 30-min max | 1 | $FEV_1$ (mL) | -57 (-102, 13)[b] |
| Ward et al. (2002) | Birmingham and Sandwell, England<br>162 children, age 9 yr | 24-h avg | 0<br>2 | Daily deviation from mean morning PEF (L/min) | -3.2 (-8.3, 2.0)[d]<br>-6.7 (-12, -1.4)[d] |
| Gold et al. (1999) | Mexico City, Mexico<br>40 children, ages 8-11 yr | 24-h avg | 0<br>1-10 avg | Intraday change PEF (% change) | -0.47 (-1.1, 0.11)<br>-3.8 (-6.7, -0.94) |

*Includes studies in Figure 6-9, plus others.

[a]Effect estimates are standardized to a 40-, 30-, and 20-ppb increase for 1-h (or 30-min) max, 8-h max, and 24-h avg $O_3$, respectively.

[b]The 95% CI was constructed using a standard error that was estimated from the p-value.

[c]Results are not presented in Figure 6-9 because sufficient data were not available to calculate percent change in $FEV_1$, or PEF was analyzed.

[d]Effect estimates are from analyses restricted to summer months.

In the limited number of studies that examined only healthy children, increases in ambient $O_3$ concentration were associated with decreases (Hoppe et al., 2003) or no change in lung function (Neuberger et al., 2004). Several studies that included small proportions (4-10%) of children with history of respiratory disease or symptoms found associations between increases in ambient $O_3$ concentration and lung function decrements (Chen et al., 1999; Ulmer et al., 1997; Scarlett et al., 1996). Based on analysis of interaction terms for $O_3$ concentration and asthma/wheeze history, Avol et al. (1998b) and Ward et al. (2002) did not find differences in $O_3$-associated lung function decrements between children with history of asthma or wheeze and healthy children. Combined, these lines of evidence suggest that the ambient $O_3$-associated

lung function decrements found in children overall were not solely due to effects in children with asthma, and that increases in ambient $O_3$ exposure may decrease lung function in healthy children.

Among the studies of children, the magnitudes of decrease in lung function per unit increase in ambient $O_3$ concentration[1] ranged from <1 to 4%, a range similar to that estimated in children with asthma. Comparable data were not adequately available to assess whether mean lung function differed between groups of children with asthma and healthy children. In contrast with studies of children with asthma, studies of children in the general population did not consistently find both $O_3$-associated decreases in lung function and $O_3$-associated increases in respiratory symptoms. For example, Gold et al. (1999) found $O_3$-associated decreases in PEF and increases in phlegm; however, the increase in phlegm was associated with lag 1 $O_3$ concentrations whereas the PEF decrement was found with single-day lags 2 to 4 of $O_3$. Also, $O_3$ was weakly associated with cough and shortness of breath among children in England (Ward et al., 2002) and was associated with a decrease in respiratory symptom score among children in California (Linn et al., 1996).

### Adults

Compared with children, in a smaller body of studies, $O_3$ was less consistently associated with lung function decrements in populations of adults not restricted to those with asthma (Table 6-13). In a study that included only healthy adults, increases in ambient $O_3$ concentration were associated with decreases and increases in lung function across the various lags of exposure examined (Steinvil et al., 2009). Contrasting results also were found in studies of older adults (Alexeeff et al., 2008; Alexeeff et al., 2007; Hoppe et al., 2003).

[1]Effect estimates were standardized to a 40-, 30-, and 20-ppb increase for 1-h max (or 30-min max), 8-h max, and 24-h avg $O_3$.

## Table 6-13 Associations between ambient $O_3$ concentration and lung function in studies of adults.

| Study[a] | Location/Population | $O_3$ Averaging Time | $O_3$ Lag | Parameter | $O_3$ Assessment Method/Subgroup | Standardized Effect Estimate (95% CI)[b] |
|---|---|---|---|---|---|---|
| Son et al. (2010) | Ulsan, Korea<br>2,102 children and adults, ages 7-97 yr | 8-h max | 0-2 avg | Change in percent predicted $FEV_1$ | All monitor avg<br>Nearest monitor<br>IDW<br>Kriging | -1.4 (-2.7, -0.08)<br>-0.76 (-1.8, 0.25)<br>-1.1 (-2.2, 0.05)<br>-1.4 (-2.6, -0.11) |
| Steinvil et al. (2009) | Tel Aviv, Israel<br>2,380 healthy adults, mean age 43 yr, 75th percentile: 52 yr | 8-h avg (10 a.m. - 6 p.m.) | 0<br>0-6 avg | $FEV_1$ (mL) | | 60 (0, 120)<br>141 (49, 234) |
| Naeher et al. (1999) | Multiple communities, VA<br>473 women, ages 19 - 43 yr | 24-h avg | 0<br>0-2 avg | Evening PEF (L/min) | | -1.7 (-3.4, 0.03)<br>-3.0 (-4.4, -1.7) |
| Hoppe et al. (2003) | Munich, Germany<br>41 older adults, ages 69 - 95 yr | 30-min max (1-4 p.m.) | 0<br>1 | % change in afternoon $FEV_1$ | | 0.75 (-2.1, 3.7)<br>1.2 (-1.3, 3.6) |
| Alexeeff et al. (2008) | Greater Boston, MA; NAS<br>1,015 older adults, mean (SD) age: 68.8 (7.2) yr at baseline | 24-h avg | 0-1 avg | % change in $FEV_1$ | GSTP1 Ile/Ile<br>GSTP1 Ile/Val or Val/Val | -1.0 (-2.2, 0.20)<br>-2.3 (-3.5, -1.0) |
| Alexeeff et al. (2007) | Greater Boston, MA; NAS<br>904 older adults, mean (SD) age: 68.8 (7.3) yr at baseline | 24-h avg | 0-1 avg | % change in $FEV_1$ | BMI <30<br>BMI ≥ 30<br>No AHR<br>AHR<br>BMI ≥ 30 and AHR | -1.5 (-2.5, -0.51)<br>-3.5 (-5.1, -1.9)<br>-1.7 (-2.7, -0.73)<br>-4.0 (-6.2, -1.8)<br>-5.3 (-8.2, -2.3) |

IDW = Inverse distance weighting, NAS = Normative Aging Study, BMI = Body mass index, AHR = airway hyperresponsiveness.
[a]Results generally are presented in order of increasing mean ambient $O_3$ concentration.
[b]Effect estimates are standardized to a 40-ppb increase for 30-min max $O_3$, 30-ppb increase for 8-h max or 8-h avg $O_3$, and 20-ppb increase for 24-h avg $O_3$.

Despite mixed results overall, studies that found ambient $O_3$-associated lung function decrements in adults used various exposure assessment methods with potentially varying degrees of measurement error. These methods included the average of multiple intra-city monitors, nearest monitor, estimates from spatial interpolation (Son et al., 2010), average of monitors in multiple towns (Alexeeff et al., 2008; Alexeeff et al., 2007), and one site for multiple towns (Naeher et al., 1999). In a large cross-sectional study, conducted in 2,102 children and adults (mean age: 45 years) living near a petrochemical plant in Ulsan, Korea, Son et al. (2010) did not find a consistent difference in the magnitude of association with lung function among ambient $O_3$ concentrations averaged across 13 city monitors, concentrations from the nearest monitor, inverse distance-weighted concentrations, and estimates from kriging across the various lags examined (Table 6-13). Ozone concentrations were similar (<10% difference) and highly correlated (r = 0.84 – 0.96) among the methods.

Although the health status of subjects was not reported, the study population mean percent predicted $FEV_1$ was 82.85%, indicating a large proportion of subjects with underlying airway obstruction. Results from this study were not adjusted for meteorological factors and thus, confounding cannot be ruled out. Importantly, the similarities among exposure assessment methods in Son et al. (2010) may apply mostly to populations living within the same region of a city. The majority of women examined by Naeher et al. (1999) lived >60 miles from the single available central site monitor. However, in the nonurban (southwest Virginia) study area, $O_3$ concentrations may be more spatially homogeneous (Section 4.6.2.1), and the concentrations measured at the single site may capture temporal variability in ambient exposures.

The inconsistent epidemiologic findings for older adults parallel observations from controlled human exposure studies (Section 6.2.1.1). In a study that followed adults ages 69-95 years during several summers in Germany, Hoppe et al. (2003) did not find decreases in lung function in association with ambient $O_3$ measured at subjects' retirement home. However, recently, the Normative Aging Study found decrements in $FEV_1$ and FVC in a group of older men (mean [SD] age = 68.9 [7.2] years at first lung function measurement) in association with ambient $O_3$ concentrations averaged from four town-specific monitors (Alexeeff et al., 2008), which may less well represent spatial heterogeneity in ambient $O_3$ exposures. Among all subjects, who were examined once every three years for ten years, associations were found with several lags of 24-h avg $O_3$ concentration, i.e., 1- to 7-day avg (Alexeeff et al., 2008). Additionally, larger effects were estimated in adults with airway hyperresponsiveness, higher BMI (≥ 30), and GSTP1 Ile/Val or Val/Val genetic variants (Val/Val variant produces enzyme with reduced oxidative metabolism activity) (Alexeeff et al., 2008; Alexeeff et al., 2007) (Table 6-13). Larger $O_3$-related decrements in $FEV_1$ and FVC also were observed in subjects with long GT dinucleotide repeats in the promoter region of the gene for the antioxidant enzyme heme oxygenase-1 (Alexeeff et al., 2008), which has been associated with reduced inducibility (Hiltermann et al., 1998). In this cohort, $O_3$ also was associated with decreases in lung function in adults without airway hyperresponsiveness and those with BMI <30, indicating effects of $O_3$ on lung function in healthy men within the cohort. However, the findings may be generalizable only to this study population of older, predominately white men.

### Confounding in epidemiologic studies of lung function

The 1996 $O_3$ AQCD noted uncertainty regarding confounding by temperature and pollen (U.S. EPA, 1996a); however, collective evidence does not indicate that these factors fully account for the associations observed between increases in ambient $O_3$ concentration and lung function decrements. Across the populations examined, most studies that found ambient $O_3$-associated lung function decrements, whether conducted in multiple seasons or only in summer, included temperature in statistical analyses. Some summer camp studies conducted detailed analysis of temperature. In most of these studies, temperature and $O_3$ were measured at the camps. In two

Northeast U.S. studies, an increase in temperature was associated with an increase in lung function (Thurston et al., 1997; Berry et al., 1991). This positive association likely accounted for the nearly 2-fold greater decrease in $O_3$-associated PEF found by Thurston et al. (1997) with temperature in the model than with $O_3$ alone. In another Northeast U.S. camp study, Spektor et al. (1988a) estimated similar effects for $O_3$ in a model with and without a temperature-humidity index. In the re-analysis of six camp studies, investigators did not include temperature in models because temperature within the normal ambient range had not been shown to affect $O_3$-induced lung function responses in controlled human exposure studies (Kinney et al., 1996).

Pollen was evaluated in far fewer studies. Camp studies that examined pollen found that pollen independently was not associated with lung function decrements (Thurston et al., 1997; Avol et al., 1990). Many studies of individuals with asthma with follow-up over multiple seasons found $O_3$-associated decrements in lung function in models that adjusted for pollen counts (Just et al., 2002; Ross et al., 2002; Jalaludin et al., 2000; Gielen et al., 1997). In these studies, large proportions of subjects had atopy (22-98%), with some studies examining large proportions of subjects specifically with pollen allergy who would be more responsive to pollen exposure (Ross et al., 2002; Gielen et al., 1997).

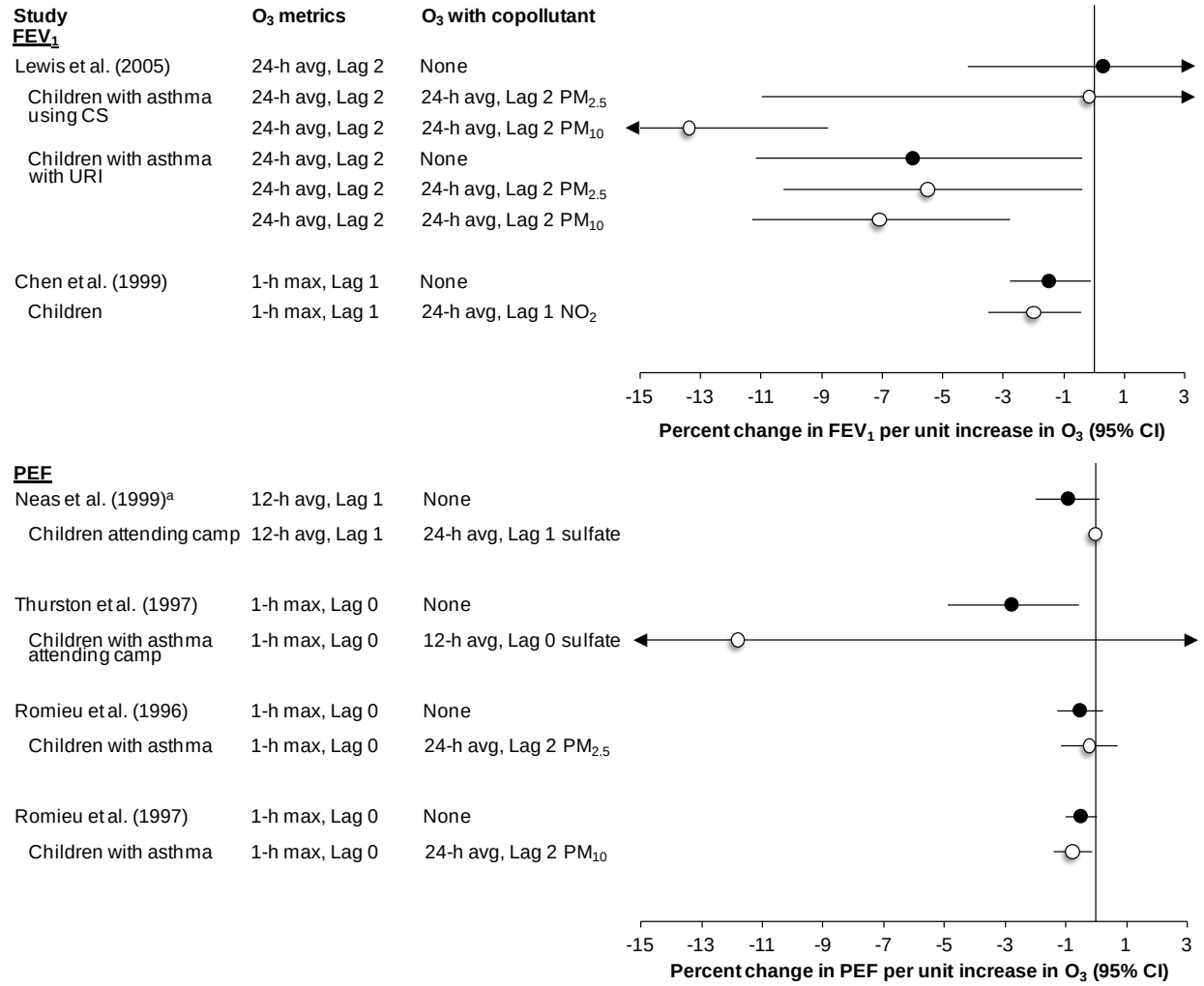
A relatively large number of studies provided information on potential confounding by copollutants such as $PM_{2.5}$, $PM_{10}$, $NO_2$, or $SO_2$. While studies were varied in how they evaluated confounding, most indicated that $O_3$-associated lung function decrements were not solely due to copollutant confounding. Some studies of subjects exercising outdoors indicated that ambient concentrations of copollutants such as $NO_2$, $SO_2$, or acid aerosol were low and thus, not likely to confound associations observed for $O_3$ (Hoppe et al., 2003; Brunekreef et al., 1994; Hoek et al., 1993). In other studies of children with increased outdoor exposures, $O_3$ was consistently associated with decreases in lung function, whereas other pollutants such as $PM_{2.5}$, sulfate, and acid aerosol individually showed variable associations across studies (Thurston et al., 1997; Castillejos et al., 1995; Berry et al., 1991; Avol et al., 1990; Spektor et al., 1988a). Most of these studies measured ambient pollutants on site of subjects' outdoor activity and related lung function changes to the pollutant concentrations measured during outdoor activity. Thus, the degree of exposure measurement error likely is comparable for $O_3$ and copollutants.

Studies that conducted copollutant modeling generally found $O_3$-associated lung function decrements to be robust; i.e., most copollutant-adjusted effect estimates fell within the 95% CI of the single-pollutant effect estimates (Figure 6-10 [and Table 6-14]). These studies used central site measurements for both $O_3$ and copollutants. There may be residual confounding because of differential exposure measurement error for $O_3$ and copollutants due to differing spatial heterogeneity and indoor-outdoor ratios; however, the limited available evidence indicates that personal $O_3$ exposures are weakly correlated with personal $PM_{2.5}$ and $NO_2$ exposures (Section 4.3.4.1). Whereas a few studies used the same averaging time for copollutants (Lewis et al., 2005; Jalaludin et al., 2000), more examined 1-h max or

8-h max $O_3$ and 24-h avg copollutant concentrations (Son et al., 2010; Chen et al., 1999; Romieu et al., 1997; Romieu et al., 1996). In a Philadelphia-area summer camp study, Neas et al. (1999) was among the few studies to find that the effect estimate for $O_3$ was attenuated to near zero in a copollutant model (24-h avg sulfate in this study) (Figure 6-10 [and Table 6-14]).

Ambient $O_3$ concentrations showed a wide range of correlations with copollutant concentrations (r = -0.31 to 0.74). In Sydney, Australia, Jalaludin et al. (2000) found low correlations of $O_3$ with $PM_{10}$ (r = 0.13) and $NO_2$ (r = -0.31), all averaged over 24 hours. In two-pollutant models, $PM_{10}$ and $NO_2$ remained associated with increases in PEF, and $O_3$ remained associated with decreases in PEF in children with asthma. In Detroit, MI, $O_3$ was moderately correlated with $PM_{2.5}$ (Pearson r = 0.57) and $PM_{10}$ (Pearson r = 0.59), all averaged over 24 hours (Lewis et al., 2005). Adjustment for $PM_{10}$ resulted in a large (more negative) change in the $O_3$-associated $FEV_1$ decrement in children with asthma, but only in CS users and not in children with concurrent URI (Figure 6-10 [and Table 6-14]). Studies conducted in Mexico City, Mexico, found small changes in $O_3$-associated PEF decrements with copollutant adjustment although different averaging times were used for copollutants (Romieu et al., 1997; Romieu et al., 1996) (Figure 6-10 [Table 6-14]). In these studies, $O_3$ was moderately correlated with copollutants such as $NO_2$ and $PM_{10}$ (range of Pearson r = 0.38 - 0.58). Studies conducted in Asia also found that associations between $O_3$ and lung function were robust to adjustment for weakly- to moderately-correlated copollutants; effect estimates for copollutants generally were attenuated, indicating that $O_3$ may confound associations of copollutants (Son et al., 2010; Chen et al., 1999).

In a summer camp study conducted in Connecticut, Thurston et al. (1997) found ambient concentrations of 1-h max $O_3$ and 12-h avg sulfate to be highly correlated (r = 0.74), making it difficult to separate their independent effects. With sulfate in the model, a larger decrease in PEF was estimated for $O_3$; however, the 95% CI was much wider (Figure 6-10 [and Table 6-14]). Investigators found that the association for sulfate was due to one day when the ambient concentrations of both pollutants were at their peak. With the removal of this peak day, the sulfate effect was attenuated, whereas $O_3$ effects remained robust (Thurston et al., 1997). Among children with asthma in Thailand, the $O_3$-associated decrease in PEF was robust to adjustment of $SO_2$; however, different lags were examined for $O_3$ (lag 5) and $SO_2$ (lag 4) (Wiwatanadate and Trakultivakorn, 2010).




Note: Results are presented first for $FEV_1$ then for PEF and within these categories, then in order of increasing mean ambient $O_3$ concentration. CS = corticosteroid, URI = Upper respiratory infection. Effect estimates are standardized to a 40-, 30-, and 20-ppb increase for 1-h max, 12-h avg, and 24-h avg $O_3$, respectively. Black circles represent $O_3$ effect estimates from single pollutant models, and open circles represent $O_3$ effect estimates from copollutant models.

[a]Information was not available to calculate 95% CI of the copollutant model.

## Figure 6-10 Comparison of $O_3$-associated changes in lung function in single- and copollutant models.

## Table 6-14 Comparison of $O_3$-associated changes in lung function in single- and copollutant models for studies presented in Figure 6-10 plus others.

| Study* | Location/Population | Parameter | $O_3$-associated Percent Change in Single-Pollutant Model (95% CI)[a] | $O_3$-associated Percent Change in Copollutant Model (95% CI)[a] |
|---|---|---|---|---|
| **$FEV_1$** | | | | |
| Lewis et al. (2005) | Detroit, MI<br>Children with asthma using CS<br>393 person-days | Lowest daily $FEV_1$ | For 24-h avg, Lag 2<br>0.29 (-4.2, 5.0) | With 24-h avg, Lag 2 $PM_{2.5}$<br>-0.18 (-11.0, 11.9)<br>With 24-h avg, Lag 2 $PM_{10}$<br>-13.4 (-17.8, -8.8) |
| | Children with asthma with URI<br>231 person-days<br>Overall mean (SD) age 9.1 (1.4 yr) | | For 24-h avg, Lag 2<br>-6.0 (-11.2, -0.41) | With 24-h avg, Lag 2 $PM_{2.5}$<br>-5.5 (-10.3, -0.42)<br>With 24-h avg, Lag 2 $PM_{10}$<br>-7.1 (-11.3, -2.8) |
| Chen et al. (1999) | 3 Taiwan communities<br>941 children, ages 8-13 yr | $FEV_1$ | For 1-h max, Lag 1<br>-1.5 (-2.8, -0.12) | With 24-h avg, Lag 1 $NO_2$<br>-2.0 (-3.5, -0.43) |
| **PEF** | | | | |
| Neas et al. (1999) | Philadelphia, PA<br>156 Children at summer camp, ages 6 - 11 yr | Morning PEF | For 12-h avg, Lag 1<br>-0.94 (-2.0, 0.08) | With 24-h avg, Lag 1 sulfate<br>-0.02[b] |
| Thurston et al. (1997) | CT River Valley<br>166 Children with asthma at summer camp, ages 7-13 yr | Intraday change PEF | For 1-h max, Lag 0<br>-2.8 (-4.9, -0.59) | With 12-h avg, Lag 0 sulfate<br>-11.8 (-31.6, 8.1) |
| Romieu et al. (1996) | Northern Mexico City, Mexico<br>71 children with asthma, ages 5-7 yr | Evening PEF | For 1-h max, Lag 2<br>-0.55 (-1.3, 0.19) | With 24-h avg, Lag 2 $PM_{2.5}$<br>-0.24 (-1.2, 0.68) |
| Romieu et al. (1997) | Southern Mexico City, Mexico<br>65 children with asthma, ages 5-13 yr | Evening PEF | For 1-h max, Lag 0<br>-0.52 (-1.0, -0.01) | With 24-h avg, Lag 0 $PM_{10}$<br>-0.79 (-1.4, -0.16) |

| Study* | Location/Population | Parameter | $O_3$-associated Percent Change in Single-Pollutant Model (95% CI)[a] | $O_3$-associated Percent Change in Copollutant Model (95% CI)[a] |
|---|---|---|---|---|
| **Results not included in Figure 6-10[c]** | | | | |
| Jalaludin et al. (2000) | Sydney, Australia<br>125 children with asthma or wheeze, mean (SD) age 9.6 (1.0) yr | Daily deviation from mean PEF | For 15-h (6 a.m.-9 p.m.) avg, Lag 0<br>-1.8 (-3.5, -0.19) | With 15-h avg, Lag 0 $PM_{10}$,<br>-1.8 (-3.5, -0.19)<br>With 15-h avg, Lag 0 $NO_2$<br>-1.8 (-3.4, -0.11) |
| Wiwatanadate and Trakultivakorn (2010) | Chiang Mai, Thailand<br>31 children with asthma, ages 4-11 yr | Daily avg PEF (L/min) | For 24-h avg, Lag 5<br>-2.6 (-5.2, 0) | With 24-h avg, Lag 4 $SO_2$<br>-3.2 (-6.2, -0.2) |
| Son et al. (2010) | Ulsan, Korea<br>2,102 children and adults, ages 7-97 yr | Change in percent predicted $FEV_1$ | For 8-h max, Lag 0-2 avg (kriging)<br>-1.4 (-2.6, -0.11) | With 24-h avg, Lag 2 $PM_{10}$ (kriging)<br>-1.8 (-3.4, -0.25) |

*Includes studies in Figure 6-10 plus others.

CS = Corticosteroid, URI = Upper respiratory infection.

[a]Effect estimates are standardized to a 40-ppb increase for 1-h max $O_3$, 30-ppb increase for 12-h avg or 8-h max $O_3$, and 20-ppb increase for 24-h avg or 15-h avg $O_3$.

[b]Information was not available to calculate 95% CI.

[c]Results are not presented in Figure 6-10 because sufficient data were not available to calculate percent change in lung function.

Some studies did not provide quantitative results but only reported that $O_3$-associated lung function decrements remained statistically significant in models that included copollutants such as $PM_{10}$, $NO_2$, sulfate, nitrate, or ammonium (Romieu et al., 1998b; Brauer et al., 1996; Linn et al., 1996; Spektor et al., 1988b).

Several studies estimated robust $O_3$-associated lung function decrements in multipollutant models that most often included $O_3$, $NO_2$, and either $PM_{2.5}$ or $PM_{10}$ (O'Connor et al., 2008; Thaller et al., 2008; Chan and Wu, 2005; Romieu et al., 2002; Korrick et al., 1998; Higgins et al., 1990). However, the independent effects of $O_3$ are more difficult to assess in relation to incremental changes in more than one copollutant.

### Summary of Epidemiologic Studies of Lung Function

The cumulative body of epidemiologic evidence indicates that short-term increases in ambient $O_3$ concentration are associated with decrements in lung function in children with asthma (Figure 6-7 [and Table 6-8]) and Figure 6-8 [and Table 6-9]) and children in the general population. In contrast with results from controlled human exposure studies, within-study epidemiologic comparisons did not consistently indicate larger ambient $O_3$-associated lung function decrements in groups with asthma (children or adults) than in groups without asthma. Notably, most epidemiologic studies were not designed to assess between-group differences. Based on comparisons between studies, differences were noted between children with and without asthma in so far as in studies of children with asthma, an increase in ambient $O_3$ concentration was associated with both lung function decrements and increases in

respiratory symptoms (Just et al., 2002; Mortimer et al., 2002; Ross et al., 2002; Gielen et al., 1997; Romieu et al., 1997; Thurston et al., 1997; Romieu et al., 1996). In studies of children in the general population, increases in ambient $O_3$ concentration were associated with decreases in lung function but not increases in respiratory symptoms (Ward et al., 2002; Gold et al., 1999; Linn et al., 1996).

Across studies of children, there was no clear indication that a particular exposure assessment method using central site measurements produced stronger findings, despite potential differences in exposure measurement error. In children, lung function was associated with ambient $O_3$ concentrations measured on site of children's daytime hours (Hoppe et al., 2003; Thurston et al., 1997), at children's schools (Chen et al., 1999; Gold et al., 1999), at the closest site (Romieu et al., 2006; Lewis et al., 2005; Romieu et al., 2004b; Romieu et al., 2002; Romieu et al., 1997; Romieu et al., 1996), at multiple community sites then averaged (O'Connor et al., 2008; Just et al., 2002; Mortimer et al., 2002; Jalaludin et al., 2000), and at a single site (Ward et al., 2002; Gielen et al., 1997; Ulmer et al., 1997; Linn et al., 1996). Among children in California, Avol et al. (1998b) found slightly larger $O_3$-associated lung function decrements for 24-h avg personal exposures than for 1-h max ambient concentrations.

As noted in the 1996 and 2006 $O_3$ AQCDs, evidence clearly demonstrates ambient $O_3$-associated lung function decrements in children and adults engaged in outdoor recreation, exercise, or work. Moreover, several results in these populations indicated associations with 10-min to 12-h avg $O_3$ concentrations <80 ppb. These studies are noteworthy for their measurement of ambient $O_3$ on site of and at the time of subjects' outdoor activity, factors that have contributed to higher $O_3$ personal exposure-ambient concentration correlations and ratios (Section 4.3.3). These epidemiologic results are well supported by observations from controlled human exposure studies of lung function decrements induced by $O_3$ exposure during exercise (Section 6.2.1.1). Although epidemiologic investigation was relatively sparse, increases in ambient $O_3$ concentration were not consistently associated with lung function decrements in adults with respiratory disease, healthy adults, or older adults.

Across the diverse populations examined, most effect estimates ranged from a <1 to 2% decrease in lung function per unit increase in $O_3$ concentration[1]. Heterogeneity in $O_3$-associated respiratory effects within populations was indicated by observations of larger decreases (3-8%) in children with asthma with CS use or concurrent URI (Lewis et al., 2005) and older adults with airway hyperresponsiveness and/or BMI >30 (Alexeeff et al., 2007). Among children in Mexico City, Mexico, higher dietary antioxidant intake attenuated $O_3$-associated lung function decrements (Romieu et al., 2004b; 2002), similar to results from controlled human exposure studies. Each of these potential effect modifiers was examined in one to two populations; thus, firm conclusions about their influences are not warranted. Adding to the evidence for heterogeneity in response, Hoppe et al. (2003) and Mortimer et al. (2002) found that

[1] Effect estimates were standardized to a 40-, 30-, and 20-ppb increase for 1-h max, 8-h max, and 24-h avg $O_3$.

increases in ambient $O_3$ concentration were associated with increased incidence of >10% decline in lung function in children with asthma.

Collectively, epidemiologic studies examined and found lung function decrements in association with single-day $O_3$ concentrations lagged from 0 to 7 days and concentrations averaged over 2-10 days. More studies found associations with $O_3$ concentrations lagged 0 or 1 day (Son et al., 2010; Alexeeff et al., 2008; Lewis et al., 2005; Ross et al., 2002; Jalaludin et al., 2000; Chen et al., 1999; Romieu et al., 1997; Brauer et al., 1996; Romieu et al., 1996; Spektor et al., 1988b) than those lagged 5-7 days (Wiwatanadate and Trakultivakorn, 2010; Hernández-Cadena et al., 2009; Steinvil et al., 2009). Associations with multiday average concentrations (Son et al., 2010; Liu et al., 2009a; Barraza-Villarreal et al., 2008; O'Connor et al., 2008; Alexeeff et al., 2007; Mortimer et al., 2002; Ward et al., 2002; Gold et al., 1999; Naeher et al., 1999; Neas et al., 1999) indicate that elevated exposures over several days may be important. Within studies, $O_3$ concentrations for multiple lag periods were associated with lung function decrements, possibly indicating that multiple modes of action may be involved in the responses. Activation of bronchial C-fibers (Section 5.3.2) may lead to decreases in lung function as an immediate response to $O_3$ exposure, and increased airway hyperresponsiveness to antigens resulting from sensitization of airways by $O_3$ (Section 5.3.5) may mediate lung function responses associated with lagged or multiday $O_3$ exposures (Peden, 2011).

For single- and multi-day average $O_3$ concentrations, lung function decrements were associated with 1-h max, 8-h max, and 24-h avg $O_3$, with no strong difference in the consistency or magnitude of association among the averaging times. For example, among studies that examined multiple averaging times, Spektor and Lippmann (1991) found a larger magnitude of association for 1-h max $O_3$ than for 24-h avg $O_3$. However, other studies found larger magnitudes of association for longer averaging times [8-h max in Chan and Wu (2005) and 12-h avg in Thaller et al. (2008)] than for 1-h max $O_3$. Other studies found no clear difference among $O_3$ averaging times (Jalaludin et al., 2000; Chen et al., 1999; Scarlett et al., 1996; Berry et al., 1991).

Several studies found that associations with lung function decrements persisted at lower ambient $O_3$ concentrations. For $O_3$ concentrations averaged up to 1 hour during outdoor recreation or exercise, associations were found in analyses restricted to $O_3$ concentrations <80 ppb (Spektor et al., 1988a; Spektor et al., 1988b), 60 ppb (Brunekreef et al., 1994; Spektor et al., 1988a), and 50 ppb (Brunekreef et al., 1994). Among outdoor workers, Brauer et al. (1996) found a robust association using daily 1-h max $O_3$ concentrations <40 ppb. Ulmer et al. (1997) found a robust association in schoolchildren using 30-min max $O_3$ concentrations <60 ppb. For 8-h avg $O_3$ concentrations, associations with lung function decrements in children with asthma were found to persist at concentrations <80 ppb in a U.S. multicity study (for lag 1-5 avg) (Mortimer et al., 2002) and <51 ppb in a study conducted in the Netherlands (for lag 2) (Gielen et al., 1997).

Evidence did not demonstrate strong confounding by meteorological factors or copollutant exposures. Most $O_3$ effect estimates for lung function were robust to adjustment for temperature, humidity, and copollutants such as $PM_{2.5}$, $PM_{10}$, $NO_2$, or

$SO_2$. Although examined in few epidemiologic studies, $O_3$ was associated with decreases in lung function with adjustment for pollen or acid aerosols.
The consistency of association in the collective body of evidence with and without adjustment for ambient copollutant concentrations and meteorological factors combined with evidence from controlled human exposure studies for the direct effects of $O_3$ exposure provide strong support for the independent effects of short-term ambient $O_3$ exposure on lung function decrements.

#### 6.2.1.3 Toxicology

The 2006 $O_3$ AQCD concluded that pulmonary function decrements occur in a number of species with acute exposures (≤ 1 week), ranging from 0.25 to 0.4 ppm $O_3$ (U.S. EPA, 2006b). Early work has demonstrated that during acute exposure of ~0.2 ppm $O_3$ in rats, the most commonly observed alterations are increased frequency of breathing and decreased tidal volume (i.e., rapid, shallow breathing). Decreased lung volumes are observed in rats with acute exposures to 0.5 ppm $O_3$. At concentrations of ≥ 1 ppm, breathing mechanics (compliance and resistance) are also affected. Exposures of 6 hours/day for 5 days create a pattern of attenuation of pulmonary function decrements in both rats and humans without concurrent attenuation of lung injury and morphological changes, indicating that the attenuation did not result in protection against all the effects of $O_3$ (Tepper et al., 1989).
A number of studies examining the effects of $O_3$ on pulmonary function in rats, mice, and dogs are described in Table 6-13 on page 6-91 (U.S. EPA, 1996m) of the 1996 $O_3$ AQCD, and Annex Table AX5-11 on page AX5-34 (U.S. EPA, 2006g) of the 2006 $O_3$ AQCD (U.S. EPA, 2006b, 1996a). Lung imaging studies using hyperpolarized $^3$He provide evidence of ventilation abnormalities in rats following exposure to 0.5 ppm $O_3$ (Crémillieux et al., 2008). Rats were exposed to 0.5 ppm $O_3$ for 2 or 6 days, either continuously (22 hours/day) or alternatingly (12 hours/day). Dynamic imaging of lung filling (2 mL/sec) revealed delayed and incomplete filling of lung segments and lobes. Abnormalities were mainly found in the upper regions of the lungs and proposed to be due to the spatial distribution of $O_3$ exposure within the lung. Although the small number of animals used in the study (n = 3 to 7/group) makes definitive conclusions difficult, the authors suggest that the delayed filling of lung lobes or segments is likely a result of an increase in airway resistance brought about by narrowing of the peripheral small airways.

### 6.2.2 Airway Hyperresponsiveness

Airway hyperresponsiveness refers to a condition in which the conducting airways undergo enhanced bronchoconstriction in response to a variety of stimuli. Airway responsiveness is typically quantified by measuring changes in pulmonary function (e.g., $FEV_1$ or specific airway resistance [sRaw]) following the inhalation of an aerosolized specific (allergen) or nonspecific (e.g., methacholine)

bronchoconstricting agent or administration of another stimulus such as exercise or cold air. People with asthma are generally more sensitive to bronchoconstricting agents than those without asthma, and the use of an airway challenge to inhaled bronchoconstricting agents is a diagnostic test in asthma. Standards for airway responsiveness testing have been developed for the clinical laboratory (ATS, 2000a), although variation in methodology for administering the bronchoconstricting agent may affect the results (Cockcroft et al., 2005). There is a wide range of airway responsiveness in people without asthma, and responsiveness is influenced by a wide range of factors, including cigarette smoke, pollutant exposures, respiratory infections, occupational exposures, and respiratory irritants. Airways hyperresponsiveness in response to $O_3$ exposure has not been examined widely in epidemiologic studies; such evidence is derived primarily from controlled human exposure and toxicological studies as described below.

#### 6.2.2.1 Controlled Human Exposures

Beyond its direct effect on lung function, experimental $O_3$ exposure has been shown to cause an increase in airway responsiveness in human subjects. Increased airway responsiveness can be an important consequence of exposure to ambient $O_3$, because the airways are then predisposed to narrowing upon inhalation of a variety of ambient stimuli.

Increases in airway responsiveness have been reported for exposures to 80 ppb $O_3$ and above. Horstman et al. (1990) evaluated airway responsiveness to methacholine in young healthy adults (22 M) exposed to 80, 100, and 120 ppb $O_3$ (6.6 hours, quasi continuous moderate exercise, 39 L/min). Dose-dependent decreases of 33, 47, and 55% in the cumulative dose of methacholine required to produce a 100% increase in sRaw after exposure to $O_3$ at 80, 100, and 120 ppb, respectively, were reported. Molfino et al. (1991) reported increased allergen-specific airway responsiveness in adults with mild asthma exposed to 120 ppb $O_3$ (1 hour resting exposure). Due to safety concerns, however, the exposures in the Molfino et al. (1991) study were not randomized with FA conducted first and $O_3$ exposure second. Attempts to reproduce the findings of Molfino et al. (1991) using a randomized exposure design have not found statistically significant changes in airway responsiveness at such low levels of $O_3$ exposure. At a considerably higher exposure to 250 ppb $O_3$ (3 hours, light-to-moderate intermittent exercise, 30 L/min), Jorres et al. (1996) found significant increases in specific and non-specific airway responsiveness of adults with mild asthma 3 hours following $O_3$ exposure. Kehrl et al. (1999) found increased reactivity to house dust mite antigen in adults with mild asthma and atopy 16-18 hours after exposure to 160 ppb $O_3$ (7.6 hours, light quasi continuous exercise, 25 L/min). Holz et al. (2002) demonstrated that repeated daily exposure to lower concentrations of 125 ppb $O_3$ (3 hours for four consecutive days; intermittent exercise, 30 L/min) causes an increased response to allergen challenge at 20 hours postexposure in allergic airway disease.

Ozone exposure of subjects with asthma, who characteristically have increased airway responsiveness at baseline relative to healthy controls (by nearly two orders of magnitude), can cause further increases in responsiveness (Kreit et al., 1989). Similar relative changes in airway responsiveness are seen in subjects with asthma and healthy control subject exposed to $O_3$ despite their markedly different baseline airway responsiveness. Several studies (Kehrl et al., 1999; Jorres et al., 1996; Molfino et al., 1991) have suggested an increase in specific (i.e., allergen-induced) airway reactivity. An important aspect of increased airway responsiveness after $O_3$ exposure is that this may provide biological plausibility for associations observed between increases in ambient $O_3$ concentration and increased respiratory symptoms in children with asthma (Section 6.2.4.1) and increased hospital admissions and ED visits for asthma (Section 6.2.7).

Changes in airway responsiveness after $O_3$ exposure appear to resolve more slowly than changes in $FEV_1$ or respiratory symptoms (Folinsbee and Hazucha, 2000). Studies suggest that $O_3$-induced increases in airway responsiveness usually resolve 18 to 24 hours after exposure, but may persist in some individuals for longer periods (Folinsbee and Hazucha, 1989). 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 $FEV_1$ (Gong et al., 1997a; Folinsbee et al., 1994; Kulle et al., 1982; Dimeo et al., 1981). Increases in airway responsiveness do not appear to be strongly associated with decrements in lung function or increases in symptoms (Aris et al., 1995). Recently, Que et al. (2011) assessed methacholine responsiveness in healthy young adults (83M, 55 F) one day after exposure to 220 ppb $O_3$ and FA for 2.25 hours (alternating 15 min periods of rest and brisk treadmill walking). Increases in airways responsiveness at 1 day post-$O_3$ exposure were not correlated with $FEV_1$ responses immediately following the $O_3$ exposure or with changes in epithelial permeability assessed 1 day post-$O_3$ exposure.

### 6.2.2.2 Toxicology

In addition to human subjects, a number of species, including nonhuman primates, dogs, cats, rabbits, and rodents, have been used to examine the effect of $O_3$ exposure on airway hyperresponsiveness (see Table 6-14 on page 6-93 (U.S. EPA, 1996n) of the 1996 $O_3$ AQCD and Annex Table AX5-12 on page AX5-36 (U.S. EPA, 2006h) of the 2006 $O_3$ AQCD). With a few exceptions, commonly used animal models have been guinea pigs, rats, or mice acutely exposed to $O_3$ concentrations of 1 to 3 ppm to induce airway hyperresponsiveness. These animal models are helpful for determining underlying mechanisms of general airway hyperresponsiveness and are relevant for understanding airway responses in humans. Although 1-3 ppm may seem like a high exposure concentration, based on $^{18}O_3$ (oxygen-18-labeled $O_3$) in the BALF of humans and rats, an exposure of 0.4 ppm $O_3$ in exercising humans appears roughly equivalent to an exposure of 2 ppm in resting rats (Hatch et al., 1994).

A limited number of studies have observed airway hyperresponsiveness in rodents and guinea pigs after exposure to less than 0.3 ppm $O_3$. As previously reported in the 2006 $O_3$ AQCD, one study demonstrated that a very low concentration of $O_3$ (0.05 ppm for 4 hours) induced airway hyperresponsiveness in some of the nine strains of rats tested (Depuydt et al., 1999). This effect occurred at a concentration of $O_3$ that was much lower than has been reported to induce airway hyperresponsiveness in any other species. Similar to the effects of $O_3$ on other endpoints, these observations suggest that a genetic component plays an important role in $O_3$-induced airway hyperresponsiveness in this species and warrants verification in other species. More recently, Chhabra et al. (2010) demonstrated that exposure of ovalbumin (OVA)-sensitized guinea pigs to 0.12 ppm for 2 hours/day for 4 weeks produced specific airway hyperresponsiveness to an inhaled OVA challenge. Interestingly, in this study, dietary supplementation of the guinea pigs with vitamins C and E ameliorated a portion of the airway hyperresponsiveness as well as indices of inflammation and oxidative stress. Larsen and colleagues conducted an $O_3$ C-R study in mice sensitized by 10 daily inhalation treatments with an OVA aerosol (Larsen et al., 2010). Although airway responsiveness to methacholine was increased in non-sensitized animals exposed to a single 3-hour exposure to 0.5, but not 0.1 or 0.25 ppm $O_3$, airway hyperresponsiveness was observed after exposure to 0.1 and 0.25 ppm $O_3$ in OVA-sensitized mice.

In order to evaluate the ability of $O_3$ to enhance specific and non-specific airway responsiveness, it is important to take into account the phenomenon of attenuation in the effects of $O_3$. Several studies have clearly demonstrated that some effects caused by acute exposure are absent after repeated or prolonged exposures to $O_3$. The ability of the pulmonary system to adapt to repeated insults to $O_3$ is complex, however, and experimental findings for attenuation to $O_3$-induced airway hyperresponsiveness are inconsistent. Airway hyperresponsiveness was observed in mice after a 3-hour exposure but not in mice exposed continuously for 72 hours to 0.3 ppm (Johnston et al., 2005b). However, the Chhabra study demonstrated $O_3$-induced airway hyperresponsiveness in guinea pigs exposed for 2 hours/day for 10 days (Chhabra et al., 2010). Besides the obvious species disparity, these studies differ in that the mice were exposed continuously for 72 hours, whereas the guinea pigs were exposed intermittently over 10 days, suggesting that attenuation might be lost with periods of rest in between $O_3$ exposures.

Overall, numerous toxicological studies have demonstrated that $O_3$-induced airway hyperresponsiveness occurs in guinea pigs, rats, and mice after either acute or repeated exposure to relevant concentrations of $O_3$. The mechanisms by which $O_3$ enhances the airway responsiveness to either specific (e.g., OVA) or non-specific (e.g., methacholine) bronchoprovocation are not clear but appear to be associated with complex cellular and biochemical changes in the conducting airways. A number of potential mediators and cells may play a role in $O_3$-induced airway hyperresponsiveness; mechanistic studies are discussed in greater detail in Section 5.3.

### 6.2.3 Pulmonary Inflammation, Injury and Oxidative Stress

In addition to physiological pulmonary responses, respiratory symptoms, and airway hyperresponsiveness, $O_3$ exposure has been shown to result in increased epithelial permeability and respiratory tract inflammation. 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 at-risk 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 only been directly observed in animals exposed to $O_3$. It is also possible that the profile of response can be altered in persons with pre-existing pulmonary disease (e.g., asthma, COPD) or smokers. Oxidative stress has been shown to play a key role in initiating and sustaining $O_3$-induced inflammation. Secondary oxidation products formed as a result of reactions between $O_3$ and components of the ELF can increase the expression of cytokines, chemokines, and adhesion molecules and enhance airway epithelium permeability (Section 5.3.3. and Section 5.3.4).

#### 6.2.3.1 Controlled Human Exposures

As reported in studies reviewed in the 1996 and 2006 $O_3$ AQCDs, acute $O_3$ exposure initiates an acute inflammatory response throughout the respiratory tract that has been observed to persist for at least 18-24 hours postexposure. A meta-analysis of 21 studies (Mudway and Kelly, 2004a) for varied experimental protocols (80-600 ppb $O_3$; 1-6.6 hours duration; light to heavy exercise; bronchoscopy at 0-24 hours post-$O_3$ exposure) showed that neutrophils (PMN) influx in healthy subjects was linearly associated ($p < 0.01$) with total $O_3$ dose (i.e., the product of $O_3$ concentration, exposure duration, and $\dot{V}_E$). As with $FEV_1$ responses to $O_3$, within-individual inflammatory responses to $O_3$ are generally reproducible and correlated between repeat exposures (Holz et al., 1999). Some individuals also appear to be intrinsically more susceptible to increased inflammatory responses to $O_3$ exposure (Holz et al., 2005).

The presence of PMNs in the lung has long been accepted as a hallmark of inflammation and is an important indicator that $O_3$ causes inflammation in the lungs. Neutrophilic inflammation of tissues indicates activation of the innate immune system and requires a complex series of events that are normally followed by processes that clear the evidence of acute inflammation. Inflammatory effects have been assessed in vivo by lavage (proximal airway and bronchoalveolar), bronchial

biopsy, and more recently, induced sputum. A single acute exposure (1-4 hours) of humans to moderate concentrations of $O_3$ (200-600 ppb) while exercising at moderate to heavy intensities results in a number of cellular and biochemical changes in the lung, including an inflammatory response characterized by increased numbers of PMNs, increased permeability of the epithelial lining of the respiratory tract, cell damage, and production of proinflammatory cytokines and prostaglandins (U.S. EPA, 2006b). These changes also occur in humans exposed to 80 and 200 ppb $O_3$ for 6-8 hours (Alexis et al., 2010; Peden et al., 1997; Devlin et al., 1991). Significant ($p = 0.002$) increases in sputum PMN (16-18 hours postexposure) relative to FA responses have been recently reported for 60 ppb $O_3$ which is the lowest exposure concentration that has been investigated in young healthy adults (Kim et al., 2011). Soluble mediators of inflammation such as the cytokines (e.g., IL-6, IL-8) and arachidonic acid metabolites (e.g., prostaglandin [PG]$E_2$, $PGF_{2\alpha}$, thromboxane, and leukotrienes [LTs] such as $LTB_4$) have been measured in the BALF 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 responsiveness following $O_3$ exposure. The possible relationship between repetitive bouts of acute inflammation in humans caused by $O_3$ and the development of chronic respiratory disease is unknown.

### Asthma

Inflammatory responses to $O_3$ exposure have also been studied in subjects with asthma. Individuals with asthma exposed to 200 ppb $O_3$ for 4-6 hours with exercise show significantly more neutrophils in BALF (18 hours postexposure) than similarly exposed healthy individuals (Scannell et al., 1996; Basha et al., 1994). In subjects with allergic asthma who tested positive for *Dermatophagoides farinae* antigen, there was an eosinophilic inflammation (2-fold increase), as well as neutrophilic inflammation (3-fold increase) 18 hours after exposure to 160 ppb $O_3$ for 7.6 hours with exercise (Peden et al., 1997). In a study of subjects with intermittent asthma exposed to 400 ppb $O_3$ for 2 hours, increases in eosinophil cationic protein, neutrophil elastase and IL-8 were found to be significantly increased 16 hours postexposure and comparable in induced sputum and BALF (Hiltermann et al., 1999). At 18 hours post-$O_3$ exposure (200 ppb, 4 hours with exercise) and corrected for FA responses, Scannell et al. (1996) found significantly increased neutrophils in 18 adults with asthma (12%) compared to 20 healthy subjects (4.5%). This difference in inflammatory response was observed despite no group differences in spirometric responses to $O_3$. Scannell et al. (1996) also reported that IL-8 tends to be higher in the BALF of subjects with asthma compared to those without asthma following $O_3$ exposure, suggesting a possible mediator for the significantly increased neutrophilic inflammation in those subjects. Bosson et al. (2003) found significantly greater epithelial expression of IL-5, IL-8, granulocyte-macrophage colony-stimulating factor and epithelial cell-derived neutrophil-activating peptide-78 in subjects with asthma compared to healthy subjects following exposure to 200 ppb $O_3$ for 2 hours. In contrast, Stenfors et al. (2002) did not detect a difference in the $O_3$-induced increases in neutrophil numbers between 15 subjects with mild asthma and 15

healthy subjects by bronchial wash at the 6 hours postexposure time point. However, the subjects with asthma were on average 5 years older than the healthy subjects in this study, and it is not yet known how age affects inflammatory responses. It is also possible that the time course of neutrophil influx differs between healthy individuals and those with asthma. Differences between subjects with asthma and healthy subjects in $O_3$-mediated activation of innate and adaptive immune responses have been observed in two studies (Hernandez et al., 2010; Bosson et al., 2003), as discussed in Section 6.2.5.4 and Section 5.4.2.2.

Vagaggini et al. (2002) investigated the effect of prior allergen challenge on responses in subjects with mild asthma exposed for 2 hours to 270 ppb $O_3$ or filtered air. At 6 hours postexposure, eosinophil numbers in induced sputum were found to be significantly greater after $O_3$ than after air exposures. Studies such as this suggest that the time course of eosinophil and neutrophil influx following $O_3$ exposure can occur at levels detectable within the airway lumen by as early as 6 hours. They also suggest that the previous or concurrent activation of pro-inflammatory pathways within the airway epithelium may enhance the inflammatory effects of $O_3$. For example, in an in vitro study of primary human nasal epithelial cells and BEAS-2B cell line, cytokine production induced by rhinovirus infection was enhanced synergistically by concurrent exposure to $O_3$ at 200 ppb for 3 hours (Spannhake et al., 2002).

A few studies have evaluated the effects of corticosteroid usage on the response of subjects with asthma to $O_3$. Vagaggini et al. (2007) evaluated whether corticosteroid usage would prevent $O_3$-induced lung function decrements and inflammatory responses in a group of subjects with mild persistent asthma (n = 9; 25 ± 7 years). In this study, subjects with asthma were randomly exposed on four occasions to 270 ppb $O_3$ or FA for 2 hours with moderate exercise. Exposures were preceded by four days of treatment with prednisone or placebo. Pretreatment with corticosteroids prevented an inflammatory response in induced sputum at 6 hours postexposure. $FEV_1$ responses were, however, not prevented by corticosteroid treatment and were roughly equivalent to those observed following placebo. Vagaggini et al. (2001) also found budesonide to decrease airway neutrophil influx in subjects with asthma following $O_3$ exposure. In contrast, inhalation of corticosteroid budesonide failed to prevent or attenuate $O_3$-induced responses in healthy subjects as assessed by measurements of lung function, bronchial reactivity and airway inflammation (Nightingale et al., 2000). High doses of inhaled fluticasone and oral prednisolone have each been reported to reduce inflammatory responses to $O_3$ in healthy individuals (Holz et al., 2005).

Stenfors et al. (2010) exposed adults with persistent asthma (n = 13; aged 33 years) receiving chronic inhaled corticosteroid therapy to 200 ppb $O_3$ for 2 hours with moderate exercise. At 18 hours postexposure, there was a significant $O_3$-induced increase in bronchioalveolar lavage (BAL) neutrophils, but not eosinophils. Bronchial biopsy also showed a significant $O_3$-induced increase in mast cells. Results from this study suggest that the protective effect of acute corticosteroid

therapy against inflammatory responses to $O_3$ in subjects with asthma demonstrated by Vagaggini et al. (2007) may be lost with continued treatment regimes.

### Associations between Inflammation and $FEV_1$ responses

Studies reviewed in the 2006 $O_3$ AQCD reported that inflammatory responses do not appear to be correlated with lung function responses in either subjects with asthma or healthy subjects. In healthy adults (14 M, 6 F) and volunteers with asthma (12 M, 6 F) exposed to 200 ppb $O_3$ (4 hours with moderate quasi continuous exercise, $\dot{V}_E$ = 44 L/min), percent PMN and total protein in BAL fluids were significantly increased in the subjects with asthma relative to the healthy controls. Spirometric measures of lung function were significantly decreased following the $O_3$ exposure in both groups, but were not significantly different between the subjects with asthma and healthy subjects. Effects of $O_3$ on PMN and total protein were not correlated with changes in $FEV_1$ or FVC (Balmes et al., 1997; Balmes et al., 1996). Devlin et al. (1991) exposed healthy adults (18 M) to 80 and 100 ppb $O_3$ (6.6-hours with moderate quasi continuous exercise, 40 L/min). In BAL fluid collected 18 hours after exposure to 100 ppb $O_3$, significant increases in PMNs, protein, PGE2, fibronectin, IL-6, lactate dehydrogenase, and α-1 antitrypsin were found compared to FA. Similar but smaller increases in all mediators were found after exposure to 80 ppb $O_3$ except for protein and fibronectin. Changes in BAL markers were not correlated with changes in $FEV_1$. Holz et al. (1999) examined inflammatory responses in healthy subjects (n = 21) and those with asthma (n = 15) exposed to 125 and 250 ppb $O_3$ (3 hours, light intermittent exercise, 26 L/min). Significantly increased percent PMN in sputum due to $O_3$ exposure was observed in both healthy subjects and those with asthma following the 250 ppb exposure. At the lower 125 ppb exposure, only the group with asthma experienced statistically significant increases in the percent PMN. Significant decrements in $FEV_1$ were only found following exposure to 250 ppb; these changes in $FEV_1$ did not differ significantly between the group with asthma and healthy group and were not correlated with changes in PMN levels. Peden et al. (1997) also found no correlation between PMN and $FEV_1$ responses in 8 individuals with asthma exposed to 160 ppb $O_3$ for 7.6 hours with light-to-moderate exercise ($\dot{V}_E$ = 25 L/min). However, a marginally significant correlation (r = -0.69, two-tailed p = 0.08, n = 7) was observed between increases in the percentage of eosinophils and $FEV_1$ responses following $O_3$ exposure.

In contrast to these earlier findings, Vagaggini et al. (2010) recently reported a significant (r = 0.61, p = 0.015) correlation between changes in $FEV_1$ and changes in sputum neutrophils in subjects with mild-to-moderate asthma (n = 23; 33 ± 11 years) exposed to 300 ppb $O_3$ for 2 hours with moderate exercise. Eight subjects were categorized as “responders” based on >10% $FEV_1$ decrements. There were no baseline differences between responders and nonresponders. However, at 6 hours post-$O_3$ exposure, sputum neutrophils were significantly increased by 15% relative to FA in responders. The neutrophil increase in responders was also significantly greater than the 0.2% increase in nonresponders. Interestingly, the nonresponders in the Vagaggini et al. (2010) study experienced a significant $O_3$-induced 11.3%

increase in sputum eosinophils, while responders had an nonsignificant 2.6% decrease.

### Time Course of the Inflammatory Response

The time course of the inflammatory response to $O_3$ in humans has not been fully characterized. Different markers exhibit peak responses at different times. Studies in which lavages were performed 1 hour after $O_3$ exposure (1 hours at 400 ppb or 4 hours at 200 ppb) have demonstrated that the inflammatory responses are quickly initiated (Torres et al., 1997; Devlin et al., 1996; Schelegle et al., 1991). Inflammatory mediators and cytokines such as IL-8, IL-6, and $PGE_2$ are greater at 1 hour than at 18 hours post-$O_3$ exposure (Torres et al., 1997; Devlin et al., 1996). However, IL-8 still remained elevated at 18 hours post-$O_3$ exposure (4 hours at 200 ppb $O_3$ versus FA) in healthy subjects (Balmes et al., 1996). Schelegle et al. (1991) found increased PMNs in the "proximal airway" lavage at 1, 6, and 24 hours after $O_3$ exposure (4 hours at 200 ppb $O_3$), with a peak response at 6 hours. However, at 18-24 hours after $O_3$ exposure, PMNs remain elevated relative to 1 hour postexposure (Torres et al., 1997; Schelegle et al., 1991).

### Genetic Polymorphisms

Alexis et al. (2010) recently reported that a 6.6-hour exposure with moderate exercise to 80 ppb $O_3$ caused increased sputum neutrophil levels at 18 hours postexposure in young healthy adults (n = 15; 24 ± 1 years). In a prior study, Alexis et al. (2009) found genotype effects on inflammatory responses to $O_3$, but not lung function responses following a 2-hour exposure to 400 ppb $O_3$. At 4 hours post-$O_3$ exposure, groups of both GSTM1 genotypes had significant increases in sputum neutrophils with a tendency for a greater increase in GSTM1-sufficient than null individuals. At 24 hours postexposure, neutrophils had returned to baseline levels in the GSTM1-sufficient individuals. In the GSTM1-null subjects, however, neutrophil levels increased further from 4 hours to 24 hours and were significantly greater than both baseline levels and 24 hours levels in GSTM1-sufficient individuals. Alexis et al. (2009) found that GSTM1-sufficient individuals (n = 19; 24 ± 3 years) had a decrease in macrophage levels at 4-24 hours postexposure to 400 ppb $O_3$ for 2 hours with exercise. These studies also provide evidence for activation of innate immunity and antigen presentation, as discussed in Section 5.3.6. Effects of the exposure apart from $O_3$ cannot be ruled out in the Alexis et al. (2010); (2009) studies, however, since no FA exposure was conducted.

Vagaggini et al. (2010) examined $FEV_1$ and sputum neutrophils in subjects with mild-to-moderate asthma (n = 23; 33 ± 11 years) exposed to 300 ppb $O_3$ for 2 hours with moderate exercise. Six of the subjects were NQO1 wild type and GSTM1 null, but this genotype was not found to be associated with $O_3$-induced changes in lung function or inflammatory responses to $O_3$. Kim et al. (2011) showed a significant ($p = 0.002$) increase in sputum neutrophil levels following a 6.6-hour exposure to

60 ppb $O_3$ relative to FA in young healthy adults (13 F, 11 M; 25.0 ± 0.5 years). There was no significant effect of GSTM1 genotype (half GSTM1-null) on the inflammatory responses observed in these individuals. Previously, inflammatory responses had only been evaluated down to a level of 80 ppb $O_3$.

### Repeated Exposures

Changes in markers from BALF following both 2-hour (Devlin et al., 1997) and 4-hour (Jorres et al., 2000; Christian et al., 1998) repeated $O_3$ exposures (up to 5 days) indicate that there is ongoing cellular damage irrespective of the attenuation of some cellular inflammatory responses of the airways, pulmonary function, and symptom responses. Devlin et al. (1997) found that several indicators of inflammation (e.g., PMN, IL-6, $PGE_2$, fibronectin) were attenuated after 5 days of exposure (i.e., values were not different from FA). However, other markers (LDH, IL-8, total protein, epithelial cells) did not show attenuation, suggesting that tissue damage probably continues to occur during repeated exposure. Some cellular responses did not return to baseline levels for more than 10-20 days following $O_3$ exposure. Christian et al. (1998) showed decreased numbers of neutrophils and a decrease in IL-6 levels in healthy adults after 4 days of exposure versus the single exposure to 200 ppb $O_3$ for 4 hours. Jorres et al. (2000) also found that both functional and BALF cellular responses to $O_3$ were abolished at 24 hours postexposure following the fourth exposure day. However, levels of total protein, IL-6, IL-8, reduced glutathione and ortho-tyrosine were still increased significantly. In addition, visual scores (bronchoscopy) for bronchitis and erythema and the numbers of neutrophils in bronchial mucosal biopsies were increased. Results indicate that, despite an attenuation of some markers of inflammation in BALF and pulmonary function decrements, inflammation within the airways persists following repeated exposure to $O_3$. The continued presence of cellular injury markers indicates a persistent effect that may not necessarily be recognized due to the attenuation of spirometric and symptom responses.

### Epithelial Permeability

A number of studies show that $O_3$ exposures increase epithelial cell permeability through direct (technetium-99m labeled diethylene triamine pentaacetic acid, $^{99m}$Tc-DTPA, clearance) and indirect (e.g., increased BALF albumin, protein) techniques. Kehrl et al. (1987) showed increased $^{99m}$Tc-DTPA clearance in healthy young adults (age 20-30 yrs) at 75 minutes postexposure to 400 ppb $O_3$ for 2 hours. Also in healthy young adults (age 26 ± 2 yrs), Foster and Stetkiewicz (1996) have shown that increased $^{99m}$Tc-DTPA clearance persists for at least 18-20 hours post-$O_3$ exposure (130 minutes to average $O_3$ concentration of 240 ppb), and the effect is greater at the lung apices than at the base. In a older group of healthy adults (mean age = 35 yrs), Morrison et al. (2006) observed $^{99m}$Tc-DTPA clearance at 1 hours and 6 hours postexposure to $O_3$ (100 and 400 ppb; 1 hour; moderate intermittent exercise,

$\dot{V}_E$ = 40 L/min) to be similar and not statistically different from $^{99m}$Tc-DTPA clearance at 1-hour postexposure to FA (1 hour; $\dot{V}_E$ = 40 L/min).

Increased BALF protein, suggesting $O_3$-induced changes in epithelial permeability, have also been reported at 1 hour and 18 hours postexposure (Devlin et al., 1997; Balmes et al., 1996). Meta-analysis of results from 21 publications (Mudway and Kelly, 2004a) for varied experimental protocols (80-600 ppb $O_3$; 1-6.6 hours duration; light to heavy exercise; bronchoscopy at 0-24 hours post-$O_3$ exposure), showed that increased BALF protein is associated with total inhaled $O_3$ dose (i.e., the product of $O_3$ concentration, exposure duration, and $\dot{V}_E$).

It has been postulated that changes in permeability associated with acute inflammation may provide increased access of inhaled antigens, particles, and other inhaled substances deposited on lung surfaces to the smooth muscle, interstitial cells, and the blood. Hence, increases in epithelial permeability following $O_3$ exposure might lead to increases in airway responsiveness to specific and nonspecific agents. Que et al. (2011) investigated this hypothesis in healthy young adults (83M, 55 F) exposed to 220 ppb $O_3$ for 2.25 hours (alternating 15 min periods of rest and brisk treadmill walking). As has been observed by others for $FEV_1$ responses, within-individual changes in permeability were correlated between sequential $O_3$ exposures. This indicates intrinsic differences in susceptibility to epithelial damage from $O_3$ exposure among individuals. Increases in epithelial permeability at 1 day post-$O_3$ exposure were not correlated with $FEV_1$ responses immediately following $O_3$ exposure or with changes in airway responsiveness to methacholine assessed 1 day post-$O_3$ exposure. The authors concluded that changes in $FEV_1$, permeability, and airway responsiveness following $O_3$ exposure were relatively constant over time in young healthy adults, although these responses appear to be mediated by differing physiologic pathways.

### 6.2.3.2 Epidemiology

In the 2006 $O_3$ AQCD, epidemiologic evidence of associations between short-term increases in ambient $O_3$ concentration (30-min or 1-h max) and changes in pulmonary inflammation was limited to a few observations of increases in nasal lavage levels of inflammatory cell counts, eosinophilic cationic protein, and myeloperoxidases (U.S. EPA, 2006b). In recent years, there has been a large increase in the number of studies assessing ambient $O_3$-related changes in pulmonary inflammation and oxidative stress, types of biological samples collected (i.e., lower airway), and types of indicators examined. Most studies collected samples every 1 to 3 weeks resulting in a total of 3 to 8 samples per subject. These recent studies form a larger base to establish coherence with findings from controlled human exposure and animal studies that have measured the same or related biological markers. Additionally, results from these studies provide further biological plausibility for the associations observed between ambient $O_3$ concentrations and respiratory symptoms and asthma exacerbations.

Despite the strengths of studies of inflammation, research in this field continues to develop, and several uncertainties are recognized that may limit inferences from results indicating the effects of ambient $O_3$ exposure. Current areas of development include examination of the clinical relevance of the observed magnitudes of changes in biological markers of pulmonary inflammation (Murugan et al., 2009; Duramad et al., 2007), characterization of the time course of changes between biomarker levels and other endpoints of respiratory morbidity, development of standardized methodologies for collection, improvement of the sensitivity and specificity of assay methods, and characterization of subject factors (e.g., asthma severity, recent medication use) that contribute to inter-individual variability. These sources of uncertainty may contribute to differences in findings among studies.

Although most of the biomarkers examined in epidemiologic studies were not specific to the lung, most studies collected exhaled breath, exhaled breath condensate (EBC), nasal lavage fluid, or induced sputum with the aim of monitoring inflammatory responses in airways, as opposed to monitoring systemic responses in blood. The biomarker most frequently measured was exhaled nitric oxide (eNO), likely related to its ease of collection in the field and automated measurement. Other biological markers were examined in EBC, induced sputum, and nasal lavage fluid, which are hypothesized to represent the fluid lining the lower or upper airways and contain cytokines, cells, and/or markers of oxidative stress that mediate inflammatory responses (Balbi et al., 2007; Howarth et al., 2005; Hunt, 2002). Table 6-15 presents the locations, time periods, and ambient $O_3$ concentrations for studies examining associations with biological markers of pulmonary inflammation and oxidative stress. Many studies found that short-term increases in ambient $O_3$ concentration were associated with increases in pulmonary inflammation and oxidative stress, in particular, studies of children with asthma conducted in Mexico City, Mexico (Figure 6-11 [and Table 6-16] and Table 6-17).

## Table 6-15 Mean and upper percentile $O_3$ concentrations in epidemiologic studies of biological markers of pulmonary inflammation and oxidative stress.

| Study* | Location | Study Period | $O_3$ Averaging Time | Mean/Median Concentration (ppb) | Upper Percentile Concentrations (ppb) |
|---|---|---|---|---|---|
| Barraza-Villarreal et al. (2008) | Mexico City, Mexico | June 2003-June 2005 | 8-h moving avg | 31.6 | Max: 86.3 |
| Berhane et al. (2011) | 13 Southern California Communities | Sept 2004-June 2005 | 8-h avg (10 a.m.-6 p.m.) | NR | NR |
| Liu et al. (2009a) | Windsor, ON, Canada | Oct-Dec 2005 | 24-h avg | 13.0 | 95th: 26.5 |
| Khatri et al. (2009) | Atlanta, GA | May-Sept 2003, 2005, 2006 | 8-h max | median: 61[a] | 75th: 74[a] |
| Qian et al. (2009) | Boston, MA; New York, NY; Denver, CO; Philadelphia, PA; San Francisco, CA; Madison, WI (SOCS) | Feb 1997 - Jan 1999 | 8-h max | 33.6 | 75th: 44.4, Max: 91.5 |
| Romieu et al. (2008) | Mexico City, Mexico | Jan-Oct 2004 | 8-h max | 31.1 | 75th: 38.3, Max: 60.7 |
| Sienra-Monge et al. (2004) | Mexico City, Mexico | All-year 1999-2000 | 8-h max | 66.2 | Max: 142.5 |
| Ferdinands et al. (2008) | Suburb of Atlanta, GA | Aug 2004 | 1-h max | 61 (median) | 75th: 67 |
| Chimenti et al. (2009) | Sicily, Italy | Nov, Feb, July, year NR | 8-h avg (7 a.m.-3 p.m.) | November: 32.7 (pre-race), 35.1 (race)[b]<br>February: 37.0 (pre-race), 30.8 (race)[b]<br>July: 51.2 (pre-race), 46.1 (race)[b] | NR |
| Nickmilder et al. (2007) | Southern Belgium | July-Aug 2002 | 1-h max | NR | Max (across 6 camps): 24.6-112.8[b] |
| | | | 8-h max | NR | Max (across 6 camps): 19.0-81.1[b] |
| Delfino et al. (2010a) | Los Angeles, CA | Warm and cold season 2005-2007 | 24-h avg | Warm season median: 32.1[c] | Max: 76.4[c] |
| | | | | Cool season median: 19.1[c] | Max: 44.9[c] |
| Adamkiewicz et al. (2004) | Steubenville, OH | Sept-Dec 2000 | 24-h avg | 15.3 | 75th: 20.2, Max: 32.2 |
| | | | 1-h avg[d] | 19.8 | 75th: 27.5, Max: 61.6 |

*Note: Studies presented in order of first appearance in the text of this section.

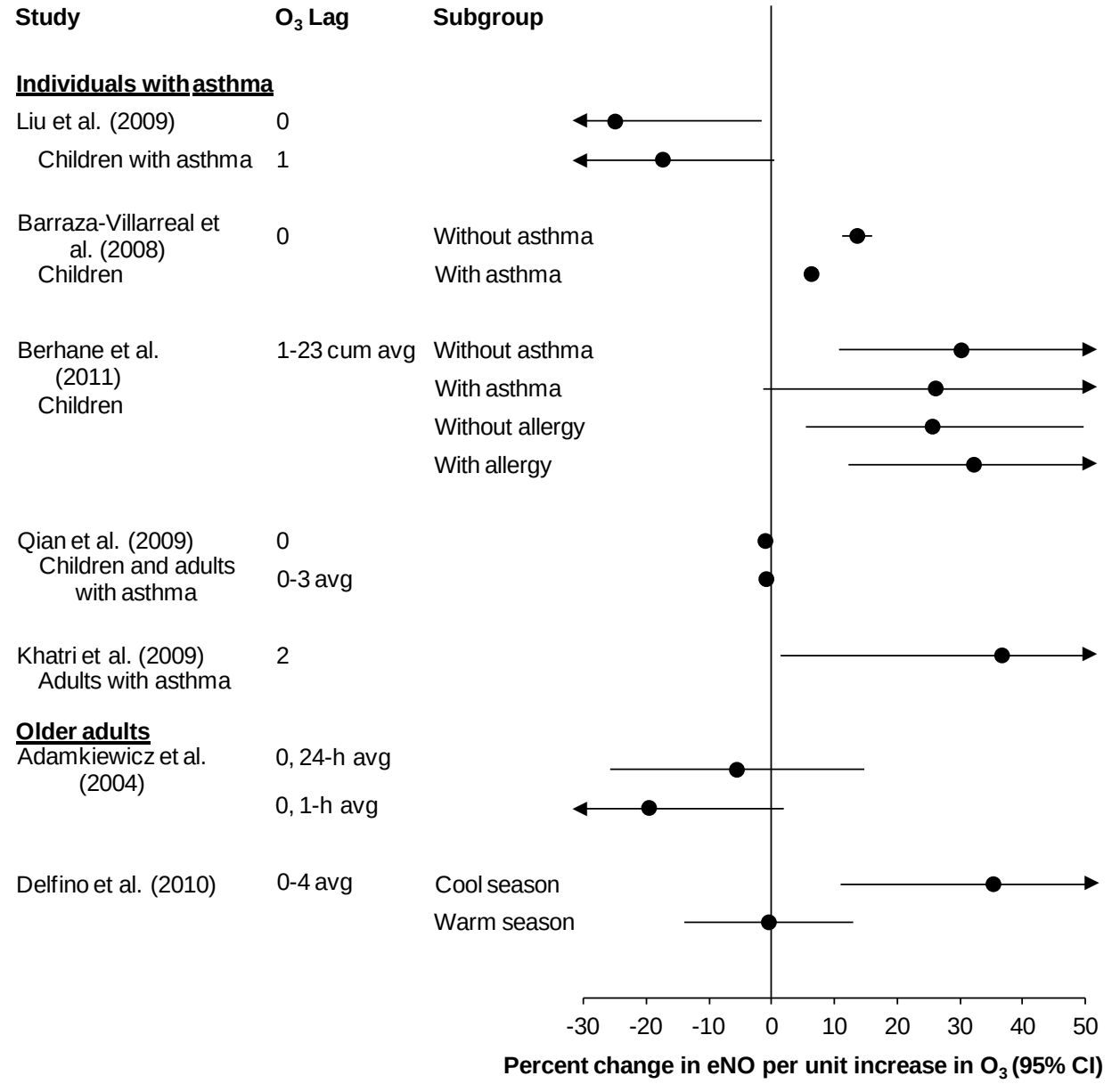
NR = Not Reported, SOCS = Salmeterol Off Corticosteroids Study.

[a]Individual-level estimates were calculated based on time spent in the vicinity of various $O_3$ monitors.

[b]Concentrations converted from μg/m$^3$ to ppb using the conversion factor of 0.51 assuming standard temperature (25°C) and pressure (1 atm).

[c]Measurements outside subject's residence (retirement home).

[d]Average $O_3$ concentration in the 1 hour preceding eNO collection.




Note: Results are presented first for children with asthma and adults with asthma then for older adults. Effect estimates are from single-pollutant models and are standardized to a 40-ppb increase for 1-h avg $O_3$ concentrations, a 30-ppb increase for 8-h max or 8-h avg $O_3$ concentrations, and a 20-ppb increase for 24-h avg $O_3$ concentrations.

**Figure 6-11 Percent change in exhaled nitric oxide (eNO) in association with ambient $O_3$ concentrations in populations with and without asthma.**

## Table 6-16 Percent change in exhaled nitric oxide (eNO) in association with ambient $O_3$ concentrations in populations with and without asthma for studies presented in Figure 6-11.

| Study* | Location/Population | $O_3$ Averaging Time | $O_3$ Lag | Subgroup | Standardized % Change (95% CI)[a] |
|---|---|---|---|---|---|
| **Studies in individuals with asthma** | | | | | |
| Liu et al. (2009a) | Windsor, ON, Canada<br>182 children with asthma, ages 9-14 yr | 24-h avg | 0<br>1 | | -25.1 (-42.9, -1.7)<br>-17.5 (-32.1, -0.24) |
| Barraza-Villarreal et al. (2008) | Mexico City, Mexico<br>208 children, ages 6-14 yr | 8-h moving avg | 0 | Without asthma<br>With asthma | 13.5 (11.2, 15.8)<br>6.2 (6.0, 6.5) |
| Berhane et al. (2011) | 13 Southern California communities<br>2,240 children, ages 6-10 yr | 8-h avg (10 a.m.-6 p.m.) | 1-23 cumulative avg | Without asthma<br>With asthma<br>Without allergy<br>With allergy | 30.1 (10.6, 53.2)<br>26.0 (-1.4, 60.9)<br>25.5 (5.3, 49.6)<br>32.1 (12.0, 55.9) |
| Qian et al. (2009) | Boston, MA; New York, NY; Denver, CO; Philadelphia, PA; San Francisco, CA; Madison, WI<br>119 children and adults with asthma, ages 12-65 yr | 8-h max | 0<br>0-3 avg | | -1.2 (-1.7, -0.64)<br>-1.0 (-1.8, -0.26) |
| Khatri et al. (2009) | Atlanta, GA<br>38 adults with asthma, ages 31-50 yr | 8-h max | 2 | | 36.6 (1.2, 72.0) |
| **Studies in older adults** | | | | | |
| Adamkiewicz et al. (2004) | Steubenville, Ohio<br>29 older adults, ages 53-90 yr | 24-h avg<br>1-h avg[b] | 0 | | -5.7 (-25.9, 14.5)<br>-19.7 (-41.3, 1.9) |
| Delfino et al. (2010a) | Los Angeles, CA<br>60 older adults, ages ≥ 65 yr | 24-h avg | 0-4 avg | Cool season<br>Warm season | 35.2 (10.9, 59.5)<br>-0.60 (-14.0, 12.8) |

*Includes studies in Figure 6-11.

[a]Effect estimates are standardized to a 40-ppb, 30-ppb, and 20-ppb increase for 1-h avg, 8-h max or 8-h avg, and 24-h avg $O_3$, respectively.

[b]Average $O_3$ concentration in the 1 hour preceding eNO collection.

## Table 6-17 Associations between short-term ambient $O_3$ exposure and biological markers of pulmonary inflammation and oxidative stress.

| Study | Location/Population | $O_3$ Averaging Time | $O_3$ Lag | Biological Marker | Subgroup | Standardized Effect Estimate (95% CI)[a] |
|---|---|---|---|---|---|---|
| Liu et al. (2009a) | Windsor, ON, Canada<br>182 children with asthma, ages 9 - 14 yr | 24-h avg | 0 | EBC 8-isoprostane (% change) | | 16.2 (-13.9, 56.8) |
| | | | | EBC TBARS (% change) | | 11.5 (-27.0, 70.1) |
| Romieu et al. (2008) | Mexico City, Mexico<br>107 children with asthma, mean (SD) age 9.5 (2.1) yr | 8-h max | 0 | EBC Malondialdehyde[b] | | 1.9 (1.1, 3.5) |
| Barraza-Villarreal et al. (2008) | Mexico City, Mexico<br>208 children, ages 6 - 14 yr | 8-h moving avg | 0 | Nasal lavage IL-8 (pg/mL) | Without asthma<br>With asthma | 1.6 (1.4, 1.9)<br>1.6 (1.4, 1.8) |
| | | | | EBC pH | Without asthma<br>With asthma | -0.10 (-0.27, 0.08)[c]<br>-0.10 (-0.20, 0.01)[c] |
| Sienra-Monge et al. (2004) | Mexico City, Mexico<br>117 children with asthma, mean age 9 yr | 8-h max | 0-2 avg | Nasal lavage IL-8[b] | Placebo<br>Antioxidant | 2.2 (1.1, 4.7)<br>1.0 (0.44, 2.3) |
| | | | | Nasal lavage IL-6[b] | Placebo<br>Antioxidant | 2.7 (1.4, 5.1)<br>1.1 (0.53, 2.2) |
| | | | | Nasal lavage Uric acid[b] | Placebo<br>Antioxidant | 0.75 (0.44, 1.3)<br>1.3 (0.68, 2.4) |
| | | | | Nasal lavage Glutathione[b] | Placebo<br>Antioxidant | 0.79 (0.63, 0.98)<br>0.80 (0.66, 0.96) |
| Khatri et al. (2009) | Atlanta, GA<br>38 adults with asthma, ages 31 - 50 yr | 8-h max | 2 | Blood eosinophils (% change) | | 2.4 (0.62, 4.2) |
| Ferdinands et al. (2008) | Atlanta, GA<br>16 children exercising outdoors, ages 14 - 17 yr | 1-h max | 0 | EBC pH (normalized score) | | 0.80 (-0.20, 1.8)[c] |

Results generally are presented in order of increasing mean ambient $O_3$ concentration. EBC = exhaled breath condensate, TBARS = thiobarbituric acid reactive substances, IL-8 = interleukin 8, IL-6 = interleukin 6, Antioxidant = group supplemented with vitamins C and E.

[a]Effect estimates are standardized to a 40-, 30- and 20-ppb increase for 1-h max, 8-h max or 8-h avg, and 24-h avg $O_3$, respectively.

[b]Effect estimates represent the ratio of the geometric means of biological marker per unit increase in $O_3$ concentration. A ratio <1 indicates a decrease in marker, and a ratio >1 indicates an increase in marker for an increase in $O_3$.

[c]Model analyzed log-transformed $O_3$. Decreases and increases in pH indicate increases and decreases in pulmonary inflammation, respectively.

### Populations with Asthma

#### Exhaled Nitric Oxide

Neither NO nor eNO has been examined in the controlled human exposure or toxicological studies of $O_3$ exposure reviewed in this ISA. However, several lines of evidence support its analysis as an indicator of pulmonary inflammation. Inducible NO synthase can be activated by pro-inflammatory cytokines, and NO can be produced by cells such as neutrophils, eosinophils, and epithelial cells in the lung during an inflammatory response (Barnes and Liew, 1995). Further, eNO commonly is higher in individuals with asthma and increases during acute exacerbations (Jones et al., 2001; Kharitonov and Barnes, 2000).

As indicated in Figure 6-10 [and Table 6-16]), short-term increases in ambient $O_3$ concentration (8-h max or avg) were associated with increases in eNO in children with asthma. These studies used different methods to assign exposures using central site $O_3$ measurements: the site closest (within 5 km) to home or school (Barraza-Villarreal et al., 2008) and a single site per community (Berhane et al., 2011). Because information on spatial homogeneity of ambient $O_3$ concentrations and time spent outdoors was not available in these studies, it is not possible to assess whether these two methods produced different personal-ambient $O_3$ ratios and correlations. Liu et al. (2009a) (described in Section 6.2.1.2) reported $O_3$-associated decreases in eNO; however, this study was restricted to winter. Results for EBC markers of oxidative stress and lung function collectively also provided weak evidence of $O_3$-associated respiratory effects in this study. As described in Section 4.3.3, in non-summer months, indoor to outdoor $O_3$ ratios are lower as are personal-ambient ratios, making it more difficult to detect associations with ambient $O_3$ concentrations.

In contrast with controlled human exposure studies (Section 6.2.3.1), epidemiologic studies did not find larger $O_3$-associated increases in pulmonary inflammation in groups with asthma than in groups without asthma (Figure 6-11 [and Table 6-16]). Among children in Southern California, Berhane et al. (2011) estimated similar associations for a 1-23 day cumulative average of 8-h avg (10 a.m.-6 p.m.) $O_3$ in children with and without asthma. Among children in Mexico City, Mexico, Barraza-Villarreal et al. (2008) found a larger association (for lag 0 [of 8-max $O_3$]) in children without asthma, most of whom had atopy.

Studies that included adults with asthma produced contrasting results (Khatri et al., 2009; Qian et al., 2009). The multicity salmeterol (β-2 agonist) trial (Boston, MA; New York, NY; Denver, CO; Philadelphia, PA; San Francisco, CA; and Madison, WI) involved serial collection of eNO from 119 subjects with asthma, 87% of whom were 20-65 years of age (Qian et al., 2009). Ambient $O_3$ concentrations were averaged from all sites within 20 miles of subjects' zipcode centroids, which in a repeated measures study, may capture the temporal variation in $O_3$ reasonably well (Darrow et al., 2011a; Gent et al., 2003). Among all subjects, increases in 8-h max $O_3$ at multiple lags (0 to 3 single-day and 0-4 avg) were associated with decreases in eNO. Results did not vary among the salmeterol-, CS-, and placebo-treated groups, indicating that the counterintuitive findings for $O_3$ were not only due to the reduction

of inflammation by medication. Qian et al. (2009) suggested that at higher concentrations, $O_3$ may transform NO in airways to reactive nitrogen species. However, this hypothesis was not supported by results from Khatri et al. (2009), who in Atlanta, GA examined overall higher 8-h max $O_3$ ambient concentrations than did Qian et al. (2009) and found that an increase in $O_3$ was associated with an increase in eNO in adults with asthma (36.6% [95% CI: 1.2, 71.9] per 30-ppb increase in lag 2 of 8-h max $O_3$). Although Khatri et al. (2009) was cross-sectional and did not adjust for any meteorological factors, it may have better characterized $O_3$ exposures because subjects were examined during warm months, and an 8-h max $O_3$ concentration was calculated for each subject using measurements at the site closest to his/her location each hour.

### Other biological markers of pulmonary inflammation and oxidative stress

Short-term increases in ambient $O_3$ concentration were associated with changes in the levels of pro-inflammatory cytokines and cells, indicators of oxidative stress, and antioxidants (Table 6-17). Importantly, any particular biomarker was examined in only one to two studies, and the evidence in individuals with asthma is derived primarily from studies conducted in Mexico City, Mexico (Barraza-Villarreal et al., 2008; Romieu et al., 2008; Sienra-Monge et al., 2004). These studies measured ambient $O_3$ concentrations at sites within 5 km of subjects' schools or homes. In a Mexico City cohort of children with asthma, school ambient $O_3$ concentrations averaged over 48 to 72 hours had a ratio and correlation with personal exposures (48- to 72-h avg) of 0.17 and 0.35, respectively (Ramírez-Aguilar et al., 2008). These observations suggest that the effects of personal $O_3$ exposure on inflammation may have been underestimated in the Mexico City studies. Despite the limited evidence, the epidemiologic findings are well supported by controlled human exposure and toxicological studies that measured the same or related endpoints.

Several of the modes of action of $O_3$ are mediated by reactive oxygen species (ROS) produced in the airways by $O_3$ (Section 5.3.3). These ROS are important mediators of inflammation as they regulate cytokine expression and inflammatory cell activity in airways (Heidenfelder et al., 2009). Controlled human exposure and toxicological studies, frequently have found $O_3$-induced increases in oxidative stress as shown by increases in prostaglandins (Section 5.3.3 and Section 6.2.3.1), which are produced by the peroxidation of cell membrane phospholipids (Morrow et al., 1990). Romieu et al. (2008) analyzed EBC malondialdehyde (MDA), a thiobarbituric acid reactive substance, which like prostaglandins, is derived from lipid peroxidation (Janero, 1990). For a 30-ppb increase in lag 0 of 8-h max $O_3$, the ratio of the geometric means of MDA was 1.9 (95% CI: 1.1, 3.5). Similar results were reported for lags 1 and 0-1 avg $O_3$. A limitation of the study was that 25% of EBC samples had nondetectable levels of MDA, and the random assignment of concentrations between 0 and 4.1 nmol to these samples may have contributed random measurement error to the estimated $O_3$ effects. Because MDA represents less than 1% of lipid peroxides and is present at low concentrations, its biological relevance has been questioned. However, Romieu et al. (2008) pointed to their observations of statistically significant

associations of EBC MDA levels with nasal lavage IL-8 levels to demonstrate its relationship with pulmonary inflammation.

Uric acid and glutathione are ROS scavengers that are present in the airway ELF. While the roles of these markers in the inflammatory cascade of asthma are not well defined, they have been observed to be consumed in response to short-term $O_3$ exposure as part of an antioxidant response in controlled human exposure and animal studies (Section 5.3.3). Results from an epidemiologic study also indicate that a similar antioxidant response may be induced by increases in ambient $O_3$ exposure. Sienra-Monge et al. (2004) found $O_3$-associated decreases in nasal lavage levels of uric acid and glutathione in children with asthma not supplemented with antioxidant vitamins (Table 6-17). The magnitudes of decrease were similar for $O_3$ concentrations lagged 2 or 3 days and averaged over 3 days.

Both controlled human exposure and toxicological studies have found $O_3$-induced increases in the cytokines IL-6 and IL-8 (Section 5.3.3, Section 6.2.3.1, and Section 6.2.3.3), which are involved in initiating an influx of neutrophils, a hallmark of $O_3$-induced inflammation (Section 6.2.3.1). Epidemiologic studies conducted in Mexico City, Mexico, had similar findings. Sienra-Monge et al. (2004) found that an increase in 8-h max $O_3$ was associated with increases in nasal lavage levels of IL-6 and IL-8 (placebo group), with larger effects estimated for lag 0-2 avg than for lag 2 or 3 $O_3$ (Table 6-17). In another cohort of children with asthma, a 30-ppb increase in lag 0 of 8-h max $O_3$ was associated with a 1.6 pg/mL increase (95% CI: 1.4, 1.8) in nasal lavage levels of IL-8 (Barraza-Villarreal et al., 2008). This study also reported a small $O_3$-associated decrease in EBC pH (Table 6-17). EBC pH, which is thought to reflect the proton-buffering capacity of ammonium in airways, decreases upon asthma exacerbation, and is negatively correlated with airway levels of pro-inflammatory cytokines (Carpagnano et al., 2005; Kostikas et al., 2002; Hunt et al., 2000).

Albeit with limited investigation, controlled human exposure studies have found $O_3$-induced increases in eosinophils in adults with asthma (Section 6.2.3.1). Eosinophils are believed to be the main effector cells that initiate and sustain inflammation in asthma and allergy (Schmekel et al., 2001). Consistent with these findings, in a cross-sectional study of adults with asthma in Atlanta, GA, a 30-ppb increase in lag 2 of 8-h max $O_3$ was associated with a 2.4% increase (95% CI: 0.62, 4.2) in blood eosinophils (Khatri et al., 2009). Potential confounding by weather was not evaluated in models.

The prominent influences demonstrated for ROS and antioxidants in mediating the respiratory effects of $O_3$ provide biological plausibility for effect modification by antioxidant capacity. Effect modification by antioxidant capacity has been described for $O_3$-associated lung function in controlled human exposure and epidemiologic studies (Section 6.2.1.1 and Section 6.2.1.2). An epidemiologic study conducted in Mexico City, Mexico, also found that vitamin C and E supplementation, which potentially increase antioxidant capacity, attenuated $O_3$-associated inflammation and oxidative stress. Among children with asthma supplemented daily with vitamin C

and E, the ratios of the geometric means of nasal lavage IL-6 and IL-8 per 30-ppb increases in lag 0-2 avg of 8-h max $O_3$ were approximately 1, reflecting no change with increases in $O_3$ concentration (Table 6-17) (Sienra-Monge et al., 2004). The results did not clearly delineate interactions among ambient $O_3$ concentrations, endogenous antioxidants, and dietary antioxidants (Table 6-17). Ozone was associated with increases in uric acid in the antioxidant group but decreases in the placebo group across the $O_3$ lags examined. Associations with glutathione were similar in the two groups. In another cohort, 8-h max $O_3$ concentrations $\geq$ 38 ppb enhanced the effects of diets high in antioxidant vitamins and/or omega-3 fatty acids in protecting against $O_3$-related increases in nasal lavage IL-8 (Romieu et al., 2009). Information on the main effects of $O_3$ or effect modification by diet was not presented.

The levels of several biological markers such as eNO, EBC pH, and MDA consistently differ between groups with and without asthma and change during an asthma exacerbation (Corradi et al., 2003; Hunt et al., 2000); however, the magnitudes of change associated with these overt effects are not well defined. Ozone-associated increases in interleukins and indicators of oxidative stress were small: 1-2% increase for each 30-ppb increase in 8-h max $O_3$ concentration (Table 6-17). Ozone-associated increases in eNO were larger: 6-36% increase per 30-ppb increase in 8-h max ambient $O_3$ concentration (Berhane et al., 2011; Delfino et al., 2010a; Khatri et al., 2009; Barraza-Villarreal et al., 2008). Some studies in populations with asthma found that increases in ambient $O_3$ concentration (the same lag) were associated with increases in pulmonary inflammation concurrently and respiratory symptoms. For example, among adults with asthma in Atlanta, GA, an increase in lag 2 ambient $O_3$ concentration was associated with increases in eNO, blood eosinophils, and a decrease in quality of life score, which incorporates indices for symptoms and activity limitations (Khatri et al., 2009). Also, among children with asthma in Mexico City, Mexico, lag 0 $O_3$ was associated with increases in eNO, nasal lavage IL-8, and concurrently assessed cough but not wheeze (Barraza-Villarreal et al., 2008).

### Children without Asthma

In the limited investigation, short-term increases in ambient $O_3$ concentration (8-h max or avg) were associated with increases in pulmonary inflammation in children without asthma (Berhane et al., 2011; Barraza-Villarreal et al., 2008) (Figure 6-11 [and Table 6-16] and Table 6-17). The study of children in Mexico City found a larger $O_3$-associated increase in eNO in the children without asthma than with asthma (13.5% versus 6.2% increase per 30-ppb increase in lag 0 of 8-h max $O_3$) (Barraza-Villarreal et al., 2008). Ozone was associated with similar magnitudes of change in IL-8 and EBC pH in children with and without asthma. A distinguishing feature of this study was that 72% of children without asthma had allergies. A study conducted in 13 Southern California communities also found that increases in ambient $O_3$ concentration (8-h avg, 10 a.m.-6 p.m.) were associated with increases in eNO in children with respiratory allergy (Berhane et al., 2011). Coherence for these

epidemiologic findings is provided by observations of $O_3$-induced allergic inflammation in animal models of allergy (Section 6.2.3.3 and Section 6.2.6).

Berhane et al. (2011) found $O_3$-associated increases in eNO in children without asthma and children without respiratory allergy, providing evidence for effects on pulmonary inflammation in healthy children. This study provided detailed information on differences in association among various lags of 8-h avg (10 a.m.-6 p.m.) $O_3$. Ozone concentrations averaged over the several hours preceding eNO collection were not significantly associated with eNO. Consistent with other studies examining pulmonary inflammation and oxidative stress, Berhane et al. (2011) found that relatively short lags of $O_3$, i.e., 1 to 5 days, were associated with increases in eNO. However, among several types of lag-based models, including unconstrained lag models, polynomial distributed lag models, spline-based distributed lag models, and cumulative lag models, a 23-day cumulative lag of $O_3$ best fit the data. Among the studies evaluated in this ISA, Berhane et al. (2011) was unique in evaluating and finding larger respiratory effects for multi-week (e.g., 13-30 days) average $O_3$ concentrations. A mechanism for the effects of $O_3$ peaking with a 23-day cumulative lag of exposure has not been delineated. Further, with examination of such long lag periods, there is greater potential for residual confounding by weather.

### Populations with Increased Outdoor Exposures

With limited investigation, increases in ambient $O_3$ concentration were not consistently associated with pulmonary inflammation in populations engaged in outdoor activity or exercise. Common limitations of these studies were the small numbers of subjects and lack of consideration for potential confounding factors. A study in 16 adolescent long-distance runners near Atlanta, GA was noteworthy for the daily collection of EBC and the likely greater extent to which ambient $O_3$ concentrations represented ambient exposures because $O_3$ concentrations were measured during outdoor running at a site less than 1 mile from the exercise track (Ferdinands et al., 2008). Increases in 1-h max $O_3$ (lags 0 to 2) were associated with increases in EBC pH, indicating $O_3$-associated decreases in pulmonary inflammation. Among 9 adult male runners in Sicily, Italy examined 3 days before and 20 hours after 3 races in fall, winter, and summer, weekly average $O_3$ concentrations (8-h avg, 7 a.m.-3 p.m.) were positively correlated with apoptosis of neutrophils (Spearman's $r = 0.70$, $p < 0.005$) and bronchial epithelial cell differential counts (Spearman's $r = 0.47$, $p < 0.05$) but not with neutrophil or macrophage cell counts or levels of the pro-inflammatory cytokines TNF-α and IL-8 (Chimenti et al., 2009). Associations with $O_3$ concentrations measured during the races (mean 35 to 89 minutes) were not examined. This study provides evidence for new endpoints; however, the implications of findings are limited due to the lack of a rigorous statistical analysis.

In a cross-sectional study of children at camps in south Belgium, although lung function was not associated with $O_3$ measured at camps during outdoor activity, an association was found for eNO (Nickmilder et al., 2007). Children at camps with lag 0 1-h max $O_3$ concentrations >85.2 ppb had greater intraday increases in eNO

compared with children at camps with $O_3$ concentrations <51 ppb. A benchmark dose analysis indicated that the threshold for an $O_3$-associated increase of 4.3 ppb eNO (their definition of increased pulmonary inflammation) was 68.6 ppb for 1-h max $O_3$ and 56.3 ppb for 8-h max $O_3$. While these results provide additional evidence for $O_3$-associated increases in pulmonary inflammation in healthy children, they should be interpreted with caution since they were not adjusted for any potential confounding factors and based on camp-level comparisons.

### Older Adults

The panel studies examining $O_3$-associated changes in eNO in older adults produced contrasting findings (Figure 6-11 [and Table 6-16]). The studies differed with respect to geographic location, inclusion of healthy subjects, exposure assessment method, and lags of $O_3$ examined. Delfino et al. (2010a) followed 60 older adults with coronary artery disease in the Los Angeles, CA area for 6 weeks each during a warm and cool season; the specific months were not specified. Ambient $O_3$ was measured at subjects' retirement homes, possibly reducing some exposure measurement error due to spatial variability. Multiday averages of $O_3$ (3- to 9-day) were associated with increases in eNO, with effect estimates increasing with increasing number of averaging days. In contrast with most other studies, an association was found in the cool season but not warm season (increase in eNO per 20-ppb increase in lag 0-4 avg of 24-h avg $O_3$: 35.2% [95% CI: 10.9, 59.5] in cool season, -0.06% [95% CI: -14.0, 12.8] in warm season). Despite these unusual findings for the cool season, they were similar to findings from another study of Los Angeles area adults with asthma, which indicated an $O_3$-associated decrease in indoor activity during the fall season (Eiswerth et al., 2005).

In a cool season (September-December) study conducted in older adults (ages 54-91 years) in Steubenville, OH, Adamkiewicz et al. (2004) found that increases in $O_3$ (1-h avg and 24-h avg before eNO collection) were associated with decreases in eNO, reflecting decreases in pulmonary inflammation (Figure 6-11 [and Table 6-16]). The study included healthy adults and those with asthma or COPD. A study in a subset of these adults illustrated why it is difficult to detect effects with central site $O_3$ concentrations in the cool season by showing that subjects spent $\geq$ 90% of time indoors and >77% at home and had a mean 24-h avg $O_3$ personal-ambient ratio of 0.27 (Sarnat et al., 2006a).

### Confounding in Epidemiologic Studies of Pulmonary Inflammation and Oxidative Stress

Except where noted in the preceding text; epidemiologic studies of pulmonary inflammation and oxidative stress accounted for potential confounding by meteorological factors. Increases in ambient $O_3$ concentration were associated with pulmonary inflammation or oxidative stress in models that adjusted for temperature and/or humidity (Delfino et al., 2010a; Barraza-Villarreal et al., 2008; Romieu et al.,

2008). Final results from Sienra-Monge et al. (2004) and Berhane et al. (2011) were not adjusted for temperature because associations were not altered by adjustment for temperature. Most studies conducted over multiple seasons adjusted for season or time trend.

In evidence limited to a small number of studies conducted in Mexico City, Mexico, $O_3$-associated pulmonary inflammation and oxidative stress were not found to be confounded by $PM_{2.5}$ or $PM_{10}$. These studies, which analyzed 8-hour averages for both $O_3$ and PM, found robust associations for $O_3$ (Barraza-Villarreal et al., 2008; Romieu et al., 2008; Sienra-Monge et al., 2004). Ozone and PM, both measured at central sites located within 5 km of subjects' schools or homes, were moderately correlated (r = 0.46 – 0.54). Weak correlations have been found between personal exposures of $O_3$ and $PM_{2.5}$ (Section 4.3.4.1). Only Romieu et al. (2008) provided quantitative results. Lag 0 of 8-h max $O_3$ was associated with a similar magnitude of increase in MDA without and with adjustment for lag 0 of 8-h max $PM_{2.5}$ (ratio of geometric means for a 30-ppb increase: 1.3 [95% CI: 1.0, 1.7]). In comparison, the $O_3$-adjusted effect estimate for $PM_{2.5}$ was cut in half.

### Summary of Epidemiologic Studies of Pulmonary Inflammation and Oxidative Stress

Many epidemiologic studies provided evidence that short-term increases in ambient $O_3$ exposure increase pulmonary inflammation and oxidative stress in children with asthma, with evidence primarily provided by studies conducted in Mexico City. By also finding that associations were attenuated with higher antioxidant intake, these studies indicated that inhaled $O_3$ may be an important source of ROS in airways and/or may increase pulmonary inflammation via oxidative stress-mediated mechanisms. Studies also found $O_3$-associated increases in pulmonary inflammation in children with allergy (Berhane et al., 2011; Barraza-Villarreal et al., 2008). The limited available evidence in children and adults with increased outdoor exposures and older adults was inconclusive. Results did not indicate confounding of $O_3$ associations by temperature or humidity. Copollutant models were analyzed in a few studies conducted in Mexico City; $O_3$ effect estimates were robust to adjustment for moderately correlated (r = 0.46 – 0.54) $PM_{2.5}$ or $PM_{10}$ (Barraza-Villarreal et al., 2008; Romieu et al., 2008; Sienra-Monge et al., 2004).

Ozone-associated increases in pulmonary inflammation and oxidative stress were found in studies that used varied exposure assessment methods: measurement on site of subjects' outdoor activity (Nickmilder et al., 2007), average of concentrations measured at the closest site each hour Khatri et al. (2009), measurement at a site within 5 km of subjects' schools or homes (Barraza-Villarreal et al., 2008; Romieu et al., 2008; Sienra-Monge et al., 2004), and measurement at single site per town (Berhane et al., 2011). While these methods may differ in the degree of exposure measurement error, in the limited body of evidence, there was not a clear indication that the method of exposure assessment influenced the strength or magnitude of associations.

Most studies examined and found associations with 8-h max or daytime 8-h avg $O_3$ concentrations, although associations also were found for 1-h max (Nickmilder et al., 2007) and 24-h avg $O_3$ (Delfino et al., 2010a). Collectively, studies examined single-day $O_3$ concentrations lagged from 0 to 5 days and concentrations averaged over 2 to 9 days. Lag 0 of 8-h max $O_3$ was most frequently examined and consistently associated with pulmonary inflammation and oxidative stress. However, in the few studies that examined multiple $O_3$ lags, multiday average 8-h max or 8-h avg concentrations were associated with larger increases in pulmonary inflammation and oxidative stress (Berhane et al., 2011; Delfino et al., 2010a; Sienra-Monge et al., 2004). These findings for multiday average $O_3$ concentrations are supported by controlled human exposure (Section 6.2.3.1) and animal studies (Section 6.2.3.3) that similarly have found that some markers of pulmonary inflammation remain elevated with $O_3$ exposures repeated over multiple days.

Several epidemiologic studies concurrently examined associations of ambient $O_3$ concentrations with biological markers of pulmonary inflammation and lung function or respiratory symptoms. Whether evaluated at the same or different lags of $O_3$, associations generally were stronger for biological markers of airway inflammation than for lung function within populations (Khatri et al., 2009; Barraza-Villarreal et al., 2008; Nickmilder et al., 2007). Controlled human exposure studies have demonstrated a lack of correlation between inflammatory and spirometric responses induced by $O_3$ exposure within subjects (Section 6.2.3.1). Evidence has suggested that $O_3$-related respiratory morbidity may occur via multiple mechanisms with varying time courses of action, and the examination of a limited number of $O_3$ lags in these aforementioned studies may explain some of the inconsistencies in associations of $O_3$ with measures of pulmonary inflammation and lung function. In contrast, based on examination in a few studies, increases in ambient $O_3$ concentration (at the same lag) were associated with increases in pulmonary inflammation and increases in respiratory symptoms or activity limitations in the same population of individuals with asthma (Khatri et al., 2009; Barraza-Villarreal et al., 2008).

### 6.2.3.3 Toxicology

The 2006 $O_3$ AQCD states that the "extensive human clinical and animal toxicological evidence, together with the limited available epidemiologic evidence, is clearly indicative of a causal role for $O_3$ in inflammatory responses in the airways" (U.S. EPA, 2006b). 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 pro-inflammatory 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. One critical aspect of inflammation is the potential for metaplasia and alterations in pulmonary morphology. Studies have observed increased thickness of the alveolar septa, presumably due to increased cellularity after acute exposure to $O_3$. Epithelial hyperplasia starts early in exposure and

increases in magnitude for several weeks, after which it plateaus until exposure ceases. When exposure persists for a month and longer, excess collagen and interstitial fibrosis are observed. This response, discussed in Chapter 7, continues to increase in magnitude throughout exposure and can even continue to increase after exposure ends (Last et al., 1984). Previously reviewed toxicological studies of the ability of $O_3$ to cause inflammation, injury, and morphological changes are described in Table 6-5 on page 6-25 (U.S. EPA, 1996f), Table 6-10 (U.S. EPA, 1996k) and Table 6-11 (U.S. EPA, 1996l) beginning on page 6-61 of the 1996 $O_3$ AQCD, and Annex Tables AX5-8 (U.S. EPA, 2006e) and AX5-9 (U.S. EPA, 2006f), beginning on page AX5-17 of the 2006 $O_3$ AQCD. Numerous recent in vitro and in vivo studies add to this very large body of evidence for $O_3$-induced inflammation and injury, and provide new information regarding the underlying mechanisms (see Section 5.3).

A number of species, including dogs, rabbits, guinea pigs, rats, and mice have been used as models to study the pulmonary effects of $O_3$, but the similarity of non-human primates to humans makes them an attractive model in which to study the pulmonary response to $O_3$. As reviewed in the 1996 and 2006 $O_3$ AQCDs, several pulmonary effects, including inflammation, changes in morphometry, and airway hyperresponsiveness, have been observed in macaque and rhesus monkeys after acute exposure to $O_3$ (Table 6-18 presents a highlight of these studies). Increases in inflammatory cells were observed after a single 8-hour exposure of adult rhesus monkeys to 1 ppm $O_3$ (Hyde et al., 1992). Inflammation was linked to morphometric changes, such as increases in necrotic cells, smooth muscle, fibroblasts, and nonciliated bronchiolar cells, which were observed in the trachea, bronchi, or respiratory bronchioles. Effects have also been observed after short-term repeated exposure to $O_3$ at concentrations that are more relevant to ambient $O_3$ concentrations. Morphometry changes in the lung, nose, and vocal cords were observed after exposure to 0.15 ppm $O_3$ for 8 hours/day for 6 days (Harkema et al., 1993; Dimitriadis, 1992; Harkema et al., 1987a).

Since 2006, however, only one study has been published regarding acute exposure of non-human primates to $O_3$ (a number of recent chronic studies in non-human primates are described in Chapter 7). In this study, a single 6-hour exposure of adult male cynomolgus monkeys to 1 ppm $O_3$ induced significant increases in inflammatory and injury markers, including BAL neutrophils, total protein, alkaline phosphatase, IL-6, IL-8, and G-CSF (Hicks et al., 2010a). Gene expression analysis confirmed the increases in the pro-inflammatory cytokine IL-8, which had been previously described in $O_3$ exposed rhesus monkeys (Chang et al., 1998). The anti-inflammatory cytokine IL-10 was also elevated, but the fold changes in IL-10 and G-CSF were relatively low and highly variable. The single exposure also caused necrosis and sloughing of the epithelial lining of the most distal portions of the terminal bronchioles and the respiratory bronchioles. Bronchiolitis, alveolitis, parenchymal and centriacinar inflammation were also observed. A second exposure protocol (two exposures with a 2-week inter-exposure period) resulted in similar inflammatory responses, with the exception of total protein and alkaline phosphatase levels which were attenuated, indicating that attenuation of some but not all lavage parameters occurred upon repeated exposure of non-human primates to $O_3$ (Hicks et

al., 2010a). This variability in attenuation is similar to the findings of earlier reports in rodents (Wiester et al., 1996c) and non-human primates (Tyler et al., 1988).

Table 6-18 describes key morphometric studies conducted in non-human primates exposed to $O_3$. Morphologic observations made by Dungworth (1976) and Dungworth et al. (1975) indicate that the rat and Bonnet monkey (*Macaca radiata*) are approximately equal in susceptibility to short-term effects of $O_3$. Mild but discernible lesions were caused in both species by exposure to 0.2 ppm $O_3$ for 8 hours/day for 7 days. The authors stated that detectable morphological effects in the rat occurred at levels as low as 0.1 ppm $O_3$. In both species, the lesion occurred at the junction of the small airways and the gaseous exchange region. In rats, the prominent features were accumulation of macrophages, replacement of necrotic Type 1 epithelial cells with Type 2 cells, and damage to ciliated and nonciliated Clara cells. The principal site of damage was the alveolar duct. In monkeys, the prominent $O_3$-induced injury was limited to the small airways. At 0.2 ppm $O_3$, the lesion was observed at the proximal portion of the respiratory bronchioles. As concentrations of $O_3$ were increased up to 0.8 ppm, the severity of the lesion increased, and the damage extended distally to involve the proximal portions of the alveolar duct.

Mellick et al. (1977) found similar but more pronounced effects when rhesus monkeys (3 to 5 years of age) were exposed to 0.5 and 0.8 ppm $O_3$, 8 hours/day for 7 days. In these experiments, the respiratory bronchioles were the most severely damaged, whereas more distal parenchymal regions were unaffected. Major effects were hyperplasia and hypertrophy of the nonciliated bronchiolar epithelial cells and the accumulation of macrophages intraluminally. In mice, continuous exposure to 0.5 ppm $O_3$ caused nodular hyperplasia of Clara cells after 7 days of exposure. Similar findings were reported by Schwartz (1976) andSchwartz et al. (1976), who exposed rats to 0.2, 0.5 or 0.8 ppm $O_3$ for 8 or 24 hours/day for 1 week. Changes observed within the proximal alveoli included infiltration of inflammatory cells and swelling and necrosis of Type 1 cells. In the terminal bronchiole, the changes reported were shortened cilia, clustering of basal bodies in ciliated cells suggesting ciliogenesis, and reduction in height or loss of cytoplasmic luminal projection of the Clara cells. Effects were seen at $O_3$ concentrations as low as 0.2 ppm. A dose-dependent pulmonary response to the three levels of $O_3$ was evident. No differences were observed in morphologic characteristics of the lesions between rats exposed continuously and those exposed intermittently for 8 hours/day.

**Table 6-18 Morphometric observations in non-human primates after acute $O_3$ exposure.**

| Reference | $O_3$ concentration (ppm) | Exposure duration | Species, Sex, Age | Observation |
|---|---|---|---|---|
| Harkema et al. (1993) | 0.15 | 8 h/day for 6 days | *Macaca radiata* (bonnet macaques) 2-6 years old | Several fold increase in thickness of surface epithelium in respiratory bronchioles; increase in interstitial mass with increase in proportion of cuboidal cells. |
| Harkema et al. (1987a); Harkema et al. (1987b) | 0.15 | 8 h/day for 6 days | *Macaca radiata*, M, F 2-6 years old | Ciliated cell necrosis, shortened cilia, and increased mucous cells in the respiratory epithelium of nose after 0.15 ppm; changes in nonciliated cells, intraepithelial leukocytes, and mucous cells in the transitional epithelium |
| Dungworth (1976) | 0.2<br>0.5<br>0.8 | 8 h/day for 7 days for monkey and rat; continuous at 0.5 ppm for 7 days for mouse | Adult Rhesus and bonnet monkeys; S-D rats; Mice | In both rats and monkeys mild but discernible lesions were observed at 0.2 ppm; similar severity between species but different site of lesions – respiratory bronchioles for monkey and damage to ciliated, Clara, and alveolar epithelial cells for rat; Clara cell hyperplasia in mice |
| Leonard et al. (1991) | 0.25 | 8 h/day for 7 days | *Macaca radiata* age not specified | The $O_3$ exposure level is not clear – the abstract states 0.64 ppm, but the text mentions only 0.25 ppm. Morphometric changes in vocal cord mucosa: disruption and hyperplasia of stratified squamous epithelium; epithelial and connective tissue thickness increased |
| Chang et al. (1998) | 0.96 | 8 h | Rhesus, M age not specified | Increase in IL-8 in airway epithelium correlated with PMN influx |
| Hyde et al. (1992) | 0.96 | 8 h | Rhesus, M 2 - 8.5 years old | Increased PMNs; morphometric changes in trachea, conducting airways, respiratory bronchioles including increased smooth muscle in bronchi and RB. |
| Hicks et al. (2010b) | 1.0 | 6 h | Cynomolgus, M 5-7 kg (Adult) | Increase in PMNs and IL-8 in lavage fluid |

Exposure of adult BALB/c mice to 0.1 ppm $O_3$ for 4 hours increased BAL levels of keratinocyte chemoattractant (KC; IL-8 homologue) (~ 6-fold), IL-6 (~12-fold), and TNF-α (~ 2-fold) (Damera et al., 2010). Additionally, $O_3$ increased BAL neutrophils by 21% without changes in other cell types. A trend of increased neutrophils with increased $O_3$ concentration (0.12-2 ppm) was observed in BALB/c mice exposed for 3 hours (Jang et al., 2005). Although alterations in the epithelium of the airways were not evident in 129J mice after 4 hours of exposure to 0.2 ppm $O_3$ (Plopper et al., 2006), detachment of the bronchiolar epithelium was observed in SD rats after 5 days or 60 days of exposure to 0.25 ppm $O_3$ (Oyarzún et al., 2005). Subacute (65 hours) exposure to 0.3 ppm $O_3$ induced pulmonary inflammation, cytokine induction, and enhanced vascular permeability in wild type mice of a mixed background (129/Ola and C57BL/6) and these effects were exacerbated in metallothionein I/II knockout

mice (Inoue et al., 2008). Three hours or 72 hours of exposure to 0.3 ppm $O_3$ resulted in similar levels of IL-6 expression in the lungs of C57BL/6 mice (Johnston et al., 2005b), along with increases in BAL protein, sTNFR1, and sTNFR2. Increased neutrophils were observed only after the 72-hour exposure, and neither exposure resulted in detectable levels of IL-6 or KC protein. Levels of BAL protein, sTNFR1, and sTNFR2 were higher in the 72-hour exposure group than in the 3-hour exposure group. In another study, the same subacute (72 hours) exposure protocol elicited increases in BALF protein, IP-10, sTNFR1, macrophages, neutrophils, and IL-6, IL-1α, and IL-1β expression (Johnston et al., 2007). Yoon et al. (2007) exposed C57BL/6J mice continuously to 0.3 ppm $O_3$ for 6, 24, 48, or 72 hours, and observed elevated levels of KC, MIP-2, metalloproteinases, and inflammatory cells in the lungs at various time points. A similar exposure protocol using C3H/HeJ and C3H/OuJ mice demonstrated elevations in protein, PMNs, and KC, which were predominantly TLR 4 pathway dependent based on their prominence in the TLR 4 sufficient C3H/OuJ strain Bauer et al. (2011). C3H/OuJ mice also had elevated levels of the heat-shock protein HSP70, and further experiments in HSP70 deficient mice indicated a role for this particular pathway in $O_3$-related injury, discussed in more detail in Chapter 5.

As reviewed in the 2006 $O_3$ AQCD, the time course for changes in BAL depends on the parameters being studied. Similarly, after exposing adult C57BL mice to 0.5 ppm $O_3$ for 3 hours, Han et al. (2008) observed early (5 hours postexposure) increases in BAL TNF-α and IL-1β, which diminished by 24 hours postexposure. Total BAL protein was elevated at 24 hours, but there were only minimal or negligible changes in LDH, total cells, or PMNs. Ozone increased BAL mucin levels (with statistical significance by 24 hours postexposure), and significantly elevated surfactant protein D at both time points. Prior intratracheal (IT) exposure to multiwalled carbon nanotubes enhanced most of these effects, but the majority of responses to the combined exposure were not greater than those to nanotubes alone. Ozone exposure did not induce markers of oxidative stress in lung tissue, BAL, or serum. Consistent with this study, Aibo et al. (2010) did not detect changes in BAL inflammatory cell numbers in the same mouse strain after a 6-hour exposure to 0.25 or 0.5 ppm. The majority of inflammatory cytokines (pulmonary or circulating) were not significantly changed (as assessed 9 hours post-$O_3$ exposure). Exposure of C57BL/6 mice to 1 ppm for 3 hours increased BAL total cells, neutrophils, and KC; these responses were greatest at 24 hours postexposure. F2-isoprostane (8-isoprostane), a marker of oxidative stress, was also elevated by $O_3$, peaking at 48 hours postexposure (Voynow et al., 2009).

Atopic asthma appears to be a risk factor for more severe airway inflammation induced by experimental $O_3$ exposure in humans (Balmes et al., 1997; Scannell et al., 1996), and allergic animal models are often used to investigate the effects of $O_3$ on this potentially at-risk population. Farraj et al. (2010) exposed allergen-sensitized adult male BALB/c mice to 0.5 ppm $O_3$ for 5 hours once per week for 4 weeks. Ovalbumin-sensitized mice exposed to $O_3$ had significantly increased BAL eosinophils by 85% and neutrophils by 103% relative to OVA sensitized mice exposed to air, but these changes were not evident upon histopathological evaluation

of the lung, and no $O_3$ induced lesions were evident in the nasal passages. Ozone increased BAL levels of N-acetyl-glucosaminidase (NAG; a marker of injury) and protein. DEP co-exposure (2.0 mg/m$^3$, nose only) inhibited these responses. These pro-inflammatory effects in an allergic mouse model have also been observed in rats. Wagner et al. (2007) exposed the relatively $O_3$-resistant Brown Norway rat strain to 1 ppm $O_3$ after sensitizing and challenging with OVA. Rats were exposed for 2 days, and airway inflammation was assessed one day later. Filtered air for controls contained less than 0.02 ppm $O_3$. Histopathology indicated that $O_3$ induced site-specific lung lesions in the centriacinar regions, characterized by wall thickening partly due to inflammatory cells influx. BAL neutrophils were elevated by $O_3$ in allergic rats, and modestly increased in non-allergic animals (not significant). A slight (but not significant) increase in macrophages was observed, but eosinophil numbers were not affected by $O_3$. Soluble mediators of inflammation (Cys-LT, MCP-1, and IL-6) were elevated by $O_3$ in allergic animals but not non-allergic rats. Treatment with γT, which neutralizes oxidized lipid radicals and protects lipids and proteins from nitrosative damage, did not alter the morphologic character or severity of the centriacinar lesions caused by $O_3$, nor did it reduce neutrophil influx. It did, however, significantly reduce $O_3$-induced soluble inflammatory mediators in allergic rats. The effects of $O_3$ in animal models of allergic asthma are discussed in Section 6.2.6.

In summary, a large number of toxicology studies have demonstrated that acute exposure to $O_3$ produces injury and inflammation in the mammalian lung, supporting the observations in controlled human exposure studies (Section 6.2.3.1) and epidemiologic studies (Section 6.2.3.2). These acute changes, both in inflammation and morphology, provide a limited amount of evidence for long term sequelae of exposure to $O_3$. Related alterations resulting from long term exposure, such as fibrotic changes, are discussed in Chapter 7.

### 6.2.4 Respiratory Symptoms and Medication Use

Controlled human exposure and toxicological studies have described modes of action through which short-term $O_3$ exposure may increase respiratory symptoms by demonstrating $O_3$-induced airway hyperresponsiveness (Section 6.2.2) and pulmonary inflammation (Section 6.2.3.1 and Section 6.2.3.3). Epidemiologic studies have not widely examined associations between ambient $O_3$ concentrations and airway hyperresponsiveness but have found $O_3$-associated increases in pulmonary inflammation and oxidative stress (Section 6.2.3.2). In addition to lung function decrements, controlled human exposure studies clearly indicate $O_3$-induced increases in respiratory symptoms including pain on deep inspiration, shortness of breath, and cough. This evidence is detailed in Section 6.2.1.1; however, salient observations include an increase in respiratory symptoms with increasing concentration and duration of $O_3$ exposure and activity level of exposed subjects (McDonnell et al., 1999b). Further, increases in total subjective respiratory symptoms have been reported following 5.6 and 6.6 hours of exposure to 60 ppb $O_3$ relative to baseline

(Adams, 2006a). At 70 ppb, Schelegle et al. (2009) observed a statistically significant $O_3$-induced $FEV_1$ decrement of 6.1% at 6.6 hours and a significant increase in total subjective symptoms at 5.6 and 6.6 hours. The findings for $O_3$-induced respiratory symptoms in controlled human exposure studies and the evidence integrated across disciplines describing underlying modes of action provide biological plausibility for epidemiologic associations observed between short-term increases in ambient $O_3$ concentration and increases in respiratory symptoms.

In epidemiologic studies, respiratory symptom data typically are collected by having subjects (or their parents) record symptoms and medication use in a diary without direct supervision by study staff. Several limitations of symptom reports are well recognized: recall error if not recorded daily, differences among subjects in the interpretation of symptoms, differential reporting by subjects with and without asthma, and occurrence in a smaller percentage of the population compared with changes in lung function and biological markers of pulmonary inflammation. Nonetheless, symptom diaries remain a convenient tool to collect individual-level data from a large number of subjects and allow modeling of associations between daily changes in $O_3$ concentration and daily changes in respiratory morbidity over multiple weeks or months. Importantly, most of the limitations described above are sources of random measurement error that can bias effect estimates to the null or increase the uncertainty around effect estimates. Furthermore, because respiratory symptoms are associated with limitations in activity and function and are the primary reason for using medication and seeking medical care, the evidence is directly coherent with the associations consistently observed between increases in ambient $O_3$ concentration and increases in asthma ED visits (Section 6.2.7.3).

Most studies of respiratory symptoms were conducted in individuals with asthma, and as was concluded in the 2006 $O_3$ AQCD (U.S. EPA, 2006b, 1996a), the collective body of epidemiologic evidence indicates that short-term increases in ambient $O_3$ concentration are associated with increases in respiratory symptoms in children with asthma. Studies also found $O_3$-associated increases in the use of asthma medication by children. In a smaller body of studies, increases in ambient $O_3$ concentration were associated with increases in respiratory symptoms in adults with asthma. Ozone-associated increases in respiratory symptoms in healthy populations were not as clearly indicated.

### 6.2.4.1 Children with Asthma

#### Respiratory Symptoms

Table 6-19 presents the locations, time periods, and ambient $O_3$ concentrations for studies examining respiratory symptoms and medication use in children with asthma. The evidence supporting associations between short-term increases in ambient $O_3$ concentration and increases in respiratory symptoms in children with asthma is derived mostly from examination of 1-h max, 8-h max, or 8-h avg $O_3$ concentrations

and strong findings from a large body of single-region or single-city studies (Figure 6-12 [and Table 6-20]). The few available U.S. multicity studies produced less consistent associations, but the overall body of epidemiologic evidence remains compelling. As detailed below, because of specific methodological distinctions, results from some multicity studies were not given greater consideration than results from single city studies in weighing the evidence for ambient $O_3$ exposure and respiratory symptoms.

Similar to lung function, associations with respiratory symptoms in children with asthma were found with ambient $O_3$ concentrations assigned to subjects using various methods with potentially different degrees of exposure measurement error. As was discussed for lung function, methods included measurement of $O_3$ on site of and at the time of outdoor activity (Thurston et al., 1997), which is associated with higher ambient-personal $O_3$ correlations and ratios (Section 4.3.3); $O_3$ concentrations measured at sites within 5 km of subjects' home or school (Escamilla-Nuñez et al., 2008; Romieu et al., 2006; Romieu et al., 1997; Romieu et al., 1996); $O_3$ measured at a single city site (Gielen et al., 1997); and $O_3$ concentrations averaged across multiple sites (Gent et al., 2003; Mortimer et al., 2002). In analyses with $O_3$ averaged across multiple sites, which were restricted to warm seasons, $O_3$ concentrations within the region were temporally correlated as indicated by high statewide correlations [median r = 0.83 in Gent et al. (2003)] or similar odds ratios for $O_3$ averaged across all within-city monitors and that averaged from the three closest sites (Mortimer et al., 2002). In these panel studies, the ambient concentrations averaged across sites may have well represented the temporal variability in subjects' ambient $O_3$ exposures.

## Table 6-19 Mean and upper percentile $O_3$ concentrations in epidemiologic studies of respiratory symptoms, medication use, and activity levels in children with asthma.

| Study* | Location | Study Period | $O_3$ Averaging Time | Mean/Median Concentration (ppb) | Upper Percentile Concentrations (ppb) |
|---|---|---|---|---|---|
| Thurston et al. (1997) | CT River Valley, CT | June 1991-1993 | 1-h max | 83.6[a] | Max: 160[a] |
| Escamilla-Nuñez et al. (2008) | Mexico City, Mexico | July-Mar 2003-2005 | 1-h max | 86.5 | NR |
| Romieu et al. (2006) | Mexico City, Mexico | Oct 1998-Apr 2000 | 8-h max<br>1-h max | 69<br>102 | Max: 184<br>Max: 309 |
| Romieu et al. (1997) | Southern Mexico City, Mexico | Apr-July 1991; Nov 1991-Feb 1992 | 1-h max | 196 | Max: 390 |
| Romieu et al. (1996) | Northern Mexico City, Mexico | Apr-July 1991; Nov 1991-Feb 1992 | 1-h max | 190 | Max: 370 |
| Gent et al. (2003) | CT, Southern MA | Apr-Sept 2001 | 8-h rolling avg<br>1-h max | 51.3, median 50.0<br>58.6, median 55.5 | Max: 99.6<br>Max: 125.5 |
| Mortimer et al. (2002); Mortimer et al. (2000) | Bronx, East Harlem, NY; Baltimore, MD; Washington, DC; Detroit, MI, Cleveland, OH; Chicago, IL; St. Louis, MO (NCICAS) | June-Aug 1993 | 8-h avg (10 a.m.-6 p.m.) | 48 | NR |
| Gielen et al. (1997) | Amsterdam, Netherlands | Apr-July 1995 | 8-h max | 34.2[b] | Max: 56.5[b] |
| Delfino et al. (2003) | Los Angeles, CA | Nov 1999-Jan 2000 | 8-h max<br>1-h max | 17.1<br>25.4 | 90th: 26.1, Max: 37<br>90th: 38.0, Max: 52 |
| Rabinovitch et al. (2004) | Denver, CO | Nov-Mar 1999-2002 | 1-h max | 28.2 | 75th: 60, Max: 70.0 |
| Schildcrout et al. (2006) | Albuquerque, NM; Baltimore, MD; Boston, MA; Denver, CO; San Diego, CA; Seattle, WA; St. Louis, MO; Toronto, ON, Canada (CAMP) | May-Sept 1994-1995 | 1-h max | Range in medians across cities: 43.0-65.8 | Range in 90th across cities: 61.5-94.7 |
| Jalaludin et al. (2004) | Sydney, Australia | Feb-Dec 1994 | 15-h avg (6 a.m.-9 p.m.) | 12 | Max: 43 |

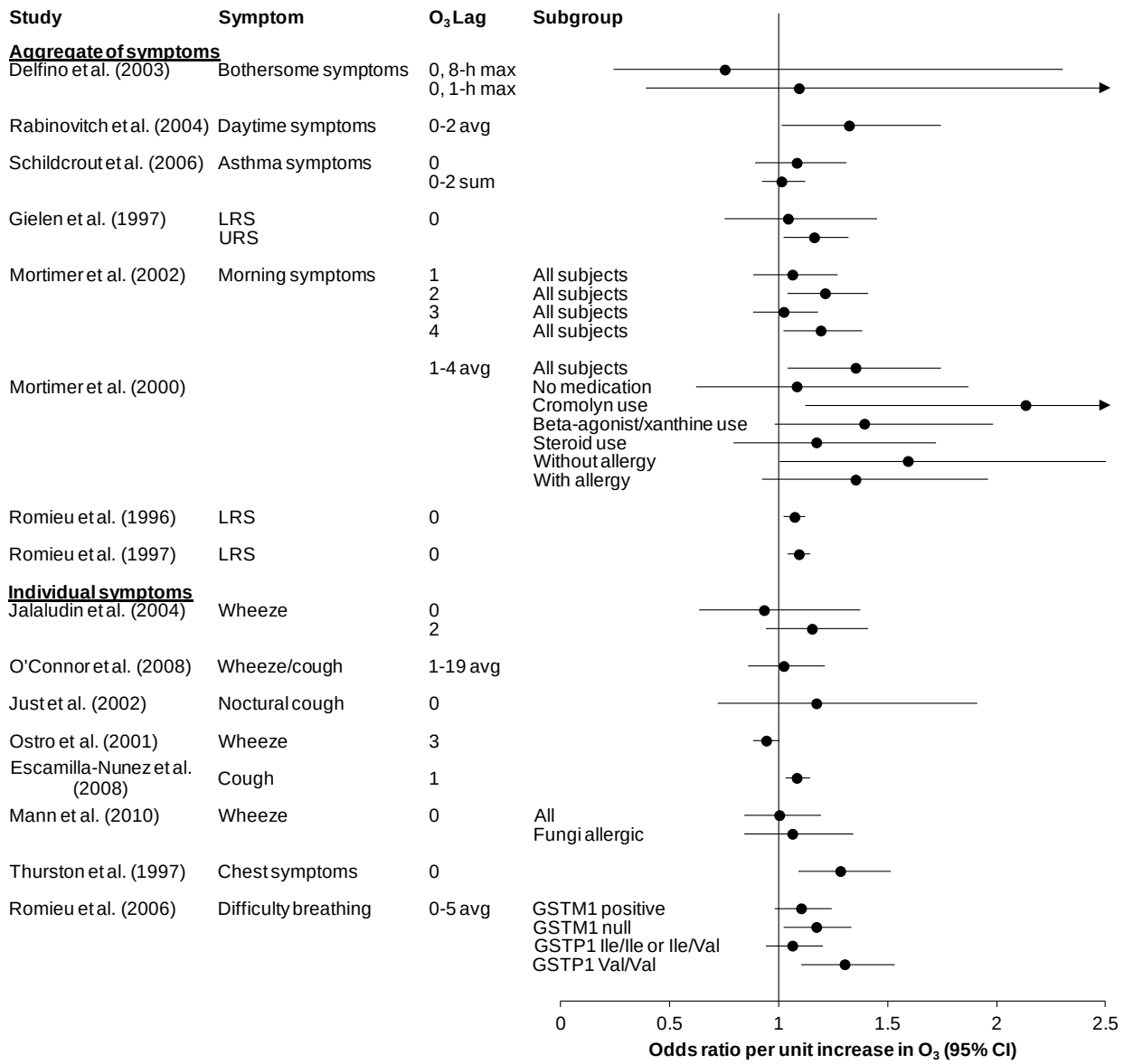
| Study* | Location | Study Period | $O_3$ Averaging Time | Mean/Median Concentration (ppb) | Upper Percentile Concentrations (ppb) |
|---|---|---|---|---|---|
| O'Connor et al. (2008) | Boston, MA; Bronx, Manhattan NY; Chicago, IL; Dallas, TX, Seattle, WA; Tucson, AZ (ICAS) | Aug 1998-July 2001 | 24-h avg | NR | NR |
| Ostro et al. (2001) | Los Angeles, CA | Aug-Oct 1993 | 1-h max | Los Angeles: 59.5<br>Pasadena: 95.8 | Max: 130<br>Max: 220 |
| Mann et al. (2010) | Fresno/Clovis, California | Winter-Summer 2000-2005 | 8-h max | 49.4 (median) | 75th: 69.5, Max: 120.0 |
| Just et al. (2002) | Paris, France | Apr-June 1996 | 24-h avg | 30.0[b] | Max: 61.7[b] |

*Note: Studies presented in order of first appearance in the text of this section.

NR = Not Reported, NCICAS = National Cooperative Inner-City Asthma Study, CAMP = Childhood Asthma Management Program, ICAS = Inner City Asthma Study.

[a]Measured on site of subjects' outdoor activity.

[b]Concentrations converted from µg/$m^3$ to ppb using the conversion factor of 0.51 assuming standard temperature (25°C) and pressure (1 atm).




Note: Results are presented first for aggregate indices of symptoms then for individual symptoms. Within each category, results generally are organized in order of increasing mean ambient $O_3$ concentration. LRS = lower respiratory symptoms, URS = upper respiratory symptoms. Odds ratios are from single-pollutant models and are standardized to a 40-, 30-, and 20-ppb increase for 1-h max, 8-h max (or 8-h avg or 15-h avg), and 24-h avg $O_3$ concentrations, respectively.

**Figure 6-12 Associations between ambient $O_3$ concentrations and respiratory symptoms in children with asthma.**

## Table 6-20 Associations between ambient $O_3$ concentrations and respiratory symptoms in children with asthma for studies presented in Figure 6-12.

| Study* | Location/Population | $O_3$ Averaging Time | $O_3$ Lag | Symptom | Subgroup | Standardized OR (95% CI)[a] |
|---|---|---|---|---|---|---|
| **Studies examining aggregates of symptoms** | | | | | | |
| Delfino et al. (2003) | Los Angeles, CA<br>22 children with asthma, ages 10-16 yr | 8-h max<br>1-h max | 0 | Bothersome symptoms | | 0.75 (0.24, 2.30)<br>1.09 (0.39, 3.03) |
| Rabinovitch et al. (2004) | Denver, CO<br>86 children with asthma, ages 6-12 yr | 1-h max | 0-2 avg | Daytime symptoms | | 1.32 (1.01, 1.74) |
| Schildcrout et al. (2006) | Albuquerque, NM; Baltimore, MD; Boston, MA; Denver, CO; San Diego, CA; Seattle, WA; St. Louis, MO; Toronto, ON, Canada<br>990 children with asthma, ages 5-12 yr | 1-h max | 0<br>0-2 sum | Asthma symptoms | | 1.08 (0.89, 1.31)<br>1.01 (0.92, 1.12) |
| Gielen et al. (1997) | Amsterdam, Netherlands<br>61 children with asthma, ages 7-13 yr | 8-h max | 0 | LRS<br>URS | | 1.04 (0.75, 1.45)<br>1.16 (1.02, 1.32) |
| Mortimer et al. (2002); Mortimer et al. (2000) | Bronx, East Harlem, NY; Baltimore, MD; Washington, DC; Detroit, MI, Cleveland, OH; Chicago, IL; St. Louis, MO<br>846 children with asthma, ages 4-9 yr | 8-h avg (10 a.m.-6 p.m.) | 1<br>2<br>3<br>4<br>1-4 avg | Morning symptoms | All subjects<br>All subjects<br>All subjects<br>All subjects<br>All subjects<br>No medication use<br>Cromolyn use<br>β-agonist/xanthine use<br>Steroid use<br>Without allergy<br>With allergy | 1.06 (0.88, 1.27)<br>1.21 (1.04, 1.41)<br>1.02 (0.88, 1.18)<br>1.19 (1.02, 1.38)<br>1.35 (1.04, 1.74)<br>1.08 (0.62, 1.87)<br>2.13 (1.12, 4.04)<br>1.39 (0.98, 1.98)<br>1.17 (0.79, 1.72)<br>1.59 (1.00, 2.52)<br>1.35 (0.92, 1.96) |
| Romieu et al. (1996) | Northern Mexico City, Mexico<br>71 children with asthma, ages 5-7 yr | 1-h max | 0 | LRS | | 1.07 (1.02, 1.12) |
| Romieu et al. (1997) | Southern Mexico City, Mexico<br>65 children with asthma, ages 5-13 yr | 1-h max | 0 | LRS | | 1.09 (1.04, 1.14) |
| **Studies examining individual symptoms** | | | | | | |
| Jalaludin et al. (2004) | Sydney, Australia<br>125 children with asthma, mean age 9.6 yr | 15-h avg (6 a.m.-9 p.m.) | 0<br>2 | Wheeze | | 0.93 (0.63, 1.37)<br>1.15 (0.94, 1.41) |
| O'Connor et al. (2008) | Boston, MA; Bronx, Manhattan NY; Chicago, IL; Dallas, TX, Seattle, WA; Tucson, AZ<br>861 children with asthma, mean (SD) age 7.7 (2.0) yr | 24-h avg | 1-19 avg | Wheeze/ cough | | 1.02 (0.86, 1.21) |

| Study* | Location/Population | $O_3$ Averaging Time | $O_3$ Lag | Symptom | Subgroup | Standardized OR (95% CI)[a] |
|---|---|---|---|---|---|---|
| Just et al. (2002) | Paris, France<br>82 children with asthma, mean (SD) age 10.9 (2.5) yr | 24-h avg | 0 | Nocturnal cough | | 1.17 (0.72, 1.91) |
| Ostro et al. (2001) | Los Angeles, CA<br>138 children with asthma, ages 6-13 yr | 1-h max | 3 | Wheeze | | 0.94 (0.88, 1.00) |
| Escamilla-Nuñez et al. (2008) | Mexico City, Mexico<br>147 children with asthma, mean (SD) age 9.6 (2.1) yr | 1-h max | 1 | Wheeze | | 1.08 (1.03, 1.14) |
| Mann et al. (2010) | Fresno/Clovia, California<br>280 children with asthma, ages 6-11 yr | 8-h max | 0 | Wheeze | All<br>Fungi allergic | 1.00 (0.84, 1.19)<br>1.06 (0.84, 1.34) |
| Thurston et al. (1997) | CT River Valley, CT<br>166 children with asthma, ages 7-13 yr | 1-h max | 0 | Chest symptoms | | 1.28 (1.09, 1.51) |
| Romieu et al. (2006) | Mexico City, Mexico<br>151 children with asthma, mean age 9 yr | 1-h max | 0-5 avg | Difficulty breathing | GSTM1 positive<br>GSTM1 null<br>GSTP1 Ile/Ile or Ile/Val<br>GSTP1 Val/Val | 1.10 (0.98, 1.24)<br>1.17 (1.02, 1.33)<br>1.06 (0.94, 1.20)<br>1.30 (1.10, 1.53) |
| Gent et al. (2003)[b] | CT, Southern MA<br>130 children with asthma on maintenance medication | 1-h max | 0 | Wheeze | $O_3$ <43.2 ppb<br>$O_3$ 43.2-51.5 ppb<br>$O_3$ 51.6-58.8 ppb<br>$O_3$ 58.9-72.6 ppb<br>$O_3$ ≥ 72.7 ppb | 1.00 (reference)<br>1.04 (0.89, 1.21)<br>1.16 (1.00, 1.35)<br>1.16 (1.00, 1.35)<br>1.22 (0.97, 1.53) |
| | | | | Chest tightness | $O_3$ <43.2 ppb<br>$O_3$ 43.2-51.5 ppb<br>$O_3$ 51.6-58.8 ppb<br>$O_3$ 58.9-72.6 ppb<br>$O_3$ ≥ 72.7 ppb | 1.00 (reference)<br>1.11 (0.91, 1.36)<br>1.01 (0.83, 1.23)<br>1.16 (0.97, 1.39)<br>1.31 (0.97, 1.77) |

*Includes studies for Figure 6-12, plus others.

LRS = Lower respiratory symptoms, URS = Upper respiratory symptoms.

[a]Effect estimates are standardized to a 40, 30, and 20 ppb increase for 1-h max, 8-h max (or 8-h avg or 15-h avg), and 24-h avg $O_3$, respectively.

[b]Results not included in Figure 6-12 because results presented per quintile of ambient $O_3$ concentration.

Among U.S. multicity studies of children with asthma, each of which examined a different $O_3$ averaging time, $O_3$ was not consistently associated with increases in respiratory symptoms (O'Connor et al., 2008; Schildcrout et al., 2006; Mortimer et al., 2002). In the NCICAS (described in Section 6.2.1.2), which analyzed greater than 11,000 person-days of data during one warm season, increases in most evaluated lags of $O_3$ (1 to 4 and 1-4 avg) were associated with increases in asthma symptoms. A 30-ppb increase in lag 1-4 avg, of 8-h avg (10 a.m.-6 p.m.), $O_3$ was associated with an increase in morning asthma symptoms with an OR of 1.35 (95% CI: 1.04, 1.69) (Mortimer et al., 2002). The OR was similar in an analysis restricted to $O_3$ concentrations <80 ppb. Associations were similarly strong for lags 2 and 4 of $O_3$ but weaker for lags 1 and 3 (Figure 6-12 [and Table 6-20]).

Like NCICAS, the U.S. multicity Childhood Asthma Management Program (CAMP, with two cities in common with NCICAS, Table 6-19) collected daily symptom data, analyzed data collected between May and September, and evaluated multiple lags of $O_3$ (Schildcrout et al., 2006). However, associations in CAMP were weaker for all evaluated lags of $O_3$. In meta-analyses that combined city-specific estimates, a 40-ppb increase in lag 0 of 1-h max $O_3$ was associated with asthma symptoms with an OR of 1.08 (95% CI: 0.89, 1.31). Odds ratios for lags 1 and 2 and the lag 0-2 sum of $O_3$ were between 1.0 and 1.03. In this study, data available from an average of 12 subjects per day per city were used to produce city-specific ORs. The person-days of data contributing to each city-specific model were likely less than those of the other multicity studies. These city-specific ORs then were combined in meta-analyses to produce study-wide ORs. Because of these methodological details of CAMP, power to detect associations with $O_3$ likely was less than that for other pollutants (which were analyzed using year-round data), other multicity studies, and several available single-city studies.

Inconsistent associations between wheeze and nighttime asthma were reported in the ICAS cohort (described in Section 6.2.1.2) (O'Connor et al., 2008); however, the results are considered separately from the other available evidence because symptom incidence was examined in association with 19-day avg (of 24-h avg) concentrations of $O_3$. Most evidence, whether from multi- or single-city studies, indicates associations of respiratory symptoms with shorter lags of $O_3$ up to a few days. The implications of ICAS results are more limited because of a lack of a well-characterized mode of action for respiratory symptoms resulting from longer lag periods of $O_3$ exposure. ICAS was precluded from examining shorter lag periods because data were collected every 2 months on the number of days with symptoms during the previous 2 weeks.

Several longitudinal studies conducted in different cohorts of children with asthma in Mexico City, Mexico examined and found increases in respiratory symptoms in association with 1-h max $O_3$ concentrations (Escamilla-Nuñez et al., 2008; Romieu et al., 2006; Romieu et al., 1997; Romieu et al., 1996). Romieu et al. (1997); (1996) found larger increases in symptoms in association with increases in 1-h max $O_3$ at lag 0 than at lag 1 or 2. Recent studies in Mexico City expanded on earlier evidence by indicating associations with multiday averages of $O_3$ concentrations. Romieu et al. (2006) and Escamilla-Nuñez et al. (2008) found that ORs for associations of ambient 1-h max $O_3$ concentrations with respiratory symptoms and medication use increased as the number of averaging days increased (up to lag 0-5 avg).

Studies of children with asthma examined factors that may modify symptom responses to ambient $O_3$ exposure but did not produce conclusive evidence. Larger $O_3$-associated (8-h avg [10 a.m.-6 p.m.] or 8-h max) increases in symptoms were found in children taking asthma medication, although the specific medications examined differed between studies. As with results for PEF, in the NCICAS multicity cohort, $O_3$-associated increases in morning symptoms were larger in children taking cromolyn (used to treat asthma with allergy) or beta-agonists/xanthines than in children taking no medication. Odds ratios were similar in

children taking steroids and children taking no medication (Figure 6-12 [and Table 6-20]) (Mortimer et al., 2000). Among children with asthma in Southern New England, $O_3$-associated increases in symptoms were limited mostly to children taking steroids, cromolyn, or leukotriene inhibitors for maintenance (Gent et al., 2003).

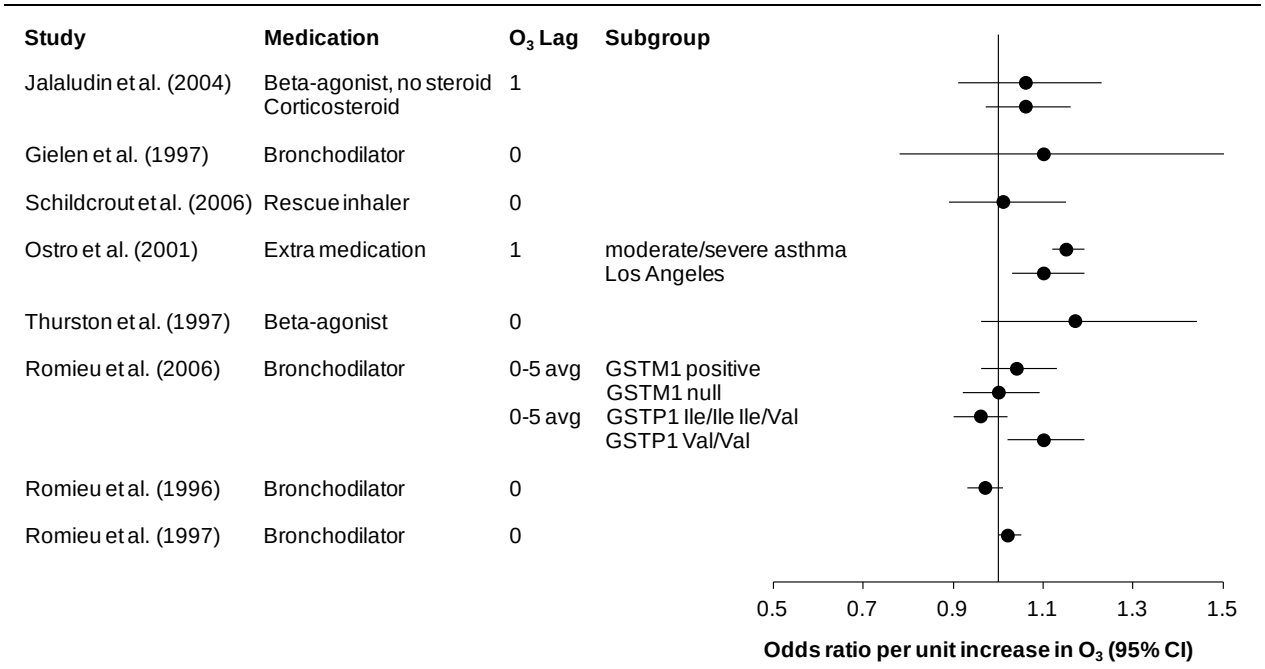
In most studies of children with asthma, a majority of subjects (52 to 100%) had atopy as determined by sensitization to any examined allergen. While other studies found $O_3$-associated increases in pulmonary inflammation in children with atopy (Section 6.2.3.2) and in animal models of allergy (Section 6.2.3.3), evidence did not indicate that the risk of $O_3$-associated respiratory symptoms differed in children with asthma with and without atopy. In the NCICAS, Mortimer et al. (2000) found that an increase in lag 1-4 avg (8-h avg, 10 a.m.-6 p.m.) $O_3$ was associated with a similar increased incidence of asthma symptoms among the 79% of subjects with atopy and the 21% of subjects without atopy (Figure 6-12 [and Table 6-20]). Odds ratios for $O_3$ did not differ by residential allergen levels. Among children with asthma in Fresno, CA, most ORs for associations of single- and multi-day lags of 8-h max $O_3$ concentrations (0-14 days) with wheeze were near or below 1.0 among all subjects. Among the various $O_3$ lags examined, increases in $O_3$ were not consistently associated with increases in wheeze in subjects with cat or fungi allergy either (Mann et al., 2010).

Romieu et al. (2006) found differences in $O_3$-associated respiratory symptoms by genetic variants in GST enzymes, particularly, GSTP1 but less so for GSTM1. Compared with GSTP1 Ile/Ile or Ile/Val subjects, larger effects were estimated for GSTP1 Val/Val subjects (Figure 6-12 [and Table 6-20]). The largest OR was found for difficulty breathing in children with asthma who had both GSTM1 null and GSTP1 Val/Val genotypes (OR: 1.49 [95% CI: 1.14, 1.93] per 40-ppb increase in lag 0-5 avg of 1-h max $O_3$). These results are consistent with those described for antioxidant capacity modifying $O_3$-associated changes in lung function (Section 6.2.1.2) and pulmonary inflammation [Section 6.2.3.2 for results in the same cohort (Sienra-Monge et al., 2004)]; however, effect modification by GSTP1 variants has not been consistent. (Romieu et al., 2006) found an $O_3$-associated decrease in $FEV_1$ only in children with GSTP1 Ile/Ile or Ile/Val genotype. Among children in southern California, GSTP1 Ile/Ile was associated with greater risk of asthma onset (Section 7.2.1). Asthma prevalence has not been consistently associated with a particular GSTP1 genotype either (Tamer et al., 2004; Mapp et al., 2002; Hemmingsen et al., 2001).

### Asthma Medication Use

Although recent studies contributed mixed evidence, the collective body of evidence supports associations between increases in ambient $O_3$ concentration and increased asthma medication use in children (Figure 6-13 [and Table 6-21]). Most studies examined and found associations with 1-h max $O_3$ concentrations lagged 0 or 1 day; however, associations also were found for multiday average $O_3$ concentrations (lag

0-5 avg in Romieu et al. (2006) and lags 0-2 avg and 0-4 avg in Just et al. (2002). Within several studies, associations of $O_3$ were similar for respiratory symptoms and asthma medication use (Escamilla-Nuñez et al., 2008; Romieu et al., 2006; Schildcrout et al., 2006; Jalaludin et al., 2004; Romieu et al., 1997; Thurston et al., 1997). As an exception, Romieu et al. (1996) found that $O_3$ was associated with an increase in respiratory symptoms but not bronchodilator use, and Rabinovitch et al. (2004) indicated statistically significant associations with symptoms but not bronchodilator use (OR not reported). A few studies found higher odds of $O_3$-associated increases in asthma medication use than in respiratory symptoms (Just et al., 2002; Ostro et al., 2001).




Note: Results generally are presented in order of increasing mean ambient $O_3$ concentration. Odds ratios are from single-pollutant models and are standardized to a 40-ppb increase for 1-h max $O_3$ and a 30-ppb increase for 8-h max or 15-h avg $O_3$.

**Figure 6-13 Associations between ambient $O_3$ concentrations and asthma medication use.**

## Table 6-21 Associations between ambient $O_3$ concentrations and asthma medication use for studies presented in Figure 6-13.

| Study* | Location/Population | $O_3$ Averaging Time | $O_3$ Lag | Medication | Subgroup | Standardized OR (95% CI)[a] |
|---|---|---|---|---|---|---|
| Jalaludin et al. (2004) | Sydney, Australia<br>125 children with asthma, mean age 9.6 yr | 15-h avg (6 a.m.-9 p.m.) | 1 | Beta-agonist, no CS | | 1.06 (0.91, 1.23) |
| | | | | Inhaled CS | | 1.06 (0.97, 1.16) |
| Gielen et al. (1997) | Amsterdam, Netherlands<br>61 children with asthma, ages 7-13 yr | 8-h max | 0 | Bronchodilator | | 1.10 (0.78, 1.55) |
| Schildcrout et al. (2006) | Albuquerque, NM; Baltimore, MD; Boston, MA; Denver, CO; San Diego, CA; Seattle, WA; St. Louis, MO; Toronto, ON, Canada<br>990 children with asthma, ages 5-12 yr | 1-h max | 0 | Rescue inhaler | | 1.01 (0.89, 1.15) |
| Ostro et al. (2001) | Los Angeles, CA<br>138 children with asthma, ages 6-13 yr | 1-h max | 1 | Any extra medication | Moderate/severe asthma | 1.15 (1.12, 1.19) |
| | | | | | Los Angeles | 1.10 (1.03, 1.19) |
| Thurston et al. (1997) | CT River Valley, CT<br>166 children with asthma, ages 7-13 yr | 1-h max | 0 | Beta-agonist | | 1.17 (0.96, 1.44) |
| Romieu et al. (2006) | Mexico City, Mexico<br>151 children with asthma, mean age 9 yr | 1-h max | 0-5 avg | Bronchodilator | GSTM1 positive | 1.04 (0.96, 1.13) |
| | | | | | GSTM1 null | 1.00 (0.92, 1.09) |
| | | | | | GSTP1 Ile/Ile or Ile/Val | 0.96 (0.90, 1.02) |
| | | | | | GSTP1 Val/Val | 1.10 (1.02, 1.19) |
| Romieu et al. (1996) | Northern Mexico City, Mexico<br>71 children with asthma, ages 5-7 yr | 1-h max | 0 | Bronchodilator | | 0.97 (0.93, 1.01) |
| Romieu et al. (1997) | Southern Mexico City, Mexico<br>65 children with asthma, ages 5-13 yr | 1-h max | 0 | Bronchodilator | | 1.02 (1.00, 1.05) |
| Just et al. (2002)[b] | Paris, France<br>82 children with asthma, mean (SD) age 10.9 (2.5) yr | 24-h avg | 0 | Beta-agonist, no steroid | | 3.95 (1.22, 12.9) |
| Gent et al. (2003)[b] | CT, southern MA<br>130 children with asthma on maintenance medication | 1-h max | 0 | Bronchodilator | $O_3$ <43.2 ppb | 1.00 (reference) |
| | | | | | $O_3$ 43.2-51.5 ppb | 1.00 (0.96, 1.05) |
| | | | | | $O_3$ 51.6-58.8 ppb | 1.04 (1.00, 1.09) |
| | | | | | $O_3$ 58.9-72.6 ppb | 1.02 (0.98, 1.07) |
| | | | | | $O_3$ ≥ 72.7 ppb | 1.05 (0.97, 1.13) |

*Includes studies in Figure 6-13, plus others.

CS = Corticosteroid

[a]Effect estimates are standardized to a 40-ppb increase for 1-h max $O_3$, a 30-ppb increase for 8-h max or 15-h avg $O_3$, and a 20-ppb increase for 24-h avg $O_3$.

[b]Results not included in Figure 6-13. Results from Just et al. (2002) were out of range of other estimates, and results from Gent et al. (2003) were presented per quintile of ambient $O_3$ concentration.

### Changes in Activity

While investigation has been limited, evidence does not consistently demonstrate $O_3$-associated diminished activity in children with asthma (O'Connor et al., 2008; Delfino et al., 2003). These studies examined different $O_3$ averaging times and lags. In the multicity ICAS cohort, O'Connor et al. (2008) found that a 20-ppb increase in lag 1-19 avg (of 24-h avg $O_3$) was associated with a 10% lower odds (95% CI: -26, 10) of slow play. In a small (n = 22) panel study conducted in children with asthma in Los Angeles CA, Delfino et al. (2003) found that a 40-ppb increase in lag 0 of 1-h max $O_3$ was associated with an increase in symptoms that interfered with daily activity with an OR of 7.14 (95% CI: 1.18, 43.2). Several studies reported increases in school absenteeism in children with asthma in association with increases in ambient $O_3$ concentration with long lag periods (14-day and 30-day distributed lags, 19-day avg) (O'Connor et al., 2008; Gilliland et al., 2001; Chen et al., 2000). Whereas Chen et al. (2000) and O'Connor et al. (2008) examined absences for any reason, Gilliland et al. (2001) found associations with absences for respiratory illnesses. Despite this evidence, several limitations are notable, including the lack of a well-characterized mode of action for respiratory effects occurring with longer lag periods of $O_3$ exposure and the potential for residual seasonal confounding with examination of long lag periods. In analyses of single-day lags, Gilliland et al. (2001) found associations with $O_3$ lagged 1 to 5 days, indicating respiratory absences may be affected by $O_3$ exposures with shorter lag periods.

## 6.2.4.2 Adults with Respiratory Disease

Within a small body of studies, several found that increases in ambient $O_3$ concentration (8-hour or 1-h max) were associated with increases in respiratory symptoms in adults with asthma (Khatri et al., 2009; Feo Brito et al., 2007; Ross et al., 2002). Details from studies of respiratory symptoms in adults with respiratory disease regarding location, time period, and ambient $O_3$ concentrations are presented in Table 6-22. These studies used different exposure assessment methods: concentrations averaged from sites closest to subjects' location each hour (Khatri et al., 2009) or concentrations measured at one (Ross et al., 2002) or multiple (Feo Brito et al., 2007) city sites. Park et al. (2005a) found inconsistent associations for 24-h avg $O_3$ measured at 10 city sites among the various symptoms and medication use examined in adults with asthma in Korea during a period of dust storms. In a study of adults with COPD in London, England, increases in lag 1 of 8-h max $O_3$ (at a single city site) were associated with higher odds of dyspnea and sputum changes but lower odds of nasal discharge, wheeze, or upper respiratory symptoms (Peacock et al., 2011).

**Table 6-22 Mean and upper percentile $O_3$ concentrations in epidemiologic studies of respiratory symptoms and medication use in adults with respiratory disease.**

| Study* | Location | Study Period | $O_3$ Averaging Time | Mean/Median Concentration (ppb) | Upper Percentile Concentrations (ppb) |
|---|---|---|---|---|---|
| Khatri et al. (2009) | Atlanta, GA | May-Sept 2003, 2005, 2006 | 8-h max | 61 (median)[a] | 75th: 74[a] |
| Feo Brito et al. (2007) | Ciudad Real and Puertollano, Spain | May-June 2000-2001 | 1-h max | 65.9 (Ciudad Real)[b]<br>56.8 (Puertollano)[b] | Max: 101.5[b] (Ciudad Real); 138.2[b] (Puertollano) |
| Eiswerth et al. (2005) | Glendora, CA | Oct-Nov 1983 | 1-h max | NR | NR |
| Ross et al. (2002) | East Moline, IL | Apr-Oct 1994 | 8-h avg | 41.5 | Max: 78.3 |
| Peacock et al. (2011) | London, England | All-year 1995-1997 | 8-h max | 15.5 | Autumn/Winter Max: 32<br>Spring/Summer Max: 74 |
| Park et al. (2005a) | Incheon, Korea | Mar-June 2002 | 24-h avg | Dust event days: 23.6<br>Control days: 25.1 | NR |
| Wiwatanadate and Liwsrisakun (2011) | Chiang Mai, Thailand | Aug 2005-June 2006 | 24-h avg | 17.5 | 90th: 26.8, Max: 34.7 |

*Note: Studies presented in order of first appearance in the text of this section.

NR = Not Reported

[a]Individual-level estimates were derived based on time spent in the vicinity of various $O_3$ monitors.

[b]Concentrations converted from μg/$m^3$ to ppb using the conversion factor of 0.51 assuming standard temperature (25°C) and pressure (1 atm).

Some studies that included adults with asthma examined populations with a high prevalence of atopy. In a study of children and adults with asthma (at least 53% with atopy), Ross et al. (2002) found that an increase in lag 1-3 avg of 8-h max $O_3$ was associated with an increase in symptom score and asthma medication use. Feo Brito et al. (2007) followed 137 adults with asthma in two central Spain cities. All subjects had pollen allergy and were examined during pollen season. In Puertollano, $O_3$ concentrations were obtained from four city monitors, and a 40-ppb increase in lag 3 of 1-h max $O_3$ was associated with a 14.3% increase (95% CI: 3.6, 26.0) in the number of subjects reporting respiratory symptoms, adjusting only for time trend. The association was imprecise in Ciudad Real (2.3% increase [95% CI: -14, 21%] per 40-ppb increase in lag 4 of 1-h max $O_3$), a city characterized by lower ambient air pollution levels and a narrower range of ambient $O_3$ concentrations as measured at a single site established by investigators.

Cross-sectional studies reported ambient $O_3$-associated decreases in activity in adults with asthma; however, due to various limitations in the collective body of evidence, firm conclusions are not warranted. Although conducted over single seasons, the studies did not consider potential confounding by meteorological factors. In a warm season study in Atlanta, GA (described in Section 6.2.1.2), Khatri et al. (2009) found

that a 30-ppb increase in lag 2 of 8-h max $O_3$ was associated with a 0.69-point decrease (95% CI: -1.3, -0.11) in the Juniper quality of life score, which incorporates indices for symptoms, mood, and activity limitations (7-point scale). In a fall study conducted in the Los Angeles, CA area in individuals with asthma (age 16 years and older), Eiswerth et al. (2005) found that a 40-ppb increase in 1-h max $O_3$ was associated with a 2.4% (95% CI: 0.83, 4) lower probability of indoor activity but higher probability of outdoor activity. The authors acknowledged that their findings were unexpected and may have been influenced by lack of control for potential confounders, but they interpreted the decrease in indoor activities as rest replacing chores. In contrast with the aforementioned studies, a panel study of individuals with asthma (ages 13-78 years) in Thailand found that a 20-ppb increase in lag 4 of 24-h avg $O_3$ was associated with a 26% (95% CI: 4, 43) lower odds of symptoms that interfered with activities (Wiwatanadate and Liwsrisakun, 2011).

#### 6.2.4.3 Populations not Restricted to Individuals with Asthma

Locations, time periods, and ambient $O_3$ concentrations for studies of respiratory symptoms in populations not restricted to individuals with asthma are presented in Table 6-23. Most studies examined children, and in contrast with lung function results (Section 6.2.1.2), short-term increases in ambient $O_3$ concentration were not consistently associated with increases in respiratory symptoms in children in the general population (Figure 6-14 [and Table 6-24]). Because examination was limited, conclusions about the effects of ambient $O_3$ exposure on respiratory symptoms in adults are not warranted.

**Table 6-23 Mean and upper percentile $O_3$ concentrations in epidemiologic studies of respiratory symptoms in populations not restricted to individuals with asthma.**

| Study* | Location | Study Period | $O_3$ Averaging Time | Mean/Median Concentration (ppb) | Upper Percentile Concentrations (ppb) |
|---|---|---|---|---|---|
| Neas et al. (1995) | Uniontown, PA | June-August 1990 | 12-h avg (8 a.m.-8 p.m.) | 50 | NR |
| Linn et al. (1996) | Rubidoux, Upland, Torrance, CA | September-June 1992-1994 | 24-h avg personal<br>24-h avg ambient | 5<br>23 | Max: 16<br>Max: 53 |
| Hoek and Brunekreef (1995) | Deurne and Enkhuizen, Netherlands | March-July 1989 | 1-h max | Deurne: 57<br>Enkhuizen: 59 | Max: 107<br>Max: 114 |
| Rodriguez et al. (2007) | Perth, Australia | All-year, 1996-2003 | 24-h avg<br>1-h max | 28<br>33 | Max: 74<br>Max: 95 |
| Moon et al. (2009) | 4 cities, South Korea | April-May 2003 | 8-h avg (10 a.m.-6 p.m.) | NR | NR |
| Ward et al. (2002) | Birmingham and Sandwell, England | January-March, May-July 1997 | 24-h avg | Winter median: 13.0<br>Summer median: 22.0 | Winter Max: 33<br>Summer Max: 41 |
| Triche et al. (2006) | Southwestern VA | June-August 1995-1996 | 24-h avg<br>8-h max<br>1-h max | 35.2<br>54.5<br>60.8 | 75th: 40.6, Max: 56.6<br>75th: 64.1, Max: 87.6<br>75th: 70.0, Max: 95.0 |
| Gold et al. (1999) | Mexico City, Mexico | January-November 1991 | 24-h avg | 52.0[a] | Max: 103[a] |
| Apte et al. (2008) | Multiple U.S. cities (NR) | Winter or summer 1994-1998 | Workday avg (8 a.m. - 5 p.m.) | 34.2[b] | Max: 86.2[b] |
| | | | 24-h avg | 25.5[b] | Max: 67.3[b] |

*Note: Studies presented in order of first appearance in the text of this section.

NR = Not Reported.

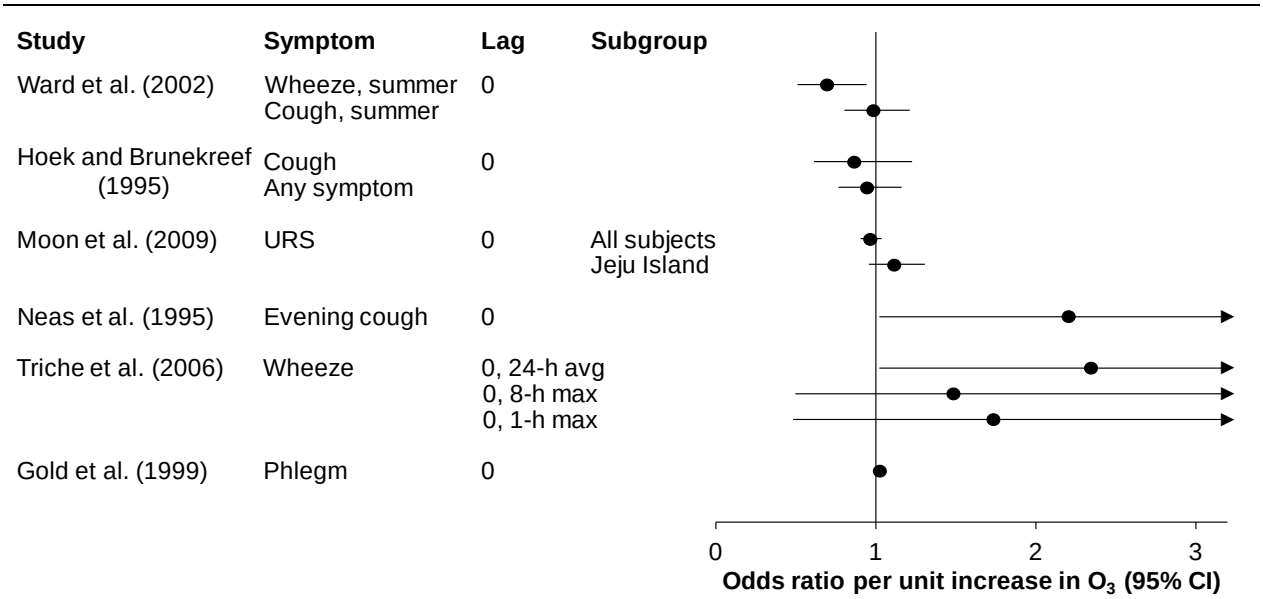
[a]Measured at subject's schools.

[b]Concentrations converted from µg/m$^3$ to ppb using the conversion factor of 0.51 assuming standard temperature (25°C) and pressure (1 atm).

### Children

Evidence of $O_3$-associated increases in respiratory symptoms in children was inconsistent, which did not appear to be attributable to the differences in exposure assessment method among studies [e.g., $O_3$ measured at a single site (Linn et al., 1996; Hoek and Brunekreef, 1995), $O_3$ averaged across multiple city sites (Rodriguez et al., 2007), $O_3$ measured at sites near schools (Moon et al., 2009; Ward et al., 2002)]. Some studies that found weak or inconsistent associations between ambient $O_3$ concentrations and respiratory symptoms in children found $O_3$-associated decrements in lung function (Ward et al., 2002; Linn et al., 1996). In their study of healthy children in Uniontown, Pennsylvania, Neas et al. (1995) found differences in association with respiratory symptoms between two estimates of $O_3$ exposure using ambient $O_3$ measurements from one central site in town. Subjects

spent a mean 5.4 hours outdoors during the 12-hour period (8 a.m.-8 p.m.) over which $O_3$ concentrations were averaged and symptoms were reported. Evening cough was more strongly associated with $O_3$ concentrations weighted by time spent outdoors (OR: 2.20 [95% CI: 1.02, 4.75] per 30-ppb increase in lag 0 of 12-h avg $O_3$) than with unweighted $O_3$ concentrations (OR: 1.36 [95% CI: 0.86, 2.13]). Time spent outdoors has been shown to influence $O_3$ personal-ambient ratios and correlations (Section 4.3.3); thus, the weighted $O_3$ concentrations may have represented personal $O_3$ exposures better.




Note: Results generally are presented in increasing order of mean ambient $O_3$ concentration. URS = Upper respiratory symptoms. Odds ratios are from single-pollutant models and are standardized to a 40-, 30-, and 20-ppb increase for 1-h max, 8-h max (or 8-h avg or 12-h avg), and 24-h avg $O_3$ concentrations, respectively.

**Figure 6-14 Associations between ambient $O_3$ concentrations and respiratory symptoms in children in the general population.**

## Table 6-24 Associations between ambient $O_3$ concentrations and respiratory symptoms in children in the general population for studies represented in Figure 6-14.

| Study* | Location/ Population | $O_3$ Lag | $O_3$ Averaging Time | Symptom | Subgroup | Standardized OR (95% CI)[a] |
|---|---|---|---|---|---|---|
| Ward et al. (2002) | Birmingham and Sandwell, England<br>162 children, age 9 yr | 0 | 24-h avg | Wheeze, summer<br>Cough, summer | | 0.69 (0.51, 0.94)<br>0.98 (0.80, 1.21) |
| Hoek and Brunekreef (1995) | Deurne and Enkhuizen, Netherlands<br>300 children, ages 7-11 yr | 0 | 1-h max | Cough<br>Any symptom | | 0.86 (0.61, 1.22)<br>0.94 (0.76, 1.16) |
| Moon et al. (2009) | 4 cities, South Korea<br>696 children, ages <13 yr | 0 | 8-h avg (10 a.m.-6 p.m.) | URS | All subjects<br>Jeju Island | 0.96 (0.90, 1.03)<br>1.11 (0.95, 1.30) |
| Neas et al. (1995) | Uniontown, PA<br>83 healthy children, 4th and 5th grades | 0 | 12-h avg (8 a.m.-8 p.m.) | Evening cough | | 2.20 (1.02, 4.75)[b] |
| Triche et al. (2006) | Southwestern VA<br>61 infants of mothers with asthma, age <1 yr | 0 | 24-h avg<br>8-h max<br>1-h max | Wheeze | | 2.34 (1.02, 5.37)<br>1.48 (0.49, 4.41)<br>1.73 (0.48, 6.22) |
| Gold et al. (1999) | Mexico City, Mexico<br>40 children, ages 8-11 yr | 1 | 24-h avg | Phlegm | | 1.02 (1.00, 1.04) |
| Linn et al. (1996)[c] | Rubidoux, Upland, Torrance, CA<br>269 children, 4th and 5th grades | 0 | 24-h avg | Evening symptom score | | -0.96 (-2.2, 0.26) |

*Includes studies in Figure 6-14, plus others.

URS = Upper respiratory symptoms

[a]Effect estimates are standardized to a 40-, 30-, and 20-ppb increase for 1-h max, 8-h max (or 8-h avg or 12-h avg), and 24-h avg $O_3$, respectively.

[b]$O_3$ concentrations were weighted by the proportion of time spent outdoors.

[c]Results not presented in Figure 6-14 because outcome is a continuous variable indicating intensity of symptoms (negative indicates improvement in symptoms).

Several other panel studies of children, in which asthma prevalence ranged from 0 to 50%, reported null or negative associations between various averaging times and lags of ambient $O_3$ concentration and respiratory symptoms (Moon et al., 2009; Rodriguez et al., 2007; Ward et al., 2002; Linn et al., 1996; Hoek and Brunekreef, 1995). Among children in Mexico City, Gold et al. (1999) reported an increase in phlegm in association with an increase in lag 1 of 24-h avg $O_3$ concentration measured at schools; however, investigators acknowledged being unable to distinguish between the effects of $O_3$ and $PM_{10}$ due to their high correlation ($r = 0.75$).

Unlike other studies that examined ambient $O_3$ concentrations from a single monitoring site, Triche et al. (2006) found respiratory symptoms to be associated with $O_3$ measured at a site that for some subjects was located >100 miles away from home (Figure 6-14 [and Table 6-24]). Subjects included infants in Southwestern VA. Odds ratios were 46-73% larger in the group who had mothers with asthma than

among all infants (Triche et al., 2006). Larger ORs were found for 24-h avg than 1-h or 8-h max $O_3$ concentrations, particularly for wheeze but less so for difficulty breathing. While these results suggested that children with mothers with asthma may be at increased risk of $O_3$-related respiratory morbidity, the authors acknowledged that mothers with asthma may be more likely to report symptoms in their children. Additionally, transient wheeze, which is common in infants, may not predict respiratory morbidity later in life. In another cohort of children with parental history of asthma that was followed to an older age (5 years), increases in ambient $O_3$ concentration (increment of effect estimate not reported) were not associated with increases in respiratory symptoms (Rodriguez et al., 2007).

#### Adults

A cross-sectional study of 4,200 adult workers from 100 office buildings across the U.S. found that multiple ambient $O_3$ metrics, including the 24-hour average, the workday average (8 a.m.-5 p.m.), and the late workday (3-6 p.m.) average, were associated with similar magnitudes of increase in building-related symptoms (Apte et al., 2008). Office workers likely have a low personal-ambient $O_3$ correlation and ratio, thus the implications of these findings compared to those of the other respiratory symptom studies are limited.

### 6.2.4.4 Confounding in Epidemiologic Studies of Respiratory Symptoms and Medication Use

Epidemiologic evidence does not indicate that confounding by meteorological factors or copollutant exposures fully accounts for associations observed between short-term increases in ambient $O_3$ concentration and respiratory symptoms and medication use. Except where specified in the text, studies found $O_3$-associated increases in respiratory symptoms or medication use in statistical models that adjusted for temperature. Thurston et al. (1997) found no independent association between temperature and respiratory symptoms among children with asthma at summer camps. A few studies additionally included humidity in models (Triche et al., 2006; Ross et al., 2002).

Several studies that examined populations with a high prevalence of atopy found $O_3$-associated increases in respiratory symptoms and asthma medication use with adjustment for daily pollen counts (Just et al., 2002; Ross et al., 2002; Gielen et al., 1997). Gielen et al. (1997) and Ross et al. (2002) examined populations with a high prevalence of grass pollen allergy (52% and 38%, respectively). In a study conducted over multiple seasons, Ross et al. (2002) found a similar magnitude of association between $O_3$ and morning symptoms and medication use with adjustment for pollen counts. Feo Brito et al. (2007) followed adults in central Spain specifically with asthma and pollen allergy. In one city, $O_3$ was associated with an increase in the number of subjects reporting symptoms. A smaller increase was estimated for pollen. Conversely, in another city, pollen was associated with an increased reporting of

respiratory symptoms, whereas $O_3$ was not. The results suggested that $O_3$ and pollen may have independent effects that vary by location, depending on the mix of ambient pollutants.

Results from copollutant models did not indicate strong confounding by copollutants such as $PM_{2.5}$, $PM_{10}$, sulfate, $SO_2$, or $NO_2$ (Table 6-25). Notably, studies examined different averaging times for $O_3$ (1-h max or 8-h avg) and copollutants (3-h avg to 24-h avg) and reported a range of correlations between $O_3$ and copollutants, which may complicate interpretation of copollutant model results. Information on potential copollutant confounding of asthma medication use results was limited.
The association between $O_3$ and bronchodilator use did not change with adjustment for $PM_{2.5}$ in Gent et al. (2003) but decreased in magnitude with adjustment for 12-h avg sulfate in Thurston et al. (1997). In Thurston et al. (1997) and Gent et al. (2003), 1-h max $O_3$ was highly correlated with 12-h avg sulfate ($r = 0.74$) and 24-h avg $PM_{2.5}$ ($r = 0.77$), respectively, making it difficult to distinguish the independent effects of $O_3$. Studies conducted concurrently in two areas of Mexico City, Mexico, examined 1-h max $O_3$ and 24-h avg $PM_{10}$ or $PM_{2.5}$ and found robust ORs for respiratory symptoms for both $O_3$ and PM (Romieu et al., 1997; Romieu et al., 1996). Romieu et al. (1997) reported a moderate correlation between 1-h max $O_3$ and 24-h avg $PM_{10}$ ($r = 0.47$). Associations between $O_3$ and respiratory symptoms were observed in NCICAS in copollutant models with $SO_2$, $NO_2$, or $PM_{10}$, which were examined with different averaging times and lags than was $O_3$ (Mortimer et al., 2002) (Table 6-25). Also difficult are interpretations of the $O_3$-associated increases in respiratory symptoms found with adjustment for two copollutants in the same model (i.e., $PM_{2.5}$ plus $NO_2$ or $PM_{10-2.5}$) (Escamilla-Nuñez et al., 2008; Triche et al., 2006).

**Table 6-25 Associations between ambient $O_3$ concentrations and respiratory symptoms in single- and copollutant models.**

| Study | Location/Population | $O_3$ Metrics | Symptom | OR for $O_3$ in Single-Pollutant Model (95% CI)[a] | OR for $O_3$ in Copollutant Model (95% CI)[a] |
|---|---|---|---|---|---|
| Mortimer et al. (2002) | Bronx, East Harlem, NY; Baltimore, MD; Washington, DC; Detroit, MI, Cleveland, OH; Chicago, IL; St. Louis, MO<br>846 children with asthma, ages 4-9 yr | 8-h avg (10 a.m.-6 p.m.)<br>Lag 1-4 avg | Morning symptoms | 8 cities with $SO_2$ data<br>1.35 (1.04, 1.74) | With lag 1-2 avg, 3-h avg $SO_2$<br>1.23 (0.94, 1.61) |
| | | | | 7 cities with $NO_2$ data<br>1.25 (0.94, 1.67) | With lag 1-6 avg, 24-h avg $NO_2$<br>1.14 (0.85, 1.55) |
| | | | | 3 cities with $PM_{10}$ data<br>1.21 (0.61, 2.41) | With lag 1-2 avg, 24-h avg $PM_{10}$<br>1.08 (0.49, 2.39) |
| Gent et al. (2003)[b] | CT, Southern MA<br>130 children with asthma on maintenance medication | 1-h max, Lag 0 | Wheeze | | with lag 0, 24-h avg $PM_{2.5}$ |
| | | <43.2 ppb | | 1.00 (reference) | 1.00 (reference) |
| | | 43.2-51.5 ppb | | 1.04 (0.89, 1.21) | 1.05 (0.90, 1.23) |
| | | 51.6-58.8 ppb | | 1.16 (1.00, 1.35) | 1.18 (1.00, 1.38) |
| | | 58.9-72.6 ppb | | 1.16 (1.00, 1.35) | 1.25 (1.05, 1.50) |
| | | ≥ 72.7 ppb | | 1.22 (0.97, 1.53) | 1.47 (1.13, 1.90) |
| Thurston et al. (1997) | CT River Valley<br>166 children with asthma, ages 7-13 yr | 1-h max<br>Lag 0 | Chest symptoms | 1.21 (1.12, 1.31)[b] | With lag 0, 12-h avg sulfate<br>1.19 (1.06, 1.35)[b] |
| | | | Beta-agonist use | 1.20 (1.09, 1.32)[b] | With lag 0, 12-h avg sulfate<br>1.07 (0.92, 1.24)[b] |
| Romieu et al. (1996) | Northern Mexico City, Mexico<br>71 children with asthma, ages 5-7 yr | 1-h max<br>Lag 0 | Lower respiratory symptoms | 1.07 (1.02, 1.12) | With lag 0, 24-h avg $PM_{2.5}$<br>1.06 (1.02, 1.10) |
| Romieu et al. (1997) | Southern Mexico City, Mexico<br>65 children with asthma, ages 5-13 yr | 1-h max<br>Lag 0 | Lower respiratory symptoms | 1.09 (1.04, 1.14) | With lag 0, 24-h avg $PM_{10}$<br>1.09 (1.01, 1.19) |

Results generally are presented in order of increasing mean ambient $O_3$ concentration.

[a]ORs are standardized to a 40- and 30-ppb increase for 1-h max and 8-h avg $O_3$, respectively.

[b]Temperature not included in models.

### 6.2.4.5 Summary of Epidemiologic Studies of Respiratory Symptoms and Asthma Medication Use

Comprising a majority of available evidence, single-city and -region epidemiologic studies provide consistent evidence for the effects of short-term increases in ambient $O_3$ exposure on increasing respiratory symptoms and asthma medication use in children with asthma (Figure 6-12 [and Table 6-20] and Figure 6-13 [and Table 6-21]). Evidence from the few available U.S. multicity studies is less consistent (O'Connor et al., 2008; Schildcrout et al., 2006; Mortimer et al., 2002) . However, methodological differences among studies make comparisons across the

multicity studies difficult. Because of fewer person-days of data (Schildcrout et al., 2006) or examination of 19-day averages of ambient $O_3$ concentrations (O'Connor et al., 2008), results from recent multicity studies were not given greater consideration than results from single-city studies in weighing the evidence for ambient $O_3$ exposure and respiratory symptoms in children with asthma. Findings from a small body of studies indicate $O_3$-associated increases in respiratory symptoms in adults with asthma. Associations between short-term increases in ambient $O_3$ concentration and reduced activity in children or adults with asthma are not clearly demonstrated. While $O_3$-associated increases in school absenteeism were found in children with asthma, evidence for respiratory-related absences and for $O_3$ exposure lags shorter than 14 days is sparse. The implications of results for multi-week averages of ambient $O_3$ concentrations are limited because of the lack of a well-characterized mode of action for such lags of $O_3$ exposure and the greater potential for residual seasonal confounding with examination of long lag periods. Short-term increases in ambient $O_3$ concentration were not consistently associated with increases in respiratory symptoms in groups comprising children with and without asthma.

Increases in respiratory symptoms and asthma medication use were associated with increases in ambient $O_3$ concentration assigned to subjects using various methods. Associations were found with methods likely to represent ambient exposures better, including $O_3$ measured on site and at the time of children's outdoor activity (Thurston et al., 1997) and concentrations weighted by time spent outdoors (Neas et al., 1995). However, associations also were found with methods that varied in their representation of ambient exposures and spatial variability in ambient concentrations, i.e., concentrations averaged among subjects' locations each hour (Khatri et al., 2009), measured within 5 km of schools or homes (Escamilla-Nuñez et al., 2008; Romieu et al., 2006; Romieu et al., 1997; Romieu et al., 1996), averaged across multiple sites (Feo Brito et al., 2007; Gent et al., 2003; Mortimer et al., 2002), and measured at a single site (Ross et al., 2002; Gielen et al., 1997).

Associations with respiratory symptoms were demonstrated most frequently for 1-h max and 8-h max or avg $O_3$, and within-study comparisons indicated similar ORs for 1-h max and 8-h max $O_3$ (Delfino et al., 2003; Gent et al., 2003). Respiratory symptoms also were associated with 12-h avg and 24-h avg $O_3$ (Jalaludin et al., 2004; Gold et al., 1999; Neas et al., 1995). Epidemiologic studies examined respiratory symptoms associated with $O_3$ concentrations lagged 0 to 5 days and those averaged over 2 to 19 days. While $O_3$ at lags 0 or 1 were consistently associated with respiratory symptoms, several studies found larger ORs for multiday averages (3- to 6-day) of $O_3$ (Escamilla-Nuñez et al., 2008; Romieu et al., 2006; Just et al., 2002; Mortimer et al., 2002; Ross et al., 2002). Epidemiologic findings for lagged or multiday average $O_3$ are supported by evidence that $O_3$ sensitizes bronchial smooth muscle to hyperreactivity and thus acts as a primer for subsequent exposure to antigens such as allergens (Section 5.3.5). Many studies examined populations with asthma with a high prevalence of atopy (52-100%). In these populations, sensitization of airways provides a biologically plausible mode of action by which increases in respiratory symptoms result from increases in $O_3$ exposure after a lag or accumulated over several days. Further support is provided by findings in controlled

human exposure studies that airway hyperresponsiveness (Section 6.2.2.1) and some indicators of inflammation (Section 6.2.3.1) remained elevated following repeated $O_3$ exposures and by observations from epidemiologic studies that increases in pulmonary inflammation were associated with multiday average $O_3$ concentrations (Section 6.2.3.2).

Epidemiologic study results did not indicate that $O_3$-associated increases in respiratory symptoms are confounded by temperature, pollen, or copollutants. In limited analysis, ambient $O_3$ was associated with respiratory symptoms with adjustment for copollutants, primarily PM. However, identifying the independent effects of $O_3$ in some studies was complicated due to the high correlations observed between $O_3$ and PM or different lags and averaging times examined for copollutants. Nonetheless, the robustness of associations in some studies of individuals with asthma with and without adjustment for ambient copollutant concentrations combined with findings from controlled human exposure studies for the direct effect of $O_3$ exposure provide substantial evidence for the independent effects of short-term ambient $O_3$ exposure on increasing respiratory symptoms.

## 6.2.5 Lung Host Defenses

The mammalian respiratory tract has a number of closely integrated defense mechanisms that, when functioning normally, provide protection from the potential health effects attributed to exposure to a wide variety of inhaled particles and microbes. For simplicity, these interrelated defenses can be divided into two major parts: (1) nonspecific (transport, phagocytosis, and bactericidal activity) 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 2006 $O_3$ AQCD stated that animal toxicological studies provide extensive evidence that acute $O_3$ exposures as low as 0.08 to 0.5 ppm can cause increases in susceptibility to infectious diseases due to modulation of lung host defenses. Table 6-6 through Table 6-9 (U.S. EPA, 1996g, h, i, j) beginning on page 6-41 of the 1996 $O_3$ AQCD (U.S. EPA, 1996a), and Annex Table AX5-7 (U.S. EPA, 2006d), beginning on page AX5-8 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b), present studies on the effects of $O_3$ on host defense mechanisms. This section discusses the various components of host defenses, such as the mucociliary escalator, the phagocytic, bactericidal, and regulatory role of the alveolar macrophages (AMs), the adaptive immune system, and integrated mechanisms that are studied by investigating the host's response to experimental pulmonary infections.

### 6.2.5.1 Mucociliary Clearance

The mucociliary system is one of the lung's primary defense mechanisms. It protects the conducting airways by trapping and quickly removing material that has been

deposited or is being cleared from the alveolar region by migrating alveolar macrophages. Ciliary movement directs particles trapped on the overlying mucous layer toward the pharynx, where the mucus is swallowed or expectorated.

The effectiveness of mucociliary clearance can be determined by measuring such biological activities as the rate of transport of deposited particles; the frequency of ciliary beating; structural integrity of the ciliated cells; and the size, number, and distribution of mucus-secreting cells. Once this defense mechanism has been altered, a buildup of both viable and nonviable inhaled substances can occur on the epithelium which may jeopardize the health of the host, depending on the nature of the uncleared substance. Impaired mucociliary clearance can result in an unwanted accumulation of cellular secretions, increased infections, chronic bronchitis, and complications associated with COPD. A number of previous studies with various animal species have examined the effect of $O_3$ exposure on mucociliary clearance and reported morphological damage to the cells of the tracheobronchial tree from acute and sub-chronic exposure to $O_3$ 0.2 ppm and higher. The cilia were either completely absent or had become noticeably shorter or blunt. Once these animals were placed in a clean-air environment, the structurally damaged cilia regenerated and appeared normal (U.S. EPA, 1986). Based on such morphological observations, related effects such as ciliostasis, increased mucus secretions, and a slowing of mucociliary transport rates might be expected. However, no measurable changes in ciliary beating activity have been reported due to $O_3$ exposure alone. Essentially no data are available on the effects of prolonged exposure to $O_3$ on ciliary functional activity or on mucociliary transport rates measured in the intact animal. In general, functional studies of mucociliary transport have observed a delay in particle clearance soon after acute exposure. Decreased clearance is more evident at higher doses (1 ppm), and there is some evidence of attenuation of these effects (U.S. EPA, 1986). However, no recent studies have evaluated the effects of $O_3$ on mucociliary clearance.

### 6.2.5.2 Alveolobronchiolar Transport Mechanism

In addition to the transport 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.
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 some studies show reduced early (tracheobronchial) clearance after $O_3$ exposure, late (alveolar) clearance of deposited material is accelerated, presumably due to macrophage influx (which in itself can be damaging due to proteases and oxidative reactions in these cells).

### 6.2.5.3 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 via destruction of microorganisms, 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. 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 for bactericidal activity and degradation of ingested material. As discussed in previous AQCDs, 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 required for killing pathogens. For example, in results from earlier work in rabbits, a 2-hour exposure to 0.1 ppm $O_3$ inhibited phagocytosis and a 3-hour exposure to 0.25 ppm decreased lysosomal enzyme activities (Driscoll et al., 1987; Hurst et al., 1970). Similarly, AMs from rats exposed to 0.1 ppm $O_3$ for 1 or 3 weeks exhibited reduced hydrogen peroxide production (Cohen et al., 2002). A controlled human exposure study reported decrements in the ability of alveolar macrophages to phagocytize yeast following exposure of healthy volunteers to 80 to 100 ppb $O_3$ for 6.6-hour during moderate exercise (Devlin et al., 1991). Although the percentage of phagocytosis-capable macrophages was unchanged by $O_3$ exposure, the number of yeast engulfed was reduced when phagocytosis was complement-dependent. However, there was no difference in the ability of macrophages to produce superoxide anion after $O_3$ exposure. These results are consistent with those from another controlled human exposure study in which no changes in the level of lysosomal enzymes or superoxide anion production were observed in macrophages lavaged from healthy human subjects exposed to 400 ppb $O_3$ for 2 hours with heavy intermittent exercise (Koren et al., 1989). More recently, Lay et al. (2007) observed no difference in phagocytic activity or oxidative burst capacity in macrophages or monocytes from sputum or blood collected from healthy volunteers after a 2-hour exposure to 400 ppb $O_3$ with moderate intermittent exercise. However, another study (Alexis et al., 2009) found that oxidative burst and phagocytic activity in macrophages increased in GSTM1 null subjects compared to GSTM1 positive subjects, who had relatively unchanged macrophage function parameters after an $O_3$ exposure identical to that of Lay et al. (2007). Collectively, these studies demonstrate that $O_3$ can affect multiple steps or aspects required for proper macrophage function, but any C-R relationship appears complex and genotype may be a consideration. A few other recent studies have evaluated the effects of $O_3$ on macrophage function, but these are of questionable relevance due to the use of in vitro exposure systems and amphibian animal models (Mikerov et al., 2008c; Dohm et al., 2005; Klestadt et al., 2005).

### 6.2.5.4 Infection and Adaptive Immunity

#### General Effects on the Immune System

The effects of $O_3$ on the immune system are complex and dependent on the exposure regimen and the observation period. According to toxicological studies it appears that the T-cell-dependent functions of the immune system are more affected than B-cell-dependent functions (U.S. EPA, 2006b). Generally, there is an early immunosuppressive effect that subsides with continued $O_3$ exposure, resulting in either a return to normal responses or an enhancement of immune responses. However, this is not always the case as Aranyi et al. (1983) showed decreased T-cell mitogen reactions in mice after subchronic (90-day) exposure to 0.1 ppm $O_3$. Earlier studies report changes in cell populations in lymphatic tissues (U.S. EPA, 2006b). A more recent study in mice demonstrated that numbers of certain T-cell subsets in the spleen were reduced after exposure to 0.6 ppm $O_3$ (10h/day x 15d) (Feng et al., 2006).

The inflammatory effects of $O_3$ involve the innate immune system, and as such, $O_3$ can affect adaptive (or acquired) immunity via alterations in antigen presentation and costimulation by innate immune cells such as macrophages and dendritic cells. Several recent controlled human exposure studies demonstrate increased expression of molecules involved in antigen presentation or costimulation. Lay et al. (2007) collected sputum monocytes from healthy volunteers exposed to 400 ppb $O_3$ for 2 hours with moderate intermittent exercise and detected increases in HLA-DR, used to present antigen to T-cells, and CD86, a costimulatory marker necessary for T-cell activation. Upregulation of HLA-DR was also observed by Alexis et al. (2009) in sputum dendritic cells and macrophages from GSTM1 null subjects exposed to 400 ppb $O_3$ for 2 hours with moderate intermittent exercise. On airway monocytes from healthy volunteers 24 hours after exposure to 80 ppb $O_3$ for 6.6 hours with moderate intermittent exercise, HLA-DR, CD86, and CD14 (a molecule involved in bacterial endotoxin reactivity) were increased, whereas CD80, a costimulatory molecule of more heterogeneous function, was decreased (Alexis et al., 2010). Patterns of expression on macrophages were similar, except that HLA-DR was found to be significantly decreased after $O_3$ exposure and CD86 was not significantly altered. An increase in IL-12p70, a macrophage and dendritic cell product that activates T-cells, was correlated with increased numbers of dendritic cells. It should be noted that these results are reported as comparisons to baseline as there was no clean air control (Alexis et al., 2010; Alexis et al., 2009). Another controlled human exposure study reported no increase in IL-12p70 in sputum from healthy subjects or those with atopy or atopy and asthma following a 2-hour exposure to 400 ppb $O_3$ with intermittent moderate exercise (Hernandez et al., 2010). Levels of HLA-DR, CD14 and CD86 were not increased on macrophages collected from any of these subjects. It is difficult to compare these results to those of Lay et al. (2007) and Alexis et al. (2010) due to differences in $O_3$ concentration, cell type examined, and timing of postexposure analysis.

Although no controlled human exposure studies have examined the effects of $O_3$ on the ability to mount antigen-specific responses, upregulation of markers associated with innate immune activation and antigen presentation could potentially enhance adaptive immunity and increase immunologic responses to antigens. While enhanced adaptive immunity may bolster defenses against infection, it also may enhance allergic responses (Section 6.2.6).

In animal models, $O_3$ has been found to alter responses 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 latter impairment may lead to lowered resistance to re-infection. The immune response is highly influenced by the temporal relationship between $O_3$ exposure and antigenic stimulation. When $O_3$ exposure preceded *Listeria* infection, there were no effects on delayed-type hypersensitivity or splenic lymphoproliferative responses; however, when $O_3$ exposure occurred during or after *Listeria* infection was initiated, these immune responses were suppressed (Van Loveren et al., 1988). In another study, a reduction in mitogen activated T-cell proliferation was observed after exposure to 0.6 ppm $O_3$ for 15 days that could be ameliorated by antioxidant supplementation. Antigen-specific proliferation decreased by 60%, indicating attenuation of the acquired immunity needed for subsequent memory responses (Feng et al., 2006). Ozone exposure also skewed the ex-vivo cytokine responses elicited by non-specific stimulation toward inflammation, decreasing IL-2 and increasing IFN-γ. Modest decreases in immune function assessed in the offspring of $O_3$-exposed dams (mice) were observed by Sharkhuu et al. (2011). The ability to mount delayed-type hypersensitivity responses was significantly suppressed in 42 day-old offspring when dams were exposed to 0.8 or 1.2 ppm $O_3$, but not 0.4 ppm, from gestational day 9-18. Humoral responses to immunization with sheep red blood cells were unaffected, as were other immune parameters such as splenic populations of CD45+ T-cells, iNKT-cells, and levels of IFN-γ, IL-4, and IL-17 in the BALF. Generally, continuous exposure to $O_3$ impairs immune responses for the first several days of exposure, followed by an adaptation to $O_3$ that allows a return of normal immune responses. Most species show little effect of $O_3$ exposures prior to immunization, but show a suppression of responses to antigen in $O_3$ exposures post-immunization.

### Microbial Infection

#### Bacterial infection

A relatively large body of evidence shows that $O_3$ increases susceptibility to bacterial infections. The majority of studies in this area were conducted before the 1996 $O_3$ AQCD was published and many are included in Table 6-9 (U.S. EPA, 1996j) on page 6-53 of that document (U.S. EPA, 1996a). Known contributing factors are impaired mucociliary streaming, altered chemotaxis/motility, defective phagocytosis of bacteria, decreased production of lysosomal enzymes or superoxide radicals by

alveolar macrophages, and decreased IFN-γ levels. In animal models of bacterial infection, exposure to 0.08 ppm $O_3$ increases streptococcus-induced mortality, regardless of whether $O_3$ exposure precedes or follows infection (Miller et al., 1978; Coffin and Gardner, 1972; Coffin et al., 1967). Increases in mortality are due to the infectious agent, thereby reflecting functional impairment of host defenses. Exercise and copollutants can enhance the effects of $O_3$ in infectivity models. Although both mice and rats exhibit impaired bactericidal macrophage activity after $O_3$ exposure, mortality due to infection is only observed in mice. Additionally, although mice and humans share many host defense mechanisms, there is little compelling evidence from epidemiologic studies to suggest an association between $O_3$ exposure and decreased resistance to bacterial infection, and the etiology of respiratory infections is not easily identified via ICD codes (Section 6.2.7.3).

#### Viral infection

Only a few studies, described in previous AQCDs, have examined the effects of $O_3$ exposure on the outcome of viral respiratory infection [see Table 6-9 on page 6-53 of the 1996 $O_3$ AQCD (U.S. EPA, 1996j)]. Some studies show increased mortality in animals, while others show diminished severity and increased survival time. There is little to no evidence from studies of animals or humans to suggest that $O_3$ increases the incidence of respiratory viral infection in humans. In human volunteers infected with rhinovirus prior to $O_3$ exposure (0.3 ppm for 5 consecutive days), no effect was observed on viral titers, IFN-γ production, or blood lymphocyte proliferative responses to viral antigen (Henderson et al., 1988). In vitro cell culture studies of human bronchial epithelial cells indicate $O_3$-induced exacerbation of human rhinovirus infection (Spannhake et al., 2002), but this is of limited relevance. More recent studies on the interactions of $O_3$ and viral infections have not been published. Natural killer (NK) cells, which destroy virally infected cells and tumors in the lung, appear to be inhibited by higher concentrations of $O_3$ and either unaffected or stimulated at lower concentrations. Several studies show decreases in NK cell activity following acute exposures ranging from 0.8 to 1 ppm (Gilmour and Jakab, 1991; Van Loveren et al., 1990; Burleson et al., 1989). However, Van Loveren et al. (1990) showed that a 1-week exposure to 0.2 or 0.4 ppm $O_3$ increased NK cell activity, and an urban pattern of exposure (base of 0.06 ppm with peaks of 0.25 ppm) had no effect on NK cell activity after 1, 3, 13, 52, or 78 weeks of exposure (Selgrade et al., 1990). A more recent study demonstrated a 35% reduction in NK cell activity after exposure of mice to 0.6 ppm $O_3$ (10h/day x 15d) (Feng et al., 2006). The defective IL-2 production demonstrated in this study may impair NK cell activation. Alternatively, NK cell surface charge may be altered by ROS, decreasing their adherence to target cells (Nakamura and Matsunaga, 1998).

### 6.2.5.5 Summary of Lung Host Defenses

Taken as a whole, the data clearly indicate that an acute $O_3$ exposure impairs the host defense capability of animals, primarily by depressing AM function and perhaps also

by decreasing mucociliary clearance of inhaled particles and microorganisms. Coupled with limited evidence from controlled human exposure studies, 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 AM function. It remains unclear how $O_3$ might affect antigen presentation and the costimulation required for T-cell activation, given the mixed results from controlled human exposure studies, but there is toxicological evidence for suppression of T-cell-dependent functions by $O_3$, including reductions in antigen-specific proliferation and antibody production, indicating the potential for impaired acquired immunity and memory responses. To date, a limited number of epidemiologic studies have examined associations between $O_3$ exposure and hospital admissions or ED visits for respiratory infection, pneumonia, or influenza. Results have been mixed, and in some cases conflicting (see Section 6.2.7.2 and Section 6.2.7.3). With the exception of influenza, it is difficult to ascertain whether cases of respiratory infection or pneumonia are of viral or bacterial etiology. A study that examined the association between $O_3$ exposure and respiratory hospital admissions in response to an increase in influenza intensity did observe an increase in respiratory hospital admissions (Wong et al., 2009), but information from toxicological studies of $O_3$ and viral infections is ambiguous.

## 6.2.6 Allergic and Asthma-Related Responses

Effects resulting from combined exposures to $O_3$ and allergens have been studied in a variety of animal species, generally as models of experimental asthma. Pulmonary function and airways hyperresponsiveness in animal models of asthma are discussed in Section 6.2.1.3 and Section 6.2.2.2. Previous evidence indicates that $O_3$ exposure skews immune responses toward an allergic phenotype. For example, Gershwin et al. (1981) reported that $O_3$ (0.8 and 0.5 ppm for 4 days) exposure caused a 34-fold increase in the number of IgE (allergic antibody)-containing cells in the lungs of mice. In general, the number of IgE-containing cells correlated positively with levels of anaphylactic sensitivity. In humans, allergic rhinoconjunctivitis symptoms are associated with increases in ambient $O_3$ concentrations (Riediker et al., 2001). Recent controlled human exposure studies have observed $O_3$-induced changes indicating allergic skewing. Airway eosinophils, which participate in allergic disease and inflammation, were observed to increase in volunteers with atopy and mild asthma 18 hours following a 7.6-hour exposure to 160 ppb $O_3$ with light intermittent exercise (Peden et al., 1997). No increase in airway eosinophils was observed 4 hours after exposure of healthy subjects or those with atopic or atopy and asthma to 400 ppb $O_3$ for 2 hours with moderate intermittent exercise (Hernandez et al., 2010). However, subjects with atopy did exhibit increased IL-5, a cytokine involved in eosinophil recruitment and activation, suggesting that perhaps these two studies observed the same effect at different time points. Epidemiologic studies describe associations between eosinophils and short- (Section 6.2.3.2) or long-term (Section 7.2.5) $O_3$ exposure, as do chronic exposure studies in non-human primates.

Hernandez et al. (2010) also observed increased expression of high and low affinity IgE receptors on sputum macrophages from atopic asthmatics, which may enhance IgE-dependent inflammation. Sputum levels of IL-4 and IL-13, both pro-allergic cytokines that aid in the production of IgE, were unaltered in all groups. The lack of increase in IL-4 levels in sputum reported by Hernandez et al. (2010), along with increased IL-5, is consistent with results from Bosson et al. (2003), in which IL-5 (but not IL-4 levels) increased in bronchial epithelial biopsy specimens following exposure of subjects with atopy and mild asthma to 200 ppb $O_3$ for 2 hours with moderate intermittent exercise. IL-5 was not elevated in specimens obtained from healthy (no asthma) $O_3$-exposed subjects. Collectively, findings from these studies suggest that $O_3$ can induce or enhance certain components of allergic inflammation in individuals with atopy or atopic asthma.

Ozone enhances inflammatory and allergic responses to allergen challenge in sensitized animals. Short-term exposure (2 days) to 1 ppm $O_3$ exacerbated allergic rhinitis and lower airway allergic inflammation in Brown Norway rats, a rat strain that is comparatively less sensitive to $O_3$ than other rats or humans (Wagner et al., 2009; 2007). OVA-sensitized rats were intranasally challenged with OVA on days 1 and 2, and exposed to 0 or 1 ppm $O_3$ (8 hours/day) on days 4 and 5. Analysis at day 6 indicated that $O_3$ exposure enhanced intraepithelial mucosubstances in the nose and airways, induced cys-LTs, MCP-1, and IL-6 production in BALF, and upregulated expression of the pro-allergic cytokines IL-5 and IL-13. These changes were not evident in non-allergic controls. All of these responses were blunted by gamma-tocopherol (γT; vitamin E) therapy. γT neutralizes oxidized lipid radicals, and protects lipids and proteins from nitrosative damage from NO-derived metabolites. Farraj et al. (2010) exposed allergen-sensitized adult male BALB/c mice to 0.5 ppm $O_3$ for 5 hours once per week for 4 weeks. Ozone exposure and $O_3$/DEP (2.0 mg/m$^3$) co-exposure of OVA-sensitized mice elicited significantly greater serum IgE levels than in DEP-exposed OVA-sensitized mice (98% and 89% increases, respectively). Ozone slightly enhanced levels of BAL IL-5, but despite increases in IgE, caused a significant decrease in BAL IL-4 levels. IL-10, IL-13, and IFN-γ levels were unaffected. Lung resistance and elastance were unaffected in allergen sensitized mice exposed solely to 0.5 ppm $O_3$ once a week for 4 weeks (Farraj et al., 2010). However, co-exposure to $O_3$ and diesel exhaust particles increased lung resistance.

In addition to exacerbating existing allergic responses, $O_3$ can also act as an adjuvant to produce sensitization in the respiratory tract. In a model of murine asthma, using OVA free of detectable endotoxin, inclusion of 1 ppm $O_3$ during the initial exposures to OVA (2 hours, days 1 and 6) enhanced the inflammatory and allergic responses to subsequent allergen challenge (Hollingsworth et al., 2010). Compared to air exposed animals, $O_3$-exposed mice exhibited significantly higher levels of total cells, macrophages, eosinophils, and PMNs in BALF, and increased total serum IgE. Pro-allergic cytokines IL-4, and IL-5 were also significantly elevated, along with pleiotropic Th2 cytokine IL-9 (associated with bronchial hyperresponsiveness) and pro-inflammatory IL-17, produced by activated T-cells. Based on lower inflammatory, IgE, and cytokine responses in Toll-like receptor 4 deficient mice, the effects of $O_3$ seem to be dependent on TLR 4 signaling, as are a number of other

biological responses to $O_3$ according to studies by Hollingsworth et al. (2004), Kleeberger et al. (2000) and Garantziotis et al. (2010). The involvement of TLR 4, along with its endogenous ligand, hyaluronan, in $O_3$-induced responses described in these studies has been corroborated by a controlled human exposure study by Hernandez et al. (2010), who found increased TLR 4 expression and elevated levels of hyaluronic acid in volunteers with atopy or atopic asthma exposed to 400 ppb $O_3$. This pathway is discussed in more detail in Chapter 5. Examination of dendritic cells (DCs) from the draining thoracic lymph nodes indicated that $O_3$ did not enhance the migration of DCs from the lungs to the lymph nodes, nor did it alter the expression of functional DC markers such as CD40, MHC class II, or CD83. However, $O_3$ did increase expression of CD86, which is generally associated with Th2 responses and was detected at higher levels on DCs from donors with allergic asthma compared to those from healthy donors Chen et al. (2006b). Increased CD86 has also been observed on airway cells collected from human subjects following exposure to $O_3$ in studies by Lay et al. (2007) and Alexis et al. (2009), but not Hernandez et al. (2010) (study details described in Section 6.2.5.4).

Ozone exposure during gestation has modest effects on allergy and asthma related endpoints in adult offspring. When dams were exposed to 1.2 ppm $O_3$ (but not 0.8 ppm) from gestational day 9-18, some allergic and inflammatory responses to OVA sensitization and challenge were reduced compared to air exposed controls. Such responses included IgE levels and eosinophils, and were observed only in mice that were immunized early in life (PND 3) as opposed to later (PND 42), perhaps due to the proximity of $O_3$ and antigen exposure. The effects of gestational $O_3$ exposure on immune function have not been widely studied, and although reductions in allergic endpoints are not generally observed in association with $O_3$, other parameters of immune function were found to be reduced, so a more global immunosuppression may underlie these effects.

In addition to pro-allergic effects, $O_3$ could also make airborne allergens more allergenic. When combined with $NO_2$, $O_3$ has been shown to enhance nitration of common protein allergens, which may increase their allergenicity Franze et al. (2005).

## 6.2.7 Hospital Admissions, Emergency Department Visits, and Physicians Visits

### 6.2.7.1 Summary of Findings from 2006 $O_3$ AQCD

The 2006 $O_3$ AQCD evaluated numerous respiratory ED visits and hospital admissions studies, which consisted primarily of time-series studies conducted in the U.S., Canada, Europe, South America, Australia and Asia. Upon collectively evaluating the scientific evidence, the 2006 $O_3$ AQCD concluded that "the overall evidence supports a causal relationship between acute ambient $O_3$ exposures and

increased respiratory morbidity resulting in increased ED visits [and hospital admissions] during the warm season" (U.S. EPA, 2006b). This conclusion was "strongly supported by the human clinical, animal toxicologic[al], and epidemiologic evidence for [$O_3$-induced] lung function decrements, increased respiratory symptoms, airway inflammation, and airway hyperreactivity" (U.S. EPA, 2006b).

Since the completion of the 2006 $O_3$ AQCD, relatively fewer studies conducted in the U.S., Canada, and Europe have examined the association between short-term exposure to ambient $O_3$ and respiratory hospital admissions and ED visits with a growing number of studies having been conducted in Asia. This section focuses primarily on multicity studies because they examine the effect of $O_3$ on respiratory-related hospital admissions and ED visits over a large geographic area using a consistent statistical methodology. Single-city studies that encompass a large number of hospital admissions or ED visits, or included a long study-duration were also evaluated because these studies have more power to detect whether an association exists between short-term $O_3$ exposure and respiratory hospital admissions and ED visits compared to smaller single-city studies. Additional single-city studies were also evaluated within this section, if they were conducted in locations not represented by the larger single-city and multicity studies, or examined population-specific characteristics not included in the larger studies that may modify the association between short-term $O_3$ exposure and respiratory-related hospital admissions or ED visits. The remaining single-city studies identified were not evaluated in this section due to factors such as inadequate study design or insufficient sample size.

It should be mentioned that when examining the association between short-term $O_3$ exposure and respiratory health effects that require medical attention, it is important to distinguish between hospital admissions and ED visits. This is because it is likely that a small percentage of respiratory ED visits will be admitted to the hospital; therefore, respiratory ED visits may represent potentially less serious, but more common outcomes. As a result, in the following sections respiratory hospital admission and ED visit studies are evaluated individually. Additionally, within each section, results are presented as either a collection of respiratory diagnoses or as individual diseases (e.g., asthma, COPD, pneumonia and other respiratory infections) in order to evaluate the potential effect of short-term $O_3$ exposure on each respiratory-related outcome. The ICD codes (i.e., ICD-9 or ICD-10) that encompass each of these endpoints are presented in Table 6-26 along with the air quality characteristics of the city, or across all cities, included in each study evaluated in this section.

## Table 6-26 Mean and upper percentile concentrations of respiratory-related hospital admission and emergency department (ED) visit studies evaluated.

| Study | Location | Type of Visit (ICD9/10) | Averaging Time | Mean Concentration (ppb)[a] | Upper Percentile Concentrations (ppb)[a] |
|---|---|---|---|---|---|
| Katsouyanni et al. (2009)[b,c] | 90 U.S. cities (NMMAPS)[d]<br>32 European cities (APHEA)[d]<br>12 Canadian cities | Hospital Admissions:<br>NMMAPS:<br>All respiratory (460-519)<br>APHEA:<br>All respiratory (460-519)<br>12 Canadian cities:<br>All respiratory (460-519)[e] | 1-h max | NMMAPS:<br>50th: 34.9-60.0<br>APHEA:<br>50th: 11.0-38.1<br>12 Canadian cities:<br>50th: 6.7-8.3 | NMMAPS:<br>75th: 46.8-68.8<br>APHEA:<br>75th: 15.3-49.4<br>12 Canadian cities:<br>75th: 8.4-12.4 |
| Cakmak et al. (2006b) | 10 Canadian cities | Hospital Admissions:<br>All respiratory (466, 480-486, 490, 491, 492, 493, 494, 496) | 24-h avg | 17.4 | Max: 38.0-79.0 |
| Biggeri et al. (2005)[c] | 4 Italian cities[f] | Hospital Admissions:<br>All respiratory (460-519) | 8-h max | Warm season (May-September):<br>5.7-60.0 | 95th: 86.1-90.0<br>Max: 107.5-115.1 |
| Dales et al. (2006) | 11 Canadian cities | Hospital Admissions:<br>Respiratory disorders (486, 768.9, 769, 770.8, 786, 799.0, 799.1) | 24-h avg | 17.0 | 95th: 24.9-46.0 |
| Lin et al. (2008a) | 11 New York regions | Hospital Admissions:<br>Respiratory diseases (466, 490-493, 496) | 8-h max[g] | 44.1 | 75th: 54.0<br>Max: 217.0 |
| Wong et al. (2009)[c] | Hong Kong | Hospital Admissions:<br>All respiratory (460-519)<br>COPD (490-496) | 8-h max[g] | 18.8 | 75th: 25.9<br>Max: 100.3 |
| Medina-Ramon et al. (2006)[h] | 36 U.S. cities | Hospital Admissions:<br>COPD (490-496, excluding 493)<br>Pneumonia (480-487) | 8-h max | Warm (May-September): 45.8<br>Cool (October-April): 27.6 | NR |
| Yang et al. (2005b) | Vancouver, Canada | Hospital Admissions:<br>COPD (490-492, 494, 496) | 24-h avg | All year: 14.1<br>Winter (January-March): 13.2<br>Spring (April-June): 19.4<br>Summer (July-September): 13.8<br>Fall (October-December): 10.0 | Max: 38.6 |
| Zanobetti and Schwartz (2006)[b] | Boston, MA | Hospital Admissions:<br>Pneumonia (480-487) | 24-h avg | 22.4 | 75th: 31.0<br>95th: 47.6 |
| Silverman and Ito (2010)[b] | New York, NY | Hospital Admissions:<br>Asthma (493) | 8-h max | Warm (April-August): 41.0 | 75th: 53<br>90th: 68 |

| Study | Location | Type of Visit (ICD9/10) | Averaging Time | Mean Concentration (ppb)[a] | Upper Percentile Concentrations (ppb)[a] |
|---|---|---|---|---|---|
| Stieb et al. (2009) | 7 Canadian cities | ED Visits:<br>Asthma (493)<br>COPD (490-492, 494-496)<br>Respiratory infection (464, 466, 480-487) | 24-h avg | 18.4 | 75th: 19.3-28.6 |
| Tolbert et al. (2007) | Atlanta, GA | ED Visits:<br>All respiratory (460-465, 460.0, 466.1, 466.11, 466.19, 477, 480-486, 491, 492, 493, 496, 786.07, 786.09) | 8-h max | Warm: 53.0 | 75th: 67.0<br>90th: 82.1<br>Max: 147.5 |
| Darrow et al. (2011a) | Atlanta, GA | ED Visits:<br>All respiratory (460-466, 477, 480-486, 491, 492, 493, 496, 786.09) | 8-h max | Warm (March-October):<br>8-h max: 53 | 8-h max:75th: 67<br>8-h max:Max: 148 |
| | | | 1-h max | Warm (March-October):<br>1-h max: 62 | 1-h max:75th: 76<br>1-h max:Max: 180 |
| | | | 24-h avg | Warm (March-October):<br>24-h avg: 30 | 24-h avg:75th: 37<br>24-h avg:Max: 81 |
| | | | Commute | Warm (March-October):<br>Commute: 35[i] | Commute:75th: 45<br>Commute:Max: 106 |
| | | | Day-time | Warm (March-October):<br>Day-time: 45[i] | Day-time:75th: 58<br>Day-time:Max: 123 |
| | | | Night-time | Warm (March-October):<br>Night-time: 14[i] | Night-time:75th: 22<br>Night-time:Max: 64 |
| Villeneuve et al. (2007)[b] | Alberta, CAN | ED Visits:<br>Asthma (493) | 8-h max | Summer (April-September): 38.0<br>Winter (October-March): 24.3 | Summer:<br>75th: 46.0<br>Winter:<br>75th: 31.5 |
| Ito et al. (2007b) | New York, NY | ED Visits:<br>Asthma (493) | 8-h max | All year: 30.4<br>Warm (April-September): 42.7<br>Cold (October-March): 18.0 | All year:<br>95th: 68.0<br>Warm months:<br>95th: 77.0<br>Cold months:<br>95th: 33.0 |
| Strickland et al. (2010) | Atlanta, GA | ED Visits:<br>Asthma (493)<br>Wheeze (786.07 after 10/1/98, 786.09 before 10/1/98) | 8-h max | All year: 45.4[j]<br>Warm (May-October): 55.2[j]<br>Cold (November-April): 34.5[j] | NR |
| Mar and Koenig (2009) | Seattle, WA | ED Visits:<br>Asthma (493-493.9) | 1-h max<br>8-h max | Warm (May-October):<br>1-h max: 38.6<br>8-h max: 32.2 | 75th:<br>1-h max: 45.5<br>8-h max: 39.2 |
| Arbex et al. (2009) | Sao Paulo, Brazil | ED Visits:<br>COPD (J40-44) | 1-h max | 48.8 | 75th: 61.0<br>Max: 143.8 |

| Study | Location | Type of Visit (ICD9/10) | Averaging Time | Mean Concentration (ppb)[a] | Upper Percentile Concentrations (ppb)[a] |
|---|---|---|---|---|---|
| Orazzo et al. (2009)[c] | 6 Italian cities | ED Visits: Wheezing | 8-h max[k] | Summer (April-September): 21.1-44.3<br>Winter (October-March): 11.5-27.9 | NR |
| Burra et al. (2009) | Toronto, Canada | Physician Visits: ED Asthma (493) | 1-h max | 33.3 | 95th: 66<br>Max: 121 |
| Villeneuve et al. (2006b) | Toronto, Canada | Physician Visits: Allergic rhinitis (177) | 8-h max | 30.0 | Max: 98.7 |
| Sinclair et al. (2010)[l] | Atlanta, GA | Physician Visits:<br>Asthma<br>Upper respiratory infection<br>Lower respiratory infection | 8-h max | Total Study Period:<br>All-year: 44.0<br>25 mo Period:<br>All-year: 47.9<br>Warm: 61.2<br>Cold: 27.8<br>28 mo Period:<br>All-year: 40.7<br>Warm: 51.8<br>Cold: 26.0 | NR |

[a]Some studies did not present an overall value for the mean, middle and/or upper percentiles of the $O_3$ distribution; as a result, the range of the mean, middle, and/or upper percentiles across all of the cities included in the study are presented.

[b]Study only presented median concentrations.

[c]Study presented concentrations as μg/m$^3$ Concentration was converted to ppb using the conversion factor of 0.51 assuming standard temperature (25°C) and pressure (1 atm).

[d]A subset of the European and U.S. cities included in the mortality analyses were used in the hospital admissions analyses: 8 of the 32 European cities and 14 of 90 U.S. cities.

[e]Hospital admission data was coded using three classifications (ICD-10-CA, ICD-9, and ICD-9-CM). Attempts were made by the original investigators to convert diagnosis from ICD-10-CA back to ICD-9.

[f]Only 4 of the 8 cities included in the study collected $O_3$ data.

[g]$O_3$ measured from 10:00 a.m. to 6:00 p.m.

[h]Only 35 of the 36 cities included in the analysis had $O_3$ data.

[i]Commute (7:00 a.m. to 10:00 a.m., 4:00 p.m. to 7:00 p.m.); day-time (8:00 a.m. to 7:00 p.m.); Night-time (12:00 a.m. to 6:00 a.m.).

[j]Means represent population-weighted $O_3$ concentrations.

[k]$O_3$ measured from 8:00 a.m. to 4:00 p.m.

[l]This study did not report the ICD codes used for the conditions examined. The 25-month period represents August 1998-August 2000, and the 28-month period represents September 2000-December 2002. This study defined the warm months as April – October and the cold months as November-March.

### 6.2.7.2 Hospital Admission Studies

#### Respiratory Diseases

The association between exposure to an air pollutant, such as $O_3$, and daily respiratory-related hospital admissions has primarily been examined using all respiratory-related hospital admissions within the range of ICD-9 codes 460-519. Recent studies published since the 2006 $O_3$ AQCD (U.S. EPA, 2006b) attempt to further examine the effect of $O_3$ exposure on respiratory-related hospital admissions through a multicity design that examines $O_3$ effects across countries using a standardized methodology; multicity studies that examine effects within one country;

and multi- and single-city studies that attempt to examine potential modifiers of the $O_3$-respiratory-related hospital admission relationship.

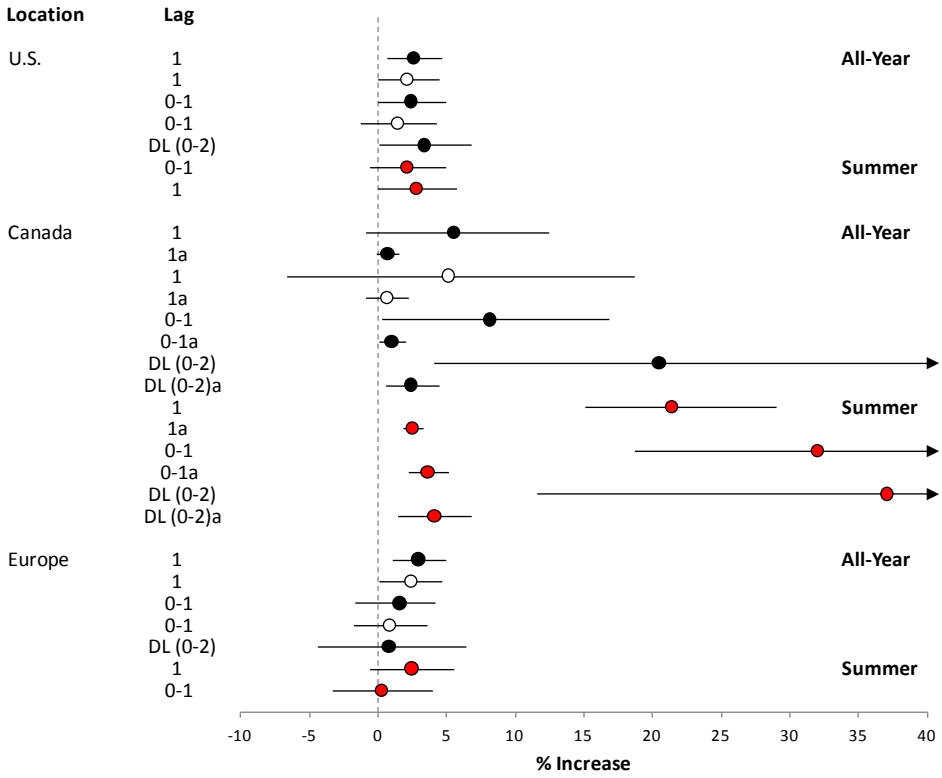
The Air Pollution and Health: A European and North American Approach (APHENA) study combined data from existing multicity study databases from Canada, Europe (APHEA2) (Katsouyanni et al., 2001), and the U.S. (NMMAPS) (Samet et al., 2000) in order to "develop more reliable estimates of the potential acute effects of air pollution on human health [and] provide a common basis for [the] comparison of risks across geographic areas" (Katsouyanni et al., 2009). In an attempt to address both of these issues, the investigators conducted extensive sensitivity analyses to evaluate the robustness of the results to different model specifications (e.g., penalized splines [PS] versus natural splines [NS]) and the extent of smoothing to control for seasonal and temporal trends. The trend analyses consisted of subjecting the models to varying extent of smoothing selected either a priori (i.e., 3 df/year, 8 df/year, and 12 df/year), which was selected through exploratory analyses using between 2 and 20 df, or by using the absolute sum of the residuals of the partial autocorrelation function (PACF). Although the investigators did not identify the model they deemed to be the most appropriate for comparing the results across study locations, they did specify that "overall effect estimates (i.e., estimates pooled over several cities) tended to stabilize at high degrees of freedom" (Katsouyanni et al., 2009). Therefore, in discussion of the results across the three study locations below, the 8 df/year results are presented for both the PS and NS models because: (1) 8 df/year is most consistent with the extent of temporal adjustment used in previous and recent large multicity studies in the U.S. (e.g., NMMAPS); (2) the risk estimates for 8 df/year and 12 df/year are comparable for all three locations; (3) the models that used the PACF method did not report the actual degrees of freedom chosen; and (4) the 3 df/year and the PACF method resulted in negative $O_3$ risk estimates, which is inconsistent with the results obtained using more aggressive seasonal adjustments and suggests inadequate control for seasonality. Additionally, in comparisons of results across studies in figures, only the results from one of the spline models (i.e., NS) are presented because it has been previously demonstrated that alternative spline models result in relatively similar effect estimates (HEI, 2003). This observation is consistent with the results of the APHENA analysis that was conducted with a higher number of degrees of freedom (e.g., $\geq$ 8 df/year) to account for temporal trends.

Katsouyanni et al. (2009) examined respiratory hospital admissions for people aged 65 years and older using 1-h max $O_3$ data. The extent of hospital admission and $O_3$ data varied across the 3 datasets: Canadian dataset included 12 cities with data for 3 years (1993-1996) per city; European dataset included 8 cities with each city having data for between 2 and 8 years from 1988-1997; and the U.S. dataset included 14 cities with each city having data for 4 to 10 years from 1985-1994 and 7 cities having only summer $O_3$ data. The investigators used a three-stage hierarchical model to account for within-city, within region, and between region variability. Results were presented individually for each region (Figure 6-15 [and Table 6-27]). Ozone and $PM_{10}$ concentrations were weakly correlated in all locations in the summer (ranging from r = 0.27 – 0.40), but not in the winter.

In the Canadian cities, using all-year data, a 40 ppb increase in 1-h max $O_3$ concentrations at lag 0-1 was associated with an increase in respiratory hospital admissions of 8.9% (95% CI: 0.79, 16.8%) in a PS model and 8.1% (95% CI: 0.24, 16.8%) in a NS model (Katsouyanni et al., 2009). The results were somewhat sensitive to the lag day selected, reduced when using a single-day lag (e.g., lag 1) (PS: 6.0%; NS: 5.5%) and increased when using a distributed lag model (PS: 18.6%; NS: 20.4%). When adjusted for $PM_{10}$, the magnitude of the effect estimate was attenuated, but remained positive with it being slightly larger in the NS model (5.1% [95% CI: -6.6, 18.6%]) compared to the PS model (3.1% [95% CI: -8.3, 15.9%]). However, in the Canadian dataset the copollutant analysis was only conducted using a 1-day lag. The large confidence intervals for both models could be attributed to the reduction in days included in the copollutant analyses as a result of the every-6th-day PM sampling schedule. When the analysis was restricted to the summer months, stronger associations were observed between $O_3$ and respiratory hospital admissions across the lags examined, ranging from ~22 to 37% (the study does not specify whether these effect estimates are from a NS or PS model). Because $O_3$ concentrations across the cities included in the Canadian dataset are low (median concentrations ranging from 6.7-8.3 ppb [Table 6-26]), the standardized increment of 40 ppb for a 1-h max increase in $O_3$ concentrations represents an unrealistic increase in $O_3$ concentrations in Canada and increases the magnitude, not direction, of the observed risk estimate. As a result, calculating the $O_3$ risk estimate using the 40 ppb increment does not accurately reflect the observed risk of $O_3$-related respiratory hospital admissions. Although this increment adequately characterizes the distribution of 1-h max $O_3$ concentrations across the U.S. and European datasets, it misrepresents the observed $O_3$ concentrations in the Canadian dataset. As a result in summary figures, for comparability, effect estimates from the Canadian dataset are presented for both a 5.1 ppb increase in 1-h max $O_3$ concentrations (i.e., an approximate interquartile range [IQR] increase in $O_3$ concentrations across the Canadian cities) as well as the 40 ppb increment used throughout the ISA.

In Europe, weaker but positive associations were also observed in year round analyses; 2.9% (95% CI: 0.63, 5.0%) in the PS model and 1.6% (95% CI: -1.7, 4.2%) in the NS model at lag 0-1 for a 40 ppb increase in 1-h max $O_3$ concentrations (Katsouyanni et al., 2009). Additionally, at lag 1, associations between $O_3$ and respiratory hospital admissions were also reduced, but in contrast to the lag 0-1 analysis, greater effects were observed in the NS model (2.9% [95% CI: 1.0, 4.9%]) compared to the PS model (1.5% [95% CI: -2.2, 5.4]). Unlike the Canadian analysis, a distributed lag model provided limited evidence of an association between $O_3$ and respiratory hospital admissions. To compare with the Canadian results, focused on adjustment for $PM_{10}$ at lag 1, $O_3$ effect estimates using the European dataset were increased in the PS model (2.5% [95% CI: 0.39-4.8%]) and remained robust in the NS model (2.4% [95% CI: 0.08, 4.6%]). However, the European analysis also examined the effect of adjusting for $PM_{10}$ at lag 0-1 and found results were attenuated, but remained positive in both models (PS: 0.8% [95% CI: -2.3, 4.0%]; NS: 0.8% [95% CI: -1.8, 3.6%]). Unlike the Canadian and U.S. datasets, the European dataset consisted of daily PM data. The investigators did not observe stronger associations in the summer-only analyses for the European cities at lag 0-1

(PS: 0.4% [95% CI: -3.2, 4.0%]; NS: 0.2% [95% CI: -3.3, 3.9%]), but did observe some evidence for larger effects during the summer, an ~2.5% increase, at lag 1 in both models (the study does not present the extent of temporal smoothing used for these models).




Note: Black circles = all-year results; open circles = all-year results in copollutant model with $PM_{10}$; and red circles = summer only results. For Canada, lag days with an "a" next to them represent the risk estimates standardized to an approximate IQR of 5.1 ppb for a 1-h max increase in $O_3$ concentrations.

**Figure 6-15 Percent increase in respiratory hospital admissions from natural spline models with 8 df/yr for a 40 ppb increase in 1-h max $O_3$ concentrations for each location of the APHENA study.**

## Table 6-27 Corresponding effect estimates for Figure 6-15.

| Location* | Season | Lag[a] | Copollutant | % Increase (95% CI)[b] |
|---|---|---|---|---|
| U.S. | All-year | 1 | | 2.62 (0.63, 4.64) |
| | | 1 | $PM_{10}$ | 2.14 (-0.08, 4.40) |
| | | 0-1 | | 2.38 (0.00, 4.89) |
| | | 0-1 | $PM_{10}$ | 1.42 (-1.33, 4.23) |
| | | DL(0-2) | | 3.34 (0.02-6.78) |
| | Summer | 0-1 | | 2.14 (-0.63, 4.97) |
| | | 1 | | 2.78 (-0.02, 5.71) |
| Canada | All-year | 1 | | 5.54 (-0.94, 12.4) |
| | | 1a | | 0.69 (-0.12, 1.50)[a] |
| | | 1 | $PM_{10}$ | 5.13 (-6.62, 18.6) |
| | | 1a | $PM_{10}$ | 0.64 (-0.87, 2.20)[a] |
| | | 0-1 | | 8.12 (0.24, 16.8) |
| | | 0-1a | | 1.00 (0.03, 2.00)[a] |
| | | DL(0-2) | | 20.4 (4.07, 40.2) |
| | | DL(0-2)[a] | | 2.4 (0.51, 4.40)[a] |
| | Summer | 1 | | 21.4 (15.0, 29.0) |
| | | 1a | | 2.50 (1.80, 3.30)[a] |
| | | 0-1 | | 32.0 (18.6, 47.7) |
| | | 0-1[a] | | 3.60 (2.20, 5.10)[a] |
| | | DL(0-2) | | 37.1 (11.5, 67.5) |
| | | DL(0-2)[a] | | 4.1 (1.40, 6.80)[a] |
| Europe | All-year | 1 | | 2.94 (1.02, 4.89) |
| | | 1 | $PM_{10}$ | 2.38 (0.08, 4.64) |
| | | 0-1 | | 1.58 (-1.71, 4.15) |
| | | 0-1 | $PM_{10}$ | 0.87 (-1.79, 3.58) |
| | | DL(0-2) | | 0.79 (-4.46, 6.37) |
| | Summer | 1 | | 2.46 (-0.63, 5.54) |
| | | 0-1 | | 0.24 (-3.32, 3.91) |

*For effect estimates in Figure 6-15.

[a]For Canada, lag days with an "a" next to them represent the risk estimates standardized to an approximate IQR of 5.1 ppb for a 1-h max increase in $O_3$ concentrations.

[b]Unless noted, risk estimates standardized to 40 ppb for a 1-h max increase in $O_3$ concentrations.

For the U.S. in year round analyses, the investigators reported a 1.4% (95% CI: -0.9, 3.9%) increase in the PS model and 2.4% (95% CI: 0.0, 4.9%) increase in the NS model in respiratory hospital admissions at lag 0-1 for a 40 ppb increase in 1-h max $O_3$ concentrations with similar results for both models at lag 1 (Katsouyanni et al., 2009). The distributed lag model provided results similar to those observed in the European dataset with the PS model (1.1% [95% CI: -3.0, 5.3%]), but larger

effects in the NS model (3.3% [95% CI: 0.02, 6.8%]), which is consistent with the Canadian results. With adjustment for $PM_{10}$ using the U.S. data (i.e., every-6th-day PM data), results were attenuated, but remained positive at lag 0-1 (PS: 0.6% [95% CI: -2.0, 3.3%]; NS: 1.4% [95% CI: -1.3, 4.2%]) which is consistent with the results presented for the European dataset. However, at lag 1, U.S. risk estimates remained robust to the inclusion of $PM_{10}$ in copollutant models as was observed in the Canadian and European datasets. Compared to the all-year analyses, the investigators did not observe stronger associations in the summer-only analysis at either lag 0-1 (~2.2%) or lag 1 (~2.8%) in both the PS and NS models (the study does not present the extent of temporal smoothing used for these models).

Several additional multicity studies examined respiratory disease hospital admissions in Canada and Europe. Cakmak et al. (2006b) evaluated the association between ambient $O_3$ concentrations and respiratory hospital admissions for all ages in 10 Canadian cities from April 1993 to March 2000. The primary objective of this study was to examine the potential modification of the effect of ambient air pollution on daily respiratory hospital admissions by education and income using a time-series analysis conducted at the city-level. The authors calculated a pooled estimate across cities for each pollutant using a random effects model by first selecting the lag day with the strongest association from the city-specific models. For $O_3$, the mean lag day across cities that provided the strongest association and for which the pooled effect estimate was calculated was 1.2 days. In this study, all-year $O_3$ concentrations were used in the analysis, and additional seasonal analyses were not conducted. Cakmak et al. (2006b) reported a 4.4% increase (95% CI: 2.2, 6.5%) in respiratory hospital admissions for a 20 ppb increase in 24-h avg $O_3$ concentrations. The investigators only examined the potential effect of confounding by other pollutants through the use of a multipollutant model (i.e., two or more additional pollutants included in the model), which is difficult to interpret due to the potential multicollinearity between pollutants. Cakmak et al. (2006b) also conducted an extensive analysis of potential modifiers, specifically sex, educational attainment, and family income, of the association between air pollution and respiratory hospital admissions. When stratified by sex, the increase in respiratory hospital admissions due to short-term $O_3$ exposure were similar in males (5.2% [95% CI: 3.0, 7.3%]) and females (4.2% [95% CI: 1.8, 6.6%]). In addition, the examination of effect modification by income found no consistent trend across the quartiles of family income. However, there was evidence that individuals with an education level less than the 9th grade were disproportionately affected by $O_3$ exposure (4.6% [95% CI: 1.8, 7.5%]) compared to individuals that completed grades 9-13 (1.7% [95% CI: -1.9, 5.3%]), some university or trade school (1.4% [95% CI: -2.0, 5.1%]), or have a university diploma (0.66% [95% CI: -3.3, 4.7%]). The association between $O_3$ and respiratory hospital admissions in individuals with an education level less than the 9th grade was the strongest association across all of the pollutants examined.

A multicity study conducted in Europe by Biggeri et al. (2005) examined the association between short-term $O_3$ exposure and respiratory hospital admissions for all ages in four Italian cities from 1990 to 1999. In this study, $O_3$ was only measured during the warm season (May-September). The authors examined associations

between daily respiratory hospital admissions and short-term $O_3$ exposure at the city-level using a time-series analysis. Pooled estimates were calculated by combining city-specific estimates using fixed and random effects models. The investigators found no evidence of an association between $O_3$ exposure and respiratory hospital admissions in the warm season in both the random (0.1% [95% CI: -5.2, 5.7%]; distributed lag 0-3) and fixed effects (0.1% [95% CI: -5.2, 5.7%]; distributed lag 0-3) models for a 30 ppb increase in 8-h max $O_3$ concentrations.

Additional studies examined associations between short-term $O_3$ exposure and respiratory hospital admissions specifically in children. In a multicity study conducted in Canada, Dales et al. (2006) examined the association between all-year ambient $O_3$ concentrations and neonatal (ages 0-27 days) respiratory hospital admissions in 11 Canadian cities from 1986 to 2000. The investigators used a statistical analysis approach similar to Cakmak et al. (2006b) (i.e., time-series analysis to examine city-specific associations, and then a random effects model to pool estimates across cities). The authors reported that for $O_3$, the mean lag day across cities that provided the strongest association was 2 days. The authors reported a 5.4% (95% CI: 2.9, 8.0%) increase in neonatal respiratory hospital admissions for a 20 ppb increase in 24-h avg $O_3$ concentrations at lag-2 days. The results from Dales et al. (2006) provide support for the associations observed in a smaller scale study that examined $O_3$ exposure and pediatric respiratory hospital admissions in New York state (Lin et al., 2008a). Lin et al. (2008a), when examining single-day lags of 0 to 3 days, observed a positive association between $O_3$ and pediatric (i.e., <18 years) respiratory admissions at lag 2 (results not presented quantitatively) in a two-stage Bayesian hierarchical model analysis of 11 geographic regions of New York state from 1991 to 2001. Additionally, in copollutant models with $PM_{10}$ collected every-6th day, the authors found region-specific $O_3$ associations with respiratory hospital admissions remained relatively robust.

Overall, the evidence from epidemiologic studies continues to support an association between short-term $O_3$ exposure and respiratory-related hospital admissions, but it remains unclear whether certain factors (individual- or population-level) modify this association. Wong et al. (2009) examined the potential modification of the relationship between ambient $O_3$ (along with $NO_2$, $SO_2$, and $PM_{10}$) and respiratory hospital admissions by influenza intensity in Hong Kong for the period 1996 – 2002. In this study air pollution concentrations were estimated by centering non-missing daily air pollution data on the annual mean for each monitor and then an overall daily concentration was calculated by taking the average of the daily centered mean across all monitors. Influenza intensity was defined as a continuous variable using the proportion of weekly specimens positive for influenza A or B instead of defining influenza epidemics. This approach was used to avoid any potential bias associated with the unpredictable seasonality of influenza in Hong Kong where there are traditionally two seasonal peaks, which is in contrast to the single peaking influenza season in the U.S. (Wong et al., 2009). In models that examined the baseline effect (i.e., without taking into consideration influenza intensity) of short-term $O_3$ exposure, the authors found a 3.6% (95% CI: 1.9, 5.3%) and 3.2% (95% CI: 1.0, 5.4%) increase in respiratory hospital admissions at lag 0-1 for a 30 ppb increase in

8-h max $O_3$ concentrations for the all age and ≥ 65 age groups, respectively. When examining influenza intensity, Wong et al. (2009) reported that the association between short-term exposure to $O_3$ and respiratory hospital admissions was stronger with higher levels of influenza intensity: additional increase in respiratory hospital admissions above baseline of 1.4% (95% CI: 0.24, 2.6%) for all age groups and 2.4% (95% CI: 0.94, 3.8%) for those 65 and older when influenza activity increased from 0% to 10%. No difference in effects was observed when stratifying by sex.

### Cause-Specific Respiratory Outcomes

In the 2006 $O_3$ AQCD a limited number of studies were identified that examined the effect of short-term $O_3$ exposure on cause-specific respiratory hospital admissions. The limited evidence "reported positive $O_3$ associations with… asthma and COPD, especially… during the summer or warm season" (U.S. EPA, 2006b). Of the studies evaluated since the completion of the 2006 $O_3$ AQCD, more have focused on identifying whether $O_3$ exposure is associated with specific respiratory-related hospital admissions, including COPD, pneumonia, and asthma, but the overall body of evidence remains small.

#### Chronic Obstructive Pulmonary Disease

Medina-Ramon et al. (2006) examined the association between short-term exposure to ambient $O_3$ and $PM_{10}$ concentrations and Medicare hospital admissions among individuals ≥ 65 years of age for COPD in 35 cities in the U.S. for the years 1986-1999. The cities included in this analysis were selected because they monitored $PM_{10}$ on a daily basis. In this study, city-specific results were obtained using a monthly time-stratified case-crossover analysis. A meta-analysis was then conducted using random effects models to combine the city-specific results. All cities measured $O_3$ from May through September, while only 16 of the cities had year-round measurements. The authors reported a 1.6% increase (95% CI: 0.48, 2.9%) in COPD admissions for lag 0-1 in the warm season for a 30 ppb increase in 8-h max $O_3$ concentrations. In examination of single-day lags, stronger associations were observed for lag 1 (2.9% [95% CI: 1.8, 4.0%]) compared to lag 0 (-1.5% [95% CI: -2.7, -0.24%]). The authors found no evidence of increased associations in cool season (-1.9% [95% CI: -3.6, -0.06%]; lag 0-1) or year round (0.24% [95% CI: -0.78, 1.2%]; lag 0-1) analyses. In a copollutant model restricted to days in which $PM_{10}$ was available, the association between $O_3$ and COPD hospital admissions remained robust. Of note, the frequency of $PM_{10}$ measurements varied across cities with measurements collected either every 2, 3, or 6 days. The authors conducted additional analyses to examine potential modification of the warm season estimates for $O_3$ and COPD admissions by several city-level characteristics: percentage living in poverty, emphysema mortality rate (as an indication of smoking), daily summer apparent temperature, and percentage of households using central air conditioning. Of the city-level characteristics examined, stronger associations were only reported for cities with a smaller variability in daily apparent summer temperature.

In a single-city study conducted in Vancouver from 1994-1998, a location with low ambient $O_3$ concentrations (Table 6-26), Yang et al. (2005b) examined the association between $O_3$ and COPD. Ozone was moderately inversely correlated with CO (r = -0.56), $NO_2$ (r = -0.32), and $SO_2$ (r = -0.34), and weakly inversely correlated with $PM_{10}$ (r = -0.09), suggesting that the observed $O_3$ effect is likely not only due to a positive correlation with other pollutants. Yang et al. (2005b) examined 1- to 7-day (e.g., (0-6 days) lagged moving averages and observed an 8.8% (95% CI: -12.5, 32.6%) increase in COPD admissions for lag 0-3 per 20 ppb increase in 24-h avg $O_3$ concentrations. In two-pollutant models with every-day data for $NO_2$, $SO_2$, or $PM_{10}$ at lag 0-3, $O_3$ risk estimates remained robust, but were increased slightly when CO was added to the model (Figure 6-20 [and Table 6-29]).

In the study discussed above, Wong et al. (2009) also examined the potential modification of the relationship between ambient $O_3$ and COPD hospital admissions by influenza intensity. The authors also found evidence of an additional increase in COPD admissions above baseline when influenza activity increased from 0% to 10% of 1.0% (95% CI: -0.82, 2.9%) for all age groups and 2.4% (95% CI: 0.41, 4.4%) for those 65 and older. The baseline increase in COPD hospital admissions at lag 0-1 for a 30 ppb increase in 8-h max $O_3$ concentrations was 8.5% (95% CI: 5.6, 11.4%) for the all age and 4.2% (95% CI: 1.1, 7.3%) $\geq$ 65 age groups.

### Pneumonia

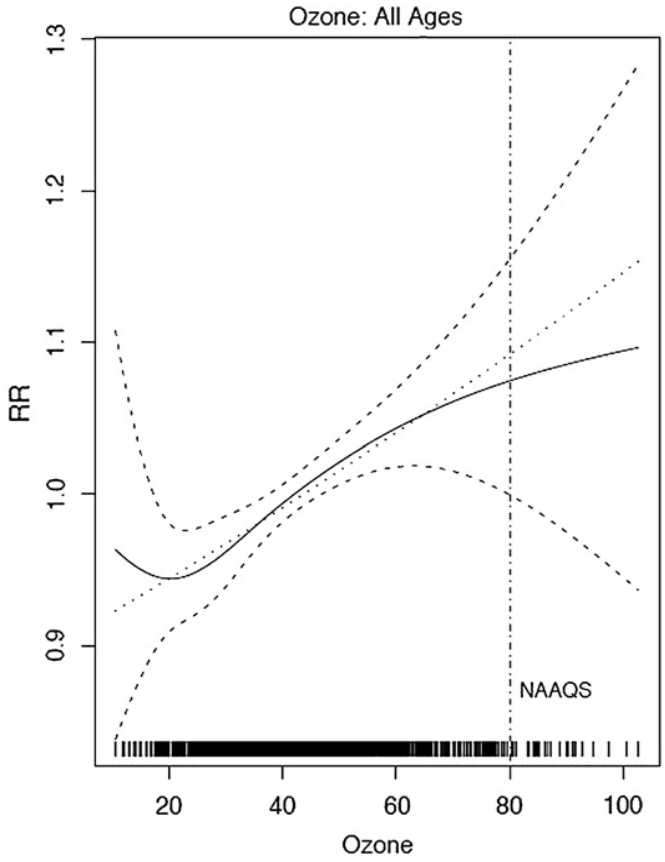
In addition to COPD, Medina-Ramon et al. (2006) examined the association between short-term exposure to ambient $O_3$ and $PM_{10}$ concentrations and Medicare hospital admissions among individuals $\geq$ 65 years of age for pneumonia (ICD-9: 480-487). The authors reported an increase in pneumonia-hospital admissions in the warm season (2.5% [95% CI: 1.6, 3.5%] for a 30 ppb increase in 8-h max $O_3$ concentrations; lag 0-1). Similar to the results observed for COPD hospital admissions, pneumonia-hospital admissions associations were stronger at lag 1 (2.6% [95% CI: 1.8, 3.4%]) compared to lag 0 (0.06% [95% CI: -0.72, 0.78%]), and no evidence of an association was observed in the cool season or year round. In two-pollutant models restricted to days for which $PM_{10}$ data was available, as discussed above, the association between $O_3$ exposure and pneumonia-hospital admissions remained robust (results not presented quantitatively). The authors also examined potential effect modification of the warm season estimates for $O_3$-related pneumonia-hospital admissions, as was done for COPD, by several city-level characteristics. Stronger associations were reported in cities with a lower percentage of central air conditioning use. Across the cities examined, the percentage of households having central air conditioning ranged from 6 to 93%. The authors found no evidence of effect modification of the $O_3$-pneumonia-hospital admission relationship when examining the other city-level characteristics.

Results from a single-city study conducted in Boston, MA, did not support the results presented by Medina-Ramon et al. (2006). Zanobetti and Schwartz (2006) examined the association of $O_3$ and pneumonia Medicare hospital admissions for the period 1995-1999. Ozone was weakly positively correlated with $PM_{2.5}$ (r = 0.20) and

weakly inversely correlated with black carbon, $NO_2$, and CO (-0.25, -0.14, and -0.30, respectively). In an all-year analysis, the investigators reported a 3.8% (95% CI: -7.9, -0.1%) decrease in pneumonia admissions for a 20 ppb increase in 24-h avg $O_3$ concentrations at lag 0 and a 6.0% (95% CI: -11.1, -1.4%) decrease for the average of lags 0 and 1. It should be noted that the mean daily counts of pneumonia admissions was low for this study, ~14 admissions per day compared to ~271 admissions per day for Medina-Ramon et al. (2006). However, in analyses with other pollutants Zanobetti and Schwartz (2006) did observe positive associations with pneumonia-hospital admissions, indicating that the low number of daily hospital admission counts probably did not influence the $O_3$ pneumonia-hospital admissions association in this study.

### Asthma

There are relatively fewer studies that examined the association between short-term exposure to $O_3$ and asthma hospital admissions, presumably due to the limited power given the relative rarity of asthma hospital admissions compared to ED or physician visits. A study from New York City examined the association of 8-h max $O_3$ concentrations with severe acute asthma admissions (i.e., those admitted to the Intensive Care Unit [ICU]) during the warm season in the years 1999 through 2006 (Silverman and Ito, 2010). In this study, $O_3$ was moderately correlated with $PM_{10}$ (r = 0.59). When stratifying by age, the investigators reported positive associations with ICU asthma admissions for the 6- to 18-year age group (26.8% [95% CI: 1.4, 58.2%] for a 30 ppb increase in 8-h max $O_3$ concentrations at lag 0-1), but little evidence of associations for the other age groups examined (<6 years, 19-49, 50+, and all ages). However, positive associations were observed for each age-stratified group and all ages for non-ICU asthma admissions, but again the strongest association was reported for the 6- to 18-years age group (28.2% [95% CI: 15.3, 41.5%]; lag 0-1). In two-pollutant models, $O_3$ effect estimates for both non-ICU and ICU hospital admissions remained robust to adjustment for $PM_{2.5}$. In an additional analysis, using a smooth function, the authors examined whether the shape of the C-R curve for $O_3$ and asthma hospital admissions (i.e., both general and ICU for all ages) is linear. To account for the potential confounding effects of $PM_{2.5}$, Silverman and Ito (2010) also included a smooth function of $PM_{2.5}$ lag 0-1. When comparing the curve to a linear fit line the authors found that the linear fit is a reasonable approximation of the C-R relationship between $O_3$ and asthma hospital admissions around and below the level of the 1997 $O_3$ NAAQS (Figure 6-16).




Note: The average of 0-day and 1-day lagged 8-hour $O_3$ was used in a two-pollutant model with $PM_{2.5}$ lag 0-1, adjusting for temporal trends, day of the week, and immediate and delayed weather effects. The solid lines are smoothed fit data, with long broken lines indicating 95% confidence bands. The density of lines at the bottom of the figure indicates sample size.

Source: Reprinted with permission of the American Academy of Allergy, Asthma & Immunology (Silverman and Ito, 2010).

**Figure 6-16 Estimated relative risks (RRs) of asthma hospital admissions for 8-h max $O_3$ concentrations at lag 0-1 allowing for possible nonlinear relationships using natural splines.**

### Averting Behavior

The studies discussed above have found consistent positive associations between short-term $O_3$ exposure and respiratory-related hospital admissions, however, the strength of these associations may be underestimated due to the studies not accounting for averting behavior. As discussed in Section 4.6.6, a recent study (Neidell and Kinney, 2010; Neidell, 2009) conducted in Southern California demonstrated that controlling for avoidance behavior increases $O_3$ effect estimates for respiratory hospital admissions, specifically for children and older adults. These analyses show that on days where no public alert was issued warning of high $O_3$ concentrations there was an increase in asthma hospital admissions. Although only one study has examined averting behavior and this study is limited to the outcome of asthma hospital admissions in one location (i.e., Los Angeles, CA) for the years

1989-1997, it does provide preliminary evidence indicating that epidemiologic studies may underestimate associations between $O_3$ exposure and health effects by not accounting for behavioral modification when public health alerts are issued.

### 6.2.7.3 Emergency Department Visit Studies

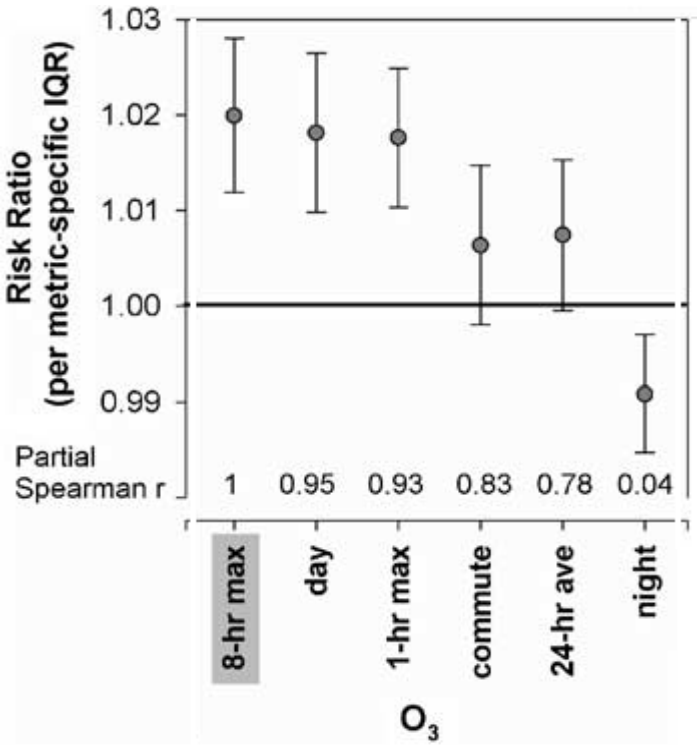
Overall, relatively fewer studies have examined the association between short-term $O_3$ exposure and respiratory-related ED visits, compared to hospital admissions. In the 2006 $O_3$ AQCD, positive, but inconsistent, associations were observed between $O_3$ and respiratory-related ED visits with effects generally occurring during the warm season. Since the completion of the previous AQCD, larger studies have been conducted, in terms of sample size, study duration, and in some cases multiple cities, to examine the association between $O_3$ and ED visits for all respiratory diseases, COPD, and asthma.

#### Respiratory Disease

A large single-city study conducted in Atlanta, GA, by Tolbert et al. (2007), and subsequently re-analyzed by Darrow et al. (2011a) using different air quality data, provides evidence for an association between short-term exposures to ambient $O_3$ concentrations and respiratory ED visits. Tolbert et al. (2007) examined the association between air pollution, both gaseous pollutants and PM and its components, and respiratory disease ED visits in all ages from 1993 to 2004. The correlations between $O_3$ and the other pollutants examined ranged from 0.2 for CO and $SO_2$ to 0.5-0.6 for the PM measures. Using an a priori average of lags 0-2 for each air pollutant examined, the authors reported a 3.9% (95% CI: 2.7, 5.2%) increase in respiratory ED visits for a 30 ppb increase in 8-h max $O_3$ concentrations during the warm season [defined as March-October in Darrow et al. (2011a)]. In copollutant models, limited to days in which data for all pollutants were available, $O_3$ respiratory ED visits associations with CO, $NO_2$, and $PM_{10}$, were attenuated, but remained positive (results not presented quantitatively).

Darrow et al. (2011a) examined the same health data as Tolbert et al. (2007), but used air quality data from one centrally located monitor instead of the average of multiple monitors. This study primarily focused on exploring whether differences exist in the association between $O_3$ exposure and respiratory-related ED visits depending on the exposure metric used (i.e., 8-h max, 1-h max, 24-h avg, commuting period [7:00 a.m. to 10:00 a.m.; 4:00 p.m. to 7:00 p.m.], day-time [8:00 a.m. to 7:00 p.m.] and night-time [12:00 a.m. to 6:00 a.m.]). An ancillary analysis of the spatial variability of each exposure metric conducted by Darrow et al. (2011a) found a rather homogenous spatial distribution of $O_3$ concentrations ($r > \sim 0.8$) as the distance from the central monitor increased from 10 km to 60 km for all exposure durations, except the night-time metric. The relatively high spatial correlation gives confidence in the use of a single monitor and the resulting risk estimates. To examine the association between the various $O_3$ exposure metrics and respiratory ED visits,

the authors conceptually used a time-stratified case-crossover framework where control days were selected as those days within the same calendar month and maximum temperature as the case day. However, instead of conducting a traditional case-crossover analysis, the authors used a Poisson model with indicator variables for each of the strata (i.e., parameters of the control days). Darrow et al. (2011a) found using an a priori lag of 1 day, the results were somewhat variable across exposure metrics. The strongest associations with respiratory ED visits were found when using the 8-h max, 1-h max, and day-time exposure metrics with weaker associations using the 24-h avg and commuting period exposure metrics; a negative association was observed when using the night-time exposure metric (Figure 6-17). These results indicate that using the 24-h avg exposure metric may lead to smaller $O_3$-respiratory ED visits risk estimates due to: (1) the dilution of relevant $O_3$ concentrations by averaging over hours (i.e., nighttime hours) during which $O_3$ concentrations are known to be low and (2) potential negative confounding by other pollutants (e.g., CO, $NO_2$) during the nighttime hours (Darrow et al., 2011a).




Source: Reprinted with permission of Nature Publishing Group (Darrow et al., 2011a).

**Figure 6-17 Risk ratio for respiratory ED visits and different $O_3$ exposure metrics in Atlanta, GA, from 1993-2004.**

In an additional study conducted in 6 Italian cities, Orazzo et al. (2009) examined respiratory ED visits for ages 0-2 years in 6 Italian cities from 1996 to 2000. However, instead of identifying respiratory ED visits using the traditional approach of selecting ICD codes as was done by Tolbert et al. (2007) and Darrow et al. (2011a), Orazzo et al. (2009) used data on wheeze extracted from medical records as an indicator of lower respiratory disease. This study examined daily counts of wheeze in relation to air pollution using a time-stratified case-crossover approach in which control days were matched on day of week in the same month and year as the case day. The authors found no evidence of an association between 8-h max $O_3$ concentrations and respiratory ED visits in children aged 0-2 years in models that examined both single-day lags and moving averages of lags from 0-6 days in year-round and seasonal analyses (i.e., warm and cool seasons). In all-year analyses, the percent increase in total wheeze ranged from -1.4% to -3.3% for a 0-1 to 0-6 day lag, respectively.

### COPD

Stieb et al. (2009) also examined the association between short-term $O_3$ exposure and COPD ED visits in 7 Canadian cities. Across cities, in an all-year analysis, $O_3$ was found to be positively associated with COPD ED visits (2.4% [95% CI: -1.9, 6.9%] at lag 1 and 4.0% [95% CI: -0.54, 8.6%] at lag 2 for a 20 ppb increase in 24-h avg $O_3$ concentrations). In seasonal analyses, larger effects were observed between $O_3$ and ED visits for COPD during the warm season (i.e., April-September) 6.8% [95% CI: 0.11, 13.9%] (lag day not specified); with no associations observed in the winter season. Stieb et al. (2009) also examined associations between respiratory-related ED visits, including COPD, and air pollution at sub-daily time scales (i.e., 3-h avg of ED visits versus 3-h avg pollutant concentrations) and found no evidence of consistent associations between any pollutant and any respiratory outcome.

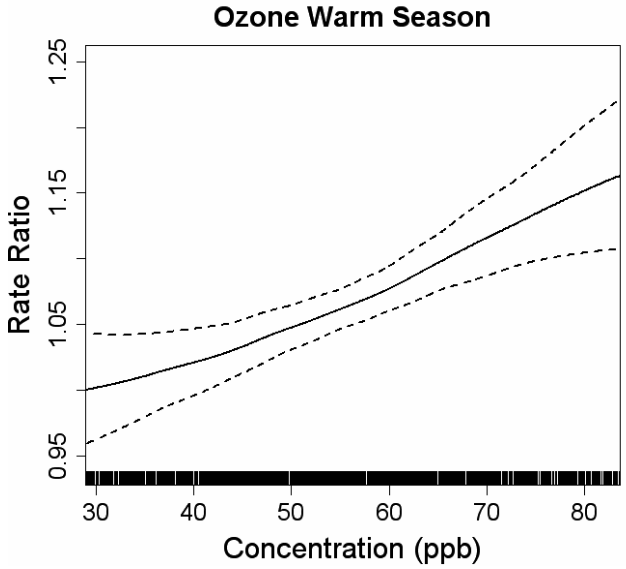
In a single-city study, Arbex et al. (2009) examined the association between COPD and several ambient air pollutants, including $O_3$, in Sao Paulo, Brazil for the years 2001-2003 for individuals over the age of 40. Associations between $O_3$ exposure and COPD ED visits were examined in both single-day lag (0-6 days) and polynomial distributed lag models (0-6 days). In all-year analyses, $O_3$ was not found to be associated with an increase in COPD ED visits (results not presented quantitatively). The authors also conducted stratified analyses to examine the potential modification of the air pollutant-COPD ED visits relationship by age (e.g., 40-64, >64) and sex. In these analyses $O_3$ was found to have an increase in COPD ED visits for women, but not for men or either of the age groups examined.

### Asthma

In a study of 7 Canadian cities, Stieb et al. (2009) also examined the association between exposure to air pollution (i.e., CO, $NO_2$, $O_3$, $SO_2$, $PM_{10}$, $PM_{2.5}$, and $O_3$) and asthma ED visits. Associations between short-term $O_3$ exposure and asthma ED visits were examined at the city level and then pooled using either fixed or random effects models depending on whether heterogeneity among effect estimates was

found to be statistically significant. Across cities, in an all-year analysis, the authors found that short-term $O_3$ exposure was associated with an increase (4.7% [95% CI: -1.4, 11.1%] at lag 1 and 3.5% [95% CI: 0.33, 6.8%] at lag 2 for a 20 ppb increase in 24-h avg $O_3$ concentrations) in asthma ED visits. The authors did not present the results from seasonal analyses for asthma but stated that no associations were observed between any pollutant and respiratory ED visits in the winter season. As stated previously, in analyses of 3-h avg $O_3$ concentrations, the authors observed no evidence of consistent associations between any pollutant and any respiratory outcome, including asthma. A single-city study conducted in Alberta, Canada Villeneuve et al. (2007) from 1992-2002 among individuals two years of age and older provides additional support for the findings from Stieb et al. (2009), but also attempts to identify those lifestages (i.e., 2-4, 5-14, 15-44, 45-64, 65-74, or 75+) at greatest risk to $O_3$-induced asthma ED visits. In a time-referent case-crossover analysis, Villeneuve et al. (2007) found an increase in asthma ED visits in an all-year analysis across all ages (12.0% [95% CI: 6.8, 17.2] for a 30 ppb increase in max 8-h avg $O_3$ concentrations at lag 0-2) with associations being stronger during the warmer months (19.0% [95% CI: 11.9, 28.1]). When stratified by age, the strongest associations were observed in the warm season for individuals 5-14 (28.1% [95% CI: 11.9, 45.1]; lag 0-2) and 15-44 (19.0% [95% CI: 8.5, 31.8]; lag 0-2). These associations were not found to be confounded by the inclusion of aeroallergens in age-specific models.

Several additional single-city studies have also provided evidence of an association between asthma ED visits and ambient $O_3$ concentrations. Ito et al. (2007b) examined the association between short-term exposure to air pollution and asthma ED visits for all ages in New York City from 1999 to 2002. Similar to Darrow et al. (2011a), when examining the spatial distribution of $O_3$ concentrations, Ito et al. (2007b) found a rather homogenous distribution ($r \geq \sim 0.80$) when examining monitor-to-monitor correlations at distances up to 20 miles. Ito et al. (2007b) used three different weather models with varying extent of smoothing to account for temporal relationships and multicollinearity among pollutants and meteorological variables (i.e., temperature and dew point) to examine the effect of model selection on the air pollutant-asthma ED visit relationship. When examining $O_3$, the authors reported a positive association with asthma ED visits, during the warm season across the models (ranging from 8.6 to 16.9%) and an inverse association in the cool season (ranging from -23.4 to -25.1%), at lag 0-1 for a 30 ppb increase in 8-h max $O_3$ concentrations. Ito et al. (2007b) conducted copollutant models using a simplified version of the weather model used in NMMAPS analyses (i.e., terms for same-day temperature and 1-3 day average temperature). The authors found that $O_3$ risk estimates were not substantially changed in copollutant models that used every-day data for $PM_{2.5}$, $NO_2$, $SO_2$, and CO during the warm season (Figure 6-20 [and Table 6-29]).




Note: The reference for the rate ratio is the estimated rate at the 5th percentile of the pollutant concentration. Estimates are presented for the 5th percentile through the 95th percentile of pollutant concentrations due to instability in the C-R estimates at the distribution tails.

Source: Reprinted with permission of American Thoracic Society (Strickland et al., 2010).

**Figure 6-18 Loess C-R estimates and twice-standard error estimates from generalized additive models for associations between 8-h max 3-day average $O_3$ concentrations and ED visits for pediatric asthma.**

Strickland et al. (2010) examined the association between $O_3$ exposure and pediatric asthma ED visits (ages 5-17 years) in Atlanta, GA, between 1993 and 2004 using air quality data over the same years as Darrow et al. (2011a) and Tolbert et al. (2007). However, unlike Darrow et al. (2011a) and Tolbert et al. (2007), which used single centrally located monitors or an average of monitors, respectively, Strickland et al. (2010) used population-weighting to combine daily pollutant concentrations across monitors. In this study, the authors developed a statistical model using hospital-specific time-series data that is essentially equivalent to a time-stratified case-crossover analysis (i.e., using interaction terms between year, month, and day-of-week to mimic the approach of selecting referent days within the same month and year as the case day). The authors observed a 6.4% (95% CI: 3.2, 9.6%) increase in ED visits for a 30 ppb increase in 8-h max $O_3$ concentrations at lag 0-2 in an all-year analysis. In seasonal analyses, stronger associations were observed during the warm season (i.e., May-October) (8.4% [95% CI: 4.4, 12.7%]; lag 0-2) than the cold season (4.5% [95% CI: -0.82, 10.0%]; lag 0-2). Strickland et al. (2011) confirmed these findings in an additional analysis using the same dataset, and found that the exposure assignment approach used (i.e., centrally located monitor, unweighted average across monitors, and population-weighted average across monitors) did not influence

pediatric asthma ED visit risk estimates for spatially homogeneous pollutants such as $O_3$.

In copollutant analyses conducted over the entire dataset for the gaseous pollutants (i.e., CO, $NO_2$), and limited to a subset of years (i.e., 1998-2004) for which daily PM data (i.e., $PM_{2.5}$ elemental carbon, $PM_{2.5}$ sulfate) were available, Strickland et al. (2010) found that $O_3$ risk estimates were not substantially changed when controlling for other pollutants (results not presented quantitatively). The authors also examined the C-R relationship between $O_3$ exposure and pediatric asthma ED visits and found that both quintile and loess C-R analyses (Figure 6-18) suggest that there are elevated associations with $O_3$ at 8-h max concentrations as low as 30 ppb. These C-R analyses do not provide evidence of a threshold level.

In a single-city study conducted on the West coast, Mar and Koenig (2009) examined the association between $O_3$ exposure and asthma ED visits (ICD-9 codes: 493-493.9) for children (<18) and adults (≥ 18) in Seattle, WA from 1998 to 2002. Of the total number of visits over the study duration, 64% of visits in the age group <18 comprised boys, and 70% of visits in the ≥ 18 age group comprised females. Mar and Koenig (2009) conducted a time-series analysis using both 1-h max and max 8-h avg $O_3$ concentrations. A similar magnitude and pattern of associations was observed at each lag examined using both metrics. Mar and Koenig (2009) presented results for single day lags of 0 to 5 days, but found consistent positive associations across individual lag days which supports the findings from the studies discussed above that examined multi-day exposures. For children, consistent positive associations were observed across all lags, ranging from a 19.1-36.8% increase in asthma ED visits for a 30 ppb increase in 8-h max $O_3$ concentrations with the strongest associations observed at lag 0 (33.1% [95% CI: 3.0, 68.5]) and lag 3 (36.8% [95% CI: 6.1, 77.2]). Ozone was also found to be positively associated with asthma ED visits for adults at all lags, ranging from 9.3-26.0%, except at lag 0. The slightly different lag times for children and adults suggest that children may be more immediately responsive to $O_3$ exposures than adults Mar and Koenig (2009).

### Respiratory Infection

Although an increasing number of studies have examined the association between $O_3$ exposure and cause-specific respiratory ED visits this trend has not included an extensive examination of the association between $O_3$ exposure and respiratory infection ED visits. Stieb et al. (2009) also examined the association between short-term $O_3$ exposure and respiratory infection ED visits in 7 Canadian cities. In an all-year analysis, there was no evidence of an association between $O_3$ exposure and respiratory infection ED visits at any lag examined (i.e., 0, 1, and 2). Across cities, respiratory infections comprised the single largest diagnostic category, approximately 32% of all the ED visits examined, which also included myocardial infarction, heart failure, dysrhythmia, asthma, and COPD.

### 6.2.7.4 Outpatient and Physician Visit Studies

Several studies have examined the association between ambient $O_3$ concentrations and physician or outpatient (non-hospital, non-ED) visits for acute conditions in various geographic locations. Burra et al. (2009) examined asthma physician visits among patients aged 1-17 and 18-64 years in Toronto, Canada from 1992 to 2001. The authors found little or no evidence of an association between asthma physician visits and $O_3$; however, seasonal analyses were not conducted. It should be noted that in this study, most of the relative risks for $O_3$ were less than one and statistically significant, perhaps due to an inverse correlation with another pollutant or an artifact of the strong seasonality of asthma visits. Villeneuve et al. (2006b) also focused on physician visits to examine the effect of short-term $O_3$ exposure on allergic rhinitis among individuals aged 65 or older in Toronto from 1995 to 2000. The authors did not observe any evidence of an association between allergic rhinitis physician visits and ambient $O_3$ concentrations in single-day lag models in an all-year analysis (results not presented quantitatively).

In a study conducted in Atlanta, GA, Sinclair et al. (2010) examined the association of acute asthma and respiratory infection (e.g., upper respiratory infections and lower respiratory infections) outpatient visits from a managed care organization with ambient $O_3$ concentrations as well as multiple PM size fractions and species from August 1998 through December 2002. The authors separated the analysis into two time periods (the first 25 months of the study period and the second 28 months of the study period), in order to compare the air pollutant concentrations and relationships between air pollutants and acute respiratory visits for the 25-month time-period examined in Sinclair and Tolsma (2004) to an additional 28-month time-period of available data from the Atlanta Aerosol Research Inhalation Epidemiology Study (ARIES). The authors found little evidence of an association between $O_3$ and asthma visits, for either children or adults, or respiratory infection visits in all-year analyses and seasonal analyses. For example, a slightly elevated RR for childhood asthma visits was observed during the 25-month period in the cold season (RR: 1.12 [95% CI: 0.86, 1.41]; lag 0-2 for a 30 ppb increase in 8-h max $O_3$), but not in the warm season (RR: 0.97 [95% CI: 0.86, 1.10]; lag 0-2). During the 28-month period at lag 0-2, a slightly larger positive effect was observed during the warm season (RR: 1.06 [95% CI: 0.97, 1.17]), compared to the cold season (RR: 1.03 [95% CI: 0.87, 1.21]). Overall, these results contradict those from Strickland et al. (2010) discussed above. Although the mean number of asthma visits and $O_3$ concentrations in Sinclair et al. (2010) and Strickland et al. (2010) are similar the difference in results between the two studies could potentially be attributed to the severity of $O_3$-induced asthma exacerbations (i.e., more severe symptoms requiring a visit to a hospital) and behavior, such as delaying a visit to the doctor for less severe symptoms.

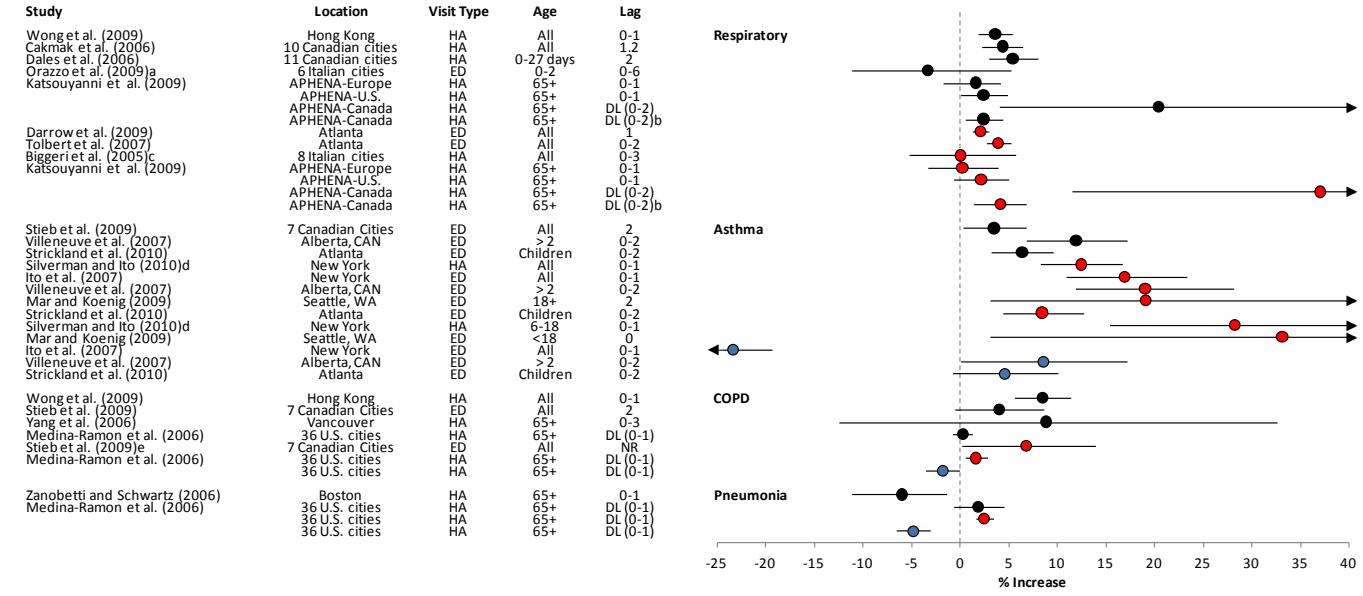
### 6.2.7.5 Summary

The results of the recent studies evaluated largely support the conclusion of the 2006 $O_3$ AQCD. While fewer studies were published overall since the previous review, several multicity studies (e.g., Cakmak et al., 2006b; Dales et al., 2006) and a multi-continent study (Katsouyanni et al., 2009) provide supporting evidence for an association between short-term $O_3$ exposure and an increase in respiratory-related hospital admissions and ED visits. Across studies, different ICD-9 codes were used to define total respiratory causes, which may contribute to some heterogeneity in the magnitude of association. These findings are supported by single-city studies that used different exposure assignment approaches (i.e., average of multiple monitors, single monitor, population-weighted average) and averaging times (i.e., 1-h max and 8-h max).

Collectively, in both single-city and multicity studies there is continued evidence for increases in both hospital admissions and ED visits when examining all respiratory outcomes combined. Additionally, recent studies published since the 2006 $O_3$ AQCD support an association between short-term $O_3$ exposure and asthma (Strickland et al., 2010; Stieb et al., 2009) and COPD (Stieb et al., 2009; Medina-Ramon et al., 2006) hospital admissions and ED visits, with more limited evidence for pneumonia-hospital admissions and ED visits (Medina-Ramon et al., 2006; Zanobetti and Schwartz, 2006). As with total respiratory causes, studies used slightly different ICD-9 codes to define specific conditions. In seasonal analyses, stronger associations were observed in the warm season or summer months compared to the cold season, particularly for asthma (Strickland et al., 2010; Ito et al., 2007b) and COPD (Medina-Ramon et al., 2006) (Figure 6-19 [and Table 6-28]), which is consistent with the conclusions of the 2006 $O_3$ AQCD. There is also continued evidence that children are particularly at greatest risk to $O_3$-induced respiratory effects (Silverman and Ito, 2010; Strickland et al., 2010; Mar and Koenig, 2009; Villeneuve et al., 2007; Dales et al., 2006). Of note, the consistent associations observed across studies for short-term $O_3$ exposure and respiratory-related hospital admissions and ED visits was not supported by studies that focused on respiratory-related outpatient or physician visits. These differences could potentially be attributed to the severity of $O_3$-induced respiratory effects requiring more immediate treatment or behavioral factors that result in delayed visits to a physician. Although the collective evidence across studies indicates a consistent positive association between $O_3$ exposure and respiratory-related hospital admissions and ED visits, the magnitude of these associations may be underestimated due to behavioral modification in response to forecasted air quality (Neidell and Kinney, 2010; Neidell, 2009) (Section 4.6.6).

The studies that examined the potential confounding effects of copollutants found that $O_3$ effect estimates remained relatively robust upon the inclusion of PM (measured using different sampling strategies ranging from every-day to every-6th day) and gaseous pollutants in two-pollutant models) (Figure 6-20 [and Table 6-29]). Additional studies that conducted copollutant analyses, but did not present quantitative results, also support these conclusions (Strickland et al., 2010; Tolbert et al., 2007; Medina-Ramon et al., 2006). Overall, recent studies provide

copollutant results that are consistent with those from the studies evaluated in the 2006 $O_3$ AQCD [(U.S. EPA, 2006b), Figure 7-12, page 7-80 of the 2006 $O_3$ AQCD], which found that $O_3$ respiratory hospital admissions risk estimates remained robust to the inclusion of PM in copollutant models.




Note: Effect estimates are for a 20 ppb increase in 24-h; 30 ppb increase in 8-h max; and 40 ppb increase in 1-h max $O_3$ concentrations. HA=hospital admission; ED=emergency department. Black=All-year analysis; Red=Summer only analysis; Blue=Winter only analysis.

[a] Wheeze used as indicator of lower respiratory disease.

[b] APHENA-Canada results standardized to approximate IQR of 5.1 ppb for 1-h max $O_3$ concentrations.

[c] Study included 8 cities; but of those 8, only 4 had $O_3$ data.

[d] non-ICU effect estimates.

[e] The study did not specify the lag day of the summer season estimate.

**Figure 6-19 Percent increase in respiratory-related hospital admission and ED visits in studies that presented all-year and/or seasonal results.**

## Table 6-28 Corresponding Effect Estimates for Figure 6-19.

| Study* | ED Visit or Hospital Admission | Location | Age | Lag | Avg Time | % Increase (95% CI) |
|---|---|---|---|---|---|---|
| **Respiratory** | | | | | | |
| **All-year** | | | | | | |
| Wong et al. (2009) | Hospital Admission | Hong Kong, China | All | 0-1 | 8-h max | 3.58 (1.90, 5.29) |
| Cakmak et al. (2006b) | Hospital Admission | 10 Canadian cities | All | 1.2 | 24-h avg | 4.38 (2.19, 6.46) |
| Dales et al. (2006) | Hospital Admission | 11 Canadian cities | 0-27 days | 2 | 24-h avg | 5.41 (2.88, 7.96) |
| Orazzo et al. (2009)[a] | ED Visit | 6 Italian cities | 0-2 | 0-6 | 8-h max | -3.34 (-11.2, 5.28) |
| Katsouyanni et al. (2009) | Hospital Admission | APHENA-europe | 65+ | 0-1 | 1-h max | 1.58 (-1.71, 4.15) |
| | | APHENA-U.S. | 65+ | 0-1 | 1-h max | 2.38 (0.00, 4.89) |
| | | APHENA-Canada | 65+ | DL(0-2) | 1-h max | 20.4 (4.07, 40.2) |
| | | APHENA-Canada | 65+ | DL(0-2)[b] | 1-h max | 2.4 (0.51, 4.40) |
| **Warm** | | | | | | |
| Darrow et al. (2011a) | ED Visit | Atlanta, GA | All | 1 | 8-h max | 2.08 (1.25, 2.91) |
| Tolbert et al. (2007) | ED Visit | Atlanta, GA | All | 0-2 | 8-h max | 3.90 (2.70, 5.20) |
| Biggeri et al. (2005)[c] | Hospital Admission | 8 Italian cities | All | 0-3 | 8-h max | 0.06 (-5.24, 5.66) |
| Katsouyanni et al. (2009) | Hospital Admission | APHENA-europe | 65+ | 0-1 | 1-h max | 0.24 (-3.32, 3.91) |
| | | APHENA-U.S. | 65+ | 0-1 | 1-h max | 2.14 (-0.63, 4.97) |
| | | APHENA-Canada | 65+ | DL(0-2) | 1-h max | 37.1 (11.5, 67.5) |
| | | APHENA-Canada | 65+ | DL(0-2)[b] | 1-h max | 4.1 (1.40, 6.80) |
| **Asthma** | | | | | | |
| **All-year** | | | | | | |
| Stieb et al. (2009) | ED Visit | 7 Canadian cities | All | 2 | 24-h avg | 3.48 (0.33, 6.76) |
| Villeneuve et al. (2007) | ED Visit | Alberta, CAN | >2 | 0-2 | 8-h max | 11.9 (6.8, 17.2) |
| Strickland et al. (2010) | ED Visit | Atlanta, GA | Children | 0-2 | 8-h max | 6.38 (3.19, 9.57) |
| **Warm** | | | | | | |
| Silverman and Ito (2010)[d] | Hospital Admission | New York, NY | All | 0-1 | 8-h max | 12.5 (8.27, 16.7) |
| Ito et al. (2007b) | ED Visit | New York, NY | All | 0-1 | 8-h max | 16.9 (10.9, 23.4) |
| Villeneuve et al. (2007) | ED Visit | Alberta, Canada | >2 | 0-2 | 8-h max | 19.0 (11.9, 28.1) |
| Mar and Koenig (2009) | ED Visit | Seattle, WA | 18+ | 2 | 8-h max | 19.1 (3.00, 40.5) |
| Strickland et al. (2010) | ED Visit | Atlanta, GA | Children | 0-2 | 8-h max | 8.43 (4.42, 12.7) |
| Silverman and Ito (2010)[d] | Hospital Admission | New York, NY | 6-18 | 0-1 | 8-h max | 28.2 (15.3, 41.5) |
| Mar and Koenig (2009) | ED Visit | Seattle, WA | <18 | 0 | 8-h max | 33.1 (3.00, 68.5) |

| Study* | ED Visit or Hospital Admission | Location | Age | Lag | Avg Time | % Increase (95% CI) |
|---|---|---|---|---|---|---|
| **Cold** | | | | | | |
| Ito et al. (2007b) | ED Visit | New York, NY | All | 0-1 | 8-h max | -23.4 (-27.3, -19.3) |
| Villeneuve et al. (2007) | ED Visit | Alberta, Canada | >2 | 0-2 | 8-h max | 8.50 (0.00, 17.2) |
| Strickland et al. (2010) | ED Visit | Atlanta, GA | Children | 0-2 | 8-h max | 4.52 (-0.82, 10.1) |
| **COPD** | | | | | | |
| **All-year** | | | | | | |
| Stieb et al. (2009) | ED Visit | 7 Canadian cities | All | 2 | 24-h avg | 4.03 (-0.54, 8.62) |
| Medina-Ramon et al. (2006) | Hospital Admission | 36 U.S. cities | 65+ | 0-1 | 8-h max | 0.24 (-0.78, 1.21) |
| Yang et al. (2005b) | Hospital Admission | Vancouver, Canada | 65+ | 0-3 | 24-h avg | 8.80 (-12.5, 32.6) |
| **Warm** | | | | | | |
| Stieb et al. (2009)[e] | ED Visit | 7 Canadian cities | All | NR | 24-h avg | 6.76 (0.11, 13.9) |
| Medina-Ramon et al. (2006) | Hospital Admission | 36 U.S. cities | 65+ | 0-1 | 8-h max | 1.63 (0.48, 2.85) |
| **Cold** | | | | | | |
| Medina-Ramon et al. (2006) | Hospital Admission | 36 U.S. cities | 65+ | 0-1 | 8-h max | -1.85 (-3.60, -0.06) |
| **Pneumonia** | | | | | | |
| **All-year** | | | | | | |
| Zanobetti and Schwartz (2006) | Hospital Admission | Boston, MA | 65+ | 0-1 | 24-h avg | -5.96 (-11.1, -1.36) |
| Medina-Ramon et al. (2006) | Hospital Admission | 36 U.S. cities | 65+ | 0-1 | 8-h max | 1.81 (-0.72, 4.52) |
| **Warm** | | | | | | |
| Medina-Ramon et al. (2006) | Hospital Admission | 36 U.S. cities | 65+ | 0-1 | 8-h max | 2.49 (1.57, 3.47) |
| **Cold** | | | | | | |
| Medina-Ramon et al. (2006) | Hospital Admission | 36 U.S. cities | 65+ | 0-1 | 8-h max | -4.88 (-6.59, -3.14) |

*Includes studies in Figure 6-19.

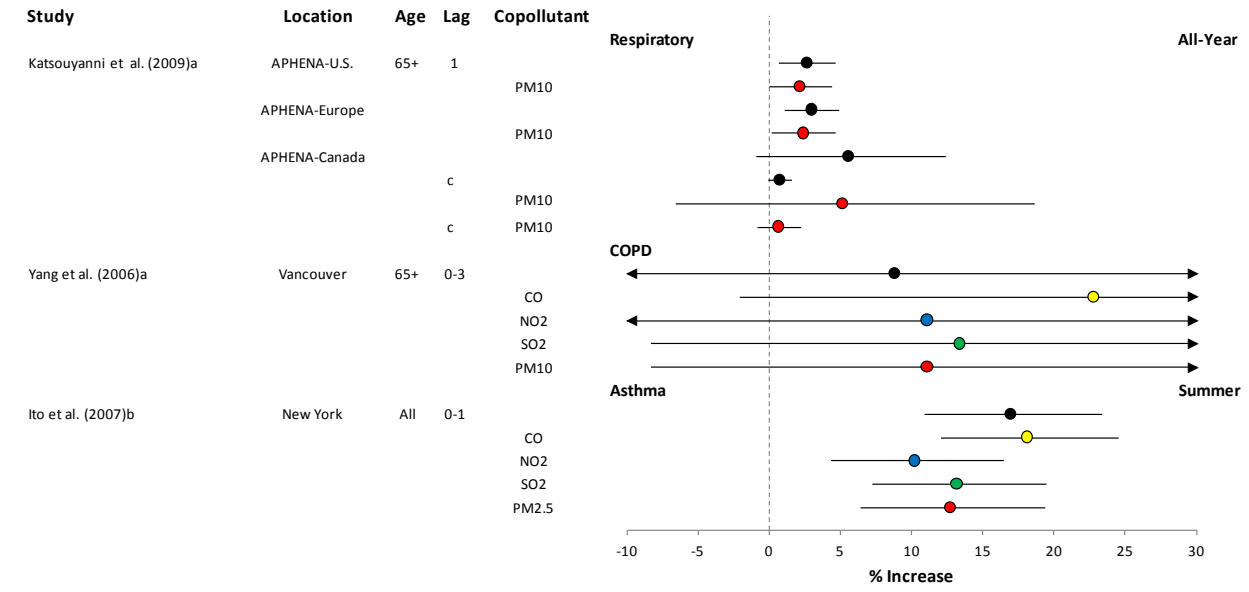
[a]Wheeze used as indicator of lower respiratory disease.

[b]APHENA-Canada results standardized to approximate IQR of 5.1 ppb for 1-h max $O_3$ concentrations.

[c]Study included 8 cities, but of those 8 only 4 had $O_3$ data.

[d]Non-ICU effect estimates.

[e]The study did not specify the lag day of the summer season estimate.




Note: Effect estimates are for a 20 ppb increase in 24-h; 30 ppb increase in 8-h max; and 40 ppb increase in 1-h max $O_3$ concentrations.

[a]Studies that examined hospital admissions.

[b]A study that examined ED visits.

[c]Risk estimates from APHENA -Canada standardized to an approximate IQR of 5.1 ppb for a 1-h max increase in $O_3$ concentrations. Black = results from single-pollutant models; Red = results from copollutant models with $PM_{10}$ or $PM_{2.5}$; Yellow = results from copollutant models with CO; Blue = results from copollutant models with $NO_2$; Green = results from copollutant models with $SO_2$.

**Figure 6-20 Percent increase in respiratory-related hospital admissions and ED visits for studies that presented single and copollutant model results.**

## Table 6-29 Corresponding effect estimates for Figure 6-20.

| Study*[a] | Location | Visit Type | Age | Lag | Copollutant | % Increase (95% CI) |
|---|---|---|---|---|---|---|
| **All-year: Respiratory** | | | | | | |
| Katsouyanni et al. (2009) | APHENA-U.S. | Hospital Admission | 65+ | 1 | | 2.62 (0.63, 4.64) |
| | | | | | $PM_{10}$ | 2.14 (-0.08, 4.40) |
| | APHENA-Europe | | | | | 2.94 (1.02, 4.89) |
| | | | | | $PM_{10}$ | 2.38 (0.08, 4.64) |
| | APHENA-Canada | | | | | 5.54 (-0.94, 12.4) |
| | | | | | | 0.69 (-0.12, 1.50)[b] |
| | | | | | $PM_{10}$ | 5.13 (-6.62, 18.6) |
| | | | | | $PM_{10}$ | 0.64 (-0.87, 2.20)[b] |
| | COPD | | | | | |
| Yang et al. (2005b) | Vancouver | Hospital Admission | 65+ | 0-3 | | 8.80 (-12.5, 32.6) |
| | | | | | CO | 22.8 (-2.14, 50.7) |
| | | | | | $NO_2$ | 11.1 (-10.4, 37.6) |
| | | | | | $SO_2$ | 13.4 (-8.40, 40.2) |
| | | | | | $PM_{10}$ | 11.1 (-8.40, 37.6) |
| **Summer: Asthma** | | | | | | |
| Ito et al. (2007b) | New York | ED | All | 0-1 | | 16.9 (10.9, 23.4) |
| | | | | | CO | 18.1 (12.1, 24.5) |
| | | | | | $NO_2$ | 10.2 (4.29, 16.4) |
| | | | | | $SO_2$ | 13.1 (7.16, 19.5) |
| | | | | | $PM_{2.5}$ | 12.7 (6.37, 19.3) |

*Studies included in Figure 6-20.

[a]Averaging times: Katsouyanni et al. (2009) = 1-h max; Yang et al. (2005b) = 24-h avg; and Ito et al. (2007b) = 8-h max.

[b]Risk estimates standardized to an approximate IQR of 5.1 ppb for a 1-h max increase in $O_3$ concentrations.

To date only a few studies have examined the C-R relationship between short-term $O_3$ exposure and respiratory-related hospital admissions and ED visits. A preliminary examination of the C-R relationship found no evidence of a deviation from linearity when examining the association between short-term $O_3$ exposure and asthma hospital admissions (Silverman and Ito, 2010). Additionally, an examination of the C-R relationship for $O_3$ exposure and pediatric asthma ED visits found no evidence of a threshold with elevated associations with $O_3$ at concentrations as low as 30 ppb (Silverman and Ito, 2010; Strickland et al., 2010). However, in both studies there is uncertainty in the shape of the C-R curve at the lower end of the distribution of $O_3$ concentrations due to the low density of data in this range.

In totality, building upon the conclusions of the 2006 $O_3$ AQCD, the evidence from recent studies continues to support an association between short-term $O_3$ exposure and respiratory-related hospital admissions and ED visits. Additional evidence also supports stronger associations during the warm season for specific respiratory outcomes such as asthma and COPD.

### 6.2.8 Respiratory Mortality

The epidemiologic, controlled human exposure, and toxicological studies discussed within this section (Section 6.2) provide evidence for multiple respiratory effects in response to short-term $O_3$ exposure. Additionally, the evidence from experimental studies indicates multiple potential pathways of $O_3$-induced respiratory effects, which support the continuum of respiratory effects that could potentially result in respiratory-related mortality. The 2006 $O_3$ AQCD found inconsistent evidence for an association between short-term $O_3$ exposure and respiratory mortality (U.S. EPA, 2006b). Although some studies reported a strong positive association between $O_3$ exposure and respiratory mortality, additional studies reported a small association or no association. The majority of recent multicity studies found consistent positive associations between short-term $O_3$ exposure and respiratory mortality, specifically during the summer months.

The APHENA study, described earlier in Section 6.2.7.2, (Katsouyanni et al., 2009) also examined associations between short-term $O_3$ exposure and mortality and found consistent positive associations for respiratory mortality in all-year analyses, except in the Canadian data set for ages $\geq 75$, with an increase in the magnitude of associations in analyses restricted to the summer season across data sets and age ranges. Additional multicity studies from the U.S. (Zanobetti and Schwartz, 2008b), Europe (Samoli et al., 2009), Italy (Stafoggia et al., 2010), and Asia (Wong et al., 2010) that conducted summer season and/or all-year analyses provide additional support for an association between short-term $O_3$ exposure and respiratory mortality (Figure 6-37).

Of the studies evaluated, only the APHENA study (Katsouyanni et al., 2009) and the Italian multicity study (Stafoggia et al., 2010) conducted an analysis of the potential for copollutant confounding of the $O_3$-respiratory mortality relationship. In the APHENA study, specifically the European dataset, focused on the natural spline model with 8 df/year (as discussed in Section 6.2.7.2) and lag 1 results (as discussed in Section 6.6.2.1), respiratory mortality risk estimates were robust to the inclusion of $PM_{10}$ in copollutant models in all-year analyses with $O_3$ respiratory mortality risk estimates increasing in the Canadian and U.S. datasets compared to single-pollutant model results. In summer season analyses, respiratory $O_3$ mortality risk estimates were robust in the U.S. dataset and attenuated in the European dataset. Similarly, in the Italian multicity study (Stafoggia et al., 2010), which was limited to the summer season, respiratory mortality risk estimates were attenuated in copollutant models with $PM_{10}$. Based on the APHENA and Italian multicity results, $O_3$ respiratory

mortality risk estimates appear to be moderately to substantially sensitive (e.g., increased or attenuated) to inclusion of $PM_{10}$. However, in the APHENA study, the mostly every-6th-day sampling schedule for $PM_{10}$ in the Canadian and U.S. datasets greatly reduced their sample size and limits the interpretation of these results.

### 6.2.9 Summary and Causal Determination

The 2006 $O_3$ AQCD concluded that there was clear, consistent evidence of a causal relationship between short-term $O_3$ exposure and respiratory effects (U.S. EPA, 2006b). This conclusion was substantiated by evidence from controlled human exposure and toxicological studies indicating a range of respiratory effects in response to short-term $O_3$ exposure, including pulmonary function decrements and increases in respiratory symptoms, lung inflammation, lung permeability, and airway hyperresponsiveness. Toxicological studies provided additional evidence for $O_3$-induced impairment of host defenses. Combined, these findings from experimental studies provided support for epidemiologic evidence, in which short-term increases in ambient $O_3$ concentration were consistently associated with decreases in lung function in populations with increased outdoor exposures, children with asthma, and healthy children; increases in respiratory symptoms and asthma medication use in children with asthma; and increases in respiratory-related hospital admissions and asthma-related ED visits. Short-term increases in ambient $O_3$ concentration also were consistently associated with increases in all-cause and cardiopulmonary mortality; however, the contribution of respiratory causes to these findings was uncertain.

Building on the large body of evidence presented in the 2006 $O_3$ AQCD, recent studies support associations between short-term $O_3$ exposure and respiratory effects. Controlled human exposure studies continue to provide the strongest evidence for lung function decrements in young healthy adults over a range of $O_3$ concentrations. Studies previously reported mean $O_3$-induced $FEV_1$ decrements of 6-8% at 80 ppb $O_3$ (Adams, 2006a, 2003a; McDonnell et al., 1991; Horstman et al., 1990), and recent evidence additionally indicates mean $FEV_1$ decrements of 6% at 70 ppb $O_3$ (Schelegle et al., 2009) and 2-3% at 60 ppb $O_3$ (Kim et al., 2011; Brown et al., 2008; Adams, 2006a) (Section 6.2.1.1). In healthy young adults, $O_3$-induced decrements in $FEV_1$ do not appear to depend on sex (Hazucha et al., 2003), body surface area or height (McDonnell et al., 1997), lung size or baseline FVC (Messineo and Adams, 1990). There is limited evidence that blacks may experience greater $O_3$-induced decrements in $FEV_1$ than do age-matched whites (Que et al., 2011; Seal et al., 1993). Healthy children experience similar spirometric responses but lesser symptoms from $O_3$ exposure relative to young adults (McDonnell et al., 1985b). On average, spirometric and symptom responses to $O_3$ exposure appear to decline with increasing age beyond about 18 years of age (McDonnell et al., 1999b; Seal et al., 1996). There is also a tendency for slightly increased spirometric responses in subjects with mild asthma and subjects with allergic rhinitis relative to healthy young adults (Jorres et

al., 1996). Spirometric responses in subjects with asthma appear to be affected by baseline lung function, i.e., responses increase with disease severity (Horstman et al., 1995).

Available information from controlled human exposure studies on recovery from $O_3$ exposure indicates that an initial phase of recovery in healthy individuals proceeds relatively rapidly, with acute spirometric and symptom responses resolving within about 2 to 4 hours (Folinsbee and Hazucha, 1989). Small residual lung function effects are almost completely resolved within 24 hours. Effects of $O_3$ on the small airways persisting a day following exposure, assessed by persistent decrement in $FEF_{25-75\%}$ and altered ventilation distribution, may be due in part to inflammation (Frank et al., 2001; Foster et al., 1997). In more responsive individuals, this recovery in lung function takes longer (as much as 48 hours) to return to baseline. Some cellular responses may not return to baseline levels in humans for more than 10-20 days following $O_3$ exposure (Devlin et al., 1997). Airway hyperresponsiveness and increased epithelial permeability are also observed as late as 24 hours post-exposure (Que et al., 2011).

With repeated $O_3$ exposures over several days, spirometric and symptom responses become attenuated in both healthy individuals and individuals with asthma, but this attenuation is lost after about a week without exposure (Gong et al., 1997a; Folinsbee et al., 1994; Kulle et al., 1982). Airway responsiveness also appears to be somewhat attenuated with repeated $O_3$ exposures in healthy individuals, but becomes increased in individuals with pre-existing allergic airway disease (Gong et al., 1997a; Folinsbee et al., 1994). Some indicators of pulmonary inflammation are attenuated with repeated $O_3$ exposures. However, other markers such as epithelial integrity and damage do not show attenuation, suggesting continued tissue damage during repeated $O_3$ exposure (Devlin et al., 1997).

Consistent with controlled human exposure study findings, epidemiologic evidence indicates that lung function decrements are related to short-term increases in ambient $O_3$ concentration (Section 6.2.1.2). As described in the 1996 and 2006 $O_3$ AQCDs, the most consistent observations were those in populations engaged in outdoor recreation, exercise, or work. Epidemiologic evidence also demonstrates that increases in ambient $O_3$ concentration are associated with decreases in lung function in children with asthma (Figure 6-7 [and Table 6-8] and Figure 6-8 [and Table 6-9]) and children in the general population (Figure 6-9 [and Table 6-12]). Evidence in adults with respiratory disease and healthy adults is inconsistent. In children with asthma, lung function mostly was found to decrease by <1-2% per unit increase in $O_3$ concentration[1]. However, within studies of children with asthma, increases in ambient $O_3$ concentration (at the same or similar lag) were associated both with decrements in lung function and increases in respiratory symptoms (Just et al., 2002; Mortimer et al., 2002; Ross et al., 2002; Gielen et al., 1997; Romieu et al., 1997; Thurston et al., 1997; Romieu et al., 1996). Biological plausibility for $O_3$-associated decrements in lung function found in controlled human exposure, epidemiologic, and

[1] Effect estimates were standardized to a 40-ppb increase for 1-h max $O_3$, a 30-ppb increase for 8-h max $O_3$, and a 20-ppb increase for 24-h avg $O_3$.

animal studies is provided by the well-documented effects of $O_3$ on activation of bronchial C-fibers (Section 5.3.2).

Across disciplines, studies have examined factors that may potentially increase the risk of $O_3$-induced decrements in lung function. In the controlled human exposure studies, there is a large degree of intersubject variability in lung function decrements, symptomatic responses, pulmonary inflammation, airway hyperresponsiveness, and altered epithelial permeability in healthy adults exposed to $O_3$ (Que et al., 2011; Holz et al., 2005; McDonnell, 1996). The magnitudes of pulmonary inflammation, airway hyperresponsiveness, and increases in epithelial permeability do not appear to be correlated, nor are these responses to $O_3$ correlated with changes in lung function, suggesting that different mechanisms may be responsible for these processes (Que et al., 2011; Balmes et al., 1997; Balmes et al., 1996; Aris et al., 1995). However, these responses tend to be reproducible within a given individual over a period of several months indicating differences in the intrinsic responsiveness of individuals (Holz et al., 2005; Hazucha et al., 2003; Holz et al., 1999; McDonnell et al., 1985c). Numerous reasons for differences in the risk of individuals to $O_3$ exposure have been reported in the literature. These include dosimetric and mechanistic differences (Section 5.4). Further, evidence in all three disciplines suggests a role for antioxidant defenses (i.e., vitamin supplementation, genetic variants in oxidative metabolizing enzymes) in modulating respiratory responses to $O_3$. The biological plausibility of these findings is provided by the well-characterized evidence for $O_3$ exposure leading to the formation of secondary oxidation products that subsequently activate neural reflexes that mediate lung function decrements (Section 5.2.3) and that initiate pulmonary inflammation (Section 5.3.3).

Recent controlled human exposure studies (Section 6.2.3.1) and toxicological studies (Section 6.2.3.3) also continue to demonstrate lung injury and inflammatory responses upon $O_3$ exposure. Evidence from more than a hundred toxicological studies clearly indicates that $O_3$ induces damage and inflammation in the lung, and studies continue to elucidate the mechanistic pathways involved (Section 5.3). Though inflammation may resolve, continued cellular damage may alter the structure and function of pulmonary tissues. Recent controlled human exposure studies support previous findings for pulmonary inflammation but demonstrate effects at 60 ppb $O_3$, the lowest concentration evaluated. Building on the extensive experimental evidence, recent epidemiologic studies, most of which were conducted in Mexico City, indicate ambient $O_3$-associated increases in pulmonary inflammation in children with asthma. Multiple studies examined and found increases in eNO (Berhane et al., 2011; Khatri et al., 2009; Barraza-Villarreal et al., 2008). In some studies of subjects with asthma, increases in ambient $O_3$ concentration at the same lag were associated with both increases in pulmonary inflammation and respiratory symptoms (Khatri et al., 2009; Barraza-Villarreal et al., 2008). Although more limited in number, epidemiologic studies also found associations with cytokines such as IL-6 or IL-8 (Barraza-Villarreal et al., 2008; Sienra-Monge et al., 2004), eosinophils (Khatri et al., 2009), antioxidants (Sienra-Monge et al., 2004), and indicators of oxidative stress (Romieu et al., 2008) (Section 6.2.3.2). This epidemiologic evidence is coherent with results from controlled human exposure and

toxicological studies that demonstrated an induction or reduction of these same endpoints after $O_3$ exposure.

The evidence for $O_3$-induced pulmonary inflammation and airway hyperresponsiveness, largely demonstrated in controlled human exposure and toxicological studies, provides mechanistic support for $O_3$-associated increases in respiratory symptoms observed in both controlled human exposure and epidemiologic studies. Controlled human exposure studies of healthy, young adults demonstrate increases in respiratory symptoms induced by $O_3$ exposures <80 ppb (Schelegle et al., 2009; Adams, 2006a) (Section 6.2.1.1). Adding to this evidence, epidemiologic studies find effects in children with asthma. Evidence from the previous large multicity NCICAS and a large body of single-city and -region studies indicates that short-term increases in ambient $O_3$ concentration are associated with increases in respiratory symptoms and asthma medication use in children with asthma (Section 6.2.4.1). Weak evidence is available from the few recent U.S. multicity studies; however, they examined either fewer person-days of data (Schildcrout et al., 2006) or longer lags of ambient $O_3$ exposure (19-day avg versus exposures lagged or averaged over a few days) than are supported by controlled human exposure, toxicological, and other epidemiologic studies (O'Connor et al., 2008). Several epidemiologic studies found associations between ambient $O_3$ concentrations and respiratory symptoms in populations with asthma that also had a high prevalence of allergy (52-100%) (Escamilla-Nuñez et al., 2008; Feo Brito et al., 2007; Romieu et al., 2006; Just et al., 2002; Mortimer et al., 2002; Ross et al., 2002; Gielen et al., 1997). The strong evidence in populations with asthma and allergy is supported by observations of $O_3$-induced inflammation in animal models of allergy (Section 6.2.3.3), and may be explained mechanistically by the action of $O_3$ to sensitize bronchial smooth muscle to hyperreactivity and thus, potentially act as a primer for subsequent exposure to antigens such as allergens (Section 5.3.5).

Modification of innate and adaptive immunity is emerging as a mechanistic pathway contributing to the effects of $O_3$ on asthma and allergic airways disease (Section 5.3.6). While the majority of evidence comes from animal studies, controlled human exposure studies have found differences between subjects with asthma and healthy controls in $O_3$-mediated innate and adaptive immune responses (Section 5.4.2.2), suggesting that these pathways may be relevant to humans and may lead to the induction and exacerbation of asthma (Alexis et al., 2010; Hernandez et al., 2010; Alexis et al., 2009; Bosson et al., 2003).

The subclinical and overt respiratory effects observed across disciplines, as described above, collectively provide support for epidemiologic studies that demonstrate consistently positive associations between short-term $O_3$ exposure and respiratory-related hospital admissions and ED visits (Section 6.2.7). Consistent with evidence presented in the 2006 $O_3$ AQCD, recent multicity studies and a multicontinent study (i.e., APHENA) (Katsouyanni et al., 2009) found risk estimates ranging from an approximate 1.6 to 5.4% increase in all respiratory-related hospital admissions and ED visits in all-year analyses per unit increase in ambient $O_3$ concentration (as described in Section 2.5). Positive associations persisted in analyses restricted to the

summer season, but the magnitude varied depending on the study location (Figure 6-19). Compared with studies reviewed in the 2006 $O_3$ AQCD, a larger number of recent studies examined hospital admissions and ED visits for specific respiratory outcomes. Although limited in number, both single- and multi-city studies consistently found positive associations between short-term $O_3$ exposures and asthma and COPD hospital admissions and ED visits, with more limited evidence for pneumonia. Consistent with the conclusions of the 2006 $O_3$ AQCD, in studies that conducted seasonal analyses, risk estimates were elevated in the warm season compared to cold season or all-season analyses, specifically for asthma and COPD. Although recent studies did not include detailed age-stratified results, the increased risk of asthma hospital admissions (Silverman and Ito, 2010; Strickland et al., 2010; Dales et al., 2006) observed for children strengthens the conclusion from the 2006 $O_3$ AQCD that children are potentially at increased risk of $O_3$-induced respiratory effects (U.S. EPA, 2006b). Although the C-R relationship has not been extensively examined, preliminary examinations found no evidence of a threshold between short-term $O_3$ exposure and asthma hospital admissions and pediatric asthma ED visits, with uncertainty in the shape of the C-R curve at the lower limit of ambient concentrations in the U.S. (Silverman and Ito, 2010; Strickland et al., 2010).

Recent evidence extends the potential range of well-established $O_3$-associated respiratory effects by demonstrating associations between short-term ambient $O_3$ exposure and respiratory-related mortality. In all-year analyses, a multicontinent (APHENA) and multicity (PAPA) study consistently found positive associations with respiratory mortality with evidence of an increase in the magnitude of associations in analyses restricted to the summer months. Further, additional multicity studies conducted in the U.S. and Europe provide evidence supporting stronger $O_3$-respiratory mortality associations during the summer season (Section 6.2.8).

Several epidemiologic studies of respiratory morbidity and mortality evaluated the potential confounding effects of copollutants, in particular, $PM_{10}$, $PM_{2.5}$, or $NO_2$. In most cases, effect estimates remained robust to the inclusion of copollutants. In some studies of lung function and respiratory symptoms, larger effects were estimated for $O_3$ when copollutants were added to models. Ozone effect estimates for respiratory-related hospital admissions and ED visits remained relatively robust upon the inclusion of PM and gaseous pollutants in two-pollutant models (Strickland et al., 2010; Tolbert et al., 2007; Medina-Ramon et al., 2006). Although copollutant confounding was not extensively examined in studies of cause-specific mortality, $O_3$-respiratory mortality risk estimates remained positive but were moderately to substantially sensitive (e.g., increased or attenuated) to the inclusion of $PM_{10}$ in copollutant models (Stafoggia et al., 2010; Katsouyanni et al., 2009). However, interpretation of these results for respiratory mortality requires caution due to the limited PM datasets used in these studies as a result of the every 3rd- or 6th-day PM sampling schedule employed in most cities. Together, these copollutant-adjusted findings across respiratory endpoints provide support for the independent effects of short-term exposures to ambient $O_3$.

Across the respiratory endpoints examined in epidemiologic studies, associations were found using several different exposure assessment methods that likely vary in how well ambient $O_3$ concentrations represent ambient exposures and between-subject variability in exposures. Evidence clearly demonstrated $O_3$-associated lung function decrements in populations with increased outdoor exposures for whom ambient $O_3$ concentrations measured on site of outdoor activity and/or at the time of outdoor activity have been more highly correlated and similar in magnitude to personal $O_3$ exposures (Section 4.3.3). However, associations with respiratory effects also were found with ambient $O_3$ concentrations expected to have weaker personal-ambient relationships, including those measured at home or school, measured at the closest site, averaged from multiple community sites, and measured at a single site. Overall, there was no clear indication that a particular method of exposure assessment produced stronger findings.

An additional consideration in the evaluation of the epidemiologic evidence is the impact of behavioral modifications on observed associations. A study demonstrated that the magnitude of $O_3$-associated asthma hospitalizations in Los Angeles, CA was underestimated due to behavioral modification in response to forecasted air quality (Section 4.6.5). It is important to note that the study was limited to one metropolitan area and used air quality data for the years 1989-1997, when the $O_3$ concentration that determines the designation of an $O_3$ action day, was much higher than it is currently.

Both panel and time-series epidemiologic studies found increases in respiratory effects in association with increases in $O_3$ concentrations using various exposure metrics (i.e., 24-h avg, 1-h max, and 8-h max $O_3$ concentrations). A majority of studies examined and found associations of respiratory symptoms with 1-h max or 8-h max $O_3$ concentrations and associations of pulmonary inflammation with 8-h max or daytime avg $O_3$. Within study comparisons of associations of lung function and respiratory symptoms among various exposure metrics yielded mixed evidence. Within some studies, larger effects were estimated for shorter $O_3$ averaging times whereas in other studies, larger effects were estimated for longer averaging times or no difference was found among averaging times. Comparisons in a limited number of time-series studies indicate rather comparable risk estimates across exposure metrics with some evidence indicating that 24-h avg $O_3$ was associated with a smaller increase in risk of respiratory ED visits (Section 6.2.7.3). Overall, there was no indication that the consistency or magnitude of the observed association was stronger for a particular $O_3$ exposure metric. In examination of the lag structure of associations, epidemiologic evidence for the range of respiratory endpoints clearly supports associations with ambient $O_3$ concentrations lagged 0 to 1 day, which is consistent with the $O_3$-induced respiratory effects observed in controlled human exposure studies. Several epidemiologic studies also found increased respiratory morbidity in association with $O_3$ concentrations averaged over multiple days (2 to 5 days). Across respiratory endpoints examined in epidemiologic studies, there was not strong evidence that the magnitude of association was larger for any particular lag.

In summary, recent studies evaluated since the completion of the 2006 $O_3$ AQCD support and expand upon the strong body of evidence that indicated a causal relationship between short-term $O_3$ exposure and respiratory health effects. Controlled human exposure studies continue to demonstrate $O_3$-induced decreases in $FEV_1$ and pulmonary inflammation at concentrations as low as 60 ppb. Epidemiologic studies provide evidence that increases in ambient $O_3$ exposure can result in lung function decrements; increases in respiratory symptoms and pulmonary inflammation in children with asthma; increases in respiratory-related hospital admissions and ED visits; and increases in respiratory mortality. Recent toxicological studies demonstrating $O_3$-induced inflammation, airway hyperresponsiveness, and impaired lung host defense have continued to support the biological plausibility and modes of action for the $O_3$-induced respiratory effects observed in the controlled human exposure and epidemiologic studies. Additionally, recent epidemiologic studies affirm that respiratory morbidity and mortality associations are stronger during the warm/summer months and remain relatively robust after adjustment for copollutants. The recent evidence integrated across toxicological, controlled human exposure, and epidemiologic studies, along with the total body of evidence evaluated in previous AQCDs, is sufficient to conclude that there **is a causal relationship between short-term $O_3$ exposure and respiratory health effects.**

## 6.3 Cardiovascular Effects

Overall, there have been a relatively small number of studies that have examined the potential effect of short-term $O_3$ exposure on the cardiovascular system. This was reflected in the 1996 $O_3$ AQCD by the limited discussion on possible $O_3$-related cardiovascular effects. The 2006 $O_3$ AQCD (U.S. EPA, 2006b) built upon the limited evidence described in the 1996 $O_3$ AQCD (U.S. EPA, 1996a) and further explored the potential relationship between short-term $O_3$ exposure and cardiovascular outcomes. The 2006 $O_3$ AQCD concluded that "$O_3$ directly and/or indirectly contributes to cardiovascular-related morbidity" but added that the body of evidence was limited. This conclusion was based on a controlled human exposure study that included hypertensive adult males, a few epidemiologic studies of physiologic effects, heart rate variability, arrhythmias, myocardial infarctions, and hospital admissions, and toxicological studies of heart rate, heart rhythm, and blood pressure.

### 6.3.1 Controlled Human Exposure

Ozone reacts rapidly on contact with respiratory tract lining fluids and is not absorbed or transported to extrapulmonary sites to any significant degree as such. Controlled human exposure studies discussed in the previous AQCDs failed to demonstrate any consistent extrapulmonary effects. Some controlled human exposure studies have attempted to identify specific markers of exposure to $O_3$ in blood. Buckley et al. (1975) reported a 28% increase in serum α-tocopherol and a 26%

increase in erythrocyte fragility in healthy males immediately following exposure to 500 ppb $O_3$ for 2.75 hours with exercise (unspecified activity level). However, in healthy adult males exposed during exercise ($\dot{V}_E$=44 L/min) to 323 ppb $O_3$ (on average) for 130 min on 3 consecutive days, Foster et al. (1996) found a 12% reduction in serum α-tocopherol 20 hours after the third day of $O_3$ exposure. Liu et al. (1999); (1997) used a salicylate metabolite, 2,3, dehydroxybenzoic acid (DHBA), to indicate increased levels of hydroxyl radical which hydroxylates salicylate to DHBA. Increased DHBA levels after exposure to 120 and 400 ppb suggest that $O_3$ increases production of hydroxyl radical. The levels of DHBA were correlated with changes in spirometry. Interestingly, simultaneous exposure of healthy adults to $O_3$ (120 ppb for 2 hours at rest) and concentrated ambient particles (CAPs) resulted in a diminished systemic IL-6 response compared with exposure to CAPs alone (Urch et al., 2010).

Devlin et al. (2012) recently evaluated systemic and cardiovascular responses in a group of young healthy adults (20 M, 3 F; median age 28.8 yrs) exposed to $O_3$ (300 ppb; 2 hours with alternating 15 min periods of rest and moderate-to-heavy exercise [$\dot{V}_E$ = 25 L/min per BSA]). Relative to FA responses, immediately following the $O_3$ exposure there was an 85% increase in blood IL-8 ($p< 0.025$). There were also trends ($p<0.10$) for $O_3$-induced increases in blood IL-1β (56%) and blood TNF-α (10%). At 24 hrs postexposure, there were significant ($p<0.025$) increases blood IL-1β (65%) and CRP (104%). Beyond these markers of systemic inflammation, there were also changes in biomarkers of vascular effects following $O_3$ exposure. There were significant ($p< 0.025$) $O_3$-induced decreases in plasminogen activator inhibitor-1 (PAI-1) by 33% immediately following exposure and by 43% at 24 hrs postexposure. Plasminogen levels were also decreased by42% at 24 hrs post exposure ($p<0.05$). Finally, there was a tendency ($p=0.065$) for a 44% increase in tissue-type plasminogen activator (tPA). Based on the combination of an increase in tPA and a decrease in PAI-1, the authors suggested that $O_3$ exposure may activate the fibronolyisis system. Until replicated at an $O_3$ concentration more typical of ambient exposures, the results of this study and other high $O_3$ concentration exposure studies should be interpreted with caution.

Gong et al. (1998) exposed hypertensive (n = 10) and healthy (n = 6) adult males, 41 to 78 years of age, to FA and on the subsequent day to 300 ppb $O_3$ for 3 hours with intermittent exercise ($\dot{V}_E$ = 30 L/min). The overall results did not indicate any major acute cardiovascular effects of $O_3$ in either the hypertensive individuals or healthy controls. Statistically significant $O_3$ effects for both groups combined were increases in heart rate, rate-pressure product, and the alveolar-to-arterial $PO_2$ gradient, suggesting that impaired gas exchange was being compensated for by increased myocardial work. The mechanism for the decrease in arterial oxygen tension in the Gong et al. (1998) study could be due to an $O_3$-induced ventilation-perfusion mismatch. Gong et al. (1998) suggested that by impairing alveolar-arterial oxygen transfer, the $O_3$ exposure could potentially lead to adverse cardiac events by decreasing oxygen supply to the myocardium. The subjects in the Gong et al. (1998) study had sufficient functional reserve so as to not experience significant ECG changes or myocardial ischemia and/or injury. In studies evaluating the exercise

performance of healthy adults, no significant effect of $O_3$ on arterial $O_2$ saturation has been observed (Schelegle and Adams, 1986).

Fakhri et al. (2009) evaluated changes in HRV among adult volunteers (n = 50; 27 ± 7 years) during 2-hour resting exposures to $PM_{2.5}$ CAPs (127 ± 62 $\mu g/m^3$) and $O_3$ (114 ± 7 ppb), alone and in combination. High frequency HRV was increased following CAPs-only ($p = 0.046$) and $O_3$-only ($p = 0.051$) exposures, but not in combination. The standard deviation of NN intervals and the square root of the mean squared differences of successive NN intervals also showed marginally significant ($0.05 < p < 0.10$) increase due to $O_3$ but not CAPS. Ten of the subjects in this study were characterized as "mildly" asthmatic, however, asthmatic status was not found to modify these effects. Power et al. (2008) also investigated HRV in a small group of mild-to-moderate allergic asthmatics (n = 5; mean age = 37 years) exposed for 4 hours during moderate intermittent exercise to FA, carbon and ammonium nitrate particles (313 ± 20 $\mu g/m^3$), and carbon and ammonium nitrate particles (255 ± 37 $\mu g/m^3$) + $O_3$ (200 ppb). Changes in frequency-domain variables for the particle and particle + $O_3$ exposures were not statistically significant compared with FA. Seemingly in contrast to Fakhri et al. (2009), the standard deviation of NN intervals and the square root of the mean squared differences of successive NN intervals also showed a significant ($p = 0.01$) decrease for both the particle and particle + $O_3$ exposures relative to FA responses. Using a similar protocol, Sivagangabalan et al. (2011) concluded that spatial dispersion of cardiac repolarization was most affected by the combined pollutant exposure of CAP + $O_3$ compared to FA in healthy adults.

In healthy young adults (20 M, 3 F; median age 28.8 yrs), Devlin et al. (2012) recently reported an $O_3$-induced reduction in high frequency HRV by 51% ($p<0.025$) immediately following $O_3$ exposure (300 ppb for 2 hr with intermittent exercise) that appeared to persist to 24 hrs postexposure (38% decrease, $p<0.10$). A small, 1.2% increase in the QT interval immediately after $O_3$ exposure relative to FA exposures was also observed. The authors suggested that changes in HRV and repolarization were likely mediated by nerve fibers that terminate in the lung. Changes in $FEV_1$ due to $O_3$ exposure are also mediated by C-fibers in the lung. There was an $O_3$-induced $FEV_1$ decrement of 11% in the Devlin et al. (2012) study, whereas the resting $O_3$ exposure used by Fakhri et al. (2009) is predicted to cause very small (<0.3%) decrements in $FEV_1$ (McDonnell et al., 2007). The induction of nerve fiber mediated responses may, in part, explain the reduction in high frequency HRV following a high level of exposure (300 ppb for 2 hr with intermittent exercise) in the Devlin et al. (2012) versus the increase in high frequency HRV observed following a lower level of exposure (120 ppb for 2hr during rest) by Fakhri et al. (2009).

Diastolic blood pressure increased by 2 mmHg following the combined $O_3$ + CAPs exposure, but was not altered by either $O_3$ or CAPs alone in the Fakhri et al. (2009) study. For a subset of the subjects without asthma in the Fakhri et al. (2009) study, Urch et al. (2005) previously reported a 6 mmHg increase in diastolic blood pressure following a 2-hour resting exposure to $O_3$ (120 ppb) + $PM_{2.5}$ CAPs (150 $\mu g/m^3$) in healthy adults (n = 23; 32 ± 10 years), which was statistically different from the 1 mmHg increase seen following FA exposure. Brook et al. (2002) found $O_3$

(120 ppb) + $PM_{2.5}$ CAPs (150 μg/$m^3$) in healthy adults (n = 25; 35 ± 10 years) caused brachial artery vasoconstriction. However, minimal change in diastolic blood pressure (0.9 mmHg increase) relative to FA (0.4 mmHg decrease) was observed. More recently, Sivagangabalan et al. (2011) observed reported a 4.2 mmHg increase in diastolic blood pressure following a 2-hour resting exposure to $O_3$ (110 ppb) + $PM_{2.5}$ CAPs (150 μg/$m^3$) in healthy adults (n = 25; 27 ± 8 years), which was statistically different from the 1.7 mmHg increase seen following the FA exposure. The CAP exposure alone also caused a 3 mmHg increase in diastolic blood pressure which was significantly more than following FA. However, similar to FA, the $O_3$ exposure alone caused a 1.8 mmHg increase in diastolic blood pressure. Overall, these studies indicate an effect of CAPs and CAP + $O_3$, but not $O_3$ alone, on diastolic blood pressure.

## 6.3.2 Epidemiology

The 2006 $O_3$ AQCD concluded that the "generally limited body of evidence is highly suggestive that $O_3$ directly and/or indirectly contributes to cardiovascular-related morbidity," including physiologic effects (e.g., release of platelet activating factor [PAF]), HRV, arrhythmias, and myocardial infarctions, although the available body of evidence reviewed during the 2006 $O_3$ AQCD does not "fully substantiate links between ambient $O_3$ exposure and adverse cardiovascular outcomes" (U.S. EPA, 2006b). Since the completion of the 2006 $O_3$ AQCD an increasing number of studies have examined the relationship between short-term $O_3$ exposure and cardiovascular morbidity and mortality. These recent studies, as well as evidence from the previous AQCDs, are presented within this section.

### 6.3.2.1 Arrhythmia

In the 2006 $O_3$ AQCD, conflicting results were observed when examining the effect of $O_3$ on arrhythmias (Dockery et al., 2005; Rich et al., 2005). A study by Dockery et al. (2005) reported no association between $O_3$ concentration and ventricular arrhythmias among patients with implantable cardioverter defibrillators (ICD) living in Boston, MA, although when $O_3$ concentration was categorized into quintiles, there was weak evidence of an association with increasing $O_3$ concentration (median $O_3$ concentration: 22.9 ppb). Rich et al. (2005) performed a re-analysis of this cohort using a case-crossover design and detected a positive association between $O_3$ concentration and ventricular arrhythmias. Recent studies were conducted in various locations and each used a different cardiac episode to define an arrhythmic event and a different time period of exposure, which may help explain observed differences across studies. Study-specific characteristics and air quality data for recent studies are reported in Table 6-30.

**Table 6-30 Characterization of $O_3$ concentrations (in ppb) from studies of arrhythmias.**

| Study* | Location | Averaging Time | Mean Concentration (Standard Deviation) | Upper Range of Concentration |
|---|---|---|---|---|
| Metzger et al. (2007) | Atlanta, GA | 8-h max<br>Summer only | 53.9 (23) | Max: 148 |
| Rich et al. (2006b) | Boston, MA | 1-h | 22.2* | 75th: 33<br>Max: 119.5 |
| | | 24-h | 22.6* | 75th: 30.9<br>Max: 77.5 |
| Rich et al. (2006a) | St. Louis, MO | 24-h | 21* | 75th: 31 |
| Anderson et al. (2010) | London, England | 8-h max | 8.08 | 75th: 11.5 |
| Sarnat et al. (2006b) | Steubenville, OH | 24-h<br>Summer and Fall only | 21.8 (12.6) | 75th: 28.5<br>Max: 74.8 |
| | | 5 days | 22.2 (9.1) | 75th: 29.1<br>Max: 44 |

Note: Median presented (information on mean not given); studies presented in order of first appearance in the text of this section.

Multiple studies examined $O_3$-related effects on individuals with ICDs. A study of 518 ICD patients who had at least 1 tachyarrythmia within a 10-year period (totaling 6,287 tachyarrhythmic event-days; 1993-2002) was conducted in Atlanta, Georgia (Metzger et al., 2007). Tachyarrhythmic events were defined as any ventricular tachyarrhythmic event, any ventricular tachyarrhythmic event that resulted in electrical therapy, and any ventricular tachyarrhythmic event that resulted in defibrillation. In the primary analysis, no evidence of an association was observed for a 30 ppb increase in 8-h max $O_3$ concentrations and tachyarrhythmic events (OR: 1.00 [95% CI: 0.92, 1.08]; lag 0). Season-specific as well as several sensitivity analyses (including the use of an unconstrained distributed lag model [lags 0-6]) were conducted resulting in similar null associations.

In a case-crossover analysis, a population of ICD patients in Boston, MA, previously examined by (Rich et al., 2005) was used to assess the association between air pollution and paroxysmal atrial fibrillation (PAF) episodes (Rich et al., 2006b). In addition to ventricular arrhythmias, ICD devices may also detect supraventricular arrhythmias, of which atrial fibrillation is the most common. Although atrial fibrillation is generally not considered lethal, it has been associated with increased premature mortality as well as hospitalization and stroke. Ninety-one electrophysiologist-confirmed episodes of PAF were ascertained among 29 patients. An association (OR: 3.86 [95% CI: 1.44, 10.28] per 40 ppb increase in 1-h max $O_3$ concentrations) was observed between increases in $O_3$ concentration during the concurrent hour (lag 0-hour) and PAF episodes. The estimated OR for the 24-hour moving average concentration was elevated (OR: 1.81 [95% CI: 0.86, 3.83] per 20 ppb), but weaker than the estimate for the shorter exposure window. The association between PAF and $O_3$ concentration in the concurrent hour during the

cold months was comparable to that during the warm months. In addition, no evidence of a deviation from linearity between $O_3$ concentration and the log odds of PAF was observed. Authors report that the difference between $O_3$ concentration and observed effect between this study (PAF and 1-hour $O_3$) and their previous study (ventricular arrhythmias and 24-hour moving average $O_3$) (Rich et al., 2005) suggest a more rapid response to air pollution for PAF (Rich et al., 2006b).

In an additional study, Rich et al. (2006a) employed a case-crossover design to examine the association between air pollution and 139 confirmed ventricular arrhythmias among 56 ICD patients in St Louis, Missouri. The authors observed a positive association with $O_3$ concentration (OR: 1.17 [95% CI: 0.58, 2.38] per 20 ppb increase in 24-hour moving avg $O_3$ concentrations [lags 0-23 hours]). Although the authors concluded these results were similar to their results from Boston, MA (Rich et al., 2005), they postulated that the pollutants responsible for the increased risk in ventricular arrhythmias are different ($O_3$ and $PM_{2.5}$ in Boston and sulfur dioxide in St Louis).

Anderson et al. (2010) used a case-crossover framework to assess air pollution and activation of ICDs among patients from all 9 ICD clinics in the London National Health Service hospitals. "Activation" was defined as tachycardias for which the defibrillator delivered treatment. Investigators modeled associations using unconstrained distributed lags from 0 to 5 days. The sample consisted of 705 patients with 5,462 activation days ($O_3$ concentration information was for 543 patients and 4,092 activation days). Estimates for the association with $O_3$ concentration were consistently positive, although weak (OR: 1.09 [95% CI: 0.76, 1.55] per 30 ppb increase in 8-h max $O_3$ concentrations at 0-1 day lag; OR: 1.04 [95% CI: 0.60, 1.81] per 30 ppb increase in 8-h max $O_3$ concentrations at 0-5 day lag) (Anderson et al., 2010).

In contrast to arrhythmia studies conducted among ICD patients, Sarnat et al. (2006b) recruited non-smoking adults (age range: 54-90 years) to participate in a study of air pollution and arrhythmias conducted over two 12-week periods during summer and fall of 2000 in a region characterized by industrial pollution (Steubenville, Ohio). Continuous ECG data acquired on a weekly basis over a 30-minute sampling period were used to assess ectopy, defined as extra cardiac depolarizations within the atria (supraventricular ectopy, SVE) or the ventricles (ventricular ectopy, VE). Increases in the 5-day moving average (days 1-5) of $O_3$ concentration were associated with an increased odds of SVE (OR: 2.17 [95% CI: 0.93, 5.07] per 20 ppb increase in 24-h avg $O_3$ concentrations). A weaker association was observed for VE (OR: 1.62 [95% CI: 0.54, 4.90] per 20 ppb increase in 24-h avg $O_3$ concentrations). The results of the effect of 5-day $O_3$ concentration on SVE were robust to the inclusion of $SO_4^{2-}$ in the model [OR: 1.62 (95% CI: 0.54, 4.90)]. The authors indicate that the strong associations observed at the 5-day moving averages, as compared to shorter time periods, suggests a relatively long-acting mechanistic pathways, such as inflammation, may have promoted the ectopic beats in this population (Sarnat et al., 2006b).

Although many studies report positive associations, collectively, studies of arrhythmias report inconsistent results. This may be due to variation in study populations, length and season of averaging time, and outcome under study.

#### 6.3.2.2 Heart Rate/Heart Rate Variability

In the 2006 $O_3$ AQCD, two large population-based studies of air pollution and HRV were summarized (Park et al., 2005b; Liao et al., 2004a). In addition, the biological mechanisms and potential importance of HRV were discussed. Briefly, the study of acute effects of air pollution on cardiac autonomic control is based on the hypothesis that increased air pollution levels may stimulate the autonomic nervous system and lead to an imbalance of cardiac autonomic control characterized by sympathetic activation unopposed by parasympathetic control (U.S. EPA, 2006b). Examples of HRV indices include the standard deviation of normal-to-normal intervals (SDNN), the square root of the mean of the sum of the squares of differences between adjacent NN intervals (r-MSSD), high-frequency power (HF), low-frequency power (LF), and the LF/HF ratio. Liao et al. (2004a) examined the association between air pollution and cardiac autonomic control in the fourth cohort examination (1996-1998) of the U.S.-based Atherosclerosis Risk in Communities Study. A decrease in log-transformed HF was associated with an increase in $O_3$ concentration among white study participants. Park et al. (2005b) examined the effects of air pollution on indices of HRV in a population-based study among men from the Normative Aging Study in Boston, Massachusetts. Several associations were observed with $O_3$ concentration and HRV outcomes. A reduction in LF was associated with increased $O_3$ concentration, which was robust to inclusion of $PM_{2.5}$. The associations with all HRV indices and $O_3$ concentration were stronger among those with ischemic heart disease and hypertension. In addition to the population-based studies included in the 2006 $O_3$ AQCD was a study by Schwartz et al. (2005), who conducted a panel study to assess the relationship between exposure to summertime air pollution and HRV. A weak association of $O_3$ concentration during the hour immediately preceding the health measures was observed with r-MSSD among a study population that consisted of mostly older female participants. In summary, these studies suggest that short-term exposures to ambient $O_3$ concentrations are predictors of decreased HRV and that the relationship may be stronger among certain subgroups. More recent studies that examined the association between $O_3$ concentration and HRV are described below. Study-specific characteristics and $O_3$ concentrations for these studies are presented in Table 6-31.

**Table 6-31 Characterization of $O_3$ concentrations (in ppb) from studies of heart rate variability.**

| Study* | Location | Averaging Time | Mean Concentration (Standard Deviation) | Upper Range of Concentration |
|---|---|---|---|---|
| Park et al. (2007) | Boston, MA | 24-h | Range of 17.0-29.1 | |
| Park et al. (2008) | Boston, MA | 24-h | 23.4 (13) | |
| Baja et al. (2010) | Boston, MA | 0 lag<br>10-h lag | 23 (16)<br>21 (15) | |
| Wheeler et al. (2006) | Atlanta, GA | 4-h<br>24-h | 18.5<br>29.4 | 75th: 22.5 |
| Zanobetti et al. (2010) | Boston, MA | 0.5-h<br>2-h<br>3-D<br>5-D | 20.7*<br>20.5*<br>21.9*<br>22.8* | 75th: 30.33<br>75th: 30.08<br>75th: 28.33<br>75th: 29.28 |
| Ruidavets et al. (2005a) | Toulouse, France | 8-h max | 38.3 (14.8) | 75th: 46.9<br>Max: 80.3 |
| Hampel et al. (2012) | Augsburg, Germany | 1-h avg | 23.4 (17.0) | 75th: 35.2<br>Max: 80.6 |
| Chan et al. (2005a) | Taipei, Taiwan | 1-h | 21.9 (15.4) | Max: 114.9 |
| Wu et al. (2010) | Taipei, Taiwan | Working period | 24.9 (14.0) | Max: 59.2 |
| Chuang et al. (2007a) | Taipei, Taiwan | 24-h<br>48-h<br>72-h | 28.4 (12.1)<br>33.3 (8.9)<br>33.8 (7.1) | Max: 49.3<br>Max: 47.8<br>Max: 48.3 |
| Chuang et al. (2007b) | Taipei, Taiwan | 1-h | 35.1 | Max: 192.0 |

*Note: Median presented (information on mean not given); studies presented in order of first appearance in the text of this section.

Several follow-up examinations of HRV were conducted among the participants of the Normative Aging Study in Boston, Massachusetts. A trajectory cluster analysis was used to assess whether pollution originating from different locations had varying relationships with HRV (Park et al., 2007). Subjects who were examined on days when air parcels originated in the west had the strongest associations with $O_3$; however, the $O_3$ concentration in this cluster was low (24-h avg, 17.0 ppb) compared to the other clusters (24-h avg of 21.3-29.1 ppb). LF and SDNN decreased with increases in the 4-hour moving average of $O_3$ concentration from the west (LF decreased by 51.2% [95% CI: 1.6, 75.9%] and SDNN decreased by 28.2% [95% CI: -0.5, 48.7%] per 30 ppb increase in 4-h avg $O_3$ concentrations) (Park et al., 2007). The Boston air mass originating in the west traveled over Illinois, Indiana, and Ohio; states typically characterized by coal-burning power plants. Due to the low $O_3$ concentrations observed in the west cluster, the authors hypothesize that $O_3$ concentration on those days could be capturing the effects of other, secondary and/or transported pollutants from the coal belt or that the relationship between ambient $O_3$ concentration and personal exposure to $O_3$ is stronger during that period (supported by a comparatively low apparent temperature which could indicate a likelihood to keep windows open and reduced air conditioning use) (Park et al., 2007).

An additional follow-up evaluation using the Normative Aging Study examined the potential for effect modification by chronic lead (Pb) exposure on the relationship between air pollution and HRV (Park et al., 2008). Authors observed graded reductions in HF and LF of HRV in relation to $O_3$ (and sulfate) concentrations across increasing quartiles of tibia and patella lead (HF: percent change 32.3% [95% CI: -32.5, 159.3] for the first quartile of tibia Pb and -59.1 [95% CI: -77.3, -26.1] for the fourth quartile of tibia Pb per 30 ppb increase in 4-h avg $O_3$ concentrations; LF: percent change 8.0% [95% CI: -36.9, 84.9] for the first quartile of tibia Pb and -59.3 [95% CI: -74.6, -34.8] for the fourth quartile of tibia Pb per 30 ppb increase in 4-h avg $O_3$ concentrations). In addition, associations were similar when education and cumulative traffic-adjusted bone Pb levels were used in analyses. Authors indicate the possibility that $O_3$ (which has low indoor concentrations) was acting as a proxy for sulfate (correlation coefficient for $O_3$ and sulfate = 0.57). Investigators of a more recent follow-up to the Normative Aging Study hypothesized that the relationships between short-term air pollution exposures and ventricular repolarization, as measured by changes in the heart-rate corrected QT interval (QTc), would be modified by participant characteristics (e.g., obesity, diabetes, smoking history) and genetic susceptibility to oxidative stress (Baja et al., 2010). No evidence of an association between $O_3$ concentration (using a quadratic constrained distributed lag model and hourly exposure lag models over a 10-hour time window preceding the visit) and QTc was reported (change in mean QTc -0.74 [95% CI: -3.73, 2.25]); therefore, potential effect modification of personal and genetic characteristics with $O_3$ concentration was not assessed (Baja et al., 2010). Collectively, the results from studies that examined the Normative Aging Study cohort found an association between increases in short-term $O_3$ concentration and decreases in HRV (Park et al., 2008; Park et al., 2007; Park et al., 2005b) although not consistently in all of the studies (Baja et al., 2010). Further, observed relationships appear to be stronger among those with ischemic heart disease, hypertension, and elevated bone lead levels, as well as when air masses arrive from the west (the coal belt). However, it is not clear if $O_3$ concentration is acting as a proxy for other, secondary particle pollutants (such as sulfate) (Park et al., 2008). In addition, since the Normative Aging Study participants were older, predominately white men, results may not be generalizable to the a large proportion of the U.S. population.

Additional studies of populations not limited to the Normative Aging Study have also examined associations between $O_3$ exposure and HRV. A panel study among 18 individuals with COPD and 12 individuals with recent myocardial infarction (MI) was conducted in Atlanta, Georgia (Wheeler et al., 2006). HRV was assessed for each participant on 7 days in fall 1999 and/or spring 2000. Ozone concentrations were not associated with HRV (SDNN) among all subjects (percent change of 2.36% [95% CI: -10.8%, 17.5%] per 30 ppb 4-hour $O_3$ increase) or when stratified by disease type (COPD, recent MI, and baseline $FEV_1$) (Wheeler et al., 2006).

HRV and air pollution was assessed in a panel study among 46 predominately white male patients (study population: 80.4% male, 93.5% white) aged 43-75 years in Boston, Massachusetts, with coronary artery disease (Zanobetti et al., 2010). Up to four home visits were made to assess HRV over the year following the index event.

Pollution lags used in analyses ranged between 30 minutes to a few hours and up to 5 days prior to the HRV assessments, calculated from hourly $O_3$ measurements averaged over three monitoring sites in Boston. Decreases in r-MSSD were reported for all averaging times of $O_3$ concentration (percent change of -5.18% [95% CI: -7.89, -2.30] per 20 ppb of 5-day moving average of $O_3$ concentration), but no evidence of an association between $O_3$ concentration and HF was observed (quantitative results not provided). In two-pollutant models with $O_3$ and either $PM_{2.5}$ or BC, $O_3$ associations remained robust.

A few recent studies were conducted outside of the U.S. in Europe (Hampel et al., 2012; Ruidavets et al., 2005a) and Asia (Wu et al., 2010; Chuang et al., 2007b; Chuang et al., 2007a; Chan et al., 2005a; Ruidavets et al., 2005a) that also examined the relationship between air pollution concentrations and heart rate and HRV. No consistent relationships were identified between $O_3$ concentration and resting heart rate among middle-aged (35-64 years) participants residing in Toulouse, France (Ruidavets et al., 2005a). A negative trend was reported for the 3-day cumulative (lag days 1-3) concentration of 8-h max $O_3$ with heart rate (p for trend = 0.02); however, the individual odds ratios comparing quintiles of exposure showed no association (OR for $O_3$ concentraction of 0.93 [95% CI: 0.86, 1.01] for the highest quintile of resting heart rate compared to the lowest). When stratified by current smoking status, non-smokers had a decreased trend with increased 3-day cumulative $O_3$ concentrations but none of the quintiles for heart rate were statistically significant. In a panel study conducted in Augsburg, Germany, Hampel et al. (2012) examined the effect of short-term $O_3$ exposures on measures of heart rate and repolarization in individuals with type 2 diabetes or impaired glucose tolerance and healthy individuals with a potential genetic predisposition. A ~1% increase in HR was observed for individuals with type 2 diabetes and impaired glucose tolerance at concurrent and lag 1-4 hours for an approximate 10 ppb increase in $O_3$ concentrations[1]; no effect was observed for healthy individuals. These associations remained robust in copollutants models with sulfate, PM, and ultrafine particles. Additionally, there was evidence of T-wave flattening across all lags in healthy individuals and those with type 2 diabetes and impaired glucose intolerance, with the effect strongest in these individuals at concurrent (-1.31% [95% CI: -2.19, -0.42]) and lag 1 hour (-1.32% [95% CI: -2.19, 0.45]). Similarly, there was evidence of an increase in T-wave complexity for all participants across all lags examined, with the strongest effects again for individuals with type 2 diabetes and impaired glucose tolerance, but at lags 1 and 2 hours. An increase in T wave complexity for healthy participants at lags of 3 and 4 hours. Hampel et al. (2012) also found evidence of effect modification for each of the heart rate and repolarization metrics when taking into consideration the location and season in which the ECG recordings were obtained, with greater effects occurring when measurements were taken outdoors during the summer.

[1] These results were not standardized to a 1-h max $O_3$ concentration of 40 ppb because the study examined hourly changes in heart rate parameters. Using an increment of 40 ppb would not appropriately represent the potential hourly change in $O_3$ concentrations.

In a study conducted in Taipei, Taiwan no associations were reported between $O_3$ concentration and HRV among CHD patients and patients with one or more major CHD risk factors (Chan et al., 2005a). Another study in Taipei, Taiwan examined mail carriers and reported $O_3$ concentration measured using personal monitors. No association was observed between $O_3$ concentration and the measures of HRV (percent change for SDNN: 0.57 [95% CI: -21.27, 28.46], r-MSSD: -7.10 [95% CI: -24.24, 13.92], HF: -1.92 [95% CI: -23.68, 26.02], LF: -4.82 [95% CI: -25.34, 21.35] per 40 ppb $O_3$) (Wu et al., 2010). A panel study was conducted in Taiwan to assess the relationship between air pollutants and inflammation, oxidative stress, blood coagulation, and autonomic dysfunction (Chuang et al., 2007b; Chuang et al., 2007a). Participants were apparently healthy college students (aged 18-25 year) who were living in a university dormitory in metropolitan Taipei. Health endpoints were measured three times from April to June in 2004 or 2005. Ozone concentration was assessed in statistical models using the average of the 24, 48, and 72 hours before the hour of each blood sampling. Decreases in HRV (measured as SDNN, r-MSSD, LF, and HF) were associated with increases in $O_3$ concentrations in single-pollutant models (percent change for SDNN: -13.45 [95% CI: -16.26, -10.60], r-MSSD -13.76 [95% CI: -21.62, -5.44], LF -9.16 [95% CI: -13.29, -4.95], HF -10.76 [95% CI: -18.88, -2.32] per 20 ppb cumulative 3-day avg $O_3$ concentrations) and remained associated with 3-day $O_3$ concentrations in two-pollutant models with sulfate. Another study in Taiwan recruited individuals with CHD or at risk for cardiovascular disease from outpatient clinics during the study period (two weeks in February) (Chuang et al., 2007b). No association was observed between $O_3$ concentration and HRV measures (SDNN, r-MSSD, LF, HF) (numerical results not provided in publication).

Overall, studies of $O_3$ concentration and HRV report inconsistent results. Multiple studies conducted in Boston, MA, observed positive associations but the authors of many of these studies postulated that $O_3$ concentration was possibly acting as a proxy for other pollutants. The majority of other studies, both in the U.S. and internationally, report null findings. The inconsistencies observed are further complicated by the different HRV measures and averaging times used by the studies.

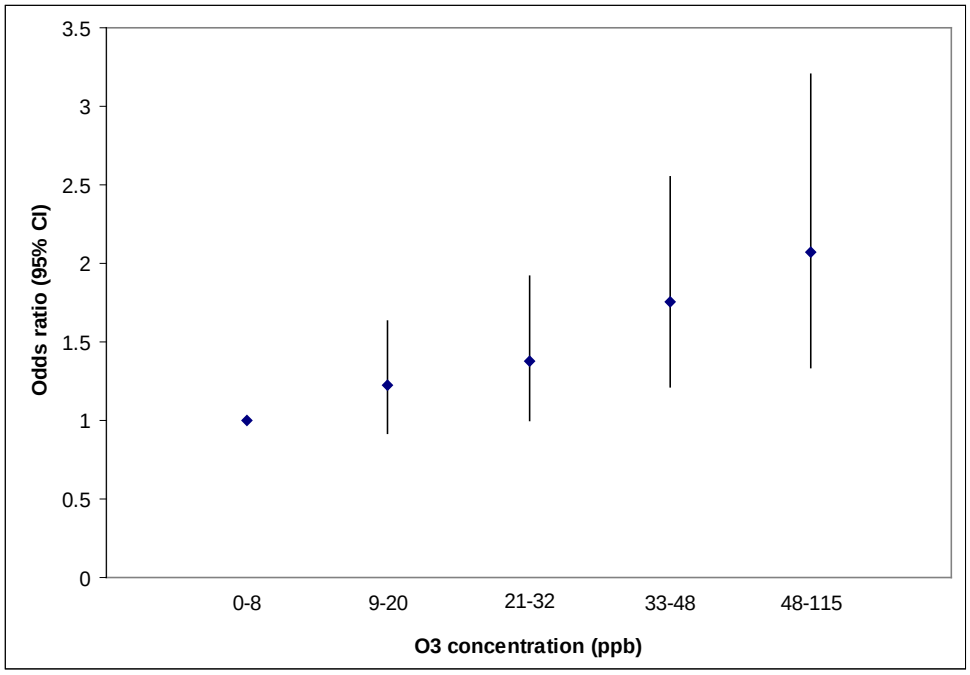
#### 6.3.2.3 Stroke

The 2006 $O_3$ AQCD did not identify any studies that examined the association between short-term $O_3$ exposure and stroke. However, recent studies have attempted to examine this relationship. Lisabeth et al. (2008) used a time-series approach to assess the relationship between daily counts of ischemic stroke and transient ischemic attack (TIA) with $O_3$ concentrations in a southeast Texas community among residents 45 years and older (2001-2005; median age of cases, 72 years). The median $O_3$ concentration (hourly average per 24-hour time-period) was 25.6 ppb (IQR 18.1-33.8). The associations between same-day $O_3$ concentrations and stroke/TIA risk were positive (RR: 1.03 [95% CI: 0.96, 1.10] per 20 ppb increase in 24-h avg $O_3$ concentrations) and previous-day (RR: 1.05 [95% CI: 0.99, 1.12] per

20 ppb increase in 24-h avg $O_3$ concentrations). Associations were robust to adjustment for $PM_{2.5}$.

A case-crossover design was used in a study conducted in Dijon, France between March 1994 and December 2004, among those 40 years of age and older who presented with first-ever stroke (Henrotin et al., 2007). The mean $O_3$ concentration, calculated over 8-hour daytime periods, was 14.95 ppb (IQR: 6-22 ppb). No association was observed between $O_3$ concentration at any of the single-day lags examined (i.e., 0-3 days) and hemorrhagic stroke. However, an association between ischemic stroke occurrence and $O_3$ concentrations with a 1-day lag was observed (OR: 1.54 [95% CI: 1.14, 2.09] per 30 ppb increase in 8-h max $O_3$ concentrations). The observed association between short-term $O_3$ exposure and ischemic stroke persisted in two-pollutant models with $PM_{10}$, $SO_2$, $NO_2$, or CO. This association was stronger among men (OR: 2.12 [95% CI: 1.36, 3.30] per 30 ppb increase in 8-h max $O_3$ concentrations) than among women (OR: 1.17 [95% CI: 0.77, 1.78] per 30 ppb increase in 8-h max $O_3$ concentrations) in single pollutant models. When stroke was examined by subtype among men, an association was observed for ischemic strokes of large arteries and for transient ischemic attacks, but not for cardioembolic or lacunar ischemic strokes. The subtype analysis was not performed for women. Additionally, for men a linear exposure-response was observed when $O_3$ concentration was assessed based on quintiles (p for trend = 0.01) (Figure 6-21). A potential limitation of this study is that 67.4% of the participating men were smokers compared to 9.3% of the women.

Another case-crossover study performed in Dijon, France examined the association between $O_3$ concentration and incidence of fatal and non-fatal ischemic cerebrovascular events (ICVE) (Henrotin et al., 2010). Mean 8-hour $O_3$ concentration was 19.1 ppb (SD 12.2 ppb). A positive association was observed between recurrent ICVE and 8-hour $O_3$ concentration with a 3-day lag (OR: 1.92 [95% CI 1.17, 3.12]), but not for other lags (0, 1, 2, 4) or cumulative days (0-1, 0-2, 1-2, 1-3). Although some ORs for incident ICVEs were elevated, none were statistically significant. Results for associations using the maximum daily 1-hour $O_3$ concentrations were similar to the 8-hour results but slightly attenuated. ORs were similar in two pollutant models with $SO_2$, $NO_2$, CO, and $PM_{10}$ (data not given). In stratified analyses, the association between 1-day lagged $O_3$ concentration and incident and recurrent ICVE was greater among individuals with diabetes or individuals with multiple pre-existing vascular conditions.




Source: Henrotin et al. (2007).

**Figure 6-21 Odds ratio (95% confidence interval) for ischemic stroke by quintiles of $O_3$ exposure.**

### 6.3.2.4 Biomarkers

An increasing number of studies have examined the relationship between air pollution and biomarkers in an attempt to elucidate the biological mechanisms linking air pollution and cardiovascular disease. A wide range of markers assessed as well as different types of study designs and locations chosen make comparisons across studies difficult. Table 6-32 provides an overview of the $O_3$ concentrations reported in each of the studies evaluated.

**Table 6-32 Characterization of $O_3$ concentrations (in ppb) from studies of biomarkers.**

| Study* | Location | Averaging Time | Mean Concentration (Standard Deviation) | Upper Range of Concentration |
|---|---|---|---|---|
| Liao et al. (2005) | 3 U.S. counties | 8-h | 40 (20) | |
| Thompson et al. (2010) | Toronto, Ontario | 1-h / 1 yr | 21.94 (15.78) | |
| Rudez et al. (2009) | Rotterdam, the Netherlands | 24-h | 22* | 75th: 31.5<br>Max: 90 |
| Chuang et al. (2007a) | Taipei, Taiwan | 24-h | 28.4 (12.1) | Max: 49.3 |
| | | 48-h | 33.3 (8.9) | Max: 47.8 |
| | | 72-h | 33.8 (7.1) | Max: 48.3 |
| Steinvil et al. (2008) | Tel-Aviv, Israel | 0.5-h | 29.2 (9.7) | 75th: 36 |
| Chen et al. (2007a) | Los Angeles and San Francisco, CA | 8-h / 2 weeks | 30.8* | Max: 47.9 |
| | | 8-h / 1 mo | 28.3* | Max: 43.1 |
| Wellenius et al. (2007) | Boston, MA | 1-h / 24-h | 25.1 (12.9) | |
| Goldberg et al. (2008) | Montreal, Quebec | 24-h | NS | |
| Baccarelli et al. (2007) | Lombardia, Italy | 1-h | 18.3* | 75th: 35.1<br>Max: 202.3 |
| Chuang et al. (2010) | Taiwan | | 26.83 (9.7) | Max: 62.1 |

*Note: Median presented (information on mean not given); studies presented in order of first appearance in the text of this section.

### Hemostasis and coagulation markers

Multiple studies used various markers to examine if associations were present between short-term $O_3$ exposure and hemostasis and coagulation. Some of the markers included in these studies were as follows: fibrinogen, von Willebrand factor (vWF), plasminogen activator fibrinogen inhibitor-1 (PAI-1), tissue-type plasminogen activator (tPA), platelet aggregation, and thrombin generation.

A population-based study in the United States was conducted to assess the relationship between short-term exposure to air pollution and markers of blood coagulation using the Atherosclerosis Risk in Communities (ARIC) study cohort (Liao et al., 2005). Significant curvilinear associations were observed for $O_3$ (1 day prior to blood draw) and fibrinogen and vWF (quantitative results not provided for regression models although adjusted means [SE] of vWF were given as 118% [0.79%] for $O_3$ concentrations <40 ppb, 117% [0.86%] for $O_3$ concentrations 40-70 ppb, and 124% [1.97%] for $O_3$ concentrations of 70 ppb). The association between short-term $O_3$ exposure and fibrinogen was more pronounced among those with a history of cardiovascular disease (CVD) and was statistically significant

among only this subgroup of the population. The curvilinear relationship between concentration and outcome suggested stronger relationships at higher concentrations of $O_3$. The authors note that the most pronounced associations occurred when the pollutant concentrations were 2-3 standard deviations above the mean. The results from this relatively large-scale cross-sectional study suggest weak associations with between short-term $O_3$ exposure and increases in fibrinogen (among those with a history of CVD) and vWF. A retrospective repeated measures analysis was performed in Toronto, Canada among adults aged 18-40 years (n = 45) between the years of 1999 and 2006 (Thompson et al., 2010). Single pollutant models were used with moving averages up to 7 days. No evidence of an association was observed between short-term $O_3$ exposure and increases in fibrinogen.

A repeated measures study was conducted among 40 healthy individuals living or working in the city center of Rotterdam, the Netherlands to assess the relationship between air pollution and markers of hemostatis and coagulation (platelet aggregation, thrombin generation, and fibrinogen) (Rudez et al., 2009). Each participant provided between 11 and 13 blood samples throughout a 1-year period (498 samples on 197 days). Examined lags ranged from 6 hours to 3 days prior to blood sampling. No consistent evidence of an association was observed between $O_3$ concentration and any of the biomarkers (percent change of max platelet aggregation: -6.87 [95% CI: -21.46, 7.70] per 20 ppb increase in 24-h avg $O_3$ concentration at 4-day average; percent change of endogenous thrombin potential: 0.95 [95% CI: -3.05, 4.95] per 20 ppb increase in 24-h avg $O_3$ concentration at 4-day avg; percent change of fibrinogen: -0.57 [95% CI: -3.05, 2.00] per 20 ppb increase in 24-h avg $O_3$ concentration at lag 1-day). Some associations with $O_3$ were in the opposite direction to that hypothesized which may be explained by the negative correlation between $O_3$ and other pollutants (correlation coefficients ranged from -0.4 to -0.6).
The statistically significant inverse effects observed in single-pollutant models with $O_3$ were no longer apparent when $PM_{10}$ was included in the model (Rudez et al., 2009).

A panel study in Taiwan measured health endpoints using blood samples from healthy individuals (n = 76) at three times from April to June in 2004 or 2005 (Chuang et al., 2007a). Increases in fibrinogen and PAI-1 were associated with increases in $O_3$ concentrations in single-pollutant models (percent change in fibrinogen: 11.76 [95% CI: 4.03, 19.71] per 20 ppb 3-day cumulative avg $O_3$ concentration; percent change in PAI-1: 6.08 [95% CI: 38.91, 84.27] per 20 ppb 3-day cumulative avg $O_3$ concentration). These associations were also observed at 1 and 2 day averaging times. Associations between PAI-1 and 3-day $O_3$ concentrations remained robust in two-pollutant models with sulfate. No association was observed between $O_3$ concentration and tPA, a fibrinolytic factor (percent change 16.15 [95% CI: -4.62, 38.34] per 20 ppb 3-day avg $O_3$ concentration).

A study in Israel examined the association between pollutant concentrations and fibrinogen among 3,659 apparently healthy individuals (Steinvil et al., 2008).
In single pollutant models, $O_3$ was associated with an increase in fibrinogen at a 4-day lag among men and a same-day $O_3$ concentration among women but results for

other lags (0 through 7 days) were mixed (i.e., some positive and some negative; none statistically significant).

### Inflammatory markers

Potential associations between short-term exposures to air pollution and inflammatory markers (C-reactive protein [CRP], white blood cell [WBC] count, albumin, and Interleukin-6 [IL-6]) were also examined in several studies.

The ARIC study cohort, which included men and women aged 45-64 years old at the start of the study, was utilized to assess the association between $O_3$ concentrations and markers of inflammation, albumin and WBC count (Liao et al., 2005). No association was observed between $O_3$ concentrations and albumin or WBC count.

Thompson et al. (2010) assessed ambient air pollution exposures and IL-6. This retrospective repeated measures analysis was conducted among 45 adults (18-40 years of age) in Toronto, Canada between the years of 1999 and 2006. Single pollutant models were used to analyze the repeated-measures data using moving averages up to 7 days. A positive association was observed between IL-6 and short-term 1-hour $O_3$ exposure with the strongest effects observed for the average of lags 0-3 days (quantitative results not provided). No association was observed for shorter averaging times (average lags of <1 day). When examined by season using 2-day moving averages, the association between short-term $O_3$ exposure and IL-6 was positive during only the spring and summer.

In Rotterdam, the Netherlands, a repeated measures study of healthy individuals living or working in the city center reported no association between short-term $O_3$ exposure and CRP (Rudez et al., 2009). Each of the 40 participants provided between 11 and 13 blood samples throughout a 1-year period (498 samples on 197 days). No consistent evidence of an association was observed between $O_3$ concentration and CRP (percent change: -0.48 [95% CI: -14.05, 13.10] per 20 ppb increase in 24-h avg $O_3$ concentration at lag 1-day). Additionally, no association was observed with 2 or 3 day lags.

The relationship between pollutant concentrations and one-time measures of inflammatory biomarkers was assessed in sex-stratified analyses among 3,659 apparently healthy individuals in Tel Aviv, Israel (Steinvil et al., 2008). No evidence of an association was observed between $O_3$ concentration and CRP or WBC for men and women.

A panel study of healthy individuals (n = 76) was conducted in Taiwan to assess the relationship between air pollutants and inflammation (Chuang et al., 2007a). Health endpoints were measured three times from April to June in 2004 or 2005. Ozone effects were assessed in statistical models using the average of the 24 hours (1 day), 48 hours (2 days), and 72 hours (3 days) before the hour of each blood sampling. Increases in CRP were associated with increases in $O_3$ concentrations in single-pollutant models (percent change in CRP: 244.38 [95% CI: 4.54, 585.15] per 20 ppb

3-day avg $O_3$ concentration). The association was also observed using a 2-day cumulative averaging time, but no association was present with a 1-day averaging time.

### Oxidative stress markers

A few studies have reported on the relationships between short-term $O_3$ exposure and increases in markers of oxidative stress. The association between $O_3$ concentration and markers of lipid peroxidation and antioxidant capacity was examined among 120 nonsmoking healthy college students, aged 18-22 years, from the University of California, Berkeley (February-June 2002) (Chen et al., 2007a). By design, students were chosen that had experienced different geographic concentrations of $O_3$ over their lifetimes and during recent summer vacation in either greater Los Angeles (LA) or the San Francisco Bay Area (SF). Long-term (based on lifetime residential history) and shorter-term (based on the moving averages of 8-h max concentrations 1-30 days prior to the day of blood collection) $O_3$ concentration were estimated (lifetime exposure results are presented in Chapter 7). A marker of lipid peroxidation, 8-isoprostane (8-iso-PGF), was assessed. This marker is formed continuously under normal physiological conditions but has been found at elevated concentrations in response to environmental exposures. A marker of overall antioxidant capacity, ferric reducing ability of plasma (FRAP), was also measured. Levels of 8-iso-PGF were associated with 2-week ($\beta$ = 0.035 [pg/mL]/8-hour ppb $O_3$, p = 0.007) and 1-month ($\beta$ = 0.031 [pg/mL]/8-hour ppb $O_3$, p = 0.006) estimated $O_3$ concentrations.
No evidence of association was observed between short-term $O_3$ exposure and increases in FRAP. A chamber study performed among a subset of study participants supported the primary study results. The concentrations of 8-iso-PGF increased immediately after the 4-hour controlled $O_3$ exposure ended (p = 0.10). However, levels returned to near baseline by 18 hours without further exposure. The authors note that $O_3$ was highly correlated with $PM_{10-2.5}$ and $NO_2$ in this study population; however, $O_3$ associations remained robust in copollutant models.

Using blood samples collected between April and June of 2004 or 2005 in Taiwan, the association between short-term $O_3$ exposure and a marker of oxidative stress (i.e., 8-hydroxy-2'-deoxyguanosine (8-OHdG)) was studied among healthy individuals (n = 76) (Chuang et al., 2007a). Increases in 8-OHdG were associated with increases in $O_3$ concentrations in single-pollutant models (percent change in 8-OHdG: 2.46 [95% CI: 1.01, 3.92] per 20 ppb increase in 24-h avg $O_3$).
The association did not persist with 2- or 3-day cumulative averaging times.

### Markers of overall cardiovascular health

Multiple studies used markers that assess overall cardiovascular well-being. Wellenius et al. (2007) examined B-type natriuretic peptide (BNP), a marker of heart failure, in a repeated-measures study conducted in Boston, MA, among 28 patients with congestive heart failure and impaired systolic function. The authors found no

evidence of an association between BNP and short-term $O_3$ exposures at lags 0-3 days (quantitative results not provided). BNP was chosen because it is directly associated with cardiac hemodynamics and symptom severity among those with heart failure and is considered a marker of functional status. However, the authors conclude that the use of BNP may not be useful in studies of the health effects of ambient air pollutants due to the large amount of within-person variability in BNP levels observed in this population.

The relationship between air pollution and oxygen saturation and pulse rate, markers of physiological well-being, was examined in a 2-month panel study among 31 congestive heart failure patients (aged 50-85 years) in Montreal, Canada from July 2002 to October 2003 (Goldberg et al., 2008). All participants had limited physical functioning (New York Heart Association Classification ≥ II) and an ejection fraction (the fraction of blood pumped out of the heart per beat) less than or equal to 35% (normal is above 55%). Daily mean $O_3$ concentrations were calculated based on hourly measures at 10 monitoring stations. There was an inverse association between $O_3$ concentration (lag-0) and oxygen saturation when adjustment was made for temporal trends. In the models incorporating personal covariates and weather factors, the association remained but was not statistically significant. The associations of $O_3$ concentration with a lag of 1 day or a 3-day mean were not statistically significant. No evidence of association was observed between $O_3$ concentration and pulse rate.

Total homocysteine (tHcy) is an independent risk factor for vascular disease and measurement of this marker after oral methionine load is used to identify individuals with mild impairment of homocysteine metabolism. The effects of air pollution on fasting and postmethionine-load tHcy levels were assessed among 1,213 apparently healthy individuals from Lombardia, Italy from January 1995 to September 2005 (Baccarelli et al., 2007). A 20-ppb increase in the 24-h avg $O_3$ concentrations was associated with an increase in fasting tHcy (percent change 6.25 [95% CI: 0.84, 11.91]) but no association was observed with postmethionine-load tHcy (percent change 3.36 [95% CI: -1.30, 8.39]). In addition, no evidence of an association was observed between 7-day cumulative averaged $O_3$ concentrations and tHcy (percent change for fasting tHcy 4.16 [95% CI: -1.76, 10.42] and percent change for postmethionine-load tHcy -0.65 [95% CI: -5.66, 4.71] per 20 ppb increase in 24-h avg $O_3$ concentrations). No evidence of effect modification by smoking was observed.

### Blood lipids and glucose metabolism markers

Chuang et al. (2010) conducted a population-based cross-sectional analysis of data collected on 7,778 participants during the Taiwanese Survey on Prevalence of Hyperglycemia, Hyperlipidemia, and Hypertension in 2001. Apolipoprotein B (ApoB), the primary apolipoprotein among low-density lipoproteins, was associated with 3-day avg $O_3$ concentration at the $p < 0.10$ level. The 5-day mean $O_3$ concentration was associated with an increase in triglycerides at $p < 0.10$. In addition, the 1-, 3-, and 5-day mean $O_3$ concentrations were associated with increased HbA1c

levels (a marker used to monitor the degree of control of glucose metabolism) at the $p < 0.05$ level. The 5-day mean $O_3$ concentration was associated with increased fasting glucose levels ($p < 0.10$). No association was observed between $O_3$ concentration and ApoA1.

### 6.3.2.5 Myocardial Infarction (MI)

The 2006 $O_3$ AQCD did not report consistent results indicating an association between short-term $O_3$ exposure and MI. One study reported a positive association between current day $O_3$ concentration and acute MI, especially among the oldest age group (55 to 64 year-olds) (Ruidavets et al., 2005b). No association was observed in a case-crossover study of $O_3$ concentration during the surrounding hours and MI (Peters et al., 2001). Since the 2006 $O_3$ AQCD, a few recent epidemiologic studies have examined the association between $O_3$ concentration and MI (Henrotin et al., 2010; Rich et al., 2010), arterial stiffness (Wu et al., 2010) and ST-segment depression (Delfino et al., 2011).

One of the studies conducted in the U.S. examined hospital admissions for first MI and reported no association with $O_3$ concentration (Rich et al., 2010). More details on this study are reported in the section on hospital admissions (Section 6.3.2.7).
A study performed in Dijon, France examined the association between $O_3$ concentration and incident and recurrent MI (Henrotin et al., 2010). The mean 8-hour $O_3$ concentration was 19.1 ppb (SD 12.2 ppb). Odds ratios for the association between cumulative $O_3$ concentrations and recurrent MIs were elevated but none of the results were statistically significant (OR: 1.71 [95% CI: 0.91, 3.20] per 20 ppb increase in 24-h avg $O_3$ concentration for a cumulative lag of 1-3 days).
No association was observed for incident MIs. In analyses stratified by vascular risk factors, positive associations were observed between 1-day lagged $O_3$ concentration and MIs (incident and recurrent combined) among those who reported having hypercholesterolaemia (OR: 1.52 [95% CI: 1.08, 2.15] per 20 ppb increase in 24-h avg $O_3$ concentration) and a slight inverse association was observed among those who reported not having hypercholesterolaemia (OR: 0.69 [95% CI: 0.50, 0.94] per 20 ppb increase in 24-h avg $O_3$ concentration). In other stratified analyses combining different vascular factors, only those containing individuals with hypercholesterolaemia demonstrated a positive association; none were inverse associations.

Wu et al. (2010) examined mail carriers aged 25-46 years and measured exposure to $O_3$ concentrations through personal monitors [mean $O_3$ 24.9 (SD 14.0) ppb]. Ozone concentration was positively associated with arterial stiffness (percent change 11.24% [95% CI: 3.67, 19.62] per 40 ppb $O_3$) and was robust to adjustment for ultrafine PM.

A study performed in the Los Angeles basin reported on the association between $O_3$ concentration and ST-segment depression, a measure representing cardiac ischemia (Delfino et al., 2011). Study participants were nonsmokers, at least 65 years old, had

a history of coronary artery disease, and were living in a retirement community. Study periods included five consecutive days in both July to mid-October and mid-October to February. Mean 24-hour $O_3$ concentrations were 27.1 ppb (SD 11.5 ppb). No association was observed between $O_3$ concentration and ST-segment depression of at least 1.0 mm during any of the exposure periods (i.e., 1-hour, 8-hours, 1-day, 2-day avg, 3-day avg, 4-day avg).

#### 6.3.2.6 Blood Pressure

In the 2006 $O_3$ AQCD, no epidemiologic studies examined $O_3$-related effects on blood pressure (BP). Recent studies have been conducted to evaluate this relationship and overall the findings are inconsistent. The $O_3$ concentrations for these studies are listed in Table 6-33.

**Table 6-33 Characterization of $O_3$ concentrations (in ppb) from studies of blood pressure.**

| Study* | Location | Averaging Time | Mean Concentration (Standard Deviation) | Upper Range of Concentration |
|---|---|---|---|---|
| Zanobetti et al. (2004) | Boston, Massachusetts | 1-h | 20 | |
| | | 5-days | 24 | |
| Delfino et al. (2010b) | Los Angeles, California | 24-h | 27.1 (11.5) | Max: 60.7 |
| Choi et al. (2007) | Incheon, South Korea | 8-h (warm season) | 26.6 (11.8) | 75th: 34.8<br>Max: 62.4 |
| | | 8-h (cold season) | 17.5 (7.3) | 75th: 22.9<br>Max: 33.9 |
| Chuang et al. (2010) | Taiwan | | 26.83 (9.7) | Max: 62.1 |

*Note: Studies presented in order of first appearance in the text of this section.

Zanobetti et al. (2004) examined the relationship between air pollutants and BP from May 1999 to January 2001 for 631 repeat visits among 62 Boston, MA, residents with CVD. In single-pollutant models, higher resting diastolic blood pressure (DBP) was associated with the 5-day (0-4 days) averages of $O_3$ concentration (RR: 1.03 [95% CI: 1.00, 1.05] per 20 ppb increase in 24-hour $O_3$ concentrations). However, this effect was no longer apparent when $PM_{2.5}$ was included in the model (data were not presented) (Zanobetti et al., 2004). Delfino et al. (2010b) examined 64 subjects 65 years and older with coronary artery disease, no tobacco smoke exposure, and living in retirement communities in the Los Angeles air basin with hourly (up to 14-hours/day) ambulatory BP monitoring for 5 days during a warm period (July-mid-October) and 5 days during a cool period (mid-October-February). Investigators assessed lags of 1, 4, and 8 hours, 1 day, and up to 9 days before each BP measure; no evidence of an association was observed for $O_3$ (change in BP associated with a

20 ppb increase in 24-h avg $O_3$ concentration was 0.67 [95% CI: -1.16, 2.51 for systolic BP [SBP] and -0.25 [95% CI: -1.25, 0.75] for DBP) (Delfino et al., 2010b). Choi et al. (2007) conducted a cross-sectional study to investigate the relationship between air pollutants and BP among 10,459 participants of the Inha University Hospital health examination from 2001 to 2003. These individuals had no medical history of cardiovascular disease or hypertension. Ozone concentration was associated with an increase in SBP for 1-day lag in the warm season and similar effect estimates were observed during the cold season but were not statistically significant (quantitative results not provided). Associations between $O_3$ concentration and DBP were present in the cold season but not the warm season (quantitative results not provided). Chuang et al. (2010) conducted a similar type of study among 7,578 participants of the Taiwanese Survey on Prevalence of Hyperglycemia, Hyperlipidemia, and Hypertension in 2001. Investigators examined 1-, 3-, and 5-day avg $O_3$ concentrations. An increase in DBP was associated with the 3-day mean $O_3$ concentration (change in BP for a 20 ppb increase in 24-h avg $O_3$ concentration was 0.61 [95% CI: 0.07, 1.14]) (Chuang et al., 2010). Associations were not observed for other days or with SBP.

#### 6.3.2.7 Hospital Admissions and Emergency Department Visits

Upon evaluating the collective evidence for $O_3$-related cardiovascular hospital admissions and emergency department (ED) visits, the 2006 $O_3$ AQCD concluded that "a few studies observed positive $O_3$ associations, largely in the warm season. Overall, however, the currently available evidence is inconclusive regarding any association between ambient $O_3$ exposure on cardiovascular hospitalizations" (U.S. EPA, 2006b). Table 6-34 provides information on the $O_3$ concentrations reported in each of the recent hospital admission and ED visit studies evaluated.

## Table 6-34 Characterization of $O_3$ concentrations (in ppb) from studies of hospital admissions and ED visits.

| Study[a] | Location | Averaging Time | Mean Concentration (Standard Deviation) | Upper Range of Concentration |
|---|---|---|---|---|
| Peel et al. (2007) | Atlanta, GA | 8-h max warm season | 55.6 (23.8) | |
| Tolbert et al. (2007) | Atlanta, GA | 8-h max warm season | 53.0 | 75th: 67.0<br>Max: 147.5 |
| Katsouyanni et al. (2009) | 12 Canadian cities | 1-h | 6.7-8.3* | 75th: 8.4-12.4 |
| | 8 European cities | 1-h | 11.0-38.1* | 75th: 15.3-49.4 |
| | 14 United Statescities | 1-h | 34.9-60.0* | 75th: 46.8-68.8 |
| Rich et al. (2010) | New Jersey | 24-h | NR | |
| Cakmak et al. (2006a) | 10 Canadian cities | 1-h max | 17.4 | |
| Stieb et al. (2009) | 7 Canadian cities | 24-h | 18.4 | |
| Szyszkowicz (2008) | Edmonton, Canada | 24-h | 18.6 (9.3) | |
| Villeneuve et al. (2006a) | Edmonton, Canada | 24-h | 17 (9.1) | 75th: 23.5 |
| | | 24-h warm season | 21.8 (8) | 75th: 27.0 |
| | | 24-h cold season | 12.2 (7.4) | 75th: 17.0 |
| Symons et al. (2006) | Baltimore, MD | 8-h warm season | 31.0 (20.0) | Max: 120.0 |
| Wellenius et al. (2005) | Allegheny County, PA | 24-h | 24.3 (12.2) | 75th: 32.0 |
| Zanobetti and Schwartz (2006) | Boston, MA | 24-h | 22.4* | 75th: 31.0 |
| Yang (2008) | Taipei, Taiwan | 24-h | 21.0 | 75th: 26.3<br>Max: 62.8 |
| Lee et al. (2007) | Kaohsiung, Taiwan | 24-h | 26.5 | 75th: 35.5<br>Max: 83.0 |
| Chan et al. (2006) | Taipei, Taiwan | 1-h max | 50.9 (26.4) | Max: 150.3 |
| Chiu and Yang (2009) | Taipei, Taiwan | 24-h | 23.0 | 75th: 28.7<br>Max: 62.8 |
| Lee et al. (2008a) | Taipei, Taiwan | 24-h | 21.0 | 75th: 26.4<br>Max: 62.8 |
| Wong et al. (2009) | Hong Kong | 8-h | 18.5 (11.5) | 75th: 25.4<br>Max: 48.3 |
| Bell et al. (2008) | Taipei, Taiwan | 24-h | 21.4 | Max: 53.4 |
| Buadong et al. (2009) | Bangkok, Thailand | 1-h | 14.4 (3.2) | Max: 41.9 |
| Lee et al. (2003b) | Seoul, Korea | 1-h max | 36.0 (18.6) | 75th: 44.9 |
| Azevedo et al. (2011) | Portugal | 1-h | NR | |
| Linares and Diaz (2010) | Madrid,Spain | 24-h | 17.4 (8.9) | |
| Middleton et al. (2008) | Nicosia, Cyprus | 8-h max | 28.7 - 54.9 | |

| Study[a] | Location | Averaging Time | Mean Concentration (Standard Deviation) | Upper Range of Concentration |
|---|---|---|---|---|
| Turner et al. (2007) | Sydney, Australia | 24-h | 28 | 75th: 33 |
| Ballester et al. (2006) | 14 Spanish cities | 8-h<br>warm season | 24.2 - 44.3 | |
| De Pablo et al. (2006) | Castilla-Leon, Spain | 24-h | 23.2-33.6 | |
| Von Klot et al. (2005) | 5 European cities | 8 h max<br>warm season | 16.4 - 28.0 | |
| Oudin et al. (2010) | Scania, Sweden | 24-h | 30.5 | |
| Halonen et al. (2009) | Helsinki, Finland | 8-h max<br>warm season | 35.7* | 75th: 42.1<br>Max: 79.6 |
| Larrieu et al. (2007) | 8 French cities | 8-h max<br>warm season | 34.2 - 53.1 | |
| Barnett et al. (2006) | 4 Australian cities | 8-h | 19.0-28.5 | Max: 58.4-86.8 |
| Hinwood et al. (2006) | Perth, Australia | 8-h max | 25.9 (6.5) | |
| Lanki et al. (2006) | 5 European cities | 8-h max<br>warm season | 31.7 - 57.2* | |
| Hosseinpoor et al. (2005) | Tehran, Iran | 8-h max | 4.9 (4.8) | 75th: 7.2<br>Max: 99.0 |
| Simpson et al. (2005) | 4 Australian cities | 1-h max | 24.4-33.8 | Max: 96.0-111.5 |
| Dennekamp et al. (2010) | Melbourne, Australia | 24-h | 13.34 | 75th: 16.93 |
| Silverman et al. (2010) | New York City, NY | 8-h max | 28* | 75th: 40 |

[a]Notes: Median presented (information on mean not given); NR: Not reported; Studies presented in order of first appearance in the text of this section.

Multiple recent studies of $O_3$ concentration and cardiovascular hospital admissions and ED visits have been conducted in the U.S. and Canada. Peel et al. (2007) used a case-crossover framework (using a time-stratified approach matching on day of the week in the calendar month of the event) to assess the relationship between air pollutants and cardiovascular disease ED visits among those with and without secondary comorbid conditions (hypertension, diabetes, chronic obstructive pulmonary disease [COPD], congestive heart failure [CHF], and dysrhythmia). Data on over 4 million ED visits from 31 hospitals were collected from January 1993 to August 2000. Ozone was monitored from March to October. This study was a re-analysis of a time series study conducted to assess the main effects of air pollutants on cardiovascular ED visits in Atlanta, GA (Tolbert et al., 2007; Metzger et al., 2004). In the initial study, no evidence of associations was observed between $O_3$ concentration and all CVD visits or visits for CVD subgroups, such as dysrhythmia, CHF, ischemic heart disease (IHD), and peripheral vascular and cerebrovascular disease. The relative risk for all CVD visits was 1.01 (95% CI: 0.98, 1.04) for a 30 ppb increase in the 3-day moving avg (lags 0-2 days) of 8-hour $O_3$ concentration (Metzger et al., 2004). Similar to the initial investigation using a time-series analysis, no evidence of an association was observed between short-term $O_3$ exposure and CVD visits at lag 0-2 among the entire population using the case-crossover design (Peel et al., 2007). However, the relationship between $O_3$ concentration and

peripheral and cerebrovascular disease visits was stronger among patients with comorbid COPD (OR: 1.29 [95% CI: 1.05-1.59] per 30 ppb, lag 0-2 days) as compared to patients without COPD (OR: 1.01 [95% CI: 0.96-1.06] per 30 ppb, lag 0-2 days). The same research group expanded upon the number of Atlanta hospitals providing ED visit data (41 hospitals) as well as the length of the study period (1993-2004) (Tolbert et al., 2007). Again, models assessing the health effects of $O_3$ concentration utilized data collected from March through October. Similar to the results presented by Metzger et al. (2004) and Peel et al. (2007) among the entire study population, no evidence of associations was observed for $O_3$ concentration and CVD visits (Tolbert et al., 2007).

Existing multicity studies in North America and Europe were evaluated under a common framework in the Air Pollution and Health: A European and North American Approach (APHENA) study (Katsouyanni et al., 2009). One component of the study examined the relationship between short-term $O_3$ exposure and CVD hospital admissions among individuals 65 years of age and older. The study presented multiple models but this section focuses on the results for the models that used 8 df to account for temporal trends and natural splines (see Section 6.2.7.2 for additional explanation). Across the study locations, no associations were observed between $O_3$ concentration and CVD hospital admissions at lags 0-1, lag 1, or a distributed lag of 0-2. Additionally, there was no evidence of an association when restricting the analysis to the summer months.

A study of hospital admissions for MI was performed using a statewide registry from New Jersey between January 2004 and December 2006 (Rich et al., 2010). Using a case-crossover design, the association between the previous 24-hours $O_3$ concentration and transmural infarction (n = 1,003) was examined. No association was observed (OR: 0.94 [95% CI: 0.79, 1.13] per 20 ppb increase in 24-h avg $O_3$ concentration) and this did not change with the inclusion of $PM_{2.5}$ in the model.

Cakmak et al. (2006a) investigated the relationship between gaseous air pollutants and cardiac hospitalizations in 10 large Canadian cities using a time-series approach. A total of 316,234 hospital discharge records for primary diagnosis of congestive heart failure, ischemic heart disease, or dysrhythmia were obtained from April 1993 through March 2000. Correlations between pollutants varied substantially across cities, which could partially explain discrepancies in effect estimates observed across the cities. In addition, pollutant lags differed across cities; the average lag for $O_3$ was 2.9 days. The pooled effect estimate for a 20 ppb increase in the daily 1-h max $O_3$ concentration and the percent change in hospitalizations among all 10 cities was 2.3 (95% CI: 0.11, 4.50) in an all-year analysis. The authors reported no evidence of effect modification by sex, neighborhood-level education, or neighborhood-level income. A similar multicity time-series study was conducted using nearly 400,000 ED visits to 14 hospitals in seven Canadian cities from 1992 to 2003 (Stieb et al., 2009). Primary analyses considered daily $O_3$ single day lags of 0-2 days; in addition, sub-daily lags of 3-h avg concentrations up to 12 hours before presentation to the ED were considered. Seasonal variation was assessed by stratifying analyses by warm and cold seasons. No evidence of associations between short-term $O_3$ exposure and

CVD ED visits was observed. One negative, statistically significant association was reported between a 1-day lag of $O_3$ concentration and visits for angina/myocardial infarction. Ozone concentration was negatively correlated with many of the other pollutants, particularly during the cold season.

The effect of air pollution on daily ED visits for ischemic stroke (n = 10,881 visits) in Edmonton, Canada was assessed from April 1992 through March 2002 (Szyszkowicz, 2008). A 26.4% (95% CI: 3.16-54.5) increase in stroke ED visits was associated with a 20 ppb increase in 24-hour average $O_3$ concentration at lag 1 among men aged 20-64 years in the warm season. No associations were present among women or among men age 65 and older. In addition, no associations were observed for the cold season or for other lags (lag 0 or lag 2). A similar investigation over the same time period in Edmonton, Canada, assessed the relationship between air pollutants and ED visits for stroke (ischemic stroke, hemorrhagic stroke, and transient ischemic attack) among those 65 years of age and older using a case-crossover framework (Villeneuve et al., 2006a). No evidence of association was reported for $O_3$ concentration and stroke hospitalization in single or copollutant models (Villeneuve et al., 2006a).

Additional studies in the U.S. reported no evidence of an association between $O_3$ concentrations and ED visits, hospitalizations, or symptoms leading to hospitalization (Symons et al., 2006; Zanobetti and Schwartz, 2006; Wellenius et al., 2005). Symons et al. (2006) used a case-crossover framework to assess the relationship between air pollutants and the onset of symptoms (dyspnea) severe enough to lead to hospitalization (through the ED) for congestive heart failure. The study was conducted from April to December of 2002 in Baltimore, Maryland. Exposures were assigned using 3 index times: 8-hour and 24-hour periods prior to symptom onset and date of hospital admission. No evidence of association was reported for $O_3$ concentrations. Although seasonal variation was not assessed, the time frame for the study did not involve an entire year (April to December). Wellenius et al. (2005) investigated the association between air pollutants and congestive heart failure hospitalization among Medicare beneficiaries in Pittsburgh, Pennsylvania from 1987 to 1999 utilizing a case-crossover framework. A total of 55,019 admissions from the emergency room with a primary discharge diagnosis of CHF were collected. No evidence of an association was reported for $O_3$ concentration and CHF hospitalization (Wellenius et al., 2005). Finally, Zanobetti and Schwartz (2006) assessed the relationship between air pollutants and hospital admissions through the ED for MI and pneumonia among patients aged 65 and older residing in the greater Boston, MA, area (1995-1999) using a case-crossover framework with control days in the same month matched on temperature. Pollution exposures were assigned for the same day and for the mean of the exposure the day of and the day before the admission. Ozone concentration was not associated with MI admissions in all-year and seasonal analyses.

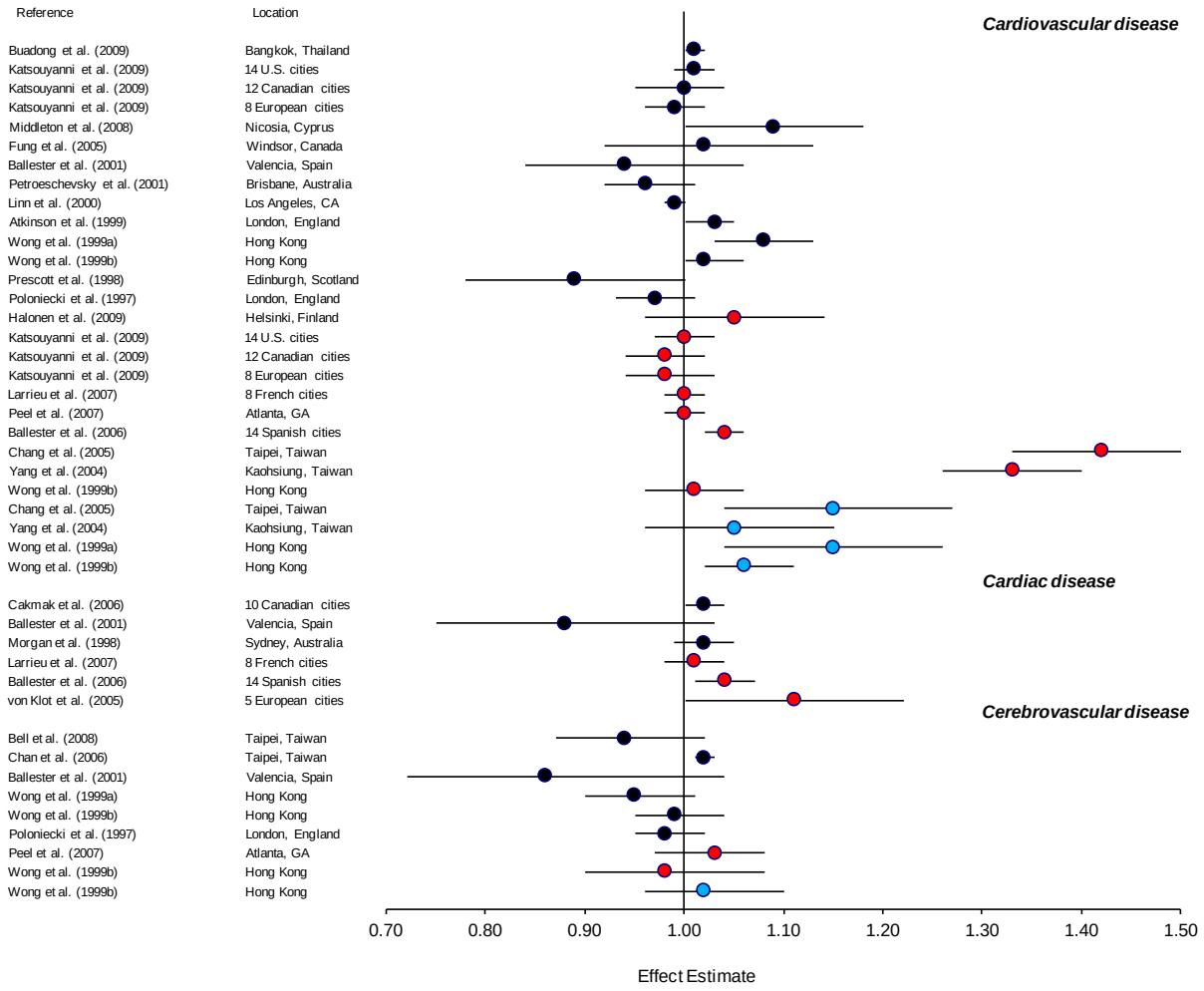
Several recent studies have examined the relationship between air pollution and CVD hospital admissions and/or emergency department visits in Asia. Of note, some areas of Asia have a more tropical climate than the U.S. and do not experience similar

seasonal changes. In Taiwan, fairly consistent positive associations have been reported for $O_3$ concentration and congestive heart failure hospital admissions (for single- and copollutant models) in Taipei on warm days (Yang, 2008) and in Kaohsiung (Lee et al., 2007); cerebrovascular disease ED visits (for lag 0 single- and two-pollutant models but not other lags) in Taipei (Chan et al., 2006); and arrhythmia ED visits in Taipei among those without comorbid conditions (Chiu et al., 2009; Lee et al., 2008a) and in Taipei on warm days among those with and without comorbid conditions (Lee et al., 2008a). However, one study in Taiwan did not show an association. Bell et al. (2008) reported no evidence of an association between $O_3$ concentration and hospital admissions for ischemic heart disease or cerebrovascular disease. Studies based in Asia but outside Taiwan were also performed. A Hong Kong-based investigation (Wong et al., 2009) reported no consistent evidence of a modifying effect of influenza on the relationship between $O_3$ concentration and CVD admissions. Among elderly populations in Thailand, $O_3$ concentration was associated with CVD visits, but this association was not detected among younger age groups (15-64) (Buadong et al., 2009). Also, a study performed in Seoul, Korea reported a positive association between $O_3$ concentration and hospital admissions for ischemic heart disease; the association was slightly greater among those over 64 years of age (Lee et al., 2003b).

Positive associations between short-term $O_3$ exposure and CVD hospital admissions and/or ED visits have been reported in other areas of the world as well (Azevedo et al., 2011; Linares and Diaz, 2010; Middleton et al., 2008; Turner et al., 2007; Ballester et al., 2006; De Pablo et al., 2006; Von Klot et al., 2005), although not consistently; some studies reported no association (Oudin et al., 2010; Halonen et al., 2009; Larrieu et al., 2007; Barnett et al., 2006; Hinwood et al., 2006; Lanki et al., 2006; Hosseinpoor et al., 2005; Simpson et al., 2005).

A couple of studies (U.S. and Australia) have examined cardiac arrests where emergency services attempted treatment/resuscitation. No evidence of an association between $O_3$ concentration and out-of-hospital cardiac arrest was observed (Dennekamp et al., 2010; Silverman et al., 2010).

An increasing number of air pollution studies have investigated the relationship between $O_3$ concentrations and CVD hospital admissions and/or ED visits. As summarized in the 2006 $O_3$ AQCD, some, especially those reporting results stratified by season (or temperature) or comorbid conditions have reported positive associations. However, even studies performing these stratified analyses are not consistent and the overall evidence remains inconclusive regarding the association between short-term $O_3$ exposure and CVD hospital admissions and ED visits. The Hospital Admission (HA) and ED visit studies evaluated in this section are summarized in Figure 6-22 through Figure 6-26, which depict the associations for studies in which quantitative data were presented. Table 6-35 through Table 6-39 provide the numerical results displayed in the figures.




Note: Change in $O_3$ standardized to 20 ppb for 24-h avg period, 30 ppb for 8-h avg period, and 40 ppb for 1-h avg period (see Section 2.5). Ozone concentrations in ppb. Seasons depicted by colors – black: all year; red: warm season; light blue: cold season. Age groups of study populations were not specified or were adults with the exception of Katsouyanni et al. (2009), Fung et al. (2005), Wong et al. (1999b), and Prescott et al. (1998), which included only individuals aged 65+. Studies organized by outcome and season and then listed in descending order of publication date.

**Figure 6-22 Effect estimate (95% CI) per increment ppb increase in $O_3$ for over all cardiovascular ED visits or hospital admissions.**

## Table 6-35 Effect estimate (95% CI) per increment ppb increase in $O_3$ for overall cardiovascular ED visits or hospital admissions in studies presented in Figure 6-22.

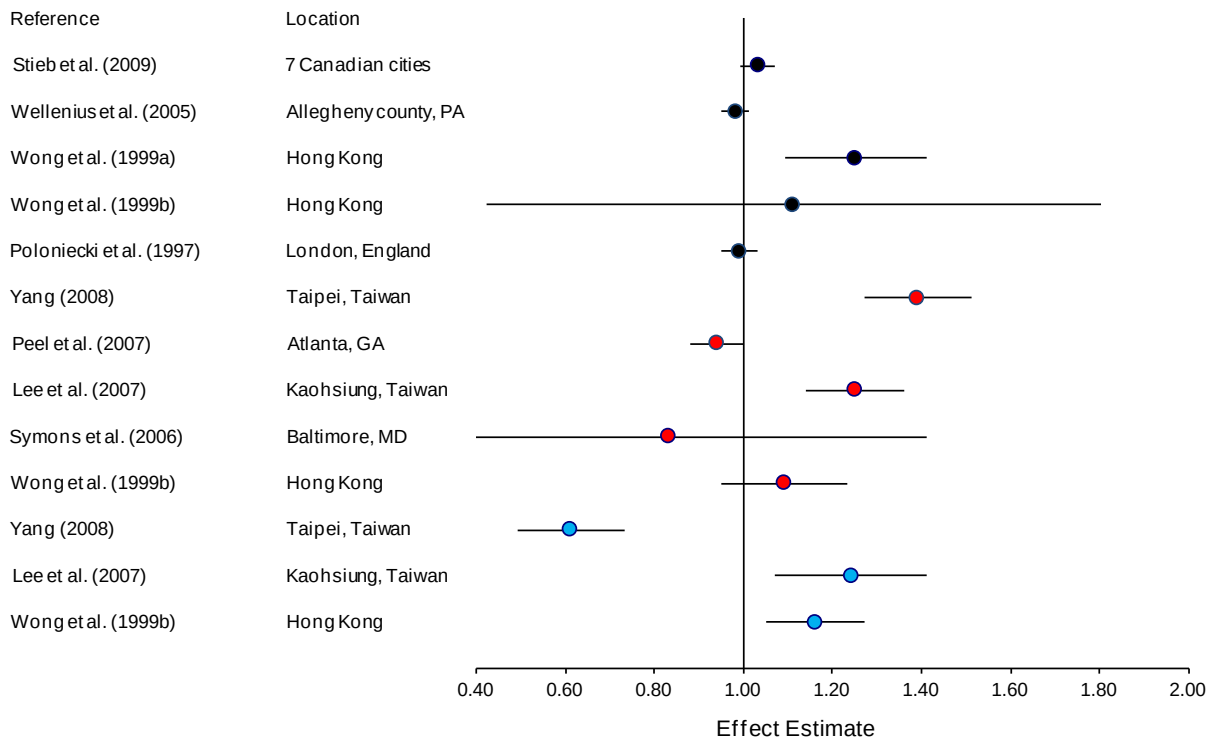
| Study* | Location | Outcome | Averaging Time | Effect Estimate (95% CI) |
|---|---|---|---|---|
| Atkinson et al. (1999) | London, England | Cardiovascular disease | 8-h | 1.03 (1.00, 1.05) |
| Ballester et al. (2006) | 14 Spanish cities | Cardiovascular disease | 8-h warm season | 1.04 (1.02, 1.06) |
| | | Cardiac disease | 8-h warm season | 1.04 (1.01, 1.07) |
| Ballester et al. (2006) | Valencia, Spain | Cardiovascular disease | 8-h | 0.94 (0.84, 1.06) |
| | | Cardiac disease | 8-h | 0.88 (0.75, 1.03) |
| | | Cerebrovascular disease | 8-h | 0.86 (0.72, 1.04) |
| Bell et al. (2008) | Taipei, Taiwan | Cerebrovascular disease | 24-h | 0.94 (0.87, 1.02) |
| Buadong et al. (2009) | Bangkok, Thailand | Cardiovascular disease | 1-h | 1.01 (1.00, 1.02) |
| Cakmak et al. (2006a) | 10 Canadian cities | Cardiac disease | 1-h max | 1.02 (1.00, 1.04) |
| Chan et al. (2006) | Taipei, Taiwan | Cerebrovascular disease | 1-h max | 1.02 (1.01, 1.03) |
| Chang et al. (2005) | Taipei, Taiwan | Cardiovascular disease | 24-h warm season | 1.42 (1.33, 1.50) |
| | | | 24-h cold season | 1.15 (1.04, 1.27) |
| Fung et al. (2005) | Windsor, Canada | Cardiovascular disease | 1-h | 1.02 (0.92, 1.13) |
| Halonen et al. (2009) | Helsinki, Finland | Cardiovascular disease | 8-h max warm season | 1.05 (0.96, 1.14) |
| Katsouyanni et al. (2009) | 14 U.S. cities | Cardiovascular disease | 1-h max | 1.01 (0.99, 1.03) |
| | | | 1-h max warm season | 1.00 (0.97, 1.03) |
| | 12 Canadian cities | Cardiovascular disease | 1-h max | 1.00 (0.95, 1.04) |
| | | | 1-h max warm season | 0.98 (0.94, 1.02) |
| | 8 European cities | Cardiovascular disease | 1-h max | 0.99 (0.96, 1.02) |
| | | | 1-h max warm season | 0.98 (0.94, 1.03) |
| Larrieu et al. (2007) | 8 French cities | Cardiac disease | 8-h max warm season | 1.01 (0.98, 1.04) |
| Linn et al. (2000) | Los Angeles, California | Cardiovascular disease | 24-h | 0.99 (0.98, 1.00) |
| Middleton et al. (2008) | Nicosia, Cyprus | Cardiovascular disease | 8-h max | 1.09 (1.00, 1.18) |
| Morgan et al. (1998) | Sydney, Australia | Cardiac disease | 1-h max | 1.02 (0.99, 1.05) |
| Peel et al. (2007) | Atlanta, GA | Cardiovascular disease | 8-h warm season | 1.00 (0.98, 1.02) |
| | | Cerebrovascular disease | 8-h warm season | 1.03 (0.97, 1.08) |
| Petroeschevsky et al. (2001) | Brisbane, Australia | Cardiovascular disease | 8-h | 0.96 (0.92, 1.01) |
| Poloniecki et al. (1997) | London, England | Cardiovascular disease | 8-h | 0.97 (0.93, 1.01) |
| | | Cerebrovascular disease | 8-h | 0.98 (0.95, 1.02) |

| Study* | Location | Outcome | Averaging Time | Effect Estimate (95% CI) |
|---|---|---|---|---|
| Prescott et al. (1998) | Edinburgh, Scotland | Cardiovascular disease | 24-h | 0.89 (0.78, 1.00) |
| Von Klot et al. (2005) | 5 European cities | Cardiac disease | 8-h max warm season | 1.11 (1.00, 1.22) |
| Wong et al. (1999b) | Hong Kong | Cardiovascular disease | 24-h | 1.08 (1.03, 1.13) |
| | | | 24-h cold season | 1.15 (1.04, 1.26) |
| | | Cerebrovascular disease | 24-h | 0.95 (0.90, 1.01) |
| Wong et al. (1999a) | Hong Kong | Cardiovascular disease | 24-h | 1.02 (1.03, 1.06) |
| | | | 24-h warm season | 1.01 (0.96, 1.06) |
| | | | 24-h cold season | 1.06 (1.02, 1.11) |
| | | Cerebrovascular disease | 24-h | 0.99 (0.95, 1.04) |
| | | | 24-h warm season | 0.98 (0.90, 1.08) |
| | | | 24-h cold season | 1.02 (0.96, 1.10) |
| Yang et al. (2004) | Kaohsiung, Taiwan | Cardiovascular disease | 24-h warm season | 1.33 (1.26, 1.40) |
| | | | 24-h cold season | 1.05 (0.96, 1.15) |

*Studies included in Figure 6-22.

Note: Change in $O_3$ standardized to 20 ppb for 24-hour averaging period, 30 ppb for 8-hour averaging period, and 40 ppb for 1-hour averaging period (see Section 2.5). Ozone concentrations in ppb. Age groups of study populations were not specified or were adults with the exception of Katsouyanni et al. (2009), Fung et al. (2005), Wong et al. (1999a), and Prescott et al. (1998), which included only individuals aged 65+. Studies listed in alphabetical order.

Warm season defined as: March-October (Peel et al., 2007), May-October (Ballester et al., 2005; Wong et al., 1999a), May-September (Halonen et al., 2009), April-September (Katsouyanni et al., 2009; Larrieu et al., 2007; Von Klot et al., 2005), ≥ 20°C (Chang et al., 2005) and ≥ 25°C (Yang et al., 2004). Cold season defined as: November-April (Wong et al., 1999a), <20°C (Chang et al., 2005) and <25°C (Yang et al., 2004), December-March (Wong et al., 1999b)




Note: Change in $O_3$ standardized to 20 ppb for 24-hour averaging period, 30 ppb for 8-hour averaging period, and 40 ppb for 1-hour averaging period (see Section 2.5). Ozone concentrations in ppb. Seasons depicted by colors: black: all year; red: warm season; light blue: cold season. Outcomes were all congestive heart failure, with the exception of Symons et al. (2006), which examined onset of congestive heart failure symptoms leading to a heart attack. Age groups of study populations were not specified or were adults with the exception of Wellenius et al. (2005) and Wong et al. (1999a), which included only individuals aged 65+. Studies organized by outcome and season and then listed in descending order of publication date.

**Figure 6-23 Effect estimate (95% CI) per increment ppb increase in $O_3$ for congestive heart failure ED visits or hospital admissions.**

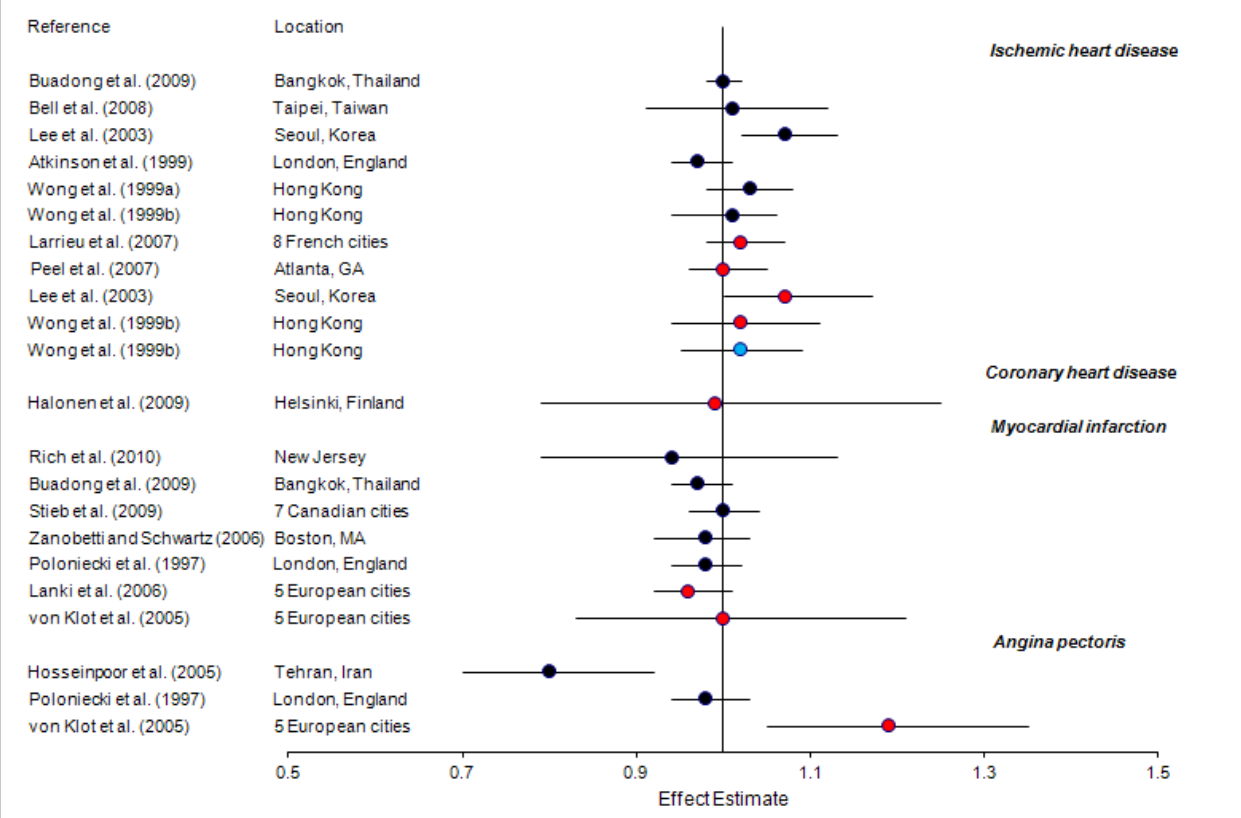
## Table 6-36 Effect estimate (95% CI) per increment ppb increase in $O_3$ for congestive heart failure ED visits or hospital admissions for studies in Figure 6-23.

| Study* | Location | Outcome | Averaging Time | Effect Estimate (95% CI) |
|---|---|---|---|---|
| Lee et al. (2007) | Kaohsiung, Taiwan | Congestive heart failure | 24-h warm season | 1.25 (1.15, 1.36) |
| | | Congestive heart failure | 24-h cold season | 1.24 (1.09, 1.41) |
| Peel et al. (2007) | Atlanta, GA | Congestive heart failure | 8-h warm season | 0.94 (0.89, 1.00) |
| Poloniecki et al. (1997) | London, England | Congestive heart failure | 8-h | 0.99 (0.95, 1.03) |
| Stieb et al. (2009) | 7 Canadian cities | Congestive heart failure | 24-h | 1.03 (0.98, 1.07) |
| Symons et al. (2006) | Baltimore, MD | Onset of congestive heart failure symptoms leading to heart attack | 8-h warm season | 0.83 (0.49, 1.41) |
| Wellenius et al. (2005) | Allegheny county, PA | Congestive heart failure | 24-h | 0.98 (0.96, 1.01) |
| Wong et al. (1999a) | Hong Kong | Congestive heart failure | 24-h | 1.11 (1.04, 1.80) |
| | | | 24-h warm season | 1.09 (0.96, 1.23) |
| | | | 24-hcold season | 1.16 (1.06, 1.27) |
| Yang (2008) | Taipei, Taiwan | Congestive heart failure | 24-h warm season | 1.39 (1.27, 1.51) |
| | | Congestive heart failure | 24-h cold season | 0.61 (0.52, 0.73) |
| Wong et al. (1999b) | Hong Kong | Congestive heart failure | 24-h | 1.25 (1.11, 1.41) |

*Studies include those from Figure 6-23.

Note: Change in $O_3$ standardized to 20 ppb for 24-hour averaging period, 30 ppb for 8-hour averaging period, and 40 ppb for 1-hour averaging period (see Section 2.5). Ozone concentrations in ppb. Outcomes were all congestive heart failure, with the exception of Symons et al. (2006), which examined onset of congestive heart failure symptoms leading to a heart attack. Age groups of study populations were not specified or were adults with the exception of Wellenius et al. (2005) and Wong et al. (1999a), which included only individuals aged 65+. Studies listed in alphabetical order.

Warm season defined as: March-October (Peel et al., 2007), April-November (Symons et al., 2006), May-October (Wong et al., 1999a) ≥ 20°C (Yang, 2008), and >25°C (Lee et al., 2007). Cold season defined as: November-April (Wong et al., 1999a), <20°C (Yang, 2008), and <25°C (Lee et al., 2007).




Note: Change in $O_3$ standardized to 20 ppb for 24-hour averaging period, 30 ppb for 8-hour averaging period, and 40 ppb for 1-hour averaging period (see Section 2.5). Ozone concentrations in ppb. Seasons depicted by colors: black: all year; red: warm season; light blue: cold season. Age groups of study populations were not specified or were adults with the exception of Wong et al. (1999a) and Atkinson et al. (1999), which included only individuals aged 65+. Studies organized by outcome and season and then listed in descending order of publication date.

**Figure 6-24 Effect estimate (95% CI) per increment ppb increase in $O_3$ for ischemic heart disease, coronary heart disease, myocardial infarction, and angina pectoris ED visits or hospital admissions.**

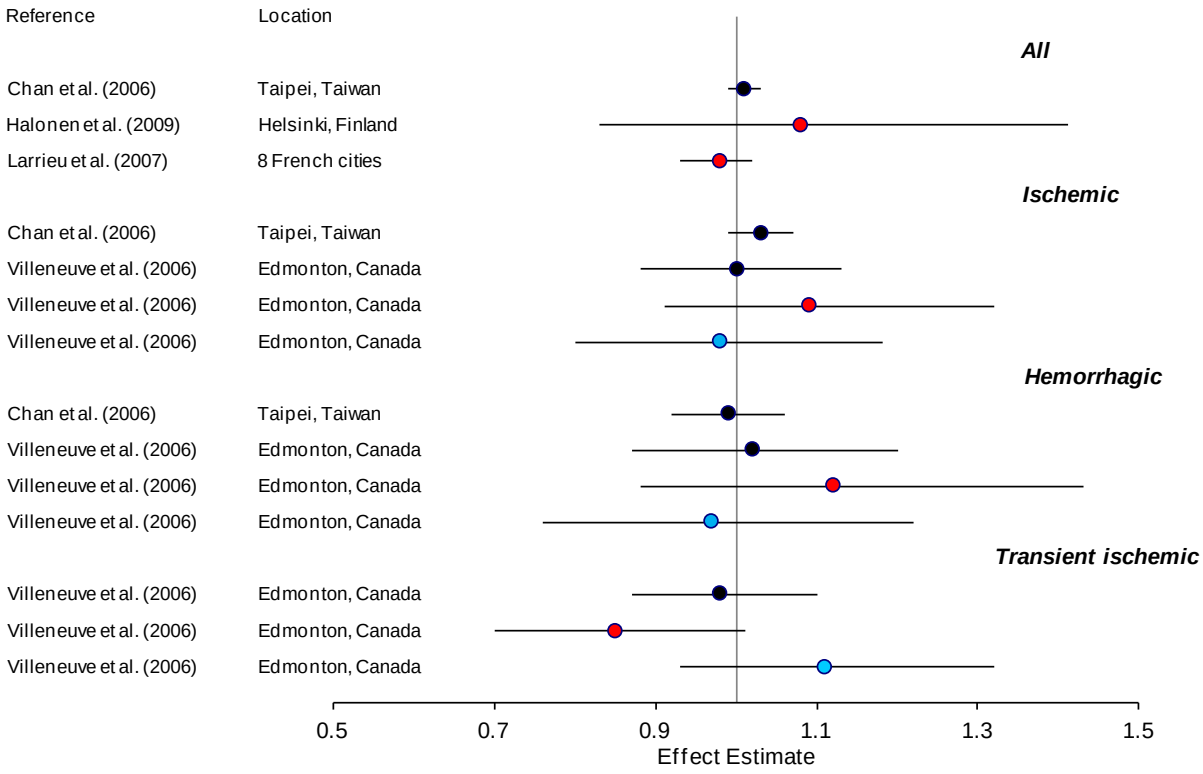
## Table 6-37 Effect estimate (95% CI) per increment ppb increase in $O_3$ for ischemic heart disease, coronary heart disease, myocardial infarction, and angina pectoris ED visits or hospital admissions for studies presented in Figure 6-24.

| Study* | Location | Outcome | Averaging Time | Effect Estimate (95% CI) |
|---|---|---|---|---|
| Atkinson et al. (1999) | London, England | Ischemic heart disease | 8-h | 0.97 (0.94, 1.01) |
| Bell et al. (2008) | Taipei, Taiwan | Ischemic heart disease | 24-h | 1.01 (0.91, 1.12) |
| Buadong et al. (2009) | Bangkok, Thailand | Ischemic heart disease | 1-h | 1.00 (0.98, 1.02) |
| | | Myocardial infarction | 1-h | 0.97 (0.94, 1.01) |
| Halonen et al. (2009) | Helsinki, Finland | Coronary heart disease | 8-h max warm season | 0.99 (0.79, 1.25) |
| Hosseinpoor et al. (2005) | Tehran, Iran | Angina | 8-h max | 0.80 (0.70, 0.92) |
| Lanki et al. (2006) | 5 European cities | Myocardial infarction | 8-h max warm season | 0.96 (0.92, 1.01) |
| Larrieu et al. (2007) | 8 French cities | Ischemic heart disease | 8-h max warm season | 1.02 (0.98, 1.07) |
| Lee et al. (2003b) | Seoul, Korea | Ischemic heart disease | 1-h max | 1.07 (1.02, 1.13) |
| | | Ischemic heart disease | 1-h max warm season | 1.07 (1.00, 1.17) |
| Peel et al. (2007) | Atlanta, GA | Ischemic heart disease | 8-h warm season | 1.00 (0.96, 1.05) |
| Poloniecki et al. (1997) | London, England | Myocardial infarction | 8-h | 0.98 (0.94, 1.02) |
| | | Angina | 8-h | 0.98 (0.94, 1.03) |
| Rich et al. (2010) | New Jersey | Myocardial infarction | 24-h | 0.94 (0.79, 1.13) |
| Stieb et al. (2009) | 7 Canadian cities | Myocardial infarction | 2-h | 1.00 (0.96, 1.04) |
| Von Klot et al. (2005) | 5 European cities | Myocardial infarction | 8-h max warm season | 1.00 (0.83, 1.21) |
| | | Angina | 8-h max warm season | 1.19 (1.05, 1.35) |
| Wong et al. (1999a) | Hong Kong | Ischemic heart disease | 24-h | 1.01 (0.94, 1.06) |
| | | | 24-h warm season | 1.02 (0.94, 1.11) |
| | | | 24-h cold season | 1.02 (0.95, 1.09) |
| Wong et al. (1999b) | Hong Kong | Ischemic heart disease | 24-h | 1.03 (0.98, 1.08) |
| Zanobetti and Schwartz (2006) | Boston, MA | Myocardial infarction | 24-h | 0.98 (0.92, 1.03) |

*Studies included from Figure 6-24.

Note: Change in $O_3$ standardized to 20 ppb for 24-hour averaging period, 30 ppb for 8-hour averaging period, and 40 ppb for 1-hour averaging period (see Section 2.5). Ozone concentrations in ppb. Age groups of study populations were not specified or were adults with the exception of Wong et al. (1999a) and Atkinson et al. (1999), which included only individuals aged 65+. Studies listed in alphabetical order.

Warm season defined as: March-October (Peel et al., 2007), June-August (Lee et al., 2003b), May-September (Halonen et al., 2009), May-October (Buadong et al., 2009), and April-September (Larrieu et al., 2007; Lanki et al., 2006; Von Klot et al., 2005). Cold season defined as: November-April (Buadong et al., 2009).




Note: Change in $O_3$ standardized to 20 ppb for 24-hour averaging period, 30 ppb for 8-hour averaging period, and 40 ppb for 1-hour averaging period (see Section 2.5). Ozone concentrations in ppb. Seasons depicted by colors: black: all year; red: warm season; light blue: cold season. Age groups of study populations were not specified or were adults with the exception of Villeneuve et al. (2006a), which included only individuals aged 65+, and.Chan et al. (2006), which included only individuals aged 50+. Studies organized by outcome and season and then listed in descending order of publication date.

**Figure 6-25 Effect estimate (95% CI) per increment ppb increase in $O_3$ for stroke ED visits or hospital admissions.**

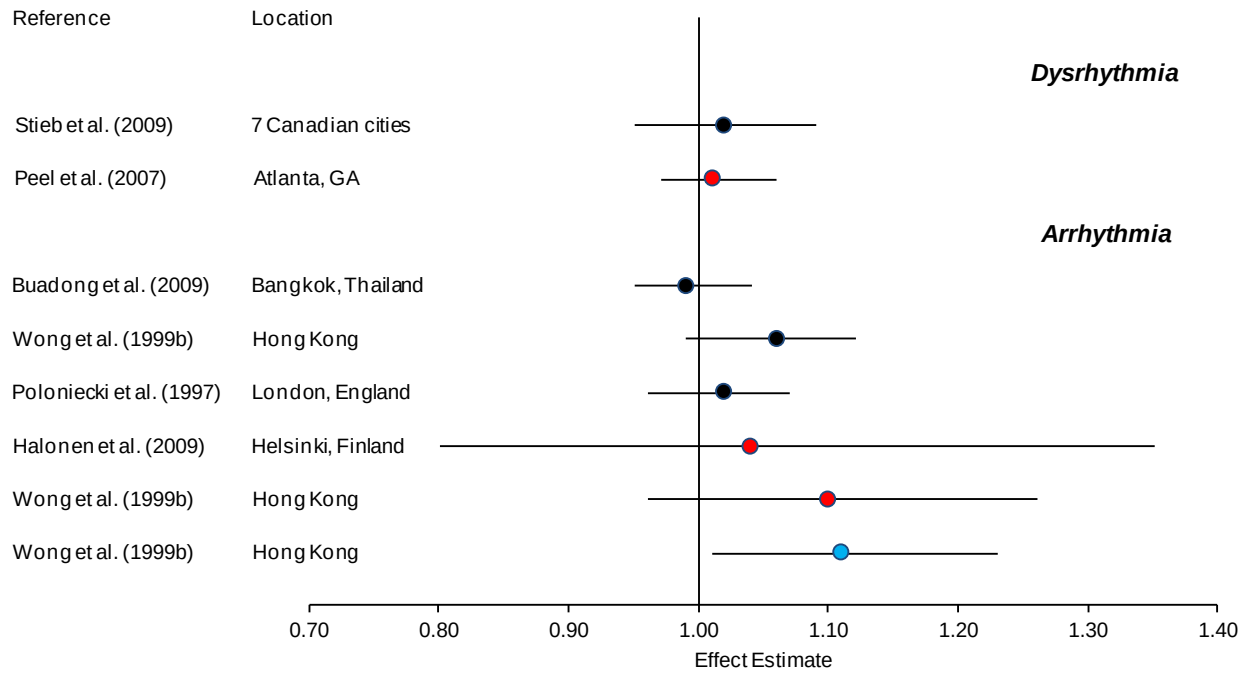
## Table 6-38 Effect estimate (95% CI) per increment ppb increase in $O_3$ for stroke ED visits or hospital admissions for studies presented in Figure 6-25.

| Study* | Location | Outcome | Averaging Time | Effect Estimate (95% CI) |
|---|---|---|---|---|
| Chan et al. (2006) | Taipei, Taiwan | All/non-specified stroke | 1-h max | 1.01 (0.99, 1.03) |
| | | Ischemic stroke | 1-h max | 1.03 (0.99, 1.07) |
| | | Hemorrhagic stroke | 1-h max | 0.99 (0.92, 1.06) |
| Halonen et al. (2009) | Helsinki, Finland | All/non-specified stroke | 8-h max warm season | 1.08 (0.83, 1.41) |
| Larrieu et al. (2007) | 8 French cities | All/non-specified stroke | 8-h max warm season | 0.98 (0.93, 1.02) |
| Villeneuve et al. (2006a) | Edmonton, Canada | Ischemic stroke | 24-h | 1.00 (0.88, 1.13) |
| | | | 24-h warm season | 1.09 (0.91, 1.32) |
| | | | 24-h cold season | 0.98 (0.80, 1.18) |
| | | Hemorrhagic stroke | 24-h | 1.02 (0.87, 1.20) |
| | | | 24-h warm season | 1.12 (0.88, 1.43) |
| | | | 24-h cold season | 0.97 (0.76, 1.22) |
| | | Transient ischemic stroke | 24-h | 0.98 (0.87, 1.10) |
| | | | 24-h warm season | 0.85 (0.70, 1.01) |
| | | | 24-h cold season | 1.11 (0.93, 1.32) |

*Studies included from Figure 6-25.

Note: Change in $O_3$ standardized to 20 ppb for 24-hour averaging period, 30 ppb for 8-hour averaging period, and 40 ppb for 1-hour averaging period (see Section 2.5). Ozone concentrations in ppb. Age groups of study populations were not specified or were adults with the exception of Villeneuve et al. (2006a), which included only individuals aged 65+, and Chan et al. (2006), which included only individuals aged 50+. Studies listed in alphabetical order.

Warm season defined as: May-September (Halonen et al., 2009), and April-September (Larrieu et al., 2007; Villeneuve et al., 2006a). Cold season defined as: October-March (Villeneuve et al., 2006a).




Note: Change in $O_3$ standardized to 20 ppb for 24-hour averaging period, 30 ppb for 8-hour averaging period, and 40 ppb for 1-hour averaging period (see Section 2.5). Ozone concentrations in ppb. Seasons depicted by colors: black: all year; red: warm season; light blue: cold season. Age groups of study populations were not specified or were adults with the exception of Wong et al. (1999a), which included only individuals aged 65+. Studies organized by outcome and season and then listed in descending order of publication date.

**Figure 6-26 Effect estimate (95% CI) per increment ppb increase in $O_3$ for arrhythmia and dysrhythmia ED visits or hospital admissions.**

**Table 6-39 Effect estimate (95% CI) per increment ppb increase in $O_3$ for arrhythmia and dysrhythmia ED visits or hospital admissions for studies presented in Figure 6-26.**

| Study | Location | Outcome | Averaging Time | Effect Estimate (95% CI) |
|---|---|---|---|---|
| Buadong et al. (2009) | Bangkok, Thailand | Arrhythmia | 1-h | 0.99 (0.95, 1.04) |
| Halonen et al. (2009) | Helsinki, Finland | Arrhythmia | 8-h max warm season | 1.04 (0.80, 1.35) |
| Peel et al. (2007) | Atlanta, GA | Dysrhythmia | 8-h warm season | 1.01 (0.97, 1.06) |
| Poloniecki et al. (1997) | London, England | Arrhythmia | 8-h | 1.02 (0.96, 1.07) |
| Stieb et al. (2009) | 7 Canadian cities | Dysrhythmia | 24-h | 1.02 (0.95, 1.09) |
| Wong et al. (1999a) | Hong Kong | Arrhythmia | 24-h | 1.06 (0.99, 1.12) |
| | | | 24-h warm season | 1.10 (0.96, 1.26) |
| | | | 24-h cold season | 1.11 (1.01, 1.23) |

*Studies included from Figure 6-26.

Note: Change in $O_3$ standardized to 20 ppb for 24-hour averaging period, 30 ppb for 8-hour averaging period, and 40 ppb for 1-hour averaging period (see Section 2.5). Ozone concentrations in ppb. Age groups of study populations were not specified or were adults with the exception of (Wong et al., 1999a), which included only individuals aged 65+. Studies listed in alphabetical order. Warm season defined as: March-October (Peel et al., 2007), May-October (Wong et al., 1999a) and May-September (Halonen et al., 2009). Cold season defined as: November-April (Wong et al., 1999a).

### 6.3.2.8 Cardiovascular Mortality

As discussed within this section (Section 6.3), epidemiologic studies provide inconsistent evidence of an association between short-term $O_3$ exposure and cardiovascular effects. However, toxicological studies have demonstrated $O_3$-induced cardiovascular effects, specifically enhanced atherosclerosis and altered vascular function, which could lead to death. The 2006 $O_3$ AQCD provided evidence, primarily from single-city studies, of consistent positive associations between short-term $O_3$ exposure and cardiovascular mortality. Recent multicity studies conducted in the U.S., Canada, and Europe further support the association between short-term $O_3$ exposure and cardiovascular mortality.

As discussed in Section 6.2.7.2, the APHENA study (Katsouyanni et al., 2009) also examined associations between short-term $O_3$ exposure and mortality and found consistent positive associations for cardiovascular mortality in all-year analyses. However, in analyses restricted to the summer season, results were more variable with no evidence of an association in the Canadian dataset in the population <75 years of age, and evidence of associations persisting or increasing in magnitude in the Canadian (population $\geq$ 75 years of age), U.S., and European datasets. Additional multicity studies from the U.S. (Zanobetti and Schwartz, 2008b), Europe (Samoli et al., 2009), Italy (Stafoggia et al., 2010), and Asia (Wong et al., 2010) that conducted summer season and/or all-year analyses provide additional support for an association between short-term $O_3$ exposure and cardiovascular mortality (Figure 6-27).

Of the studies evaluated, only the APHENA study (Katsouyanni et al., 2009) and the Italian multicity study (Stafoggia et al., 2010) conducted an analysis of the potential for copollutant confounding of the $O_3$-cardiovascular mortality relationship. In the European dataset, when focusing on the natural spline model with 8 df/year (Section 6.2.7.2) and lag 1 results in order to compare results across study locations (Section 6.6.2.1), cardiovascular mortality risk estimates were robust to the inclusion of $PM_{10}$ in copollutant models in all-year analyses with more variability in the Canadian and U.S. datasets (i.e., cardiovascular $O_3$ mortality risk estimates were reduced or increased in copollutant models). In summer season analyses, cardiovascular $O_3$ mortality risk estimates were robust in the European dataset and attenuated but remained positive in the U.S. dataset. Similarly, in the Italian multicity study (Stafoggia et al., 2010), which was limited to the summer season, cardiovascular mortality risk estimates were robust to the inclusion of $PM_{10}$ in copollutant models. Based on the APHENA and Italian multicity results, $O_3$ cardiovascular mortality risk estimates appear to be robust to inclusion of $PM_{10}$ in copollutant models. However, in the U.S. and Canadian datasets there was evidence that $O_3$ cardiovascular mortality risk estimates are moderately to substantially sensitive (e.g., increased or attenuated) to $PM_{10}$. The mostly every-6th-day sampling schedule for $PM_{10}$ in the Canadian and U.S. datasets greatly reduced their sample size and limits the interpretation of these results.

### 6.3.2.9 Summary of Epidemiologic Studies

Overall, the available body of evidence examining the relationship between short-term exposures to $O_3$ concentrations and cardiovascular morbidity is inconsistent. Across studies, different definitions, i.e., ICD-9 diagostic codes were used for both all-cause and cause-specific cardiovascular morbidity (Table 6-35, Table 6-36, Table 6-37, Table 6-38, and Table 6-39), which may contribute to inconsistency in results. However, within diagnostic categories, no consistent pattern of association was found with $O_3$. Generally, the studies summarized in this section used nearest air monitors to assess $O_3$ concentrations, with a few exceptions that used modeling or personal exposure monitors (these exceptions were noted throughout the previous sections). The inconsistencies in the associations observed between short-term $O_3$ and CVD morbidities are unlikely to be explained by the different exposure assignment methods used (see Section 4.6). The wide variety of biomarkers considered and the lack of consistency among definitions used for specific cardiovascular disease endpoints (e.g., arrhythmias, HRV) make comparisons across studies difficult. Despite the inconsistent evidence for an association between $O_3$ concentration and CVD morbidity, mortality studies indicate a consistent positive association between short-term $O_3$ exposure and cardiovascular mortality in multicity studies and a multicontinent study.

### 6.3.3 Toxicology

In the previous $O_3$ AQCDs (U.S. EPA, 2006b, 1996a) experimental animal studies have reported relatively few cardiovascular system alterations after exposure to $O_3$ and other photochemical oxidants. The limited amount of research directed at examining $O_3$-induced cardiovascular effects has primarily found alterations in heart rate (HR), heart rhythm, and BP after $O_3$ exposure. Although $O_3$ induced changes in HR and core temperature ($T_{CO}$) in a number of rat studies, these responses have not been reported or extensively studied in humans exposed to $O_3$ and may be unique to rodents.

According to recent animal toxicology studies, short-term $O_3$ exposure induces vascular oxidative stress and proinflammatory mediators, alters HR and HRV, and disrupts the regulation of the pulmonary endothelin system (study details are provided in Table 6-40. A number of these effects were variable between strains examined, suggesting a genetic component to development of $O_3$ induced cardiovascular effects. Further, recent studies provide evidence that extended $O_3$ exposure enhances the risk of ischemia-reperfusion (I/R) injury and atherosclerotic lesion development. Still, few studies have investigated the role of $O_3$ reaction products in these processes, but more evidence is provided for elevated inflammatory and reduction-oxidation (redox) cascades known to initiate these cardiovascular pathologies.

#### Heart Rate, Rhythm, and Heart Rate Variability

Studies (Arito et al., 1992; Arito et al., 1990; Uchiyama and Yokoyama, 1989; Yokoyama et al., 1989; Uchiyama et al., 1986) report $O_3$ exposure (0.2-1.0 ppm, 3 hours to 3 days) in rats decreased $T_{CO}$, HR, and mean arterial pressure (MAP). In addition, $O_3$ exposure (0.1 – 1.0 ppm, 3 hours to 3 days) in rats induced arrhythmias, including increased PR interval and QRS complex, premature atrial contraction, and incomplete A-V block (Arito et al., 1990; Yokoyama et al., 1989; Uchiyama et al., 1986). The effects were more pronounced in adult and awake rats than in younger or sleeping animals, whereas no sex-related differences were noted in these $O_3$ induced outcomes (Uchiyama et al., 1986). However, these cardiovascular responses to $O_3$, including decreased $T_{CO}$ and HR, could be attenuated by increased ambient temperatures and environmental stress and exhibited adaptation (Watkinson et al., 2003; Watkinson et al., 1993). These studies suggest that these responses to $O_3$ were the result of the rodent hypothermic response, which serves as a physiological and behavioral defense mechanism to minimize the irritant effects of $O_3$ inhalation, (Iwasaki et al., 1998; Arito et al., 1997). As humans do not appear to exhibit decreased HR, MAP, and $T_{CO}$ with routine environmental (Section 6.3.2) or controlled laboratory (Section 6.3.1) exposures to $O_3$, caution must be used in extrapolating the results of these animal studies to humans.

Other studies have shown that $O_3$ can increase BP in animal models. Rats exposed to 0.6 ppm $O_3$ for 33 days had increased systolic pressure and HR (Revis et al., 1981).

Increased BP triggers the release of atrial natriuretic factor (ANF), which has been found in increased levels in the heart, lungs, and circulation of $O_3$ exposed (0.5 ppm) rats (Vesely et al., 1994a, b, c). Exposures to high concentrations of $O_3$ (1.0 ppm) have also been found to lead to heart and lung edema (Friedman et al., 1983), which could be the result of increased ANF levels. Thus, $O_3$ may increase blood pressure and HR, leading to increased ANF and tissue edema.

Recent studies report strain differences in HR and HRV in response to a 2-hour $O_3$ pretreatment followed by exposure to carbon black (CB) in mice (C3H/HeJ [HeJ], C57BL/6J [B6], and C3H/HeOuJ [OuJ]) (Hamade and Tankersley, 2009; Hamade et al., 2008). These mice strains were chosen from prior studies on lung inflammatory and hyperpermeability responses to be at increased risk (B6 and OuJ) or resistant (HeJ) to $O_3$-induced health effects (Kleeberger et al., 2000). HR decreased during $O_3$ pre-exposure for all strains, but recovered during the CB exposure (Hamade et al., 2008). Percent change in HRV parameters, SDNN (indicating total HRV) and rMSSD (indicating beat-to-beat HRV), were increased in both C3H mice strains, but not B6 mice, during $O_3$ pre-exposure and recovered during CB exposure when compared to the filtered air group. The two C3H strains differ by a mutation in the Toll-like receptor 4 (TLR4) gene, but these effects did not seem to be related to this mutation since similar responses were observed. Hamade et al. (2008) speculate that the B6 and C3H strains differ in mechanisms of HR response after $O_3$ exposure between withdrawal of sympathetic tone and increase of parasympathetic tone; however, no direct evidence for this conclusion was reported. The strain differences observed in HR and HRV suggest that genetic variability affects cardiac responses after acute air pollutant exposures.

Hamade and Tankersley (2009) continued this investigation of gene-environment interactions on cardiopulmonary adaptation of $O_3$ and CB induced changes in HR and HRV using the previously described (Hamade et al., 2008) daily exposure scheme for 3 consecutive days. By comparing day-1 interim values it is possible to observe that $O_3$ exposure increased SDNN and rMSSD, but decreased HR in all strains. Measures of HR and HRV in B6 and HeJ mice recovered to levels consistent with filtered air treated mice by day 3; however, these responses in OuJ mice remained suppressed. B6 mice had no change in respiratory rate (RR) after $O_3$ treatment, whereas HeJ mice on days 1 and 2 had increased RR and OuJ mice on days 2 and 3 exhibited increased RR. $V_T$ did not change with treatment among the strains. Overall, B6 mice were mildly responsive with rapid adaptation, whereas C3 mice were highly responsive with adaptation only in HeJ mice with regards to changes in cardiac and respiratory responses. HR and HRV parameters were not equally correlated with $V_T$ and RR between the three mice strains, which suggest that strains vary in the integration of the cardiac and respiratory systems. These complex interactions could help explain variability in interindividual responses to air pollution.

Hamade et al. (2010) expanded their investigation to explore the variation of these strain dependent cardiopulmonary responses with age. As was observed previously, all experimental mouse strains (B6, HeJ, and OuJ) exhibited decreased HR and

increased HRV after $O_3$ exposure. Younger $O_3$-exposed mice had a significantly lower HR compared to older exposed mice, indicating an attenuation of the bradycardic effect of $O_3$ with age. Younger mice also had a greater increase in rMSSD in HeJ and OuJ strains and SDNN in HeJ mice. Conversely, B6 mice had a slightly greater increase in SDNN in aged mice compared to the young mice. No change was observed in the magnitude of the $O_3$ induced increase of SDNN in OuJ mice or rMSSD in B6 mice. The B6 and HeJ mice genetically vary in respect to the nuclear factor erythroid 2-related factor 2 (Nrf-2). The authors propose that the genetic differences between the mice strains could be altering the formation of ROS, which tends to increase with age, thus modulating the changes in cardiopulmonary physiology after $O_3$ exposure.

Strain and age differences in HR and heart function were further investigated in B6 and 129S1/SvlmJ (129) mice in response to a sequential $O_3$ and filtered air or CB exposure (Tankersley et al., 2010). Young 129 mice showed a decrease in HR after $O_3$ or $O_3$ and CB exposure. This bradycardia was not observed in B6 or older animals in this study, suggesting a possible alteration or adaptation of the autonomic nervous system activity with age. However, these authors did previously report bradycardia in similarly aged young B6 mice (Hamade et al., 2010; Hamade and Tankersley, 2009; Hamade et al., 2008). Ozone exposure in 129 mice also resulted in an increase in left ventricular chamber dimensions at end diastole (LVEDD) in young and old mice and a decrease in left ventricular posterior wall thickness at end systole (PWTES) in older mice. The increase in LVEDD caused a decrease in fractional shortening, which can be used as a rough indicator of left ventricular function. Regression analysis revealed a significant interaction between age and strain on HR and PWTES, which implies that aging affects HR and heart function in response to $O_3$ differently between mouse strains.

### Vascular Disease and Injury

A recent study in young mice (C57Bl/6) and rhesus monkeys examined the effects of short-term $O_3$ exposure (0.5 ppm, 1 or 5 days) on a number of cardiovascular endpoints (Chuang et al., 2009). Mice exposed to $O_3$ for 5 days had increased HR as well as mean and diastolic blood pressure. This is in contrast to the bradycardia that was reported in 18-20 week-old B6 mice treated with $O_3$, as described above (Hamade and Tankersley, 2009; Hamade et al., 2008). Increased blood pressure could be explained by the inhibition in endothelial-dependent (acetylcholine) vasorelaxation from decreased bioavailability of aortic nitric oxide (·NO). Ozone caused a decrease in aortic $NO_X$ (nitrite and nitrate levels) and a decrease in total, but not phosphorylated, endothelial nitric oxide synthase (eNOS). Ozone also increased vascular oxidative stress in the form of increased aortic and lung lipid peroxidation (F2-isoprostane), increased aortic protein nitration (3-nitrotyrosine), decreased aortic superoxide dismutase (SOD2) protein and activity, and decreased aortic aconitase activity, indicating specific inactivation by $O_2^-$ and $ONOO^-$. Mitochondrial DNA (mtDNA) damage was also used as a measure of oxidative and nitrative stress in mice and infant rhesus monkeys exposed to $O_3$. Chuang et al. (2009) observed that

mtDNA damage accumulated in the lung and aorta of mice after 1 and 5 days of $O_3$ exposure and in the proximal and distal aorta of $O_3$ treated nonhuman primates. Additionally, genetically hyperlipidemic mice exposed to $O_3$ (0.5 ppm) for 8 weeks had increased aortic atherosclerotic lesion area (Section 7.3.1), which may be associated with the short-term exposure changes discussed. Overall, this study suggests that $O_3$ initiates an oxidative environment by increasing $O_2^-$ production, which leads to mtDNA damage and ·NO consumption, known to perturb endothelial function (Chuang et al., 2009). Endothelial dysfunction is characteristic of early and advanced atherosclerosis and coincides with impaired vasodilation and blood pressure regulation.

Vascular occlusion resulting from atherosclerosis can block blood flow causing ischemia. The restoration of blood flow in the vessel or reperfusion can cause injury to the tissue from subsequent inflammation and oxidative damage. Perepu et al. (2010) observed that $O_3$ exposure (0.8 ppm, 28 or 56 days) enhanced the sensitivity to myocardial I/R injury in Sprague-Dawley rats while increasing oxidative stress levels and pro-inflammatory mediators and decreasing production of anti-inflammatory proteins. Ozone was also found to decrease the left ventricular developed pressure, rate of change of pressure development, and rate of change of pressure decay while increasing left ventricular end diastolic pressure in isolated perfused hearts. In this ex vivo heart model, $O_3$ induced oxidative stress by decreasing SOD enzyme activity and increasing malondialdehyde levels. Ozone also elicited a proinflammatory state which was evident by an increase in TNF-α and a decrease in the anti-inflammatory cytokine IL-10. Perepu et al. (2010) concluded that $O_3$ exposure may result in a greater I/R injury.

### Effects on Cardiovascular-Related Proteins

Increased BP, changes in HRV, and increased atherosclerosis may be related to increases in the vasoconstrictor peptide, endothelin-1 (amino acids 1-21, ET-$1_{[1-21]}$). Regulation of the pulmonary endothelin system can be affected in rats by inhalation of PM (0, 5, 50 mg/$m^3$, EHC-93) and $O_3$ (Thomson et al., 2006; Thomson et al., 2005). Exposure to either $O_3$ (0.8 ppm) or PM increased plasma ET-$1_{[1-21]}$, ET-$3_{[1-21]}$, and the ET-1 precursor peptide, bigET-1. Increases in circulating ET-$1_{[1-21]}$ could be a result of a transient increase in the gene expression of lung preproET-1 and endothelin converting enzyme-1 (ECE-1) immediately following inhalation of $O_3$ or PM. These latter gene expression changes (e.g., preproET-1 and ECE-1) were additive with co-exposure to $O_3$ and PM. Conversely, preproET-3 decreased immediately after $O_3$ exposure, suggesting the increase in ET-$3_{[1-21]}$ was not through de novo production. A recent study also found increased ET-1 gene expression in the aorta of $O_3$-exposed rats (Kodavanti et al., 2011). These rats also exhibited an increase in $ET_BR$ after $O_3$ exposure; however, they did not demonstrate increased biomarkers for vascular inflammation, thrombosis, or oxidation.

Ozone can oxidize protein functional groups and disturb the affected protein. For example, the soluble plasma protein fibrinogen is oxidized by $O_3$ (0.01-0.03 ppm) in

vitro, creating fibrinogen and fibrin aggregates, characteristically similar to defective fibrinogen (Rosenfeld et al., 2009; Rozenfeld et al., 2008). In these studies, oxidized fibrinogen retained the ability to form fibrin gels that are involved in coagulation, however the aggregation time increased and the gels were rougher than normal with thicker fibers. Oxidized fibrinogen also developed the ability to self assemble creating fibrinogen aggregates that may play a role in thrombosis. Since $O_3$ does not readily translocate past the ELF and pulmonary epithelium and fibrinogen is primarily a plasma protein, it is uncertain if $O_3$ would have the opportunity to react with plasma fibrinogen. However, fibrinogen can be released from the basolateral face of pulmonary epithelial cells during inflammation, where the deposition of fibrinogen could lead to lung injury (Lawrence and Simpson-Haidaris, 2004).

### Studies on Ozone Reaction Products

Although toxicological studies have demonstrated $O_3$-induced effects on the cardiovascular system, it remains unclear if the mechanism is through a reflex response or the result of effects from $O_3$ reaction products (U.S. EPA, 2006b, 1996a). Oxysterols derived from cholesterol ozonation, such as β-epoxide and 5β,6β-epoxycholesterol (and its metabolite cholestan-6-oxo-3,5-diol), have been implicated in inflammation associated with cardiovascular disease (Pulfer et al., 2005; Pulfer and Murphy, 2004). Two other cholesterol ozonolysis products, atheronal-A and -B (e.g., cholesterol secoaldehyde), have been found in human atherosclerotic plaques and shown in vitro to induce foam cell formation and induce cardiomyocyte apoptosis and necrosis (Sathishkumar et al., 2005; Wentworth et al., 2003); however, these products have not been found in the lung compartment or systemically after $O_3$ exposure. The ability to form these cholesterol ozonation products in the circulation in the absence of $O_3$ exposure complicates their implication in $O_3$ induced cardiovascular disease.

Although it has been proposed that $O_3$ reaction products released after the interaction of $O_3$ with ELF constituents (see Section 5.2.3) on $O_3$ interaction with ELF) are responsible for systemic effects, it is not known whether they gain access to the vascular space. Alternatively, extrapulmonary release of diffusible mediators, such as cytokines or endothelins, may initiate or propagate inflammatory responses in the vascular or systemic compartments (Cole and Freeman, 2009) (Section 5.3.8). Ozone reacts within the lung to amplify ROS production, induce pulmonary inflammation, and activate inflammatory cells, resulting in a cascading proinflammatory state and extrapulmonary release of diffusible mediators that could lead to cardiovascular injury.

A recent study that examined $O_3$ reaction byproducts has shown that cholesterol secoaldehyde (e.g., atheronal A) induces apoptosis in vitro in mouse macrophages (Gao et al., 2009b) and cardiomyocytes (Sathishkumar et al., 2009). Additionally, atheronal-A and -B has been found to induce in vitro macrophage and endothelial cell proinflammatory events involved in the initiation of atherosclerosis (Takeuchi et al., 2006). These $O_3$ reaction products when complexed with low density lipoprotein

upregulate scavenger receptor class A and induce dose-dependent macrophage chemotaxis. Atheronal-A increases expression of the adhesion molecule, E-selectin, in endothelial cells, while atheronal-B induces monocyte differentiation. These events contribute to both monocyte recruitment and foam cell formation in atherosclerotic vessels. It is unknown whether these $O_3$ reaction products gain access to the vascular space from the lungs. Alternative explanations include the extrapulmonary release of diffusible mediators that may initiate or propagate inflammatory responses in the vascular or systemic compartments.

## Table 6-40 Characterization of study details for Section 6.3.3.

| Study[a]* | Model | $O_3$ (ppm) | Exposure Duration | Effects |
|---|---|---|---|---|
| Chuang et al. (2009) | Mice; C57Bl/6; M; 6 weeks | 0.5 | 1 or 5 days, 8-h/day | Increased HR and blood pressure. Initiated an oxidative environment by increasing vascular $O_2^-$ production, which lead to mtDNA damage and ·NO consumption, known to perturb endothelial function. |
| | Monkey; rhesus *Macaca mulatta*; M; Infant (180 days old) | 0.5 | 5 days, 8-h/day | Increased aortic mtDNA damage. |
| Perepu et al. (2010) | Rat; Sprague-Dawley; 50-75 g | 0.8 | 28 days, 8-h/day | Enhanced the sensitivity to myocardial I/R injury while increasing oxidative stress and pro-inflammatory mediators and decreasing production of anti-inflammatory proteins. |
| Hamade et al. (2008) | Mice; C57Bl/6J, C3H/HeJ, and C3H/HeOuJ; M; 18-20 weeks | 0.6 (subsequent CB exposure, 536 µg/m$^3$) | 2-h followed by 3 h of CB | Decreased HR. Strain differences observed in HRV suggest that genetic variability affects cardiac responses. |
| Hamade and Tankersley (2009) | Mice; C57Bl/6J, C3H/HeJ, and C3H/HeOuJ; M; 18-20 weeks | 0.6 (subsequent CB exposure, 536 µg/m$^3$) | 3 days, 2-h/day followed by 3-h of CB | Strains varied in integration of the cardiac and respiratory systems, implications in interindividual variability. B6 mice were mildly responsive with rapid adaptation, whereas C3 mice were highly responsive with adaptation only in HeJ mice with regards to changes in cardiac and respiratory responses. |
| Hamade et al. (2010) | Mice; C57Bl/6J, C3H/HeJ, and C3H/HeOuJ; M; 5 or 12 mo old | 0.6 (subsequent CB exposure, 536 µg/m$^3$) | 2-h followed by 3-h of CB | Aged mice exhibited attenuated changes in cardiopulmonary physiology after $O_3$ exposure. Genetic differences between mice strains could be altering formation of ROS, which tends to increase with age, thus modulating $O_3$ induced effects. |
| Tankersley et al. (2010) | Mice; C57Bl/6J, 129S1/SvlmJ; M/F; 5 or 18 mo old | 0.6 (subsequent CB exposure, 556 µg/m$^3$) | 2-h followed by 3-h of CB | Significant interaction between age and strain on HR and PWTES, which implies that aging affects the HR and function in response to $O_3$ differently between mouse strains. |
| Thomson et al. (2005) | Rat; Fischer-344; M; 200-250 g | 0.4 or 0.8 | 4-h | Activation of the vasoconstricting ET system. Increased plasma ET-1 through higher production and slower clearance. |
| Thomson et al. (2006) | Rat; Fischer-344; M; 200-250 g | 0.8 | 4-h | Increased plasma ET-3 not due to de novo synthesis, unlike ET-1. |
| Kodavanti et al. (2011) | Rat; Wistar; M; 10-12 weeks | 0.5 or 1.0 | 2 days, 5-h/day | No changes to aortic genes of thrombosis, inflammation, or proteolysis, except ET-1 and ETBR (1.0 ppm). |

[a]Results from previous studies are presented in Annex Table AX5-14 of the 2006 $O_3$ AQCD and Table 6-23 of the 1996 $O_3$ AQCD.

*Study details for Section 6.3.3.

### Summary of Toxicological Studies

Overall, animal studies suggest that $O_3$ exposure may result in $O_3$ induced cardiovascular effects. Studies provide evidence for both increased and decreased HR, however it is uncertain if $O_3$-induced bradycardia would also occur in humans or if it is due solely to a rodent hypothermic response. Animal studies also provide evidence for increased HRV, arrhythmias, vascular disease, and injury following short-term $O_3$ exposure. In addition, a series of studies highlight the role of gene-environment interactions and age in the induction of effects and attenuation of responses to $O_3$ exposure.

Biologically plausible mechanisms are present for the cardiovascular effects observed in animal exposure studies. Further discussion of the modes of action that may lead to cardiovascular effects can be found in Section 5.3.8. Recent studies suggest that $O_3$ exposure may disrupt both the $NO^{\cdot}$ and endothelin systems, which can result in an increase in HR, HRV, and ANF. The observed bradycardia following $O_3$ exposure may be the result of reflex reactions, including the trigeminocardiac reflex, evoked following the stimulation of sensory receptors lining the nose and RT. These mechanisms of parasympathetically-derived cardiac effects are described in more detail in Section 5.3.2. Additionally, $O_3$ may increase oxidative stress and vascular inflammation promoting the progression of atherosclerosis and leading to increased susceptibility to I/R injury. As $O_3$ reacts quickly with the ELF and does not translocate to the heart and large vessels, studies suggest that the cardiovascular effects exhibited could be caused by reaction byproducts of $O_3$ exposure. However, direct evidence of translocation of $O_3$ reaction products to the cardiovascular system has not been demonstrated in vivo. Alternatively, extrapulmonary release of diffusible mediators, such as cytokines or endothelins, may initiate or propagate inflammatory responses in the vascular or systemic compartments leading to the reported cardiovascular pathologies.

## 6.3.4 Summary and Causal Determination

In previous $O_3$ reviews (U.S. EPA, 2006b, 1996a), very few studies were described which examined the effect of short-term $O_3$ exposure on the cardiovascular system. More recently, the body of scientific evidence available that has examined the effect of $O_3$ on the cardiovascular system has expanded.

Toxicological studies, although limited in number, provide evidence of $O_3$-induced cardiovascular effects. These include enhanced I/R injury, disrupted NO-induced vascular reactivity, decreased cardiac function, increased vascular disease, and increased HRV following short-term $O_3$ exposure. A number of these effects have also been observed following long-term $O_3$ exposure (see Section 7.3.1.2). Results of studies investigating the role of $O_3$ in heart rate regulation are mixed with both bradycardic and tachycardic responses observed in animal models.
The cardiovascular effects of $O_3$ found in animals may, in part, correspond to alteration of the autonomic nervous system or to the development and maintenance

of systemic oxidative stress and a proinflammatory environment that may result from pulmonary inflammation.

Controlled human exposure studies also suggest cardiovascular effects in response to short-term $O_3$ exposure and provide some coherence with evidence from animal toxicology studies. Increases and decreases in high frequency HRV have been reported following relatively low (120 ppb during rest) and high (300 ppb with exercise) $O_3$ exposures, respectively. These changes in cardiac function observed in animal and human studies provide preliminary evidence for $O_3$-induced modulation of the autonomic nervous system through the activation of neural reflexes in the lung (see Section 5.3.2). Controlled human exposure studies also support the animal toxicology studies by demonstrating $O_3$-induced effects on blood biomarkers of systemic inflammation and oxidative stress as well as changes in biomarkers suggestive of a prothrombogenic response to $O_3$.

The experimental evidence provides initial biological plausibility for the consistently positive associations observed in epidemiologic studies of short-term $O_3$ exposure and cardiovascular mortality. These include studies reviewed in the 2006 $O_3$ AQCD, recent multicity studies, and the multicontinent APHENA study. The few studies that examined copollutant confounding found that associations with cardiovascular mortality remain robust in copollutant models with PM. However, epidemiologic studies generally do not observe associations between short-term exposure to $O_3$ and cardiovascular morbidity; studies of cardiovascular-related hospital admissions and ED visits and other various cardiovascular effects did not find consistent evidence of a relationship with $O_3$ exposure. The lack of coherence between the results from studies that examined associations between short-term $O_3$ exposure and cardiovascular morbidity and cardiovascular mortality complicate the interpretation of the overall evidence for $O_3$-induced cardiovascular effects.

In conclusion, animal toxicological studies demonstrate $O_3$-induced cardiovascular effects, and support the strong body of evidence indicating $O_3$-induced cardiovascular mortality. Animal toxicological and controlled human exposure studies provide evidence for biologically plausible mechanisms underlying these $O_3$-induced cardiovascular effects. However, a lack of coherence with epidemiologic studies of cardiovascular morbidity remains an important uncertainty. Taken together, the overall body of evidence across disciplines is sufficient to conclude that there **is likely to be a causal relationship between relevant short-term exposures to $O_3$ and cardiovascular effects.**

## 6.4 Central Nervous System Effects

The 2006 $O_3$ AQCD (U.S. EPA, 2006b) included toxicological evidence indicating that acute exposures to $O_3$ are associated with alterations in neurotransmitters, motor activity, short and long term memory, and sleep patterns. Additionally, histological signs of neurodegeneration have been observed. Reports of headache, dizziness, and irritation of the nose with $O_3$ exposure are common complaints in humans, and some

behavioral changes in animals may be related to these symptoms rather than indicative of neurotoxicity. Peterson and Andrews (1963) and Tepper et al. (1983) showed that mice would alter their behavior to avoid $O_3$ exposure. Murphy et al. (1964) and Tepper et al. (1982) showed that running-wheel behavior was suppressed, and Tepper et al. (1985) subsequently demonstrated the effects of a 6-hour exposure to $O_3$ on the suppression of running-wheel behavior in rats and mice, with the lowest effective concentration being about 0.12 ppm $O_3$ in the rat and about 0.2 ppm in the mouse. The suppression of active behavior by 6 hours of exposure to 0.12 ppm $O_3$ has recently been confirmed by Martrette et al. (2011) in juvenile female rats, and the suppression of three different active behavior parameters was found to become more pronounced after 15 days of exposure. A table of studies examining the effects of $O_3$ on behavior can be found on p 6-128 of the 1996 $O_3$ AQCD. Generally speaking, transient changes in behavior in rodent models appear to be 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). Many behavioral changes are likely to result from avoidance of irritation, but more recent studies indicate that $O_3$ also directly affects the CNS.

Research in the area of $O_3$-induced neurotoxicity has notably increased over the past few years, with the majority of the evidence coming from toxicological studies that examined the association between $O_3$ exposure, neuropathology, and neurobehavioral effects. As discussed below, these studies demonstrated that exposure to $O_3$ can produce a variety of CNS effects including behavioral deficits, morphological changes, and oxidative stress in the brains of rodents.In these rodent studies, interestingly, CNS effects were reported at $O_3$ concentrations that were generally lower than those concentrations commonly observed to produce pulmonary or cardiac effects in rats. A recent epidemiologic study provides new evidence, which is coherent with the toxicological evidence indicating that ambient $O_3$ exposure may result in cognitive function decrements. This study is discussed in detail in Chapter 7 (Section 7.5.1) because it focuses on long-term exposures to $O_3$.

A number of new studies demonstrate various perturbations in neurologic function or histology, including changes similar to those observed with Parkinson's and Alzheimer's disease pathologies occurring in similar regions of the brain (Table 6-41). Many of these include exposure durations ranging from short-term to long-term, and as such are discussed here and in Chapter 7 with emphasis on the effects resulting from exposure durations relevant to the respective chapter. Several studies assess short- and long-term memory acquisition via passive avoidance behavioral testing and find decrements in test performance after $O_3$ exposure, consistent with the aforementioned observation made in humans by Chen and Schwartz (2009). Impairment of long-term memory has been previously described in rats exposed to 0.2 ppm $O_3$ for 4 hours (Rivas-Arancibia et al., 1998) and in other studies of 4-hour exposures at concentrations of 0.7 to 1 ppm (Dorado-Martinez et al., 2001; Rivas-Arancibia et al., 2000; Avila-Costa et al., 1999). More recently,

statistically significant decreases in both short and long-term memory were observed in rats after 15 days of exposure to 0.25 ppm $O_3$ (Rivas-Arancibia et al., 2010).

The central nervous system is very sensitive to oxidative stress, due in part to its high content of polyunsaturated fatty acids, high rate of oxygen consumption, and low antioxidant enzyme capacity. Oxidative stress has been identified as one of the pathophysiological mechanisms underlying neurodegenerative disorders such as Parkinson's and Alzheimer's disease, among others (Simonian and Coyle, 1996). It is also believed to play a role in altering hippocampal function, which causes cognitive deficits with aging (Vanguilder and Freeman, 2011). A particularly common finding in studies of $O_3$-exposed rats is lipid peroxidation in the brain, especially in the hippocampus, which is important for higher cognitive function including contextual memory acquisition. Performance in passive avoidance learning tests is impaired when the hippocampus is injured, and the observed behavioral effects are well correlated with histological and biochemical changes in the hippocampus, including reduction in spine density in the pyramidal neurons (Avila-Costa et al., 1999), lipoperoxidation (Rivas-Arancibia et al., 2010; Dorado-Martinez et al., 2001), progressive neurodegeneration, and activated and phagocytic microglia (Rivas-Arancibia et al., 2010). The hippocampus is also one of the main regions affected by age-related neurodegenerative diseases, including Alzheimer's disease, and it may be more sensitive to oxidative damage in aged rats. In a study of young (47 days) and aged (900 days) rats exposed to 1 ppm $O_3$ for 4 hours, $O_3$-induced lipid peroxidation occurred to a greater extent in the striatum of young rats, whereas it was highest in the hippocampus in aged rats (Rivas-Arancibia et al., 2000). Martínez-Canabal and Angora-Perez (2008) showed exposure of rats to 0.25 ppm, 4h/day, for 7, 15, or 30 days increased lipoperoxides in the hippocampus. This effect was observed at day 7 and continued to increase with time, indicating cumulative oxidative damage. $O_3$-induced changes in lipid peroxidation, neuronal death, and COX-2 positive cells in the hippocampus could be significantly inhibited by daily treatment with growth hormone (GH), which declines with age in most species. The protective effect of GH on -induced oxidative stress was greatest at 15 days of exposure and was non-significant at day 30. Consistent with these findings, lipid peroxidation in the hippocampus of rats was observed to increase significantly after a 30-day exposure to 0.25 ppm, but not after a single 4-hour exposure to the same concentration (Mokoena et al., 2010). However, 4 hours of exposure was sufficient to cause significant increases in lipid peroxidation when the concentration was increased to 0.7 ppm, and another study observed lipid peroxidation after a 4-hour exposure to 0.4 ppm (Dorado-Martinez et al., 2001).

Other commonly affected areas of the brain include the striatum, substantia nigra, cerebellum, olfactory bulb, and frontal/prefrontal cortex. The striatum and substantia nigra are particularly sensitive to oxidative stress because the metabolism of dopamine, central to their function, is an oxidative process perturbed by redox imbalance. Oxidative stress has been implicated in the premature death of substantia nigra dopamine neurons in Parkinson's disease. Angoa-Pérez et al. (2006) have shown progressive lipoperoxidation in the substantia nigra and a decrease in nigral dopamine neurons in ovariectomized female rats exposed to 0.25 ppm $O_3$, 4h/day,

for 7, 15, or 30 days. Estradiol, an antioxidant, attenuated $O_3$-induced oxidative stress and nigral neuronal death, and the authors note that in humans, estrogen therapy can ameliorate symptoms of Parkinson's disease, which is more prevalent in men. Progressive oxidative stress has also been observed in the striatum and substantia nigra of rats after 15 and 30 days of exposure to 0.25 ppm $O_3$ for 4 hours/day, along with a loss of dopaminergic neurons from the substantia nigra (Pereyra-Muñoz et al., 2006). Decreases in motor activity were also observed at 15 and 30 days of exposure, consistent with other reports (Martrette et al., 2011; Dorado-Martinez et al., 2001). Using a similar $O_3$ exposure protocol, Santiago-López et al. (2010) also observed a progressive loss of dopaminergic neurons within the substantia nigra, accompanied by alterations in the morphology of remaining cells and an increase in p53 levels and nuclear translocation.

The olfactory bulb also undergoes oxidative damage in $O_3$ exposed animals, in some cases altering olfactory-dependent behavior. Lipid peroxidation was observed in the olfactory bulbs of ovariectomized female rats exposed to 0.25 ppm $O_3$ (4 hours/day) for 30 or 60 days (Guevara-Guzmán et al., 2009). Ozone also induced decrements in a selective olfactory recognition memory test, and the authors note that early deficits in odor perception and memory are components of human neurodegenerative diseases. The decrements in olfactory memory were not due to damaged olfactory perception based on other tests. However, deficits in olfactory perception emerged with longer exposures (discussed in Chapter 7). As with the study by Angoa-Pérez et al. (2006) described above, a protective effect for estradiol was demonstrated for both lipid peroxidation and olfactory memory defects. The role of oxidative stress in memory deficits and associated morphological changes has also been demonstrated via attenuation by other antioxidants as well, such as α-tocopherol (Guerrero et al., 1999) and taurine (Rivas-Arancibia et al., 2000).It is unclear how persistent these effects might be. One study of acute exposure, using 1 ppm $O_3$ for 4 hours, observed morphological changes in the olfactory bulb of rats at 2 hours, and 1 and 10 days, but not 15 days, after exposure (Colín-Barenque et al., 2005).

Other acute studies also report changes in the CNS. Lipid peroxidation was observed in multiple regions of the brain after a 1- to 9-hour exposure to 1 ppm $O_3$ (Escalante-Membrillo et al., 2005). Ozone has also been shown to alter gene expression of endothelin-1 (pituitary) and inducible nitric oxide synthase (cerebral hemisphere) after a single 4-hour exposure to 0.8 ppm $O_3$, indicating potential cerebrovascular effects. This concentration-dependent effect was not observed at 0.4 ppm $O_3$ (Thomson et al., 2007). Vascular endothelial growth factor was upregulated in astroglial cells in the central respiratory areas of the brain of rats exposed to 0.5 ppm $O_3$ for 3 hours (Araneda et al., 2008). The persistence of CNS changes after a single exposure was also examined and the increase in vascular endothelial growth factor was present after a short (3 hours) recovery period. Thus, there is evidence that $O_3$-induced CNS effects are both concentration- and time-dependent.

Because $O_3$ can produce a disruption of the sleep-wake cycle (U.S. EPA, 2006b), Alfaro-Rodríguez and González-Piña (2005) examined whether acetylcholine in a region of the brain involved in sleep regulation was altered by $O_3$. After a 24-hour

exposure to 0.5 ppm $O_3$, the acetylcholine concentration in the medial preoptic area was decreased by 58% and strongly correlated with a disruption in paradoxical sleep. Such behavioral-biochemical effects of $O_3$ are confirmed by a number of studies which have demonstrated morphological and biochemical changes in rats.

CNS effects have also been demonstrated in newborn and adult rats whose only exposure to $O_3$ occurred in utero. Several neurotransmitters were assessed in male offspring of dams exposed to 1 ppm $O_3$ during the entire pregnancy (Gonzalez-Pina et al., 2008). The data showed that catecholamine neurotransmitters were affected to a greater degree than indole-amine neurotransmitters in the cerebellum. CNS changes, including behavioral, cellular, and biochemical effects, have also been observed after in utero exposure to 0.5 ppm $O_3$ for 12 hours/day from gestational days 5-20 (Boussouar et al., 2009). Tyrosine hydroxylase labeling in the nucleus tractus solatarius was increased after in utero exposure to $O_3$ whereas Fos protein labeling did not change. When these offspring were challenged by immobilization stress, neuroplasticity pathways, which were activated in air-exposed offspring, were inhibited in $O_3$-exposed offspring. Although an $O_3$ exposure C-R was not studied in these two in utero studies, it has been examined in one study. Santucci et al. (2006) investigated behavioral effects and gene expression after in utero exposure of mice to as little as 0.3 ppm $O_3$. Increased defensive/submissive behavior and reduced social investigation were observed in both the 0.3 and 0.6 ppm $O_3$ groups. Changes in gene expression of brain-derived neurotrophic factor (BDNF, increased in striatum) and nerve growth factor (NGF, decreased in hippocampus) accompanied these behavioral changes. Thus, these three studies demonstrate that CNS effects can occur as a result of in utero exposure to $O_3$, and although the mode of action of these effects is not known, it has been suggested that circulating lipid peroxidation products may play a role (Boussouar et al., 2009). Importantly, these CNS effects occurred in rodent models after in utero only exposure to relevant concentrations of $O_3$.

## Table 6-41 Central nervous system and behavioral effects of short-term $O_3$ exposure in rats.

| Study | Model | $O_3$ (ppm) | Exposure Duration | Effects |
|---|---|---|---|---|
| Martrette et al. (2011) | Rat; Wistar; F; Weight: 152g; 7 weeks old | 0.12 | 1-15 days, 6 h/day | Significant decrease in rearing, locomotor activity, and jumping activity at day 1, with a further decrease in these activities by day 15. |
| Angoa-Pérez et al. (2006) | Rat; Wistar; F; Weight: 300g; ovariectomized | 0.25 | 7 to 60 days, 4-h/day, 5 days/week | Progressive lipid peroxidation and loss of tyrosine hydrolase-immunopositive neurons in the substantia nigra starting at 7 days. |
| Guevara-Guzmán et al. (2009) | Rat; Wistar; F; 264g; ovariectomized | 0.25 | 30 and 60 days, 4h/day | Estradiol treatment protected against lipid peroxidation and decreases in estrogen receptors and dopamine β-hydroxylase in olfactory bulbs along with deficits in olfactory recognition memory. |
| Martínez-Canabal and Angora-Perez (2008) | Rat; Wistar; M; Weight: 300g | 0.25 | 7 to 30 days, 4-h/day | Growth hormone inhibited $O_3$-induced increases in lipoperoxidation and COX-2 positive cells in the hippocampus. |
| Pereyra-Muñoz et al. (2006) | Rat; Wistar; M; 250-300g | 0.25 | 15 and 30 days, 4-h/day | Decreased motor activity, increased lipid peroxidation, altered morphology, and loss of dopamine neurons in substantia nigra and striatum, increased expression of DARPP-32, iNOS, and SOD. |
| Rivas-Arancibia et al. (2010) | Rat; Wistar; M; 250-300g | 0.25 | 15 to 90 days, 4-h/ day | Ozone produced significant increases in lipid peroxidation in the hippocampus, and altered the number of p53 positive immunoreactive cells, activated and phagocytic microglia cells, GFAP immunoreactive cells, and doublecortine cells, and short- and long-term memory-retention latency. |
| Santiago-López et al. (2010) | Rat; Wistar; M; 250-300g | 0.25 | 15, 30, and 60 days, 4-h/day | Progressive loss of dopamine reactivity in the substantia nigra, along with morphological changes. Increased p53 levels and nuclear translocation. |
| Thomson et al. (2007) | Rat; Fischer-344; M; 200-250g | 0.4; 0.8 | 4-h; assays at 0 and 24 h postexposure | At 0.8 ppm, $O_3$ produced rapid perturbations in the ET-NO pathway gene expression in the brain. Ozone induced a small but significant time- and concentration-dependent increase in prepro-endothelin-1 mRNA levels in the cerebral hemisphere and pituitary, whereas TNFa and iNOS mRNA levels were decreased at 0 h and unchanged or increased, respectively, at 24 h. |
| Alfaro-Rodríguez and González-Piña (2005) | Rat; Wistar; M; 292g | 0.5 | 24-h | During the light phase, $O_3$ caused a significant decrease in paradoxical sleep accompanied by a significant decrease in Ach levels in the hypothalamic medial preoptic area. The same effects occurred during the dark phase exposure to $O_3$ in addition to a significant increase in slow-wave sleep and decrease in wakefulness. |
| Araneda et al. (2008) | Rats; Sprague-Dawley; M; 280-320g | 0.5 | 3-h (measurements taken at 0 h and 3 h after exposure) | Ozone upregulated VEGF in astroglial cells located in the respiratory center of the brain. VEGF co-located with IL-6 and TNF in cells near blood vessel walls, and blood vessel area was markedly increased. |

| Study | Model | $O_3$ (ppm) | Exposure Duration | Effects |
|---|---|---|---|---|
| Boussouar et al. (2009) | Rat; Sprague-Dawley; M; adult offspring of prenatally exposed dams; 403-414g | 0.5 | From embryonic day E5 to E20 for 1-h/day; immobilization stress | Prenatal $O_3$ exposure had a long term impact on the nucleus tractus solitarius of adult rats, as revealed during immobilization stress. |
| Soulage et al. (2004) | Rat; Sprague-Dawley; M; Approx. 7 weeks old | 0.7 | 5-h | Ozone produced differential effects on peripheral and central components of the sympatho-adrenal system. While catecholamine biosynthesis was increased in portions of the brain, the catecholamine turnover rate was significantly increased in the heart and cerebral cortex and inhibited in the lung and striatum. |
| Calderón Guzmán et al. (2006); Calderón Guzmán et al. (2005) | Rat; Wistar; M; 21 days old; well-nourished and malnourished groups | 0.75 | 15 successive days for 4-h/day | A significant decrease in body weight was observed in both well nourished (WN) and malnourished (MN) rats after $O_3$ exposure. Localized ATPase, TBARS, and GSH levels changed in response to $O_3$ in certain brain areas and the $O_3$-induced changes were dependent on nutritional condition. |
| Colín-Barenque et al. (2005) | Rats; Wistar; M; 250-300g | 1.0 | 4-h; assays at 2-h, 24-h, 10 days, and 15 days after exposure | A significant loss of dendritic spines in granule cells of the olfactory bulb occurred at 2 hrs to 10 days after exposure. Cytological and ultrastructural changes returned toward normal morphology by 15 days. |
| Escalante-Membrillo et al. (2005) | Rats; Wistar; M; 280-320g | 1.0 | 1-, 3-, 6-, or 9-h | Significant increases in TBARS occurred in hypothalamus, cortex, striatum, midbrain, thalamus, and pons. Partial but significant recovery was observed by 3 h after the 9 h exposure. |
| Gonzalez-Pina et al. (2008) | Rat; Wistar; M; | 1 | 12-h/day, 21 days of gestation; assays at 0, 5, & 10 days postnatal | Prenatal $O_3$ exposure produced significant decreases in cerebellar monoamine but not indolamine content at 0 and 5 days after birth with a partial recovery by 10 days. 5-hydroxy-indole-acetic acid levels were significantly increased at 10 days. |

### 6.4.1 Neuroendocrine Effects

According to the 2006 $O_3$ AQCD, early studies suggested an interaction of $O_3$ with the pituitary-thyroid-adrenal axis, because thyroidectomy, hypophysectomy, and adrenalectomy protected against the lethal effects of $O_3$. Concentrations of 0.7-1.0 ppm $O_3$ for a 1-day exposure in male rats caused changes in the parathyroid, thymic atrophy, decreased serum levels of thyroid hormones and protein binding, and increased prolactin. Increased toxicity to $O_3$ was reported in hyperthyroid rats and T3 supplementation was shown to increase metabolic rate and pulmonary injury in the lungs of $O_3$-treated animals. The mechanisms by which $O_3$ affects neuroendocrine function are not well understood, but previous work suggests that high ambient levels of $O_3$ can produce marked neural disturbances in structures involved in the integration of chemosensory inputs, arousal, and motor control, effects that may be

responsible for some of the behavioral effects seen with $O_3$ exposure. A more recent study exposing immature female rats to 0.12 ppm $O_3$ demonstrated significantly increased serum levels of the thyroid hormone free $T_3$ after 15 days of exposure, whereas free $T_4$ was unchanged (Martrette et al., 2011). These results are in contrast to those previously presented whereby 1 ppm $O_3$ for 1 day significantly decreased $T_3$ and $T_4$ (Clemons and Garcia, 1980), although comparisons are made difficult by highly disparate exposure regimens along with sex differences. Martrette et al. (2011) also demonstrated significantly increased corticosterone levels after 15 days of exposure, suggesting a stress related response.

### 6.4.2 Summary and Causal Determination

In rodents, $O_3$ exposure has been shown to cause physicochemical changes in the brain indicative of oxidative stress and inflammation. Newer toxicological studies add to earlier evidence that acute exposures to $O_3$ can produce a range of effects on the central nervous system and behavior. Previously observed effects, including neurodegeneration, alterations in neurotransmitters, short and long term memory, and sleep patterns, have been further supported by recent studies. In instances where pathology and behavior are both examined, animals exhibit decrements in behaviors tied to the brain regions or chemicals found to be affected or damaged. For example, damage in the hippocampus, which is important for memory acquisition, was correlated with impaired performance in tests designed to assess memory. Thus the brain is functionally affected by $O_3$ exposure. The single epidemiologic study conducted showed an association between $O_3$ exposure and memory deficits in humans as well, albeit on a long-term exposure basis. Notably, exposure to $O_3$ levels as low as 0.25 ppm for 7 days has resulted in progressive neurodegeneration and deficits in both short and long-term memory in rodents. Examination of changes in the brain at lower exposure concentrations or at 0.25 ppm for shorter durations has not been reported, but 0.12 ppm $O_3$ has been shown to alter behavior. It is possible that some behavioral changes may reflect avoidance of irritation as opposed to functional changes in brain morphology or chemistry, but in many cases functional changes are related to oxidative stress and damage. In some instances, changes were dependent on the nutritional status of the rats (high versus low protein diet). For example, $O_3$ produced an increase in glutathione in the brains of rats fed the high protein diet but decreases in glutathione in rats fed low protein chow (Calderón Guzmán et al., 2006). The hippocampus, one of the main regions affected by age-related neurodegenerative diseases, appears to be more sensitive to oxidative damage in aged rats (Rivas-Arancibia et al., 2000), and growth hormone, which declines with age in most species, may be protective (Martínez-Canabal and Angora-Perez, 2008). Developing animals may also be sensitive, as changes in the CNS, including biochemical, cellular, and behavioral effects, have been observed in juvenile and adult animals whose sole exposure occurred in utero, at levels as a low as 0.3 ppm. A number of studies demonstrate $O_3$-induced changes that are also observed in human neurodegenerative disorders such as Alzheimer's and Parkinson's disease, including signs of oxidative stress, loss of neurons/neuronal death, reductions in

dopamine levels, increased COX-2 expression, and increases in activated microglia in important regions of the brain (hippocampus, substantia nigra).

Thus, evidence for neurological effects from epidemiologic and controlled human exposure studies is lacking. However, the toxicological evidence for the impact of $O_3$ on the brain and behavior is strong, and **suggestive of a causal relationship between $O_3$ exposure and effects on the central nervous system.**

## 6.5 Effects on Other Organ Systems

### 6.5.1 Effects on the Liver and Xenobiotic Metabolism

Early investigations of the effects of $O_3$ on the liver centered on xenobiotic metabolism, and the prolongation of drug-induced sleeping time, which was observed at 0.1 ppm $O_3$ (Graham et al., 1981). In some species, only adults and especially females were affected. In rats, high (1.0-2.0 ppm for 3 hours) acute $O_3$ exposures caused increased production of NO by hepatocytes and enhanced protein synthesis (Laskin et al., 1996; Laskin et al., 1994). Except for the earlier work on xenobiotic metabolism, the responses occurred only after very high acute $O_3$ exposures. One study, conducted at 1 ppm $O_3$ exposure, has been identified (Last et al., 2005) in which alterations in gene expression underlying $O_3$-induced cachexia and downregulation of xenobiotic metabolism were examined. A number of the downregulated genes are known to be interferon (IFN) dependent, suggesting a role for circulating IFN. A more recent study by Aibo et al. (2010) demonstrates exacerbation of acetaminophen-induced liver injury in mice after a single 6-hour exposure to 0.25 or 0.5 ppm $O_3$. Data indicate that $O_3$ may worsen drug-induced liver injury by inhibiting hepatic repair. The $O_3$-associated effects shown in the liver are thought to be mediated by inflammatory cytokines or other cytotoxic mediators released by activated macrophages or other cells in the lungs (Laskin and Laskin, 2001; Laskin et al., 1998; Vincent et al., 1996a). Recently, increased peroxidated lipids were detected in the plasma of $O_3$ exposed animals (Santiago-López et al., 2010).

In summary, mediators generated by $O_3$ exposure may cause effects on the liver in laboratory rodents. Ozone exposures as low as 0.1 ppm have been shown to affect drug-induced sleeping time, and exposure to 0.25 ppm can exacerbate liver injury induced by a common analgesic. However, very few studies at relevant concentrations have been conducted, and no data from controlled human exposure or epidemiologic studies are currently available. Therefore the collective evidence **is inadequate to determine if a causal relationship exists between short-term $O_3$ exposure and effects on the liver and metabolism.**

### 6.5.2 Effects on Cutaneous and Ocular Tissues

In addition to the lungs, the skin is highly exposed to $O_3$ and contains $O_3$ reactive targets (polyunsaturated fatty acids) that can produce lipid peroxides. The 2006 $O_3$ AQCD (U.S. EPA, 2006b) reported that although there is evidence of oxidative stress at near ambient $O_3$ concentrations, skin and eyes are only affected at high concentrations (greater than 1-5 ppm). Ozone exposure (0.8 ppm for 7 days) induces oxidative stress in the skin of hairless mice, along with proinflammatory cytokines (Valacchi et al., 2009). A recent study demonstrated that 0.25 ppm $O_3$ differentially alters expression of metalloproteinases in the skin of young and aged mice, indicating that age may potentially increase risk of oxidative stress (Fortino et al., 2007). In young mice, healing of skin wounds is not significantly affected by $O_3$ exposure (Lim et al., 2006). However, exposure to 0.5 ppm $O_3$ for 6 hours/day significantly delays wound closure in aged mice. As with effects on the liver described above, the effects of $O_3$ on the skin and eyes have not been widely studied, and information from controlled human exposure or epidemiologic studies is not currently available. Therefore **the collective evidence is inadequate to determine if a causal relationship exists between short-term $O_3$ exposure and effects on cutaneous and ocular tissues.**

## 6.6 Mortality

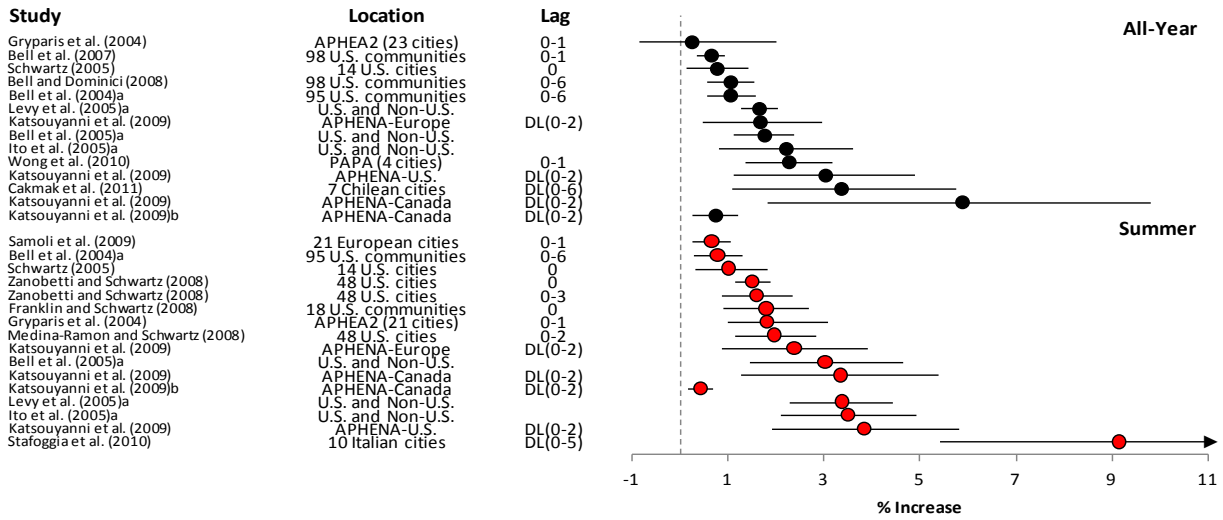
### 6.6.1 Summary of Findings from 2006 $O_3$ AQCD

The 2006 $O_3$ AQCD (U.S. EPA, 2006b) reviewed a large number of time-series studies consisting of single- and multicity studies, and meta-analyses. In the large U.S. multicity studies that examined all-year data, summary effect estimates corresponding to single-day lags ranged from a 0.5-1% increase in all-cause (nonaccidental) mortality per a standardized unit increase in $O_3$ of 20 ppb for 24-h avg, 30 ppb for 8-h max, and 40 ppb for 1-h max as discussed in Section 2.5. The association between short-term $O_3$ exposure and mortality was substantiated by a collection of meta-analyses and international multicity studies. The studies evaluated found some evidence for heterogeneity in $O_3$ mortality risk estimates across cities and studies. Studies that conducted seasonal analyses, although more limited in number, reported larger $O_3$ mortality risk estimates during the warm or summer season. Overall, the 2006 $O_3$ AQCD identified robust associations between various measures of daily ambient $O_3$ concentrations and all-cause mortality, with additional evidence for associations with cardiovascular mortality, which could not be readily explained by confounding due to time, weather, or copollutants. However, it was noted that multiple uncertainties remain regarding the $O_3$-mortality relationship including: the extent of residual confounding by copollutants; factors that modify the $O_3$-mortality association; the appropriate lag structure for identifying $O_3$-mortality effects (e.g., single-day lags versus distributed lag model); the shape of

the $O_3$-mortality C-R function and whether a threshold exists; and the identification of populations at-risk to $O_3$-related health effects. Collectively, the 2006 $O_3$ AQCD concluded that "the overall body of evidence is highly suggestive that $O_3$ directly or indirectly contributes to non-accidental and cardiopulmonary-related mortality."

### 6.6.2 Associations of Mortality and Short-Term $O_3$ Exposure

Recent studies that examined the association between short-term $O_3$ exposure and mortality further confirmed the associations reported in the 2006 $O_3$ AQCD. New multicontinent and multicity studies reported consistent positive associations between short-term $O_3$ exposure and all-cause mortality in all-year analyses, with additional evidence for larger mortality risk estimates during the warm or summer months (Figure 6-27 [and Table 6-42]). These associations were reported across a range of ambient $O_3$ concentrations that were in some cases quite low (Table 6-43).




Note: Effect estimates are for a 40 ppb increase in 1-h max, 30 ppb increase in 8-h max, and 20 ppb increase in 24-h avg $O_3$ concentrations. An "a" represent multicity studies and meta-analyses from the 2006 $O_3$ AQCD. Bell et al. (2005), Ito et al. (2005), and Levy et al. (2005) used a range of lag days in the meta-analysis: Lag 0, 1, 2, or average 0-1 or 1-2; single-day lags from 0 to 3; and lag 0 and 1-2; respectively. A "b" represents risk estimates from APHENA-Canada standardized to an approximate IQR of 5.1 ppb for a 1-h max increase in $O_3$ concentrations (see explanation in Section 6.2.7.2).

**Figure 6-27 Summary of mortality risk estimates for short-term $O_3$ exposure and all-cause (nonaccidental) mortality from all-year and summer season analyses.**

## Table 6-42 Corresponding effect estimates for Figure 6-27.

| Study* | Location | Lag | Avg Time | % Increase (95% CI) |
|---|---|---|---|---|
| **All-year** | | | | |
| Gryparis et al. (2004) | APHEA2 (23 cities) | 0-1 | 1-h max | 0.24 (-0.86, 1.98) |
| Bell et al. (2007) | 98 U.S. communities | 0-1 | 24-h avg | 0.64 (0.34, 0.92) |
| Schwartz (2005a) | 14 U.S. cities | 0 | 1-h max | 0.76 (0.13, 1.40) |
| Bell and Dominici (2008) | 98 U.S. communities | 0-6 | 24-h avg | 1.04 (0.56, 1.55) |
| Bell et al. (2004)[a] | 95 U.S. communities | 0-6 | 24-h avg | 1.04 (0.54, 1.55) |
| Levy et al. (2005)[a] | U.S. and Non-U.S. | --- | 24-h avg | 1.64 (1.25, 2.03) |
| Katsouyanni et al. (2009) | APHENA-europe | DL(0-2) | 1-h max | 1.66 (0.47, 2.94) |
| Bell et al. (2005)[a] | U.S. and Non-U.S. | --- | 24-h avg | 1.75 (1.10, 2.37) |
| Ito et al. (2005)[a] | U.S. and Non-U.S. | --- | 24-h avg | 2.20 (0.80, 3.60) |
| Wong et al. (2010) | PAPA (4 cities) | 0-1 | 8-h avg | 2.26 (1.36, 3.16) |
| Katsouyanni et al. (2009) | APHENA-U.S. | DL(0-2) | 1-h max | 3.02 (1.10, 4.89) |
| Cakmak et al. (2011) | 7 Chilean cities | DL(0-6) | 8-h max | 3.35 (1.07, 5.75) |
| Katsouyanni et al. (2009) | APHENA-Canada | DL(0-2) | 1-h max | 5.87 (1.82, 9.81) |
| Katsouyanni et al. (2009)[b] | APHENA-Canada | DL(0-2) | 1-h max | 0.73 (0.23, 1.20) |
| **Summer** | | | | |
| Samoli et al. (2009) | 21 European cities | 0-1 | 8-h max | 0.66 (0.24, 1.05) |
| Bell et al. (2004)[a] | 95 U.S. communities | 0-6 | 24-h avg | 0.78 (0.26, 1.30) |
| Schwartz (2005a) | 14 U.S. cities | 0 | 1-h max | 1.00 (0.30, 1.80) |
| Zanobetti and Schwartz (2008a) | 48 U.S. cities | 0 | 8-h max | 1.51 (1.14, 1.87) |
| Zanobetti and Schwartz (2008b) | 48 U.S. cities | 0-3 | 8-h max | 1.60 (0.84, 2.33) |
| Franklin and Schwartz (2008) | 18 U.S. communities | 0 | 24-h avg | 1.79 (0.90, 2.68) |
| Gryparis et al. (2004) | APHEA2 (21 cities) | 0-1 | 8-h max | 1.80 (0.99, 3.06) |
| Medina-Ramón and Schwartz (2008) | 48 U.S. cities | 0-2 | 8-h max | 1.96 (1.14, 2.82) |
| Katsouyanni et al. (2009) | APHENA-europe | DL(0-2) | 1-h max | 2.38 (0.87, 3.91) |
| Bell et al. (2005)[a] | U.S. and Non-U.S. | --- | 24-h avg | 3.02 (1.45, 4.63) |
| Katsouyanni et al. (2009) | APHENA-Canada | DL(0-2) | 1-h max | 3.34 (1.26, 5.38) |
| Katsouyanni et al. (2009) | APHENA-Canada | DL(0-2) | 1-h max | 0.42 (0.16, 0.67) |
| Levy et al. (2005)[a] | U.S. and Non-U.S. | --- | 24-h avg | 3.38 (2.27, 4.42) |
| Ito et al. (2005)[a] | U.S. and Non-U.S. | --- | 24-h avg | 3.50 (2.10, 4.90) |
| Katsouyanni et al. (2009) | APHENA-U.S. | DL(0-2) | 1-h max | 3.83 (1.90, 5.79) |
| Stafoggia et al. (2010) | 10 Italian cities | DL(0-5) | 8-h max | 9.15 (5.41, 13.0) |

*Studies included from Figure 6-27.

[a]Multicity studies and meta-analyses from the 2006 $O_3$ AQCD. Bell et al. (2005)[a], Ito et al. (2005)[a], and Levy et al. (2005)[a] used a range of lag days in the meta-analysis: Lag 0, 1, 2, or average 0-1 or 1-2; Single-day lags from 0-3; and Lag 0 and 1-2; respectively.

[b]Risk estimates from APHENA-Canada standardized to an approximate IQR of 5.1 ppb for a 1-h max increase in $O_3$ concentrations (see explanation in Section 6.2.7.2).

## Table 6-43 Range of mean and upper percentile $O_3$ concentrations in previous and recent multicity studies.

| Study | Location | Years | Averaging Time | Mean Concentration (ppb)[a] | Upper Percentile Concentrations (ppb)[a] |
|---|---|---|---|---|---|
| Gryparis et al. (2004)[b] | 23 European cities (APHEA2) | 1990-1997 | 1-h max<br>8-h max | Summer:<br>1-h max: 44-117<br>8-h max: 30-99<br>Winter:<br>1-h max: 11-57<br>8-h max: 8-49 | Summer:<br>1-h max: 62-173<br>8-h max: 57-154<br>Winter:<br>1-h max: 40-88<br>8-h max: 25-78 |
| Schwartz (2005a)[b] | 14 U.S. cities | 1986-1993 | 1-h max | 35.1-60 | 25th: 26.5-52<br>75th: 46.3-69 |
| Bell et al. (2004) | 95 U.S. communities (NMMAPS) | 1987-2000 | 24-h avg | 26.0 | NR |
| Bell et al. (2007) | 98 U.S. communities (NMMAPS) | 1987-2000 | 24-h avg | 26.0[d] | NR |
| Bell and Dominici (2008) | 98 U.S. communities (NMMAPS) | 1987-2000<br>(All year and May-Sept) | 24-h avg | All year: 26.8<br>May-September: 30.0 | Maximum:<br>All year: 37.3<br>May-September: 47.2 |
| Franklin and Schwartz (2008) | 18 U.S. communities | 2000-2005<br>(May-Sept) | 24-h avg | 21.4-48.7 | NR |
| Katsouyanni et al. (2009)[b,e] | NMMAPS<br>12 Canadian cities<br>(APHEA2) | 1987-1996 (Canada and U.S.) varied by city for Europe | 1-h max | U.S.: 13.3-38.4<br>Canada: 6.7-8.4<br>Europe:18.3-41.9 | 75th:<br>U.S.: 21.0-52.0<br>Canada: 8.7-12.5<br>Europe: 24.0-65.8 |
| Medina-Ramón and Schwartz (2008)[b] | 48 U.S. cities | 1989-2000<br>(May-Sept) | 8-h max | 16.1-58.8 | NR |
| Samoli et al. (2009)[b] | 21 European cities (APHEA2) | 1990-1997<br>(June-Aug) | 8-h max | 20.0-62.8 | 75th: 27.2-74.8 |
| Stafoggia et al. (2010) | 10 Italian cities | 2001-2005<br>(Apr-Sept) | 8-h max | 41.2-58.9 | 75th: 47.0-71.6 |
| Cakmak et al. (2011) | 7 Chilean cities | 1997-2007 | 8-h max | 59.0-87.6 | NR |
| Wong et al. (2010) | PAPA (4 cities) | 1999-2003 (Bangkok)<br>1996-2002 (Hong Kong)<br>2001-2004 (Shanghai)<br>2001-2004 (Wuhan) | 8-h avg | 18.7-43.7 | 75th: 38.4 - 60.4<br>Max: 92.1 - 131.8 |
| Zanobetti and Schwartz (2008b) | 48 U.S. cities | 1989-2000<br>(June-Aug) | 8-h max | 15.1-62.8 | Max: 34.3-146.2<br>75th: 19.8-75.9 |

| Study | Location | Years | Averaging Time | Mean Concentration (ppb)[a] | Upper Percentile Concentrations (ppb)[a] |
|---|---|---|---|---|---|
| Zanobetti and Schwartz (2008a) | 48 U.S. citiesc | 1989-2000<br>(Winter: Dec-Feb)<br>(Spring: Mar-May)<br>(Summer: June-Aug)<br>(Autumn: Sept-Nov) | 8-h max | Winter: 16.5<br>Spring: 41.6<br>Summer: 47.8<br>Autumn: 33.5 | Max:<br>Winter: 40.6<br>Spring: 91.4<br>Summer: 103.0<br>Autumn: 91.2 |

[a]Ozone concentrations were converted to ppb if the study presented them as μg/m$^3$ by using the conversion factor of 0.51 assuming standard temperature (25° C) and pressure (1 atm).

[b]Study only reported median $O_3$ concentrations.

[c]Cities with less than 75% observations in a season excluded. As a result, 29 cities examined in winter, 32 in spring, 33 in autumn, and all 48 in the summer.

[d]Bell et al. (2007) did not report mean $O_3$ concentrations, however, it used a similar dataset as Bell et al. (2004) which consisted of 95 U.S. communities for 1987-2000. For comparison purposes the 24-h avg $O_3$ concentrations for the 95 communities from Bell et al. (2004) are reported here.

[e]Study did not present air quality data for the summer months.

In addition to examining the relationship between short-term $O_3$ exposure and all-cause mortality, recent studies attempted to address the uncertainties that remained upon the completion of the 2006 $O_3$ AQCD. As a result, given the robust associations between short-term $O_3$ exposure and mortality presented across studies in the 2006 $O_3$ AQCD and supported in the new multicity studies (Figure 6-27), the following sections primarily focus on the examination of previously identified uncertainties in the $O_3$-mortality relationship, specifically: confounding, effect modification (i.e., sources of heterogeneity in risk estimates across cities), the $O_3$-mortality C-R relationship including lag structure (e.g., multiday effects and mortality displacement), and $O_3$ associations with cause-specific mortality. Focusing specifically on these uncertainties allows for a more detailed characterization of the relationship between short-term $O_3$ exposure and mortality.

### 6.6.2.1 Confounding

Recent epidemiologic studies examined potential confounders of the $O_3$-mortality relationship. These studies specifically focused on whether PM and its constituents or seasonal trends confounded the association between short-term $O_3$ exposure and mortality.

#### Confounding by PM and PM Constituents

An important question in the evaluation of the association between short-term $O_3$ exposure and mortality is whether the relationship is confounded by particulate matter, particularly the PM chemical components that are found in the "summer haze" mixture which also contains $O_3$. However, because of the temporal correlation

among these PM components and $O_3$, and their possible interactions, the interpretation of results from copollutant models that attempt to disentangle the health effects associated with each pollutant is challenging. Further complicating the interpretation of copollutant results, at times, is the every-3rd or -6th day PM sampling schedule employed in most locations, which limits the number of days where both PM and $O_3$ data is available.

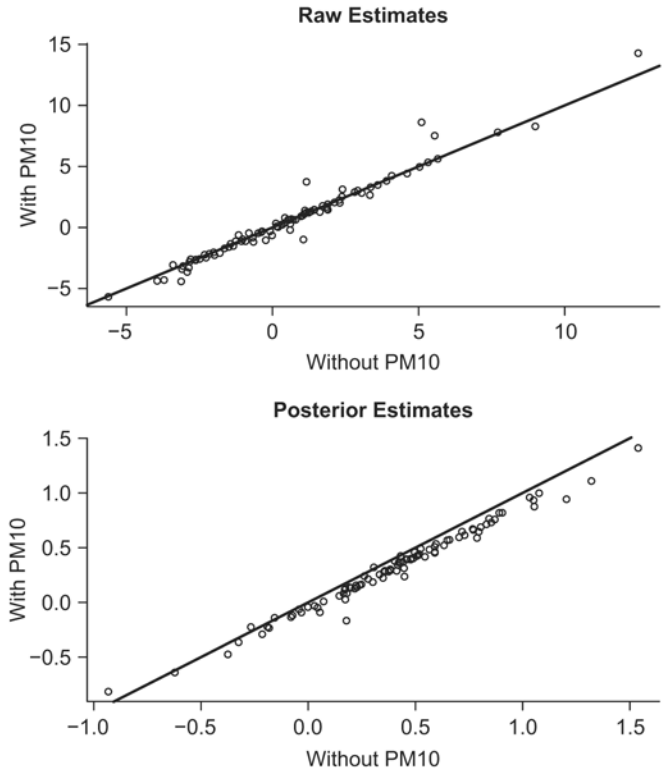
The potential confounding effects of $PM_{10}$ and $PM_{2.5}$ on the $O_3$-mortality relationship were examined by Bell et al. (2007) using data on 98 U.S. urban communities for the years 1987-2000 from the National Morbidity, Mortality, and Air Pollution Study (NMMAPS). In this analysis the authors included PM as a covariate in time-series models, and also examined $O_3$-mortality associations on days when $O_3$ concentrations were below a specified value. This analysis was limited by the small fraction of days when both PM and $O_3$ data were available, due to the every-3rd - or 6th -day sampling schedule for the PM indices, and the limited amount of city-specific data for $PM_{2.5}$ because it was only collected in most cities since 1999. As a result, of the 91 communities with $PM_{2.5}$ data, only 9.2% of days in the study period had data for both $O_3$ and $PM_{2.5}$, resulting in the use of only 62 communities in the $PM_{2.5}$ analysis. An examination of the correlation between PM ($PM_{10}$ and $PM_{2.5}$) and $O_3$ across various strata of daily $PM_{10}$ and $PM_{2.5}$ concentrations found that neither PM size fraction was highly correlated with daily $O_3$ concentrations across any of the strata examined. These results were also observed when using 8-h max and 1-h max $O_3$ exposure metrics. National and community-specific effect estimates of the association between short-term $O_3$ exposure and mortality were robust to inclusion of $PM_{10}$ or $PM_{2.5}$ in time-series models through the range of $O_3$ concentrations (i.e., <10 ppb, 10-20, 20-40, 40-60, 60-80, and >80 ppb). Even with the small number of days in which both $PM_{2.5}$ and $O_3$ data was available, the percent increases in nonaccidental deaths per 10 ppb increase 24-h avg $O_3$ concentrations at lag 0-1 day were 0.22% (95% CI: -0.22, 0.65) without $PM_{2.5}$ and 0.21% (95% CI: -0.22, 0.64) with $PM_{2.5}$ in 62 communities.

Although strong correlations between PM and $O_3$ were not reported by Bell et al. (2007) the patterns observed suggest regional differences in their correlation (Table 6-44). Both $PM_{10}$ and $PM_{2.5}$ show positive correlations with $O_3$ in the Industrial Midwest, Northeast, Urban Midwest, and Southeast, especially in the summer months, presumably, because of the summer peaking sulfate. However, the mostly negative or weak correlations between PM and $O_3$ in the summer in the Southwest, Northwest, and southern California could be due to winter-peaking nitrate. Thus, the potential confounding effect of PM on the $O_3$-mortality relationship could be influenced by the relative contribution of sulfate and nitrate, which varies regionally and seasonally.

## Table 6-44 Correlations between PM and $O_3$ by season and region.

| | No. of Communities | Winter | Spring | Summer | Fall | Yearly |
|---|---|---|---|---|---|---|
| **$PM_{10}$** | | | | | | |
| Industrial Midwest | 19 | 0.37 | 0.44 | 0.44 | 0.39 | 0.41 |
| Northeast | 15 | 0.34 | 0.44 | 0.36 | 0.44 | 0.40 |
| Urban Midwest | 6 | 0.24 | 0.25 | 0.22 | 0.26 | 0.24 |
| Southwest | 9 | 0.00 | 0.02 | -0.02 | 0.10 | 0.03 |
| Northwest | 11 | -0.17 | -0.20 | -0.13 | -0.11 | -0.16 |
| Southern California | 7 | 0.19 | 0.08 | 0.12 | 0.19 | 0.14 |
| Southeast | 25 | 0.33 | 0.35 | 0.31 | 0.31 | 0.32 |
| U.S. | 93 | 0.23 | 0.26 | 0.24 | 0.26 | 0.25 |
| **$PM_{2.5}$** | | | | | | |
| Industrial Midwest | 19 | 0.18 | 0.39 | 0.43 | 0.44 | 0.36 |
| Northeast | 13 | 0.05 | 0.26 | 0.16 | 0.43 | 0.25 |
| Urban Midwest | 4 | 0.22 | 0.31 | 0.15 | 0.32 | 0.20 |
| Southwest | 9 | -0.15 | -0.08 | -0.17 | -0.15 | -0.14 |
| Northwest | 11 | -0.32 | -0.34 | -0.39 | -0.24 | -0.31 |
| Southern California | 7 | -0.25 | -0.22 | -0.25 | -0.15 | -0.23 |
| Southeast | 26 | 0.38 | 0.47 | 0.30 | 0.37 | 0.39 |
| U.S. | 90 | 0.09 | 0.21 | 0.12 | 0.22 | 0.16 |

Source: Bell et al. (2007).



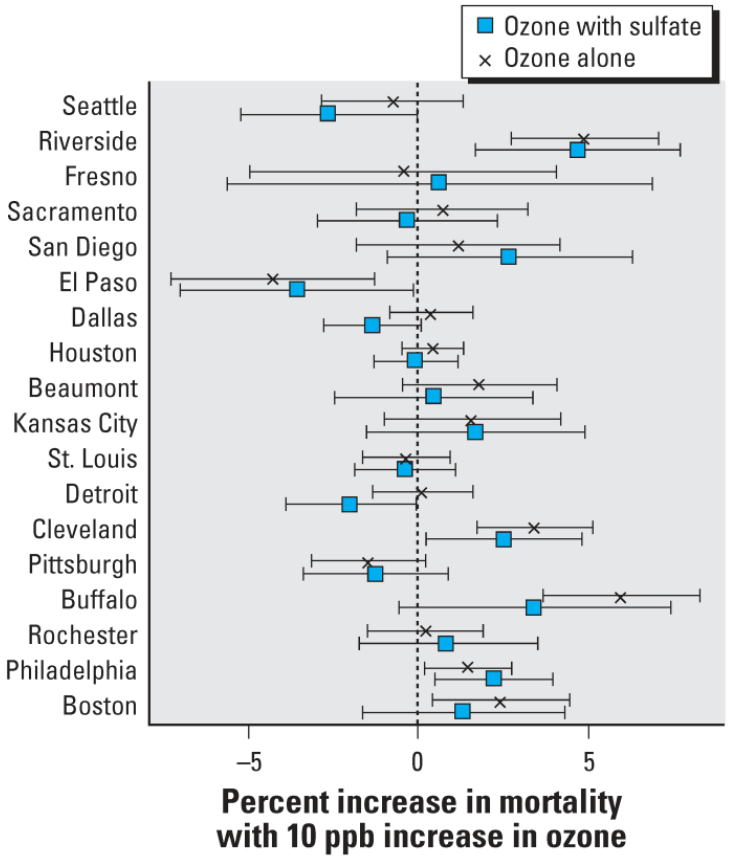

Note: The diagonal line indicates 1:1 ratio.
Source: Reprinted with permission of Informa UK Ltd, (Smith et al., 2009b).

**Figure 6-28 Scatter plots of $O_3$ mortality risk estimates with versus without adjustment for $PM_{10}$ in NMMAPS cities.**

In an attempt to reassess a number of issues associated with the $O_3$-mortality relationship, including confounding, Smith et al. (2009b) re-analyzed the publicly available NMMAPS database for the years 1987-2000. Similar to Bell et al. (2007), the $PM_{10}$ data used in the Smith et al. (2009b) analysis consisted primarily of every-6th day data. In analyses conducted to examine the potential confounding effects of $PM_{10}$, the authors reported that, in most cases, $O_3$ mortality risk estimates were reduced by between 22% and 33% in copollutant models. This is further highlighted in Figure 6-28, which shows scatter plots of $O_3$-mortality risk estimates with adjustment for $PM_{10}$ versus without adjustment for $PM_{10}$. Smith et al. (2009b) point out that a larger fraction (89 out of 93) of the posterior estimates lie below the diagonal line (i.e., estimates are smaller with $PM_{10}$ adjustment) compared to the raw estimates (56 out of 93). This observation could be attributed to both sets of posterior estimates being calculated by "shrinking towards the mean" along with the small number of days where both $PM_{10}$ and $O_3$ data was available. However, the most prominent feature of these plots is that the variation of $O_3$-mortality risk estimates across cities is much larger than the impact of $PM_{10}$ adjustment on the $O_3$-mortality relationship.

Franklin and Schwartz (2008) examined the sensitivity of $O_3$ mortality risk estimates to the inclusion of $PM_{2.5}$ or PM chemical components associated with secondary aerosols (e.g., sulfate [$SO_4^{2-}$], organic carbon [OC], and nitrate [$NO_3$-]) in copollutant models. This analysis consisted of between 3 and 6 years of data from May through September 2000-2005 from 18 U.S. communities. The association between $O_3$ and non-accidental mortality was examined in single-pollutant models and after adjustment for $PM_{2.5}$, sulfate, organic carbon, or nitrate concentrations. The single-city effect estimates were combined into an overall estimate using a random-effects model. In the single-pollutant model, the authors found a 0.89% (95% CI: 0.45, 1.33%) increase in nonaccidental mortality with a 10 ppb increase in same-day 24-hour summertime $O_3$ concentrations across the 18 U.S. communities. Adjustment for $PM_{2.5}$ mass, which was available for 84% of the days, decreased the $O_3$-mortality risk estimate only slightly (from 0.88% to 0.79%), but the inclusion of sulfate in the model reduced the risk estimate by 31% (from 0.85% to 0.58%). However, sulfate data were only available for 18% of the days. Therefore, a limitation of this study is the limited amount of data for $PM_{2.5}$ chemical components due to the every-3rd-day or every-6th-day sampling schedule. For example, when using a subset of days when organic carbon measurements were available (i.e., 17% of the available days), $O_3$ mortality risk estimates were reduced to 0.51% (95% CI: -0.36 to 1.36) in a single-pollutant model.

Consistent with the studies previously discussed, the results from Franklin and Schwartz (2008) also demonstrate that the interpretation of the potential confounding effects of copollutants on $O_3$ mortality risk estimates is not straightforward as a result of the PM sampling schedule employed in most cities. However, Franklin and Schwartz (2008) find that $O_3$-mortality risk estimates, although attenuated in some cases (i.e., sulfate), remain positive. As presented in Figure 6-29, the regional and city-to-city variations in $O_3$ mortality risk estimates appear greater than the impact of adjusting for copollutants. In addition, in some cases, a negative $O_3$ mortality risk estimate becomes even more negative with the inclusion of sulfate (e.g., Seattle) in a copollutant model, or a null $O_3$ mortality risk estimate becomes negative when sulfate is included (e.g., Dallas and Detroit). Thus, the reduction in the overall $O_3$ mortality risk estimate (i.e., across cities) needs to be assessed in the context of the heterogeneity in the single-city estimates.




Source: Franklin and Schwartz (2008).

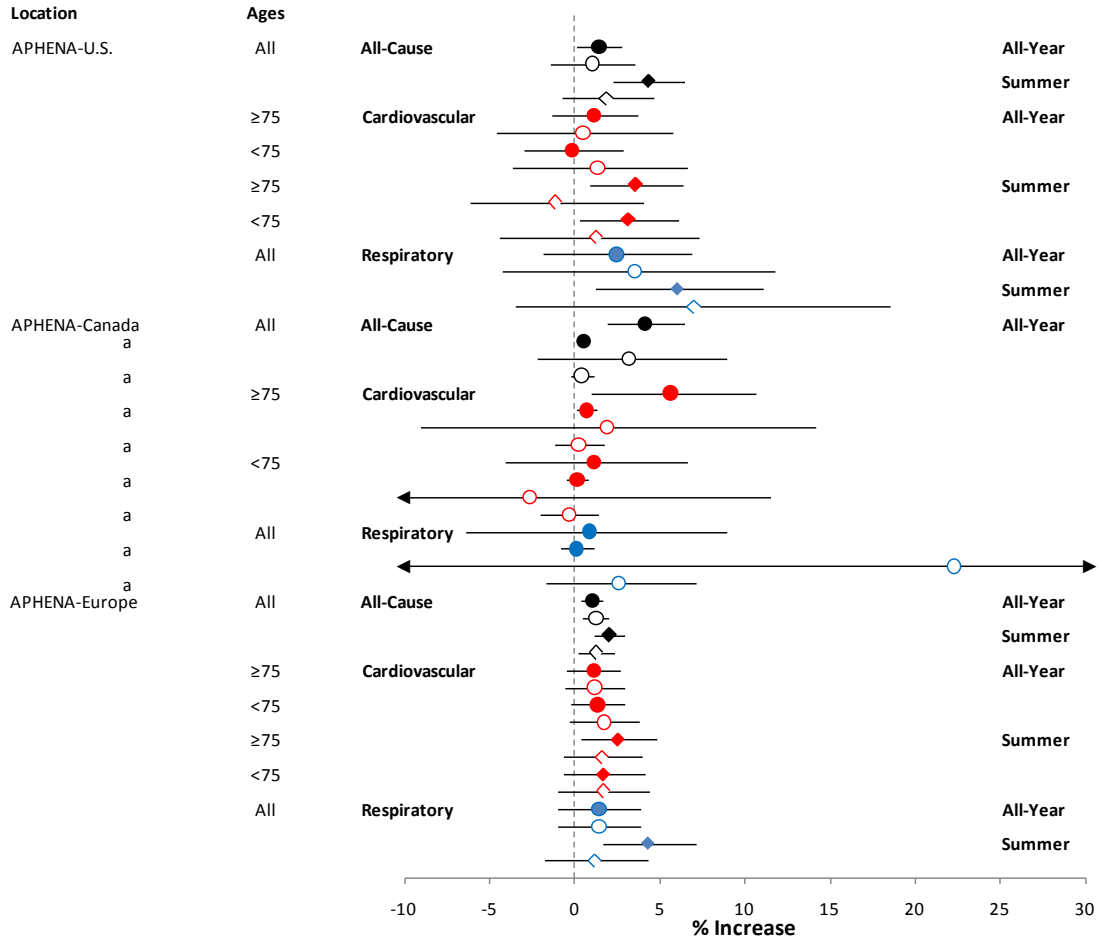
**Figure 6-29 Community-specific $O_3$-mortality risk estimates for nonaccidental mortality per 10 ppb increase in same-day 24-h average summertime $O_3$ concentrations in single-pollutant models and copollutant models with sulfate.**

In the APHENA study, the investigators from the U.S. (NMMAPS), Canadian, and European (APHEA2) multicity studies collaborated and conducted a joint analysis of $PM_{10}$ and $O_3$ using each of these datasets (Katsouyanni et al., 2009). For mortality, each dataset consisted of a different number of cities and years of air quality data: U.S. encompassed 90 cities with daily $O_3$ data from 1987-1996 of which 36 cities had summer only $O_3$ measurements; Europe included 23 cities with 3-7 years of daily $O_3$ data during 1990-1997; and Canada consisted of 12 cities with daily $O_3$ data from 1987 to 1996. As discussed in Section 6.2.7.2, the APHENA study conducted extensive sensitivity analyses, of which the 8 df/year results for both the penalized spline (PS) and natural spline (NS) models are presented in the text for comparison purposes, but only the NS results are presented in figures because alternative spline models have previously been shown to result in similar effect estimates (HEI, 2003).

Additionally, for the Canadian results, figures contain risk estimates standardized to a 40 ppb increment for 1-h max $O_3$ concentrations, consistent with the rest of the ISA, but also standardized to the approximate IQR across the Canadian cities as discussed previously (Section 6.2.7.2).

In the three datasets, the authors found generally positive associations between short-term $O_3$ exposure and all-cause, cardiovascular, and respiratory mortality. The estimated excess risks for $O_3$ were larger for the Canadian cities than for the U.S. and European cities. When examining the potential confounding effects of $PM_{10}$ on $O_3$ mortality risk estimates, the sensitivity of the estimates varied across the data sets and age groups. In the Canadian dataset, $O_3$ risk estimates were modestly reduced, but remained positive, when adjusting for $PM_{10}$ for all-cause mortality for all ages in the PS (4.5% [95% CI: 2.2, 6.7%]) and NS (4.2% [95% CI: 1.9, 6.5%]) models to 3.8% (95% CI: -1.4, 9.8%) and 3.2% (95% CI: -2.2, 9.0%), respectively, at lag 1 for a 40 ppb increase in 1-h max $O_3$ concentrations (Figure 6-30 [and Table 6-45]). However, adjusting for $PM_{10}$ reduced $O_3$ mortality risk estimates in the $\geq$ 75-year age group, but increased the risk estimates in the <75-year age group. For cardiovascular and respiratory mortality more variable results were observed with $O_3$ risk estimates being reduced and increased, respectively, in copollutant models with $PM_{10}$ (Figure 6-30 [and Table 6-45]). Unlike the European and U.S. datasets, the Canadian dataset only conducted copollutant analyses at lag 1; as a result, to provide a comparison across study locations only the lag 1 results are presented for the European and U.S. datasets in this section.

In the European data, $O_3$ risk estimates were robust when adjusting for $PM_{10}$ in the year-round data for all-cause, cardiovascular and respiratory mortality. When restricting the analysis to the summer months moderate reductions were observed in $O_3$ risk estimates for all-cause mortality with more pronounced reductions in respiratory mortality. In the U.S. data, adjusting for $PM_{10}$ moderately reduced $O_3$ risk estimates for all-cause mortality in a year-round analysis at lag 1 (e.g., both the PS and NS models were reduced from 0.18% to 0.13%) (Figure 6-30 [and Table 6-45]). Similar to the European data, when restricting the analysis to the summer months, in the United States. Ozone mortality risk estimates were moderately reduced, but remained positive, when adjusting for $PM_{10}$ for all-cause mortality. However, when examining cause-specific mortality risk estimates, consistent with the results from the Canadian dataset, which employed a similar PM sampling strategy (i.e., every-6th-day sampling), $O_3$ risk estimates for cardiovascular and respiratory mortality were more variable (i.e., reduced or increased in all-year and summer analyses). Overall, the estimated $O_3$ risks appeared to be moderately to substantially sensitive to inclusion of $PM_{10}$ in copollutant models. Despite the multicity approach, the mostly every-6th-day sampling schedule for $PM_{10}$ in the Canadian and U.S. datasets greatly reduced the sample size and limits the interpretation of these results.




Note: Effect estimates are for a 40 ppb increase in 1-h max $O_3$ concentrations at lag 1. All estimates are for the 8 df/year model with natural splines. Circles represent all-year analysis results while diamonds represent summer season analysis results. Open circles and diamonds represent copollutant models with $PM_{10}$. Black = all-cause mortality; red = cardiovascular mortality; and blue = respiratory mortality.

[a]Risk estimates from APHENA-Canada standardized to an approximate IQR of 5.1 ppb for a 1-h max increase in $O_3$ concentrations (see explanation in Section 6.2.7.2).

**Figure 6-30 Percent increase in all-cause (nonaccidental) and cause-specific mortality from natural spline models with 8 df/yr from the APHENA study for single- and copollutant models.**

## Table 6-45 Corresponding effect estimates for Figure 6-30.

| Location* | Mortality | Ages | Season | Copollutant | % Increase (95% CI) |
|---|---|---|---|---|---|
| **APHENA-U.S.** | All-Cause | All | All-year | | 1.42 (0.08, 2.78) |
| | | | | $PM_{10}$ | 1.02 (-1.40, 3.50) |
| | | | Summer | | 4.31 (2.22, 6.45) |
| | | | | $PM_{10}$ | 1.90 (-0.78, 4.64) |
| | Cardiovascular | ≥ 75 | All-year | | 1.10 (-1.33, 3.67) |
| | | | | $PM_{10}$ | 0.47 (-4.61, 5.79) |
| | | <75 | All-year | | -0.16 (-3.02, 2.86) |
| | | | | $PM_{10}$ | 1.34 (-3.63, 6.61) |
| | | ≥ 75 | Summer | | 3.58 (0.87, 6.37) |
| | | | | $PM_{10}$ | -1.17 (-6.18, 4.07) |
| | | <75 | Summer | | 3.18 (0.31, 6.12) |
| | | | | $PM_{10}$ | 1.26 (-4.46, 7.28) |
| | Respiratory | All | All-year | | 2.46 (-1.87, 6.86) |
| | | | | $PM_{10}$ | 3.50 (-4.23, 11.8) |
| | | | Summer | | 6.04 (1.18, 11.1) |
| | | | | $PM_{10}$ | 7.03 (-3.48, 18.5) |
| **APHENA-Canada** | All-Cause | All | All-year | | 4.15 (1.90, 6.45) |
| | | | | | 0.52 (0.24, 0.80)[a] |
| | | | | $PM_{10}$ | 3.18 (-2.18, 8.96) |
| | | | | $PM_{10}$ | 0.40 (-0.28, 1.10)[a] |
| | Cardiovascular | ≥ 75 | All-year | | 5.62 (0.95, 10.7) |
| | | | | | 0.70 (0.12, 1.30)[a] |
| | | | | $PM_{10}$ | 1.90 (-9.03, 14.1) |
| | | | | $PM_{10}$ | 0.24 (-1.20, 1.70)[a] |
| | | <75 | All-year | | 1.10 (-4.08, 6.61) |
| | | | | | 0.14 (-0.53, 0.82)[a] |
| | | | | $PM_{10}$ | -2.64 (-14.7, 11.5) |
| | | | | $PM_{10}$ | -0.34 (-2.00, 1.40)[a] |
| | Respiratory | All | All-year | | 0.87 (-6.40, 8.96) |
| | | | | | 0.11 (-0.84, 1.10)[a] |
| | | | | $PM_{10}$ | 22.3 (-12.6, 71.3) |
| | | | | $PM_{10}$ | 2.60 (-1.70, 7.10)[a] |

| Location* | Mortality | Ages | Season | Copollutant | % Increase (95% CI) |
|---|---|---|---|---|---|
| APHENA-Europe | All-Cause | All | All-year | | 1.02 (0.39, 1.66) |
| | | | | $PM_{10}$ | 1.26 (0.47, 1.98) |
| | | | Summer | | 2.06 (1.10, 2.94) |
| | | | | $PM_{10}$ | 1.26 (0.16, 2.30) |
| | Cardiovascular | ≥ 75 | All-year | | 1.10 (-0.47, 2.70) |
| | | | | $PM_{10}$ | 1.18 (-0.55, 2.94) |
| | | <75 | All-year | | 1.34 (-0.24, 2.94) |
| | | | | $PM_{10}$ | 1.74 (-0.31, 3.75) |
| | | ≥ 75 | Summer | | 2.54 (0.39, 4.80) |
| | | | | $PM_{10}$ | 1.58 (-0.70, 3.99) |
| | | <75 | Summer | | 1.66 (-0.70, 4.15) |
| | | | | $PM_{10}$ | 1.66 (-1.02, 4.40) |
| | Respiratory | All | All-year | | 1.42 (-1.02, 3.83) |
| | | | | $PM_{10}$ | 1.42 (-1.02, 3.83) |
| | | | Summer | | 4.31 (1.66, 7.11) |
| | | | | $PM_{10}$ | 1.18 (-1.79, 4.31) |

*Effect estimates from Figure 6-30.

[a]Risk estimates from APHENA-Canada standardized to an approximate IQR of 5.1 ppb for a 1-h max increase in $O_3$ concentrations (see explanation in Section 6.2.7.2).

Stafoggia et al. (2010) examined the potential confounding effects of $PM_{10}$ on the $O_3$-mortality relationship in individuals 35 years of age and older in 10 Italian cities from 2001 to 2005. In a time-stratified case-crossover analysis, using data for the summer months (i.e., April-September), the authors examined $O_3$-mortality associations across each city, and then obtained a pooled estimate through a random-effects meta-analysis. Stafoggia et al. (2010) found a strong association with nonaccidental mortality (9.2% [95% CI: 5.4, 13.0%] for a 30 ppb increase in 8-h max $O_3$ concentrations) in an unconstrained distributed lag model (lag 0-5) that persisted in copollutant models with $PM_{10}$ (9.2% [95% CI: 5.4, 13.7%]). Additionally, when examining cause-specific mortality, the authors found positive associations between short-term $O_3$ exposure and cardiovascular (14.3% [95% CI: 6.7, 22.4%]), cerebrovascular (8.5% [95% CI: 0.1, 16.3%]), and respiratory (17.6% [95% CI: 1.8, 35.6%]) mortality in single-pollutant models. In copollutant models, $O_3$-mortality effect estimates for cardiovascular and cerebrovascular mortality were robust to the inclusion of $PM_{10}$ (9.2% [95% CI: 5.4, 13.7%]) and 7.3% [95% CI: -1.2, 16.3%], respectively), and attenuated, but remained positive, for respiratory mortality (9.2% [95% CI: -6.9, 28.8%]). Of note, the correlations between $O_3$ and $PM_{10}$ across cities were found to be generally low, ranging from (-0.03 to 0.49). The authors do not specify the sampling strategy used for $PM_{10}$ in this analysis.

### Confounding by Seasonal Trend

The APHENA study (Katsouyanni et al., 2009), mentioned above, also conducted extensive sensitivity analyses to identify the appropriate: (1) smoothing method and basis functions to estimate smooth functions of time in city-specific models; and (2) degrees of freedom to be used in the smooth functions of time, to adjust for seasonal trends. Because $O_3$ peaks in the summer and mortality peaks in the winter, not adjusting or not sufficiently adjusting for the seasonal trend would result in an apparent negative association between the $O_3$ and mortality time-series. Katsouyanni et al. (2009) examined the effect of the extent of smoothing for seasonal trends by using models with 3 df/year, 8 df/year (the choice for their main model), 12 df/year, and df/year selected using the sum of absolute values of partial autocorrelation function of the model residuals (PACF) (i.e., choosing the degrees of freedom that minimizes positive and negative autocorrelations in the residuals). Table 6-46 presents the results of the degrees of freedom analysis using alternative methods to calculate a combined estimate: the Berkey et al. (1998) meta-regression and the two-level normal independent sampling estimation (TLNISE) hierarchical method. The results show that the methods used to combine single-city estimates did not influence the overall results, and that neither 3 df/year nor choosing the df/year by minimizing the sum of absolute values of PACF of regression residuals was sufficient to adjust for the seasonal negative relationship between $O_3$ and mortality. However, it should be noted, the majority of studies in the literature that examined the mortality effects of short-term $O_3$ exposure, particularly the multicity studies, used 7 or 8 df/year to adjust for seasonal trends, and in both methods a positive association was observed between $O_3$ exposure and mortality.

**Table 6-46 Sensitivity of $O_3$ risk estimates per 10 μg/m$^3$ increase in 24-h average $O_3$ concentrations at lag 0-1 to alternative methods for adjustment of seasonal trend, for all-cause mortality using Berkey MLE and TLNISE Hierarchical Models.**

| Seasonality Control | Berkey | TLNISE |
|---|---|---|
| 3 df/year | -0.54 (-0.88, 0.20) | -0.55 (-0.88, -0.22) |
| 8 df/year | 0.30 (0.11, 0.50) | 0.31 (0.09, 0.52) |
| 12 df/year | 0.34 (0.15, 0.53) | 0.33 (0.12, 0.54) |
| PACF | -0.62 (-1.01, -0.22) | -0.62 (-0.98, -0.27) |

Source: Reprinted with permission of Health Effects Institute (Katsouyanni et al., 2009).

## 6.6.2.2 Effect Modification

Epidemiologic studies have examined potential effect modifiers of the $O_3$-mortality relationship through the use of either: (1) time-invariant factors or (2) time-variant factors. There have been several multicity studies that examined potential effect

modifiers, or time-invariant factors, which may modify $O_3$ mortality risk estimates. These effect modifiers can be categorized into either individual-level or community-level characteristics, which are traditionally examined in second stage regression models. The results from these analyses also inform upon whether certain populations are at greater risk of an $O_3$-related health effect (Chapter 8). In addition to potentially modifying the association between short-term $O_3$ exposure and mortality, both individual-level and community-level characteristics may contribute to the geographic pattern of spatial heterogeneity in $O_3$ mortality risk estimates. As a result, the geographic pattern of $O_3$ mortality risk estimates is also evaluated in this section. Although less common, this section also evaluates those studies that examine effect modification by using time-variant factors, such as temperature and copollutants that are included in first stage time-series regression models.

## Time-Invariant Factors

### Individual-Level Characteristics

Medina-Ramón and Schwartz (2008) conducted a case-only study in 48 U.S. cities to identify populations potentially at increased risk to $O_3$-related mortality for the period 1989-2000 (May through September of each year [i.e., warm season]). A case-only design predicts the occurrence of time-invariant characteristics among cases as a function of the exposure level (Armstrong, 2003). For each potential effect modifier (time-invariant individual-level characteristics), city-specific logistic regression models were fitted, and the estimates were pooled across all cities. Furthermore, the authors examined potential differences in individual effect modifiers according to several city characteristics (e.g., mean $O_3$ level, mean temperature, households with central air conditioning, and population density) in a meta-regression. Across cities, the authors found a 1.96% (95% CI: 1.14-2.82%) increase in mortality at lag 0-2 for a 30 ppb increase in 8-h max $O_3$ concentrations. Additionally, Medina-Ramón and Schwartz (2008) examined a number of individual-level characteristics (e.g., age, race) and chronic conditions (e.g., secondary causes of death) as effect modifiers of the association between short-term $O_3$ exposure and mortality. The authors found that older adults (i.e., $\geq$ 65), women >60 years of age, black race, and secondary atrial fibrillation showed the greatest additional percent change in $O_3$-related mortality (Table 6-47). When examining city-level characteristics, the authors found that older adults, black race, and secondary atrial fibrillation had a larger effect on $O_3$ mortality risk estimates in cities with lower mean $O_3$ concentrations. Of note, a similar case-only study (Schwartz, 2005b) examined potential effect modifiers of the association between temperature and mortality, which would be expected to find results consistent with the Medina-Ramón and Schwartz (2008) study due to the high correlation between temperature and $O_3$. However, when stratifying days by temperature Schwartz (2005b) found strong evidence that diabetes modified the temperature-mortality association on hot days, which was not as evident when examining the $O_3$-mortality association in Medina-Ramón and Schwartz (2008). This difference could be due to the study design and populations included in both studies, a multicity study including all ages (Medina-Ramón and Schwartz, 2008) compared

to a single-city study of individuals ≥ 65 years of age (Schwartz, 2005b). However, when examining results stratified by race, nonwhites were found to have higher mortality risks on both hot and cold days, which provide some support for the additional risk found for black race in Medina-Ramón and Schwartz (2008).

Individual-level factors that may result in increased risk of $O_3$-related mortality were also examined by Stafoggia et al. (2010). As discussed above, using a time-stratified case-crossover analysis, the authors found an association between short-term $O_3$ exposure and nonaccidental mortality in an unconstrained distributed lag model in 10 Italian cities (9.2% [95% CI: 5.4, 13.0%; lag 0-5 for a 30 ppb increase in 8-h max $O_3$ concentrations). Stafoggia et al. (2010) conducted additional analyses to examine whether age, sex, income level, location of death, and underlying chronic conditions increased the risk of $O_3$-related mortality, but data were only available for nine of the cities for these analyses. Of the individual-level factors examined, the authors found the strongest evidence for increased risk of $O_3$-related mortality in individuals ≥ 85 years of age (22.4% [95% CI: 15.0, 30.2%]), women (13.7% [95% CI: 8.5, 19.7%]), and out-of-hospital deaths (13.0% [95% CI: 6.0, 20.4%]). When focusing specifically on out-of hospital deaths and the subset of individuals with chronic conditions, Stafoggia et al. (2010) found the strongest association for individuals with diabetes, which is consistent with the potentially increased risk of diabetics on hot days observed in Schwartz (2005b).

**Table 6-47 Additional percent change in $O_3$-related mortality for individual-level characteristics.**

| | Percentage | (95% CI) |
|---|---|---|
| **Socio-demographic characteristics** | | |
| Age 65 yr or older | 1.10 | 0.44, 1.77 |
| Women | 0.58 | 0.18, 0.98 |
| Women <60 yr old[b] | -0.09 | -0.76, 0.58 |
| Women ≥ 60 yr old[b] | 0.60 | 0.25, 0.96 |
| Black race | 0.53 | 0.19, 0.87 |
| Low education | -0.29 | -0.81, 0.23 |
| **Chronic conditions (listed as secondary cause)** | | |
| **Respiratory system diseases** | | |
| Asthma | 1.35 | -0.31, 3.03 |
| COPD | 0.01 | -0.49, 0.52 |
| **Circulatory system diseases** | | |
| Atherosclerosis | -0.72 | -1.89, 0.45 |
| Atherosclerotic CVD | 0.74 | -0.86, 2.37 |
| Atherosclerotic heart disease | -0.38 | -1.70, 0.96 |
| Congestive heart disease | -0.04 | -0.39, 0.30 |
| Atrial fibrillation | 1.66 | 0.03, 3.32 |
| Stroke | 0.17 | -0.28, 0.62 |
| **Other diseases** | | |
| Diabetes | 0.19 | -0.46, 0.84 |
| Inflammatory diseases | 0.18 | -1.09, 1.46 |

[a]These estimates represent the additional percent change in mortality for persons who had the characteristic being examined compared to persons who did not have the characteristic, when the mean $O_3$ level of the previous 3 days increased 10 ppb. These values were not standardized because they do not represent the actual effect estimate for the characteristic being evaluated, but instead, the difference between effect estimates for persons with versus without the condition.
[b]Compared with males in the same age group.

Source: Reprinted with permission of Lippincott Williams & Wilkins (Medina-Ramón and Schwartz, 2008).

Additionally, Cakmak et al. (2011) examined the effect of individual-level characteristics that may modify the $O_3$-mortality relationship in 7 Chilean cities. In a time-series analysis using a constrained distributed lag of 0-6 days, Cakmak et al. (2011) found evidence for larger $O_3$ mortality effects in individuals >75 years of age compared to younger ages, which is similar to Medina-Ramón and Schwartz (2008) and Stafoggia et al. (2010). Unlike the studies discussed above $O_3$-mortality risk estimates were found to be slightly larger in males (3.71% [95% CI: 0.79, 6.66] for a 40 ppb increase in max 8-h avg $O_3$ concentrations), but were not significantly different than those observed for females (3.00% [95% CI: 0.43, 5.68]). The major focus of Cakmak et al. (2011) is the examination of the influence of SES indicators (i.e., educational attainment, income level, and employment status) on the $O_3$-

mortality relationship. The authors found the largest risk estimates in the lowest SES categories for each of the indicators examined this includes: primary school not completed when examining educational attainment; the lowest quartile of income level; and unemployed individuals when comparing employment status.

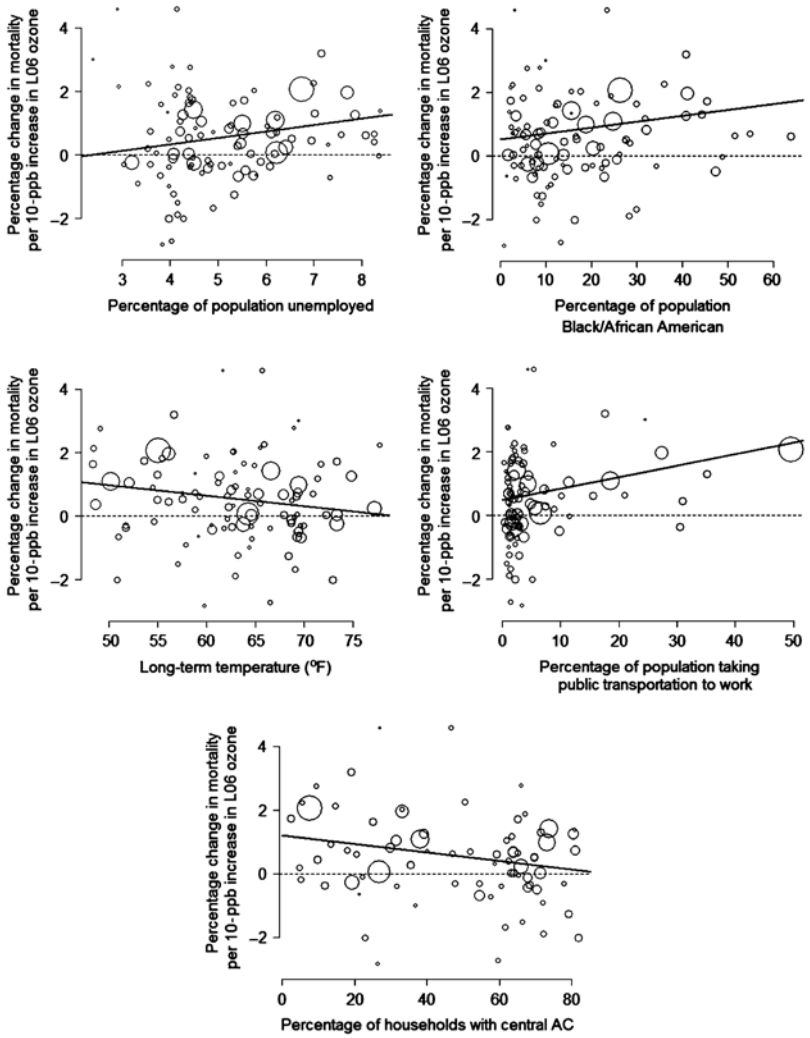
Overall, uncertainties exist in the interpretation of the potential effect modifiers identified in Medina-Ramón and Schwartz (2008), Stafoggia et al. (2010), and Cakmak et al. (2011) of the $O_3$-mortality relationship due to the heterogeneity in $O_3$-mortality risk estimates across cities as highlighted in Smith et al. (2009b) (Figure 6-28) and Franklin and Schwartz (2008) (Figure 6-29). In addition, it is likely that individual-level factors identified in Medina-Ramón and Schwartz (2008), Stafoggia et al. (2010), and Cakmak et al. (2011) only modify the $O_3$-mortality relationship and do not entirely explain the observed regional heterogeneity in $O_3$-mortality risk estimates.

### Community-level Characteristics

Several studies also examined city-level (i.e., ecological) variables in an attempt to explain the observed city-to-city variation in estimated $O_3$-mortality risk estimates. Bell and Dominici (2008) investigated whether community-level characteristics, such as race, income, education, urbanization, transportation use, PM and $O_3$ concentrations, number of $O_3$ monitors, weather, and air conditioning use could explain the heterogeneity in $O_3$-mortality risk estimates across cities. The authors analyzed 98 U.S. urban communities from NMMAPS for the period 1987-2000. In the all-year regression model that included no community-level variables, a 20 ppb increase in 24-h avg $O_3$ concentrations during the previous week was associated with a 1.04% (95% CI: 0.56, 1.55) increase in mortality. Bell and Dominici (2008) found that higher $O_3$-mortality effect estimates were associated with an increase in: percent unemployment, fraction of the population Black/African-American, percent of the population that take public transportation to work; and with a reduction in: temperatures and percent of households with central air conditioning (Figure 6-31). The modification of $O_3$-mortality risk estimates reported for city-specific temperature and prevalence of central air conditioning in this analysis confirm the result from the meta-analyses reviewed in the 2006 $O_3$ AQCD.

The APHENA project (Katsouyanni et al., 2009) examined potential effect modification of $O_3$ risk estimates in the Canadian, European, and U.S. data sets using a consistent set of city-specific variables. Table 6-48 presents the results from all age analyses for all-cause mortality using all-year $O_3$ data for the average of lag 0-1 day. While there are several significant effect modifiers in the U.S. data, the results are mostly inconsistent with the results from the Canadian and European data sets. The positive effect modification by percentage unemployed and the negative effect modification by mean temperature (i.e., a surrogate for air conditioning rate) are consistent with the results reported by Bell and Dominici (2008) discussed above. However, the lack of consistency across the data sets, even between the Canadian and U.S. data, makes it difficult to interpret the results. Some of these associations

may be due to coincidental correlations with other unmeasured factors that vary regionally (e.g., mean $SO_2$ tend to be higher in the eastern U.S.).




Note: The size of each circle corresponds to the inverse of the standard error of the community's maximum likelihood estimate. Risk estimates are for a 10 ppb increase in 24-h avg $O_3$ concentrations during the previous week.

Source: Reprinted with permission of Johns Hopkins Bloomberg School of Public Health (Bell and Dominici, 2008).

**Figure 6-31 Ozone mortality risk estimates and community-specific characteristics, U.S., 1987-2000.**

**Table 6-48 Percent change in all-cause mortality, for all ages, associated with a 40ppb increase in 1-h max $O_3$ concentrations at Lag 0–1 at the 25th and 75th percentile of the center-specific distribution of selected effect modifiers.**

| | Canada | | | Europe | | | U.S. | | |
|---|---|---|---|---|---|---|---|---|---|
| Effect Modifier | 25th Percentile Estimate (95% CI) | 75th Percentile Estimate (95% CI) | t-value | 25th Percentile Estimate (95% CI) | 75th Percentile Estimate (95% CI) | t-value | 25th Percentile Estimate (95% CI) | 75th Percentile Estimate (95% CI) | t-value |
| $NO_2$ CV | 3.10 (1.90, 4.40) | 3.99 (2.38, 5.62) | 1.33 | 1.66 (0.71, 2.62) | 1.34 (-0.08, 2.78) | -0.49 | 1.26 (0.47, 1.98) | 0.08 (-0.78, 0.95) | -2.87 |
| Mean $SO_2$ | 2.22 (0.71, 3.83) | 4.72 (2.94, 6.61) | 2.16 | 1.58 (0.47, 2.62) | 1.66 (0.39, 2.86) | 0.16 | 0.47 (-0.47, 1.42) | 1.98 (1.10, 2.94) | 2.79 |
| $O_3$ CV | 2.86 (0.79, 5.05) | 3.50 (2.14, 4.89) | 0.60 | 2.62 (1.50, 3.75) | 1.10 (0.24, 1.98) | -2.65 | 0.16 (-0.70, 1.10) | 1.50 (0.71, 2.22) | 2.68 |
| Mean $NO_2/PM_{10}$ | 3.91 (2.54, 5.29) | 2.54 (0.95, 4.15) | -1.58 | 1.74 (0.87, 2.70) | 1.50 (0.47, 2.62) | -0.43 | -0.08 (-1.02, 0.95) | 1.26 (0.47, 2.06) | 2.64 |
| Mean Temperature | 2.86 (0.95, 4.72) | 3.50 (2.22, 4.89) | 0.83 | 1.58 (0.39, 2.86) | 1.58 (0.31, 2.78) | -0.04 | 2.14 (1.34, 2.94) | 0.00 (-0.78, 0.79) | -4.40 |
| % ≥ 75 yr | 2.22 (0.79, 3.58) | 4.23 (3.02, 5.54) | 2.68 | 1.50 (0.55, 2.46) | 1.82 (0.55, 3.10) | 0.52 | 1.02 (0.24, 1.90) | 1.02 (0.31, 1.74) | -0.02 |
| Age-standardized Mortality | 2.62 (0.79, 4.48) | 4.07 (2.22, 5.87) | 1.14 | 1.10 (-0.16, 2.38) | 1.98 (0.79, 3.26) | 1.07 | 0.00 (-0.94, 0.87) | 1.58 (0.87, 2.38) | 3.81 |
| % Unemployed | 2.78 (1.42, 4.07) | 3.75 (2.54, 4.89) | 1.88 | 1.42 (-0.47, 3.34) | 1.34 (-0.47, 3.18) | -0.07 | 0.16 (-0.78, 1.18) | 1.50 (0.71, 2.30) | 2.45 |

Source: Adapted with permission of Health Effects Institute; Katsouyanni et al. (2009).

### Regional Pattern of Ozone-Mortality Risk Estimates

In addition to examining whether individual- and community-level factors modify the $O_3$-mortality association, studies have also examined whether these associations varied regionally within the United States. Bell and Dominici (2008), in the study discussed above, also noted that $O_3$-mortality risk estimates were higher in the Northeast (1.44% [95% CI: 0.78, 2.10%]) and Industrial Midwest (0.73% [95% CI: 0.11, 1.35%]), while null associations were observed in the Southwest and Urban Midwest (Table 6-49). The regional heterogeneity in $O_3$-mortality risk estimates was further reflected by Bell and Dominici (2008) in a map of community-specific Bayesian $O_3$-mortality risk estimates (Figure 6-32). It is worth noting that in the analysis of $PM_{10}$ using the same data set, Peng et al. (2005) also found that both the Northeast and Industrial Midwest showed particularly elevated effects, especially during the summer months. As mentioned above, although no evidence for confounding of $O_3$ mortality risk estimates by $PM_{10}$ was observed, Bell et al. (2007) did find regional differences in the correlation between $O_3$ and $PM_{10}$. Thus, the heterogeneity in $O_3$ mortality risk estimates may need to be examined as a function of the correlation between PM and $O_3$.

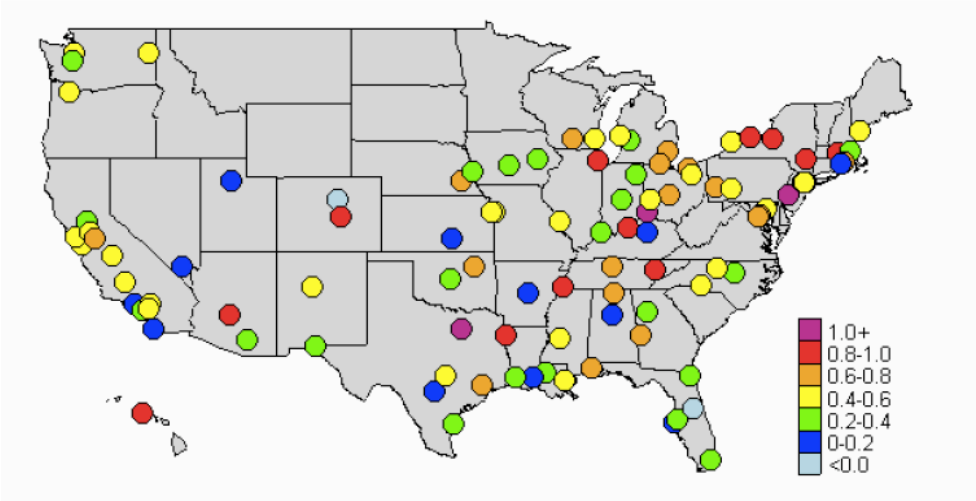
Smith et al. (2009b), as discussed earlier, also examined the regional difference in $O_3$ mortality risk estimates across the same seven regions and similarly found evidence for regional heterogeneity. In addition, Smith et al. (2009b) constructed spatial maps of the risk estimates by an extension of a hierarchical model that allows for spatial auto-correlation among the city-specific random effects. Figure 6-33 presents the spatial map of $O_3$ mortality coefficients from the Smith et al. (2009b) analysis that used 8-h max $O_3$ concentrations during the summer. The results from the Bell and Dominici (2008) analysis (Figure 6-32) shows much stronger apparent heterogeneity in $O_3$-mortality risk estimates across cities than the smoothed map from Smith et al. (2009b) (Figure 6-33), but both maps generally show larger risk estimates in the eastern region of the U.S.

**Table 6-49 Percentage increase in daily mortality for a 10 ppb increase in 24-h average $O_3$ concentrations during the previous week by geographic region in the U.S., 1987-2000.**

| | No. of Communities | Regional Estimate | 95% PI* |
|---|---|---|---|
| **Regional results** | | | |
| Industrial Midwest | 20 | 0.73 | 0.11, 1.35 |
| Northeast | 16 | 1.44 | 0.78, 2.10 |
| Northwest | 12 | 0.08 | -0.92, 1.09 |
| Southern California | 7 | 0.21 | -0.46, 0.88 |
| Southeast | 26 | 0.38 | -0.07, 0.85 |
| Southwest | 9 | -0.06 | -0.92, 0.81 |
| Urban Midwest | 7 | -0.05 | -1.28, 1.19 |
| **National results** | | | |
| All continental communities | 97 | 0.51 | 0.27, 076 |
| All communities | 98 | 0.52 | 0.28, 0.77 |

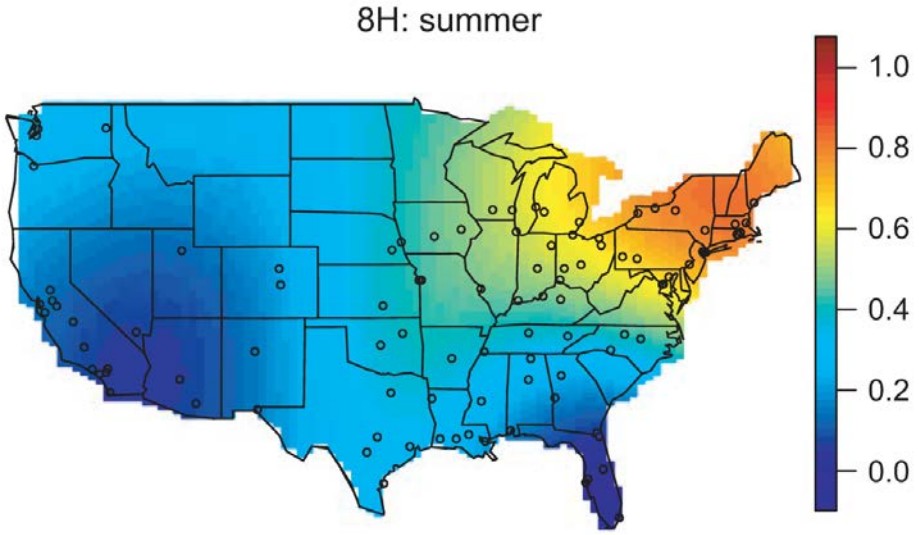
*PI, posterior interval.

Source: Reprinted with permission of Johns Hopkins Bloomberg School of Public Health (Bell and Dominici, 2008).




Source: Reprinted with permission of Johns Hopkins Bloomberg School of Public Health, (Bell and Dominici, 2008).

**Figure 6-32 Community-specific Bayesian $O_3$-mortality risk estimates in 98 U.S. communities.**




Source: Reprinted with permission of Informa UK Ltd. (Smith et al., 2009b).

**Figure 6-33 Map of spatially dependent $O_3$-mortality coefficients for 8-h max $O_3$ concentrations using summer data.**

### Time-Variant Factors

To date, only a few time-series studies have investigated the potential interaction between $O_3$ exposure and copollutants or weather variables in first stage regression models. This can be attributed to the moderate to high correlation between $O_3$ and these covariates, which makes such investigations methodologically challenging.

Ren et al. (2008) examined the possible synergistic effect between $O_3$ and temperature on mortality in the 60 largest eastern U.S. communities from the NMMAPS data during the warm months (i.e., April to October) from 1987-2000. This analysis was restricted to the eastern areas of the U.S. (i.e., Northeast, Industrial Midwest and Southeast) because a previous study which focused specifically on the eastern U.S. found that temperature-mortality patterns differ between the northeast and southeast regions possibly due to climatic differences (Curriero et al., 2002). To examine possible geographic differences in the interaction between temperature and $O_3$, Ren et al. (2008) further divided the NMMAPS regions into the Northeast, which included the Northeast and Industrial Midwest regions (34 cities), and the Southeast, which included the Southeast region (26 cities). The potential synergistic effects between $O_3$ and temperature were examined using two different models. Model 1 included an interaction term in a Generalized Additive Model (GAM) for $O_3$ and maximum temperature (3-day avg values were used for both terms) to examine the bivariate response surface and the pattern of interaction between the two variables in each community. Model 2 consisted of a Generalized Linear Model (GLM) that used interaction terms to stratify by "low," "moderate," and "high"

temperature days using the first and third quartiles of temperature as cut-offs to examine the percent increase in mortality in each community. Furthermore, a two-stage Bayesian hierarchical model was used to estimate the overall percent increase in all-cause mortality associated with short-term $O_3$ exposure across temperature levels and each region using model 2. The same covariates were used in both model 1 and 2. The bivariate response surfaces from model 1 suggest possible interactive effects between $O_3$ and temperature although the interpretation of these results is not straightforward due to the high correlation between these terms. The apparent interaction between temperature and $O_3$ as evaluated in model 2 varied across geographic regions. In the northeast region, a 20 ppb increase in 24-h avg $O_3$ concentrations at lag 0-2 was associated with an increase of 4.49% (95% posterior interval [PI]: 2.39, 6.36%), 6.21% (95% PI: 4.47, 7.66%) and 12.8% (95% PI: 9.77, 15.7%) in mortality at low, moderate and high temperature levels, respectively. The corresponding percent increases in mortality in the southeast region were 2.27% (95% PI: -2.23, 6.46%) for low temperature, 3.02% (95% PI: 0.44, 5.70%) for moderate temperature, and 2.60% (95% PI: -0.66, 6.01%) for high temperature.

When examining the relationship between temperature and $O_3$-related mortality, the results reported by Ren et al. (2008) (i.e., higher $O_3$-mortality risks on days with higher temperatures) may appear to contradict the results of Bell and Dominici (2008) described earlier (i.e., communities with higher temperature have lower $O_3$-mortality risk estimates). However, the observed difference in results can be attributed to the interpretation of effect modification in a second-stage regression which uses long-term average temperatures, as was performed by Bell and Dominici (2008), compared to a first-stage regression that examines the interaction between daily temperature and $O_3$-related mortality. In this case, the second-stage regression results from Bell and Dominici (2008) indicate that a city with lower temperatures, on average, tend to show a stronger $O_3$ mortality effect, whereas, in the first-stage regression performed by Ren et al. (2008), the days with higher temperature tend to show a larger $O_3$-mortality effect. This observed difference may in part reflect the higher air conditioning use in communities with higher long-term average temperatures. Therefore, the findings from Ren et al. (2008) indicating generally lower $O_3$ risk estimates in the southeast region where the average temperature is higher than in the northeast region is consistent with the regional results reported by Bell and Dominici (2008). As demonstrated by the results from both Ren et al. (2008) and Bell and Dominici (2008) caution is required when interpreting results from studies that examined interactive effects using two different approaches because potential effect modification as suggested in a second-stage regression generally does not provide evidence for a short-term interaction examined in a first-stage regression. Overall, further examination of the potential interactive (synergistic) effects of $O_3$ and covariates in time-series regression models is required to more clearly understand the factors that may influence $O_3$ mortality risk estimates.

### 6.6.2.3 Evaluation of the $O_3$-Mortality C-R Relationship and Related Issues

Evaluation of the $O_3$-mortality C-R relationship is not straightforward because the evidence from multicity studies (using log-linear models) suggests that $O_3$-mortality associations are highly heterogeneous across regions. In addition, there are numerous issues that may influence the shape of the $O_3$-mortality C-R relationship and the observed association between short-term $O_3$ exposure and mortality that warrant examination including: multi-day effects (distributed lags), mortality displacement (i.e., hastening of death by a short period), potential adaptation, and the exposure metric used to compute risks (e.g., 1-hour daily max versus 24-h avg). The following section presents the recent studies identified that conducted an initial examination of these issues.

#### Multiday Effects, Mortality Displacement, and Adaptation

The pattern of positive lagged associations followed by negative associations in a distributed lag model may be considered an indication of "mortality displacement" (i.e., deaths are occurring in frail individuals and exposure is only moving the day of death to a day slightly earlier). Zanobetti and Schwartz (2008b) examined this issue in 48 U.S. cities during the warm season (i.e., June-August) for the years 1989-2000. In an initial analysis, the authors applied a GLM to examine same-day $O_3$-mortality effects, and in the model included an unconstrained distributed lag for apparent temperature to take into account the effect of temperature on the day death occurred and the previous 7 days. To examine mortality displacement Zanobetti and Schwartz (2008b) refit models using two approaches: an unconstrained and a smooth distributed lag each with 21-day lags for $O_3$. In this study, all-cause mortality as well as cause-specific mortality (i.e., cardiovascular, respiratory, and stroke) were examined for evidence of mortality displacement. The authors found a 0.96% (95% CI: 0.60, 1.30%) increase in all-cause mortality across all 48 cities for a 30 ppb increase in 8-h max $O_3$ concentrations at lag 0 whereas the combined estimate of the unconstrained distributed lag model (lag 0-20) was 1.54% (95% CI: 0.15, 2.91%). Similarly, when examining the cause-specific mortality results (Table 6-50), larger risk estimates were observed for the distributed lag model compared to the lag 0 day estimates. However, for stroke a slightly larger effect was observed at lags 4-20 compared to lags 0-3 suggesting a larger window for $O_3$-induced stroke mortality. This is further supported by the sum of lags 0 through 20 days showing the greatest effect. Overall, these results suggest that estimating the mortality risk using a single day of $O_3$ exposure may underestimate the public health impact, but the extent of multi-day effects appear to be limited to a few days. This is further supported by the shape of the combined smooth distributed lag (Figure 6-34). It should be noted that the proportion of total variation in the effect estimates due to the between-cities heterogeneity, as measured by $I^2$ statistic, was relatively low (4% for the lag 0 estimates and 21% for the distributed lag), but 21 out of the 48 cities exhibited null or negative estimates. As a result, the estimated shape of the distributed lag cannot be

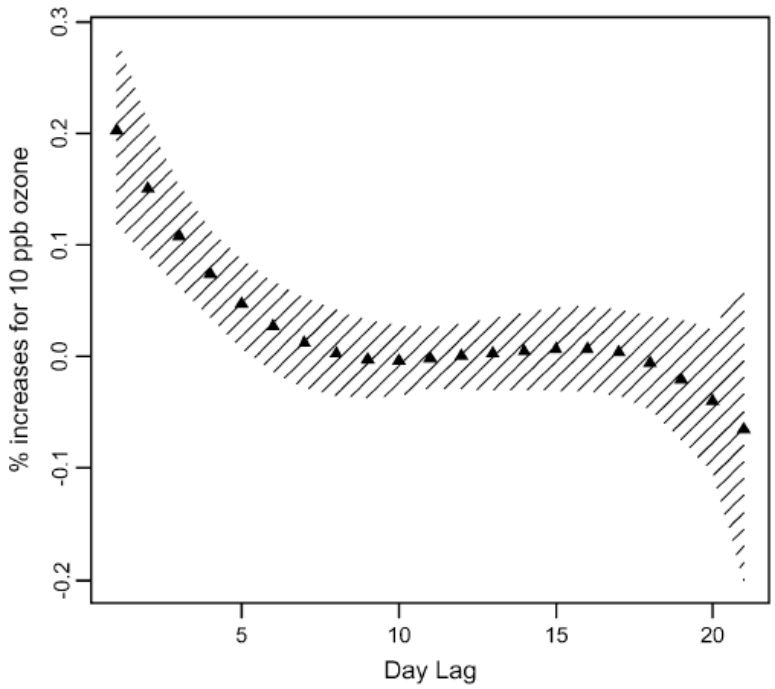
interpreted as a general form of lag structure of associations applicable to all the cities included in this analysis.

Samoli et al. (2009) also investigated the temporal pattern of mortality effects in response to short-term exposure to $O_3$ in 21 European cities that were included in the APHEA2 project. Using a method similar to Zanobetti and Schwartz (2008b), the authors applied unconstrained distributed lag models with lags up to 21 days in each city during the summer months (i.e., June through August) to examine the effect of $O_3$ on all-cause, cardiovascular, and respiratory mortality. They also applied a generalized additive distributed lag model to obtain smoothed distributed lag coefficients. However, unlike Zanobetti and Schwartz (2008b), Samoli et al. (2009) controlled for temperature using a linear term for humidity and an unconstrained distributed lag model of temperature at lags 0-3 days. The choice of 0- through 3-day lags of temperature was based on a previous European multicity study (Baccini et al., 2008), which suggested that summer temperature effects last only a few days. Upon combining the individual city estimates across cities in a second stage regression, Samoli et al. (2009) found that the estimated effects on respiratory mortality were extended for a period of two weeks. However, for all-cause and cardiovascular mortality, the 21-day distributed lag models yielded null or (non-significant) negative estimates (Table 6-51). Figure 6-35 shows the distributed lag coefficients for all-cause mortality, which exhibit a declining trend and negative coefficients beyond 5-day lags. The authors' interpretation of these results was that "using single-day exposures may have overestimated the effects on all-cause and cardiovascular mortality, but underestimated the effects on respiratory mortality." Thus, the results in part suggest evidence of mortality displacement for all-cause and cardiovascular mortality.

**Table 6-50 Estimated effect of a 10 ppb increase in 8-h max $O_3$ concentrations on mortality during the summer months for single-day and distributed lag models.**

| | % (Percentage) | 95% CI |
|---|---|---|
| **Total mortality** | | |
| Lag 0 | 0.32 | 0.20, 0.43 |
| Sum lags 0-20 | 0.51 | 0.05, 0.96 |
| Sum lags 0-3 | 0.53 | 0.28, 0.77 |
| Sum lags 4-20 | -0.02 | -0.35, 0.31 |
| **Cardiovascular mortality** | | |
| Lag 0 | 0.47 | 0.30, 0.64 |
| Sum lags 0-20 | 0.49 | -0.01, 1.00 |
| Sum lags 0-3 | 0.80 | 0.48, 1.13 |
| Sum lags 4-20 | -0.23 | -0.67, 0.22 |
| **Respiratory mortality** | | |
| Lag 0 | 0.54 | 0.26, 0.81 |
| Sum lags 0-20 | 0.61 | -0.41, 1.65 |
| Sum lags 0-3 | 0.83 | 0.38, 1.28 |
| Sum lags 4-20 | -0.24 | -1.08, 0.60 |
| **Stroke** | | |
| Lag 0 | 0.37 | 0.01, 0.74 |
| Sum lags 0-20 | 2.20 | 0.76, 3.67 |
| Sum lags 0-3 | 0.92 | 0.26, 1.59 |
| Sum lags 4-20 | 1.26 | 0.05, 2.49 |

Source: Reprinted with permission of American Thoracic Society, Zanobetti and Schwartz (2008b).




Source: Reprinted with permission of American Thoracic Society (Zanobetti and Schwartz, 2008b).

Note: The triangles represent the percent increase in all-cause mortality for a 10 ppb increase in 8-h max $O_3$ concentrations at each lag while the shaded areas are the 95% point-wise confidence intervals.

**Figure 6-34 Estimated combined smooth distributed lag for 48 U.S. cities during the summer months.**

**Table 6-51 Estimated percent increase in cause-specific mortality (and 95% CIs) for a 10-μg/m$^3$ increase in 8-h daily max $O_3$ during June-August.**

| | Fixed effects % (95% CI) | Random effects % (95% CI) |
|---|---|---|
| **Total mortality**[a] | | |
| Lag 0 | 0.28 (0.11, 0.45) | 0.28 (0.07, 0.48) |
| Average lags 0-1 | 0.24 (0.15, 0.34) | 0.22 (0.08, 0.35) |
| Sum lags 0-20, unconstrained | 0.01 (-0.40, 0.41) | -0.54 (-1.28, 0.20) |
| Sum lags 0-20, penalized | 0.01 (-0.41, 0.42) | -0.56 (-1.30, 0.19) |
| **Cardiovascular mortality**[a] | | |
| Lag 0 | 0.43 (0.18, 0.69) | 0.37 (0.05, 0.69) |
| Average lags 0-1 | 0.33 (0.19, 0.48) | 0.25 (0.03, 0.47) |
| Sum lags 0-20, unconstrained | -0.33 (-0.93, 0.29) | -0.62 (-1.47, 0.24) |
| Sum lags 0-20, penalized | -0.32 (-0.92, 0.28) | -0.57 (-1.39, 0.26) |
| **Respiratory mortality**[a] | | |
| Lag 0 | 0.36 (-0.21, 0.94) | 0.36 (-0.21, 0.94) |
| Average lags 0-1 | 0.40 (0.11, 0.70) | 0.40 (0.11, 0.70) |
| Sum lags 0-20, unconstrained | 3.35 (1.90, 4.83) | 3.35 (1.90, 4.83) |
| Sum lags 0-20, penalized | 3.66 (2.25, 5.08) | 3.66 (2.25, 5.08) |

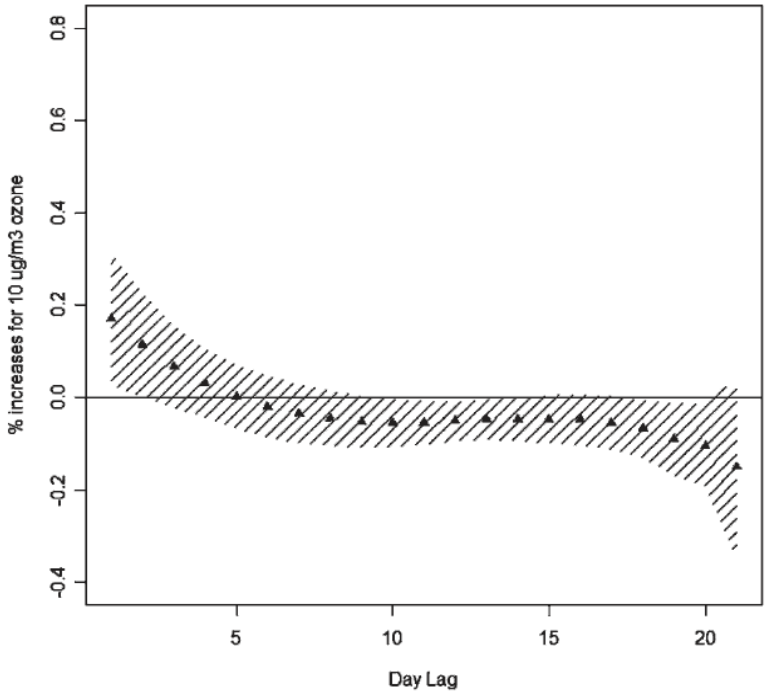
[a]Analysis for the same day (lag 0), the average of the same and previous day (lag 0-1), the unconstrained distributed lag model for the sum of 0-20 days and the penalized distributed lag model (lag 0-20)

Source: Used with permission of BMJ Group (Samoli et al., 2009).

Although the APHENA project (Katsouyanni et al., 2009) did not specifically investigate mortality displacement and therefore did not consider longer lags (e.g., lag >3 days), the study did present $O_3$ risk estimates for lag 0-1, lag 1, and a distributed lag model of 0-2 days in the Canadian, European, and U.S. datasets. Katsouyanni et al. (2009) found that the results vary somewhat across the regions, but, in general, there was no indication that the distributed lag model with up to a 2-day lag yielded meaningfully larger $O_3$ mortality risk estimates than the lag 0-1 and lag 1 results. For example, for all-cause mortality, using the model with natural splines and 8 df/year to adjust for seasonal trends, the reported percent excess risk for mortality for a 40 ppb increase in 1-h max $O_3$ concentrations for lag 0-1, lag 1, and the distributed lag model (lag 0-2) was 2.70% (95% CI: 1.02, 4.40%), 1.42% (95% CI: 0.08, 2.78%), and 3.02% (95% CI: 1.10, 4.89%), respectively. Thus, the observed associations appear to occur over a short time period, (i.e., a few days). Similarly, the Public Health and Air Pollution in Asia (PAPA) study (Wong et al., 2010) also examined multiple lag days (i.e., lag 0, lag 0-1, and lag 0-4), and although it did not specifically examine mortality displacement it does provide additional evidence regarding the timing of mortality effects proceeding $O_3$ exposure. In a combined analysis using data from all four cities examined (Bangkok, Hong Kong, Shanghai, and Wuhan), excess risk estimates at lag 0-4 were larger than those at lag

0 or lag 0-1 in both fixed and random effect models (results not presented quantitatively). The larger risk estimates at lag 0-4 can primarily be attributed to the strong associations observed in Bangkok and Shanghai. However, it is worth noting that Bangkok differs from the three Chinese cities included in this analysis in that it has a tropical climate and does not exhibit seasonal patterns of mortality. As a result, Wong et al. (2010) examined the $O_3$-mortality associations at lag 0-1 in only the three Chinese cities and found that risk estimates were slightly reduced from 2.26% (95% CI: 1.36, 3.16) in the 4 city analysis to 1.84% (0.77, 2.86) in the 3 city analysis for a 30 ppb increase in 8-h max $O_3$ concentrations. Overall, the PAPA study further supports the observation of the APHENA study that associations between $O_3$ and mortality occur over a relatively short-time period, but also indicates that it may be difficult to interpret $O_3$-mortality associations across cities with different climates and mortality patterns.

When comparing the studies that explicitly examined the potential for mortality displacement in the $O_3$-mortality relationship, the results from Samoli et al. (2009), which provide evidence that suggests mortality displacement, are not consistent with those reported by Zanobetti and Schwartz (2008b). However, the shapes of the estimated smooth distributed lag associations are similar (Figure 6-34 and Figure 6-35). A closer examination of these figures shows that in the European data beyond a lag of 5 days the estimates remain negative whereas in the U.S. data the results remain near zero for the corresponding lags. These observed difference could be due to the differences in the model specification between the two studies, specifically the use of: an unconstrained distributed lag model for apparent temperature up to 7 previous days (Zanobetti and Schwartz, 2008b) versus a linear term for humidity and an unconstrained distributed lag model of temperature up to 3 previous days (Samoli et al., 2009); and natural cubic splines with 2 df per season (Zanobetti and Schwartz, 2008b) versus dummy variables per month per year to adjust for season (Samoli et al., 2009). It is important to note that these differences in model specification may have also influenced the city-to-city variation in risk estimates observed in these two studies (i.e., homogenous estimates across cities in Zanobetti and Schwartz (2008b) and heterogeneous estimates across cities in Samoli et al. (2009). Overall, the evidence of mortality displacement remains unclear, but Samoli et al. (2009), Zanobetti and Schwartz (2008b), and Katsouyanni et al. (2009) all suggest that the positive associations between $O_3$ and mortality are observed mainly in the first few days after exposure.




Note: The triangles represent the percent increase in all-cause mortality for a 10 µg/m$^3$ increase in 8-h max $O_3$ concentrations at each lag; the shaded area represents the 95% CIs.

Source: Reprinted with permission of BMJ Group (Samoli et al., 2009).

**Figure 6-35 Estimated combined smooth distributed lag in 21 European cities during the summer (June-August) months.**

### Adaptation

Controlled human exposure studies have demonstrated an adaptive response to $O_3$ exposure for respiratory effects, such as lung function decrements, but this issue has not been examined in the epidemiologic investigation of mortality effects of $O_3$. Zanobetti and Schwartz (2008a) examined if there was evidence of an adaptive response in the $O_3$-mortality relationship in 48 U.S. cities from 1989 to 2000 (i.e., the same data analyzed in Zanobetti and Schwartz (2008b). The authors examined all-cause mortality using a case-crossover design to estimate the same-day (i.e., lag 0) effect of $O_3$, matched on referent days from every-3rd-day in the same month and year as the case. Zanobetti and Schwartz (2008a) examined $O_3$-mortality associations by: season, month in the summer season (i.e., May through September), and age categories in the summer season (Table 6-52). The estimated $O_3$ mortality risk estimate at lag 0 was found to be highest in the summer (1.51% [95% CI: 1.14, 1.87%]; lag 0 for a 30 ppb increase in 8-h max $O_3$ concentrations), and, within the warm months, the association was highest in July (1.96% [95% CI: 1.42, 2.48%];

lag 0).[1] Upon further examination of the summer months, the authors also observed diminished effects in August (0.84% [95% CI: 0.33, 1.39%]; lag 0). Based on these results, the authors concluded that the mortality effects of $O_3$ appear diminished later in the $O_3$ season.

**Table 6-52 Percent excess all-cause mortality per 10 ppb increase in daily 8-h max $O_3$ on the same day, by season, month, and age groups.**

| | % | 95% CI |
|---|---|---|
| **By Season** | | |
| Winter | -0.13 | -0.56, 0.29 |
| Spring | 0.35 | 0.16, 0.54 |
| Summer | 0.50 | 0.38, 0.62 |
| Fall | 0.05 | -0.14, 0.24 |
| **By Month** | | |
| May | 0.48 | 0.28, 0.68 |
| June | 0.46 | 0.24, 0.68 |
| July | 0.65 | 0.47, 0.82 |
| August | 0.28 | 0.11, 0.46 |
| September | -0.09 | -0.35, 0.16 |
| **By Age Group** | | |
| 0-20 | 0.08 | -0.42, 0.57 |
| 21-30 | 0.10 | -0.67, 0.87 |
| 31-40 | 0.07 | -0.38, 0.52 |
| 41-50 | 0.08 | -0.27, 0.43 |
| 51-60 | 0.54 | 0.19, 0.89 |
| 61-70 | 0.38 | 0.16, 0.61 |
| 71-80 | 0.50 | 0.32, 0.67 |
| 80 | 0.29 | 0.13, 0.44 |

Source: Zanobetti and Schwartz (2008a).

To further evaluate the potential adaptive response observed in Zanobetti and Schwartz (2008a) the distribution of the $O_3$ concentrations across the 48 U.S. cities during July and August was examined. Both July and August were found to have comparable means of 48.6 and 47.9 ppb with a reported maximum value of 97.9 and 96.0 ppb, respectively. Thus, the observed reduction in $O_3$-related mortality effect estimates in August (0.84%) compared to July (1.96%) appears to support the existence of an adaptive response. However, unlike an individual's adaptive response

[1] These values have been standardized to the increment used throughout the ISA for max 8-h avg increase in $O_3$ concentrations of 30 ppb. These values differ from those presented in Table 6-52; from Zanobetti and Schwartz (2008a) because the authors presented values for a 10 ppb increase in max 8-h avg $O_3$ concentrations.

to decrements in lung function from short-term $O_3$ exposure, an examination of mortality prevents a direct observation of adaptation. Rather, for mortality the adaptation hypothesis is tested with a tacit assumption that, whatever the mechanism for $O_3$-induced mortality, the risk of death from short-term $O_3$ exposure is reduced over the course of the summer months through repeated exposures. This idea would translate to a smaller population that would die from $O_3$ exposure toward the end of summer. This may complicate the interpretation of the distributed lag coefficients with long lag periods because the decreased coefficients may reflect diminished effects of the late summer, rather than diminished effects that are constant across the summer. These intertwined issues need to be investigated together in future research.

### Exposure Metric

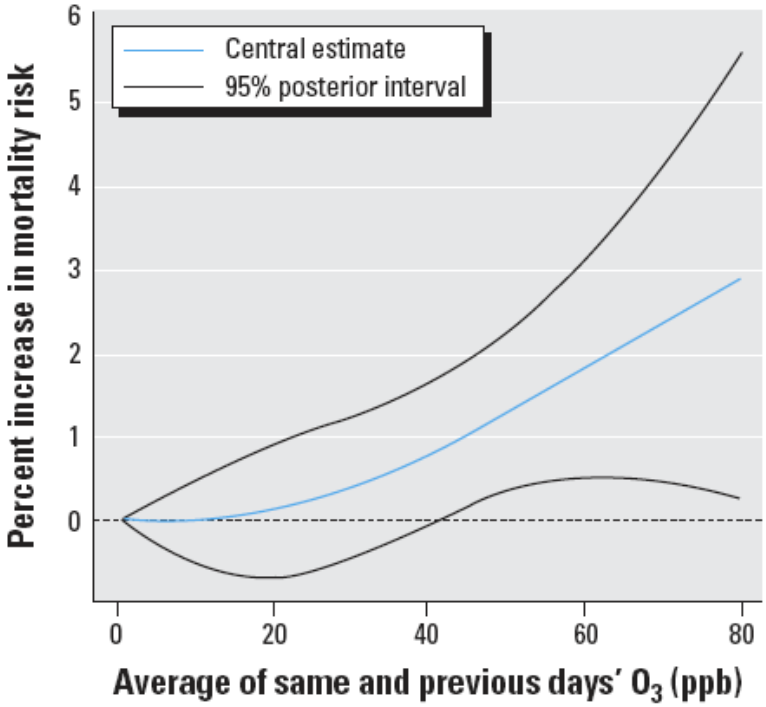
When examining the association between short-term $O_3$ exposure and mortality it is also important to consider the exposure metric used (i.e., 24-h avg, 8-h max, and 1-h max). To date, only a few studies have conducted analyses to examine the impact of different exposure metrics on $O_3$ mortality risk estimates. In Smith et al. (2009b), the authors examined the effect of different exposure metrics (i.e., 24-h avg, 8-h max, and 1-h max) on $O_3$-mortality regression coefficients. When examining whether there are differences in city-specific risk estimates when using different exposure metrics, Smith et al. (2009b) found a rather high correlation ($r = 0.7 – 0.8$) between risk estimates calculated using 24-h avg versus 8-h max and 1-h max versus 8-h max averaging times. These results are consistent with the correlations reported by Darrow et al. (2011a) (Section 6.2.7.3) between the 8-h max and 24- avg exposure metrics.

In addition to these recent studies published since the 2006 $O_3$ AQCD, Gryparis et al. (2004) also supports the high correlation between 1-h max and 8-h max $O_3$ concentrations reported in Smith et al. (2009b) and Darrow et al. (2011a) and the subsequent high degree of similarity between mortality risk estimates calculated using either metric. Although only a limited number of studies have examined the effect of different exposure metrics on $O_3$-mortality risk estimates, these studies suggest relatively comparable results across the exposure metrics used.

### Ozone-Mortality C-R Relationship and Threshold Analyses

Several of the recent studies evaluated have applied a variety of statistical approaches to examine the shape of the $O_3$-mortality C-R relationship and whether a threshold exists. The approach used by Bell et al. (2006) consisted of applying four statistical models to the NMMAPS data, which included 98 U.S. communities for the period 1987-2000. These models included: a linear analysis (i.e., any change in $O_3$ concentration can be associated with mortality) (Model 1); a subset analysis (i.e., examining $O_3$-mortality relationship below a specific 24- avg concentration, ranging from 5 to 60 ppb) (Model 2); a threshold analysis (i.e., assuming that an association between $O_3$ and mortality is observed above a specific concentration and

not below it, using the threshold values set at an increment of 5 ppb between 0 to 60 ppb and evaluating a presence of a local minima in AICs computed at each increment) (Model 3); and nonlinear models using natural cubic splines with boundary knots placed at 0 and 80 ppb, and interior knots placed at 20 and 40 ppb (Model 4). A two-stage Bayesian hierarchical model was used to examine these models and $O_3$-mortality risk estimates at the city-level in the first stage analysis and aggregate estimates across cities in the 2nd stage analysis using the average of 0- and 1-day lagged 24-h avg $O_3$ concentrations. The results from all of these models suggest that if a threshold exists it does so well below the current $O_3$ NAAQS. When restricting the analysis to all days when the 1997 $O_3$ NAAQS 8-hour standard (i.e., 84 ppb daily 8-h max) is met in each community, Bell et al. (2006) found there was still a 0.60% (95% PI: 0.30, 0.90%) increase in mortality per 20 ppb increase in 24-h avg $O_3$ concentrations at lag 0-1. Figure 6-36 shows the combined C-R curve obtained using the nonlinear model (Model 4). Although these results suggest the lack of threshold in the $O_3$-mortality relationship, it is difficult to interpret such a curve because: (1) there is uncertainty around the shape of the C-R curve at 24-h avg $O_3$ concentrations generally below 20 ppb, and (2) the C-R curve does not take into consideration the heterogeneity in $O_3$-mortality risk estimates across cities.




Source: Bell et al. (2006)

**Figure 6-36 Estimated combined C-R curve for nonaccidental mortality and 24-hour average $O_3$ concentrations at lag 0-1 using the nonlinear (spline) model.**

Using the same NMMAPS dataset as Bell et al. (2006), Smith et al. (2009b) further examined the $O_3$-mortality C-R relationship. Similar to Bell et al. (2006), Smith et al. (2009b) conduct a subset analysis, but instead of restricting the analysis to days with $O_3$ concentrations below a cutoff the authors only include days above a defined cutoff in the analysis. The results of this "reversed subset" approach are in line with those reported by Bell et al. (2006); consistent positive associations at all cutoff points up to a defined concentration where the total number of days with 24-h avg $O_3$ concentrations above a value are so limited that the variability around the central estimate is increased. In the Smith et al. (2009b) analysis this observation was initially observed at 45 ppb, with the largest variability at 60 ppb; however, unlike Bell et al. (2006) where 73% of days are excluded when subsetting the data to less than 20 ppb, the authors do not detail the number of days of data included in the subset analyses at higher concentrations. In addition to the subset analysis, Smith et al. (2009b) examined the shape of the C-R curve using a piecewise linear approach with cutpoints at 8-h avg concentrations of 40 ppb, 60 ppb, and 80 ppb. Smith et al. (2009b) found that the shape of the C-R curve is similar to that reported by Bell et al. (2006) (Figure 6-36), but argue that slopes of the β for each piece of the curve are highly variable with the largest variation in the 60-80 ppb range. However, the larger

variability around the β between 60-80 would be expected due to the small number of days with $O_3$ concentrations within that range in an all-year analysis. This result is consistent with that observed by Bell et al. (2006), which is presented in Figure 6-36.

The APHENA project (Katsouyanni et al., 2009) also analyzed the Canadian and European datasets (the U.S. data were analyzed for $PM_{10}$ only) for evidence of a threshold, using the threshold analysis method (Model 3) applied in Bell et al. (2006) study described above. There was no evidence of a threshold in the Canadian data (i.e., the pattern of AIC values for each increment of a potential threshold value varied across cities, most of which showed no local minima). Likewise, the threshold analysis conducted using the European data also showed no evidence of a threshold.

The PAPA study, did not examine whether a threshold exists in the $O_3$-mortality C-R relationship, but instead the shape of the C-R curve individually for each city (Bangkok, Hong Kong, Shanghai, and Wuhan) (Wong et al., 2010). Using a natural spline smoother with 3df for the $O_3$ term, Wong et al. (2010) examined whether non-linearity was present by testing the change in deviance between the smoothed, non-linear model and an unsmoothed linear model with 1 df. For each of the cities, both across the full range of the $O_3$ distribution and specifically within the range of the 25th to 75th percentile of each city's 24-h avg $O_3$ concentrations (i.e., a range of 9.7 ppb to 60.4 ppb across the cities) there was no evidence of a non-linear relationship in the $O_3$-mortality C-R curve. It should be noted that the range of the 25th to 75th percentiles of $O_3$ concentrations in all of the cities, except Wuhan, was at the lower end of the distribution observed in the U.S. using all-year data, where the range from the 25th to 75th percentiles is 30 ppb to 50 ppb (Table 3-6).

Additional threshold analyses were conducted using NMMAPS data, by Xia and Tong (2006) and Stylianou and Nicolich (2009). Both studies used a new statistical approach developed by Xia and Tong (2006) to examine thresholds in the $O_3$ mortality C-R relationship. The approach consisted of an extended GAM model, which accounted for the cumulative and nonlinear effects of air pollution using a weighted cumulative sum for each pollutant, with the weights (non-increasing further into the past) derived by a restricted minimization method. The authors did not use the term distributed lag model, but their model has the form of distributed lag model, except that it allows for nonlinear functional forms. Using NMMAPS data for 1987-1994 for 3 U.S. cities (Chicago, Pittsburgh, and El Paso), Xia and Tong (2006) found that the extent of cumulative effects of $O_3$ on mortality were relatively short. While the authors also note that there was evidence of a threshold effect around 24-h avg concentrations of 25 ppb, the threshold values estimated in the analysis were sometimes in the range where data density was low. Thus, this threshold analysis needs to be replicated in a larger number of cities to confirm this observation.
It should be noted that the model used in this analysis did not include a smooth function of days to adjust for unmeasured temporal confounders, and instead adjusted for season using a temperature term. As a result, these results need to be viewed with caution because some potential temporal confounders (e.g., influenza) do not always follow seasonal patterns of temperature.

Stylianou and Nicolich (2009) examined the existence of thresholds following an approach similar to Xia and Tong (2006) for all-cause, cardiovascular, and respiratory mortality using data from NMMAPS for nine major U.S. cities (i.e., Baltimore, MD, Chicago, IL, Dallas/Fort Worth,TX, Los Angeles, CA, Miami, FL, New York, NY, Philadelphia, PA, Pittsburgh, PA, and Seattle, WA) for the years 1987-2000. The authors found that $PM_{10}$ and $O_3$ were the two important predictors of mortality. Stylianou and Nicolich (2009) found that the estimated $O_3$-mortality risks varied across the nine cities with the models exhibiting apparent thresholds, in the 10-45 ppb range for $O_3$ (3-day accumulation). However, given the city-to-city variation in risk estimates, combining the city-specific estimates into an overall estimate complicates the interpretation of a threshold. Unlike the Xia and Tong (2006) analysis, Stylianou and Nicolich (2009) included a smooth function of time to adjust for seasonal/temporal confounding, which could explain the difference in results between the two studies.

In conclusion, the evaluation of the $O_3$-mortality C-R relationship did not find any evidence that supports a threshold in the relationship between short-term exposure to $O_3$ and mortality within the range of $O_3$ concentrations observed in the United States. Additionally, recent evidence suggests that the shape of the $O_3$-mortality C-R curve remains linear across the full range of $O_3$ concentrations. However, the studies evaluated demonstrated that the heterogeneity in the $O_3$-mortality relationship across cities (or regions) complicates the interpretation of a combined C-R curve and threshold analysis. Given the effect modifiers identified in the mortality analyses that are also expected to vary regionally (e.g., temperature, air conditioning prevalence), a national or combined analysis may not be appropriate to identify whether a threshold exists in the $O_3$-mortality C-R relationship. Overall, the studies evaluated support a linear $O_3$-mortality C-R relationship and continue to support the conclusions from the 2006 $O_3$ AQCD, which stated that "if a population threshold level exists in $O_3$ health effects, it is likely near the lower limit of ambient $O_3$ concentrations in the United States" (U.S. EPA, 2006b).

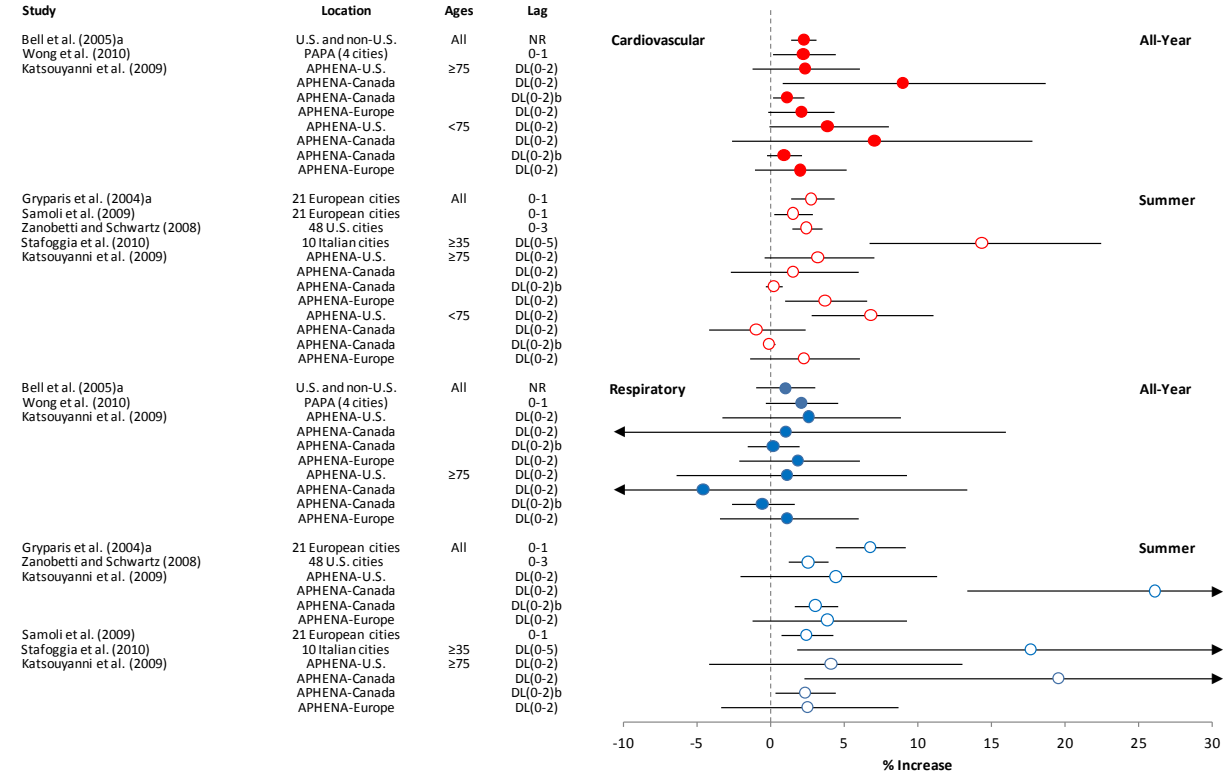
#### 6.6.2.4 Associations of Cause-Specific Mortality and Short-term $O_3$ Exposure

In the 2006 $O_3$ AQCD, an evaluation of studies that examined cause-specific mortality found consistent positive associations between short-term $O_3$ exposure and cardiovascular mortality, with less consistent evidence for associations with respiratory mortality. The majority of the evidence for associations between $O_3$ exposure and cause-specific mortality were from single-city studies, which had small daily mortality counts and subsequently limited statistical power to detect associations.

New multicity studies evaluated in this review build upon and confirm the associations between short-term $O_3$ exposure and cause-specific mortality identified in the 2006 $O_3$ AQCD (U.S. EPA, 2006b) (Figure 6-37 [and Table 6-53]).

In APHENA, a multicontinent study that consisted of the NMMAPS, APHEA2 and Canadian multicity datasets, consistent positive associations were reported for both cardiovascular and respiratory mortality in all-year analyses when focusing on the natural spline model with 8 df/year (Figure 6-37 [and Table 6-53]). The associations between $O_3$ exposure and cardiovascular and respiratory mortality in all-year analyses were further supported by the multicity PAPA study (Wong et al., 2010). The magnitude of cardiovascular mortality associations were primarily larger in analyses restricted to the summer season compared to those observed in all-year analyses (Figure 6-37 [and Table 6-53]). Additional multicity studies from the U.S. (Zanobetti and Schwartz, 2008b) and Europe (Stafoggia et al., 2010; Samoli et al., 2009) that conducted summer season analyses provide evidence supporting associations between $O_3$ exposure and cardiovascular and respiratory mortality that are similar or larger in magnitude compared to those observed in all-year analyses.

Of the studies evaluated, only the APHENA study (Katsouyanni et al., 2009) and an Italian multicity study (Stafoggia et al., 2010) conducted an analysis of the potential for copollutant confounding of the $O_3$ cause-specific mortality relationship. When focusing on the natural spline model with 8 df/year and lag 1 results (as discussed in Section 6.6.2.1), the APHENA study found that $O_3$ cause-specific mortality risk estimates were fairly robust to the inclusion of $PM_{10}$ in copollutant models in the European dataset with more variability in the U.S. and Canadian datasets (i.e., copollutant risk estimates increased and decreased for respiratory and cardiovascular mortality). In summer season analyses cardiovascular $O_3$ mortality risk estimates were robust in the European dataset and attenuated but remained positive in the U.S. datasets; whereas, respiratory $O_3$ mortality risk estimates were attenuated in the European dataset and robust in the U.S. dataset. The authors did not examine copollutant models during the summer season in the Canadian dataset (Figure 6-30 [and Table 6-45]). Interpretation of these results requires caution; however, due to the different PM sampling schedules employed in each of these study locations (i.e., primarily every-6th day in the U.S. and Canadian datasets and every-day in the European dataset). The results of the summer season analyses from the APHENA study (Katsouyanni et al., 2009) are consistent with those from a study of 10 Italian cities during the summer months (Stafoggia et al., 2010). Stafoggia et al. (2010) found that cardiovascular (14.3% [95% CI: 6.7, 22.4%]) and cerebrovascular (8.5% [95% CI: 0.06, 16.3%]) mortality $O_3$ effect estimates were robust to the inclusion of $PM_{10}$ in copollutant models (14.3% [95% CI: 6.7, 23.1%] and 7.3% [95% CI: -1.2, 16.3], respectively), while respiratory mortality $O_3$ effects estimates (17.6% [95% CI: 1.8, 35.5%]) were attenuated, but remained positive (9.2% [95% CI: -6.9, 28.8%]).




Effect estimates are for a 20 ppb increase in 24-h avg; 30 in 8-h max; and 40ppb increase in 1-h max $O_3$ concentrations. Red = cardiovascular; blue = respiratory; closed circles = all-year analysis; and open circles = summer-only analysis. An "a" represents studies from the 2006 $O_3$ AQCD. A "b" represents risk estimates from APHENA-Canada standardized to an approximate IQR of 5.1 ppb for a 1-h max increase in $O_3$ concentrations (Section 6.2.7.2).

## Figure 6-37 Percent increase in cause-specific mortality.

## Table 6-53 Corresponding effect estimates for Figure 6-37.

| Study* | Location | Ages | Lag | Avg Time | %Increase (95% CI) |
|---|---|---|---|---|---|
| **Cardiovascular** | | | | | |
| **All-year - Cardiovascular** | | | | | |
| Bell et al. (2005)[a] | U.S. and non-U.S. | All | NR | 24-h avg | 2.23 (1.36,3.08) |
| Wong et al. (2010) | PAPA (4 cities) | | 0-1 | 8-h max | 2.20 (0.06, 4.37) |
| Katsouyanni et al. (2009) | APHENA-U.S. | ≥ 75 | DL(0-2) | 1-h max | 2.30 (-1.33, 6.04) |
| | APHENA-Canada | | DL(0-2) | | 8.96 (0.75,18.6) |
| | APHENA-Canada | | DL(0-2)[b] | | 1.1 (0.10,2.20) |
| | APHENA-Europe | | DL(0-2) | | 2.06 (-0.24, 4.31) |
| | APHENA-U.S. | <75 | DL(0-2) | | 3.83 (-0.16, 7.95) |
| | APHENA-Canada | | DL(0-2) | | 7.03 (-2.71, 17.7) |
| | APHENA-Canada | | DL(0-2)[b] | | 0.87 (-0.35, 2.10) |
| | APHENA-Europe | | DL(0-2) | | 1.98 (-1.09, 5.13) |
| **Summer – Cardiovascular** | | | | | |
| Gryparis et al. (2004)[a] | 21 European cities | All | 0-1 | 8-h max | 2.7 (1.29,4.32) |
| Samoli et al. (2009) | 21 European cities | | 0-1 | 8-h max | 1.48 (0.18, 2.80) |
| Zanobetti and Schwartz (2008b) | 48 U.S. cities | | 0-3 | 8-h max | 2.42 (1.45, 3.43) |
| Stafoggia et al. (2010) | 10 Italian cities | ≥ 35 | DL(0-5) | 8-h max | 14.3 (6.65, 22.4) |
| Katsouyanni et al. (2009) | APHENA-U.S. | ≥ 75 | DL(0-2) | 1-h max | 3.18 (-0.47, 6.95) |
| | APHENA-Canada | | DL(0-2) | | 1.50 (-2.79, 5.95) |
| | APHENA-Canada | | DL(0-2)[b] | | 0.19 (-0.36, 0.74) |
| | APHENA-Europe | | DL(0-2) | | 3.67 (0.95, 6.53) |
| | APHENA-U.S. | <75 | DL(0-2) | | 6.78 (2.70, 11.0) |
| | APHENA-Canada | | DL(0-2) | | -1.02 (-4.23, 2.30) |
| | APHENA-Canada | | DL(0-2)[b] | | -0.13 (-0.55, 0.29) |
| | APHENA-Europe | | DL(0-2) | | 2.22 (-1.48, 6.04) |
| **Respiratory** | | | | | |
| **All-years - Respiratory** | | | | | |
| Bell et al. (2005)[a] | U.S. and non-U.S. | All | NR | 24-h avg | 0.94 (-1.02, 2.96) |
| Wong et al. (2010) | PAPA (4 cities) | | 0-1 | 8-h max | 2.02 (-0.41, 4.49) |
| Katsouyanni et al. (2009) | APHENA-U.S. | | DL(0-2) | 1-h max | 2.54 (-3.32, 8.79) |
| | APHENA-Canada | | DL(0-2) | | 1.02 (-11.9, 15.9) |
| | APHENA-Canada | | DL(0-2)[b] | | 0.13 (-1.60, 1.90) |
| | APHENA-Eeurope | | DL(0-2) | | 1.82 (-2.18, 6.04) |
| | APHENA-U.S. | ≥ 75 | DL(0-2) | | 1.10 (-6.48, 9.21) |
| | APHENA-Canada | | DL(0-2) | | -4.61 (-19.3, 13.3) |
| | APHENA-Canada | | DL(0-2)[b] | | -0.60 (-2.70, 1.60) |
| | APHENA-Europe | | DL(0-2) | | 1.10 (-3.48, 5.95) |

| Study* | Location | Ages | Lag | Avg Time | %Increase (95% CI) |
|---|---|---|---|---|---|
| **Summer - Respiratory** | | | | | |
| Gryparis et al. (2004)[a] | 21 European cities | All | 0-1 | 8-h max | 6.75 (4.38, 9.10) |
| Zanobetti and Schwartz (2008b) | 48 U.S. cities | | 0-3 | 8-h max | 2.51 (1.14, 3.89) |
| Katsouyanni et al. (2009) | APHENA-U.S. | | DL(0-2) | 1-h max | 4.40 (-2.10, 11.3) |
| | APHENA-Canada | | DL(0-2) | | 26.1 (13.3, 41.2) |
| | APHENA-Canada | | DL(0-2)[b] | | 3.00 (1.60, 4.50) |
| | APHENA-Europe | | DL(0-2) | | 3.83 (-1.33, 9.21) |
| Samoli et al. (2009) | 21 European cities | | 0-1 | 8-h max | 2.38 (0.65, 4.19) |
| Stafoggia et al. (2010) | 10 Italian cities | ≥ 35 | DL(0-5) | 8-h max | 17.6 (1.78, 35.5) |
| Katsouyanni et al. (2009) | APHENA-U.S. | ≥ 75 | DL(0-2) | 1-h max | 4.07 (-4.23, 13.0) |
| | APHENA-Canada | | DL(0-2) | | 19.5 (2.22, 40.2) |
| | APHENA-Canada | | DL(0-2)[b] | | 2.30 (0.28, 4.40) |
| | APHENA-Europe | | DL(0-2) | | 2.46 (-3.40, 8.62) |

*Studies from Figure 6-37, plus others.

[a]Studies from the 2006 $O_3$ AQCD.

[b]Risk estimates from APHENA-Canada standardized to an approximate IQR of 5.1 ppb for a 1-h max increase in $O_3$ concentrations (Section 6.2.7.2).

Collectively, the results from the new multicity studies provide evidence of associations between short-term $O_3$ exposure and cardiovascular and respiratory mortality with additional evidence indicating these associations persist, and in some cases the magnitude of associations are increased, in the summer season. Although copollutant analyses of cause-specific mortality are limited, the APHENA study found that $O_3$ cause-specific mortality risk estimates were fairly robust to the inclusion of $PM_{10}$ in copollutant models when focusing on the dataset with daily $PM_{10}$ data (i.e., the European dataset), which is supported by the results from Stafoggia et al. (2010). Additionally, APHENA found that $O_3$ cause-specific mortality risk estimates were moderately to substantially sensitive (e.g., increased or attenuated) to inclusion of $PM_{10}$ in the U.S. and Canadian datasets. However, the mostly every-6th-day sampling schedule for $PM_{10}$ in the U.S. and Canadian datasets greatly reduced their sample size and limits the interpretation of these results.

### 6.6.3 Summary and Causal Determination

The evaluation of new multicity studies that examined the association between short-term $O_3$ exposure and mortality found evidence which supports the conclusions of the 2006 $O_3$ AQCD. These new studies reported consistent positive associations between short-term $O_3$ exposure and all-cause (nonaccidental) mortality, with associations persisting or increasing in magnitude during the warm season, and provide additional support for associations between $O_3$ exposure and cardiovascular and respiratory mortality.

Recent studies further examined potential confounders (e.g., copollutants and seasonality) of the $O_3$-mortality relationship. Because the PM-$O_3$ correlation varies across regions, due to the difference in PM chemical constituents, interpretation of the combined effect of PM on the relationship between $O_3$ and mortality is not straightforward. Unlike previous studies that were limited to primarily examining the confounding effects of $PM_{10}$, the new studies expanded their analyses to include multiple PM indices (e.g., $PM_{10}$, $PM_{2.5}$, and PM components). An examination of copollutant models found evidence that associations between $O_3$ and all-cause mortality were robust to the inclusion of $PM_{10}$ or $PM_{2.5}$ (Stafoggia et al., 2010; Katsouyanni et al., 2009; Bell et al., 2007), while other studies found evidence for a modest reduction (~20-30%) when examining $PM_{10}$ (Smith et al., 2009b). Additional evidence suggests potential sensitivity (e.g., increases and attenuation) of $O_3$ mortality risk estimates to copollutants by age group or cause-specific mortality (e.g., respiratory and cardiovascular) (Stafoggia et al., 2010; Katsouyanni et al., 2009). An examination of PM components, specifically sulfate, found evidence for reductions in $O_3$-mortality risk estimates in copollutant models (Franklin and Schwartz, 2008). Overall, across studies, the potential impact of PM indices on $O_3$-mortality risk estimates tended to be much smaller than the variation in $O_3$-mortality risk estimates across cities suggesting that $O_3$ effects are independent of the relationship between PM and mortality. However, interpretation of the potential confounding effects of PM on $O_3$-mortality risk estimates requires caution. This is because the PM-$O_3$ correlation varies across regions, due to the difference in PM components, complicating the interpretation of the combined effect of PM on the relationship between $O_3$ and mortality. Additionally, the limited PM or PM component datasets used as a result of the every-3rd- and 6th-day PM sampling schedule instituted in most cities limits the overall sample size employed to examine whether PM or one of its components confounds the $O_3$-mortality relationship.

An examination of potential seasonal confounding of the $O_3$-mortality relationship found that the extent of smoothing or the methods used for adjustment can influence $O_3$ risk estimates when not applying enough degrees of freedom to control for temporal/season trends (Katsouyanni et al., 2009). This is because of the opposing seasonal trends between $O_3$ and mortality.

The multicity studies evaluated within this section also examined the regional heterogeneity observed in $O_3$-mortality risk estimates. These studies provide evidence which suggests generally higher $O_3$-mortality risk estimates in northeastern U.S. cities with some regions showing no associations between $O_3$ exposure and mortality (e.g., Southwest, Urban Midwest) (Smith et al., 2009b; Bell and Dominici, 2008). Multicity studies that examined individual- and community-level characteristics identified characteristics that may explain the observed regional heterogeneity in $O_3$-mortality risk estimates as well as characteristics of populations potentially at greatest risk for $O_3$-related health effects. An examination of community-level characteristics found an increase in the $O_3$-mortality risk estimates in cities with higher unemployment, percentage of the population Black/African-American, percentage of the working population that uses public transportation, lower temperatures, and lower prevalence of central air conditioning (Medina-Ramón

and Schwartz, 2008). Additionally, a potential interactive, or synergistic, effect on the $O_3$-mortality relationship was observed when examining differences in the $O_3$-mortality association across temperature levels (Ren et al., 2008).
An examination of individual-level characteristics found evidence that older age, female sex, Black race, having atrial fibrillation, SES indicators (i.e., educational attainment, income level, and employment status), and out-of hospital deaths, specifically in those individuals with diabetes, modify $O_3$-mortality associations (Cakmak et al., 2011; Stafoggia et al., 2010; Medina-Ramón and Schwartz, 2008), and lead to increased risk of $O_3$-related mortality. Overall, additional research is warranted to further confirm whether these characteristics, individually or in combination, can explain the observed regional heterogeneity.

Additional studies were evaluated that examined factors that may influence the shape of the $O_3$-mortality C-R curve, such as multi-day effects, mortality displacement, adaptation, the use of different exposure metrics (i.e., 24-h avg, 8-h max or 1-h max), and whether a threshold exists in the $O_3$-mortality relationship. An examination of multiday effects in a U.S. and European multicity study found conflicting evidence for mortality displacement, but both studies suggest that the positive associations between $O_3$ and mortality are observed mainly in the first few days after exposure (Samoli et al., 2009; Zanobetti and Schwartz, 2008b). A U.S. multicity study found evidence of an adaptive response to $O_3$ exposure, with the highest risk estimates earlier in the $O_3$ season (i.e., July) and diminished effects later (i.e., August) (Zanobetti and Schwartz, 2008a). However, the evidence of adaptive effects has an implication for the interpretation of multi-day effects, and requires further analysis. The limited number of studies conducted that examined the effect of using different exposure metrics (i.e., 1-h max, 8-h max, and 24-h avg) when examining the $O_3$-mortality relationship found relatively comparable $O_3$-mortality risk estimates across the exposure metrics used (Smith et al., 2009b; Gryparis et al., 2004). Analyses that specifically focused on the $O_3$-mortality C-R relationship supported a linear $O_3$-mortality relationship and found no evidence of a threshold within the range of $O_3$ concentrations in the U.S., but did observe evidence for potential differences in the C-R relationship across cities (Katsouyanni et al., 2009; Stylianou and Nicolich, 2009; Bell et al., 2006). Collectively, these studies support the conclusions of the 2006 $O_3$ AQCD that "if a population threshold level exists in $O_3$ health effects, it is likely near the lower limit of ambient $O_3$ concentrations in the U.S."

Studies that examined the association between short-term $O_3$ exposure and cause-specific mortality confirm the associations with both cardiovascular and respiratory mortality reported in the 2006 $O_3$ AQCD (Stafoggia et al., 2010; Wong et al., 2010; Katsouyanni et al., 2009; Samoli et al., 2009; Zanobetti and Schwartz, 2008b). These associations were primarily larger in magnitude during the summer season compared to all-year analyses. Of the studies that examined the potential confounding effects of PM [i.e., Stafoggia et al. (2010); Katsouyanni et al. (2009)], $O_3$ mortality associations remained relatively robust in copollutant models, but interpretation of these studies was complicated by the different PM sampling schedules (e.g., every-6th-day) employed in each study. Overall, the strong evidence for respiratory effects due to short-term $O_3$ exposure (Section 6.2) are consistent across disciplines and

provides coherence for the respiratory mortality associations observed across studies. The strong evidence for $O_3$-induced cardiovascular mortality is supported by controlled human exposure and animal toxicological studies that provide initial evidence for a biologically plausible mechanism for $O_3$-induced cardiovascular mortality. However, a lack of coherence with epidemiologic studies of cardiovascular morbidity that do not demonstrate consistent evidence of $O_3$-induced cardiovascular effects complicate the evidence for a biological pathway of events leading to mortality (Section 6.3).

In conclusion, the recent epidemiologic studies build upon and confirm the associations between short-term $O_3$ exposure and all-cause and cause-specific mortality reported in the 2006 $O_3$ AQCD. However, there is a lack of coherence across disciplines and consistency across health outcomes for $O_3$-induced cardiovascular morbidity (Section 6.3) which do not support the relatively strong epidemiologic evidence for $O_3$-related cardiovascular mortality. Overall, recent studies have provided additional information regarding key uncertainties (previously identified - including the potential confounding effects of copollutants and seasonal trend), individual- and community-level factors that may lead to increased risk of $O_3$-induced mortality and the heterogeneity in $O_3$-mortality risk estimates, and continued evidence of a linear no-threshold C-R relationship. Although some uncertainties still remain, the collective body of evidence is sufficient to conclude there **is likely to be a causal relationship between short-term $O_3$ exposure and total mortality**.

## 6.7 Overall Summary

The evidence reviewed in this chapter describes the recent findings regarding the health effects of short-term exposure to ambient $O_3$ concentrations. Table 6-54 provides an overview of the causal determinations for each of the health categories evaluated.

**Table 6-54 Summary of causal determinations for short-term exposures to $O_3$.**

| Health Category | Causal Determination |
|---|---|
| Respiratory Effects | Causal relationship |
| Cardiovascular Effects | Likely to be a causal relationship |
| Central Nervous System Effects | Suggestive of a causal relationship |
| Effects on Liver and Xenobiotic Metabolism | Inadequate to infer a causal relationship |
| Effects on Cutaneous and Ocular Tissues | Inadequate to infer a causal relationship |
| Total Mortality | Likely to be a causal relationship |

## References


Adamkiewicz, G; Ebelt, S; Syring, M; Slater, J; Speizer, FE; Schwartz, J; Suh, H; Gold, DR. (2004). Association between air pollution exposure and exhaled nitric oxide in an elderly population. Thorax 59: 204-209. http://dx.doi.org/10.1136/thorax.2003.006445

Adams, WC. (1998). Dose-response effect of varied equivalent minute ventilation rates on pulmonary function responses during exposure to ozone. Washington, DC: American Petroleum Institute.

Adams, WC. (2000). Ozone dose-response effects of varied equivalent minute ventilation rates. J Expo Sci Environ Epidemiol 10: 217-226. http://dx.doi.org/10.1038/sj.jea.7500086

Adams, WC. (2002). Comparison of chamber and face-mask 6.6-hour exposures to ozone on pulmonary function and symptoms responses. Inhal Toxicol 14: 745-764. http://dx.doi.org/10.1080/08958370290084610

Adams, WC. (2003a). Comparison of chamber and face mask 6.6-hour exposure to 0.08 ppm ozone via square-wave and triangular profiles on pulmonary responses. Inhal Toxicol 15: 265-281.

Adams, WC. (2003b). Relation of pulmonary responses induced by 66-h exposures to 0.08 ppm ozone and 2-h exposures to 0.30 ppm ozone via chamber and face-mask inhalation. Inhal Toxicol 15: 745-759.

Adams, WC. (2006a). Comparison of chamber 6.6-h exposures to 0.04-0.08 ppm ozone via square-wave and triangular profiles on pulmonary responses. Inhal Toxicol 18: 127-136. http://dx.doi.org/10.1080/08958370500306107

Adams, WC. (2006b). Human pulmonary responses with 30-minute time intervals of exercise and rest when exposed for 8 hours to 0.12 ppm ozone via square-wave and acute triangular profiles. Inhal Toxicol 18: 413-422. http://dx.doi.org/10.1080/08958370600563599

Adams, WC; Ollison, WM. (1997). Effects of prolonged simulated ambient ozone dosing patterns on human pulmonary function and symptomatology. Pittsburgh, PA: Air & Waste Management Association.

Adams, WC; Schelegle, ES. (1983). Ozone and high ventilation effects on pulmonary function and endurance performance. J Appl Physiol 55: 805-812.

Aibo, DI; Birmingham, NP; Lewandowski, R; Maddox, JF; Roth, RA; Ganey, PE; Wagner, JG; Harkema, JR. (2010). Acute exposure to ozone exacerbates acetaminophen-induced liver injury in mice. Toxicol Sci 115: 267-285. http://dx.doi.org/10.1093/toxsci/kfq034

Alexeeff, SE; Litonjua, AA; Suh, H; Sparrow, D; Vokonas, PS; Schwartz, J. (2007). Ozone exposure and lung function: Effect modified by obesity and airways hyperresponsiveness in the VA Normative Aging Study. Chest 132: 1890-1897. http://dx.doi.org/10.1378/chest.07-1126

Alexeeff, SE; Litonjua, AA; Wright, RO; Baccarelli, A; Suh, H; Sparrow, D; Vokonas, PS; Schwartz, J. (2008). Ozone exposure, antioxidant genes, and lung function in an elderly cohort: VA Normative Aging Study. Occup Environ Med 65: 736-742. http://dx.doi.org/10.1136/oem.2007.035253

Alexis, N; Urch, B; Tarlo, S; Corey, P; Pengelly, D; O'Byrne, P; Silverman, F. (2000). Cyclooxygenase metabolites play a different role in ozone-induced pulmonary function decline in asthmatics compared to normals. Inhal Toxicol 12: 1205-1224.

Alexis, NE; Lay, JC; Hazucha, M; Harris, B; Hernandez, ML; Bromberg, PA; Kehrl, H; Diaz-Sanchez, D; Kim, C; Devlin, RB; Peden, DB. (2010). Low-level ozone exposure induces airways inflammation and modifies cell surface phenotypes in healthy humans. Inhal Toxicol 22: 593-600. http://dx.doi.org/10.3109/08958371003596587

Alexis, NE; Zhou, H; Lay, JC; Harris, B; Hernandez, ML; Lu, TS; Bromberg, PA; Diaz-Sanchez, D; Devlin, RB; Kleeberger, SR; Peden, DB. (2009). The glutathione-S-transferase Mu 1 null genotype modulates ozone-induced airway inflammation in human subjects. J Allergy Clin Immunol 124: 1222-1228. http://dx.doi.org/10.1016/j.jaci.2009.07.036

Alfaro-Rodríguez, A; González-Piña, R. (2005). Ozone-induced paradoxical sleep decrease is related to diminished acetylcholine levels in the medial preoptic area in rats. Chem Biol Interact 151: 151-158. http://dx.doi.org/10.1016/j.cbi.2004.10.001

Anderson, HR; Armstrong, B; Hajat, S; Harrison, R; Monk, V; Poloniecki, J; Timmis, A; Wilkinson, P. (2010). Air pollution and activation of implantable cardioverter defibrillators in London. Epidemiology 21: 405-413. http://dx.doi.org/10.1097/EDE.0b013e3181d61600

Angoa-Pérez, M; Jiang, H; Rodríguez, AI; Lemini, C; Levine, RA; Rivas-Arancibia, S. (2006). Estrogen counteracts ozone-induced oxidative stress and nigral neuronal death. Neuroreport 17: 629-633.

Apte, MG; Buchanan, IS; Mendell, MJ. (2008). Outdoor ozone and building-related symptoms in the BASE study. Indoor Air 18: 156-170. http://dx.doi.org/10.1111/j.1600-0668.2008.00521.x

Araneda, S; Commin, L; Atlagich, M; Kitahama, K; Parraguez, VH; Pequignot, JM; Dalmaz, Y. (2008). VEGF overexpression in the astroglial cells of rat brainstem following ozone exposure. Neurotoxicology 29: 920-927. http://dx.doi.org/10.1016/j.neuro.2008.09.006

Aranyi, C; Vana, SC; Thomas, PT; Bradof, JN; Fenters, JD; Graham, JA; Miller, FJ. (1983). Effects of subchronic exposure to a mixture of O3, SO2, and (NH4)2SO4 on host defenses of mice. J Toxicol Environ Health 12: 55-71. http://dx.doi.org/10.1080/15287398309530407

Arbex, AM; de Souza Conceicao, GM; Perez Cendon, S; Arbex, FF; Lopes, AC; Providello Moyses, E; Santiago, SL; Saldiva, PHN; Pereira, LAA; Ferreira Braga, AL. (2009). Urban air pollution and COPD-related emergency room visits. J Epidemiol Community Health 966: 777-783. http://dx.doi.org/10.1136/jech.2008.078360

Aris, RM; Tager, I; Christian, D; Kelly, T; Balmes, JR. (1995). Methacholine responsiveness is not associated with O3-induced decreases in FEV1. Chest 107: 621-628.

Arito, H; Takahashi, M; Iwasaki, T; Uchiyama, I. (1997). Age-related changes in ventilatory and heart rate responses to acute ozone exposure in the conscious rat. Ind Health 35: 78-86.

Arito, H; Uchiyama, I; Arakawa, H; Yokoyama, E. (1990). Ozone-induced bradycardia and arrhythmia and their relation to sleep-wakefulness in rats. Toxicol Lett 52: 169-178. http://dx.doi.org/10.1016/0378-4274(90)90151-B

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

Armstrong, BG. (2003). Fixed factors that modify the effects of time-varying factors: Applying the case-only approach. Epidemiology 14: 467-472. http://dx.doi.org/10.1097/01.ede.0000071408.39011.99

Atkinson, RW; Bremner, SA; Anderson, HR; Strachan, DP; Bland, JM; Ponce de Leon, A. (1999). Short-term associations between emergency hospital admissions for respiratory and cardiovascular disease and outdoor air pollution in London. Arch Environ Occup Health 54: 398-411.

ATS (American Thoracic Society). (1991). Lung function testing: Selection of reference values and interpretative strategies. Am J Respir Crit Care Med 144: 1202-1218.

ATS (American Thoracic Society). (2000a). Guidelines for methacholine and exercise challenge testing-1999. Am J Respir Crit Care Med 161: 309-329.

Avila-Costa, MR; Colin-Barenque, L; Fortoul, TI; Machado-Salas, JP; Espinosa-Villanueva, J; Rugerio-Vargas, C; Rivas-Arancibia, S. (1999). Memory deterioration in an oxidative stress model and its correlation with cytological changes on rat hippocampus CA1. Neurosci Lett 270: 107-109.

Avissar, NE; Reed, CK; Cox, C; Frampton, MW; Finkelstein, JN. (2000). Ozone, but not nitrogen dioxide, exposure decreases glutathione peroxidases in epithelial lining fluid of human lung. Am J Respir Crit Care Med 162: 1342-1347.

Avol, EL; Linn, WS; Venet, TG; Shamoo, DA; Hackney, JD. (1984). Comparative respiratory effects of ozone and ambient oxidant pollution exposure during heavy exercise. J Air Waste Manag Assoc 34: 804-809.

Avol, EL; Navidi, WC; Rappaport, EB; Peters, JM. (1998b). Acute effects of ambient ozone on asthmatic, wheezy, and healthy children. (82). Topsfield, MA: Health Effects Institute; Flagship Press.

Avol, EL; Trim, SC; Little, DE; Spier, CE; Smith, MN; Peng, RC; Linn, WS; Hackney, JD; Gross, KB; D'Arcy, JB; Gibbons, D; Higgins, ITT. (1990). Ozone exposure and lung function in children attending a southern California summer camp. In Proceedings of the 83rd A&WMA Annual Meeting. Pittsburgh, PA: Air & Waste Management Association.

Azevedo, JM; Gonçalves, FL; de Fátima Andrade, M. (2011). Long-range ozone transport and its impact on respiratory and cardiovascular health in the north of Portugal. Int J Biometeorol 55: 187-202. http://dx.doi.org/10.1007/s00484-010-0324-2

Baccarelli, A; Zanobetti, A; Martinelli, I; Grillo, P; Hou, L; Lanzani, G; Mannucci, PM; Bertazzi, PA; Schwartz, J. (2007). Air pollution, smoking, and plasma homocysteine. Environ Health Perspect 115: 176-181. http://dx.doi.org/10.1289/ehp.9517

Baccini, M; Biggeri, A; Accetta, G; Kosatsky, T; Katsouyanni, K; Analitis, A; Anderson, HR; Bisanti, L; D'Ippoliti, D; Danova, J; Forsberg, B; Medina, S; Paldy, A; Rabczenko, D; Schindler, C; Michelozzi, P. (2008). Heat effects on mortality in 15 European cities. Epidemiology 19: 711-719. http://dx.doi.org/10.1097/EDE.0b013e318176bfcd

Baja, ES; Schwartz, JD; Wellenius, GA; Coull, BA; Zanobetti, A; Vokonas, PS; Suh, HH. (2010). Traffic-related air pollution and QT interval: Modification by diabetes, obesity, and oxidative stress gene polymorphisms in the Normative Aging Study. Environ Health Perspect 118: 840-846. http://dx.doi.org/10.1289/ehp.0901396

Balbi, B; Pignatti, P; Corradi, M; Baiardi, P; Bianchi, L; Brunetti, G; Radaeli, A; Moscato, G; Mutti, A; Spanevello, A; Malerba, M. (2007). Bronchoalveolar lavage, sputum and exhaled clinically relevant inflammatory markers: Values in healthy adults [Review]. Eur Respir J 30: 769-781. http://dx.doi.org/10.1183/09031936.00112306

Ballester, F; Rodriguez, P; Iniguez, C; Saez, M; Daponte, A; Galan, I; Taracido, M; Arribas, F; Bellido, J; Cirarda, FB; Canada, A; Guillen, JJ; Guillen-Grima, F; Lopez, E; Perez-Hoyos, S; Lertxundi, A; Toro, S. (2006). Air pollution and cardiovascular admisisons association in Spain: Results within the EMECAS project. J Epidemiol Community Health 60: 328-336.

Ballester, F; Saez, M; Daponte, A; Ordonez, JM; Taracido, M; Cambra, K; Arribas, F; Bellido, JB; Guillen, JJ; Aguinaga, I; Canada, A; Lopez, E; Iniguez, C; Rodriguez, P; Perez-Hoyos, S; Barcelo, MA; Ocana, R; Aranguez, E. (2005). [The EMECAS Project: Spanish multicentre study on short-term health effects of air pollution]. Rev Esp Salud Publica 79: 229-242.

Balmes, JR; Aris, RM; Chen, LL; Scannell, C; Tager, IB; Finkbeiner, W; Christian, D; Kelly, T; Hearne, PQ; Ferrando, R; Welch, B. (1997). Effects of ozone on normal and potentially sensitive human subjects. Part I: Airway inflammation and responsiveness to ozone in normal and asthmatic subjects (pp. 1-37; discussion 81-99). (ISSN 1041-5505). Boston, MA: Health Effects Institute.

Balmes, JR; Chen, LL; Scannell, C; Tager, I; Christian, D; Hearne, PQ; Kelly, T; Aris, RM. (1996). Ozone-induced decrements in FEV1 and FVC do not correlate with measures of inflammation. Am J Respir Crit Care Med 153: 904-909.

Barnes, PJ; Liew, FY. (1995). Nitric oxide and asthmatic inflammation [Review]. Immunol Today 16: 128-130. http://dx.doi.org/10.1016/0167-5699(95)80128-6

Barnett, AG; Williams, GM; Schwartz, J; Best, TL; Neller, AH; Petroeschevsky, AL; Simpson, RW. (2006). The effects of air pollution on hospitalizations for cardiovascular disease in elderly people in Australian and New Zealand cities. Environ Health Perspect 114: 1018-1023. http://dx.doi.org/10.1289/ehp.8674

Barraza-Villarreal, A; Sunyer, J; Hernandez-Cadena, L; Escamilla-Nunez, MC; Sienra-Monge, JJ; Ramirez-Aguilar, M; Cortez-Lugo, M; Holguin, F; Diaz-Sanchez, D; Olin, AC; Romieu, I. (2008). Air pollution, airway inflammation, and lung function in a cohort study of Mexico City schoolchildren. Environ Health Perspect 116: 832-838. http://dx.doi.org/10.1289/ehp.10926

Basha, MA; Gross, KB; Gwizdala, CJ; Haidar, AH; Popovich, J, Jr. (1994). Bronchoalveolar lavage neutrophilia in asthmatic and healthy volunteers after controlled exposure to ozone and filtered purified air. Chest 106: 1757-1765.

Bauer, AK; Rondini, EA; Hummel, KA; Degraff, LM; Walker, C; Jedlicka, AE; Kleeberger, SR. (2011). Identification of candidate genes downstream of TLR4 signaling after ozone exposure in mice: A role for heat shock protein 70. Environ Health Perspect 119: 1091-1097. http://dx.doi.org/10.1289/ehp.1003326

Bell, ML; Dominici, F. (2008). Effect modification by community characteristics on the short-term effects of ozone exposure and mortality in 98 US communities. Am J Epidemiol 167: 986-997. http://dx.doi.org/10.1093/aje/kwm396

Bell, ML; Dominici, F; Samet, JM. (2005). A meta-analysis of time-series studies of ozone and mortality with comparison to the national morbidity, mortality, and air pollution study. Epidemiology 16: 436-445. http://dx.doi.org/10.1097/01.ede.0000165817.40152.85

Bell, ML; Kim, JY; Dominici, F. (2007). Potential confounding of particulate matter on the short-term association between ozone and mortality in multisite time-series studies. Environ Health Perspect 115: 1591-1595. http://dx.doi.org/10.1289/ehp.10108

Bell, ML; Levy, JK; Lin, Z. (2008). The effect of sandstorms and air pollution on cause-specific hospital admissions in Taipei, Taiwan. Occup Environ Med 65: 104-111. http://dx.doi.org/10.1136/oem.2006.031500

Bell, ML; McDermott, A; Zeger, SL; Samet, JM; Dominici, F. (2004). Ozone and short-term mortality in 95 US urban communities, 1987-2000. JAMA 292: 2372-2378. http://dx.doi.org/10.1001/jama.292.19.2372

Bell, ML; Peng, RD; Dominici, F. (2006). The exposure-response curve for ozone and risk of mortality and the adequacy of current ozone regulations. Environ Health Perspect 114: 532-536.

Bennett, WD; Hazucha, MJ; Folinsbee, LJ; Bromberg, PA; Kissling, GE; London, SJ. (2007). Acute pulmonary function response to ozone in young adults as a function of body mass index. Inhal Toxicol 19: 1147-1154. http://dx.doi.org/10.1080/08958370701665475

Bergamaschi, E; De Palma, G; Mozzoni, P; Vanni, S; Vettori, MV; Broeckaert, F; Bernard, A; Mutti, A. (2001). Polymorphism of quinone-metabolizing enzymes and susceptibility to ozone-induced acute effects. Am J Respir Crit Care Med 163: 1426-1431.

Berhane, K; Zhang, Y; Linn, WS; Rappaport, EB; Bastain, TM; Salam, MT; Islam, T; Lurmann, F; Gilliland, FD. (2011). The effect of ambient air pollution on exhaled nitric oxide in the Children's Health Study. Eur Respir J 37: 1029-1036. http://dx.doi.org/10.1183/09031936.00081410

Berkey, CS; Hoaglin, DC; Antczak-Bouckoms, A; Mosteller, F; Colditz, GA. (1998). Meta-analysis of multiple outcomes by regression with random effects. Stat Med 17: 2537-2550. http://dx.doi.org/10.1002/(SICI)1097-0258(19981130)17:22<2537::AID-SIM953>3.0.CO;2-C

Berry, M; Lioy, PJ; Gelperin, K; Buckler, G; Klotz, J. (1991). Accumulated exposure to ozone and measurement of health effects in children and counselors at two summer camps. Environ Res 54: 135-150.

Biggeri, A; Baccini, M; Bellini, P; Terracini, B. (2005). Meta-analysis of the Italian studies of short-term effects of air pollution (MISA), 1990-1999. Int J Occup Environ Health 11: 107-122.

Blomberg, A; Mudway, IS; Nordenhall, C; Hedenstrom, H; Kelly, FJ; Frew, AJ; Holgate, ST; Sandstrom, T. (1999). Ozone-induced lung function decrements do not correlate with early airway inflammatory or antioxidant responses. Eur Respir J 13: 1418-1428.

Bosson, J; Stenfors, N; Bucht, A; Helleday, R; Pourazar, J; Holgate, ST; Kelly, FJ; Sandstrom, T; Wilson, S; Frew, AJ; Blomberg, A. (2003). Ozone-induced bronchial epithelial cytokine expression differs between healthy and asthmatic subjects. Clin Exp Allergy 33: 777-782.

Boussouar, A; Araneda, S; Hamelin, C; Soulage, C; Kitahama, K; Dalmaz, Y. (2009). Prenatal ozone exposure abolishes stress activation of Fos and tyrosine hydroxylase in the nucleus tractus solitarius of adult rat. Neurosci Lett 452: 75-78.

Brauer, M; Blair, J; Vedal, S. (1996). Effect of ambient ozone exposure on lung function in farm workers. Am J Respir Crit Care Med 154: 981-987.

Brauer, M; Brook, JR. (1997). Ozone personal exposures and health effects for selected groups residing in the Fraser Valley. Atmos Environ 31: 2113-2121.

Braun-Fahrlander, C, h; Kunzli, N; Domenighetti, G; Carell, CF; Ackermann-Liebrich, U. (1994). Acute effects of ambient ozone on respiratory function of Swiss schoolchildren after a 10-minute heavy exercise. Pediatr Pulmonol 17: 169-177. http://dx.doi.org/10.1002/ppul.1950170306

Brook, RD; Brook, JR; Urch, B; Vincent, R; Rajagopalan, S; Silverman, F. (2002). Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. Circulation 105: 1534-1536.

Brooks, EG. (2010). Correspondence from Dr. Brooks - Re: Clarifications in 2008 J Occup Environ Med article. Brooks, EG.

Brown, JS; Bateson, TF; McDonnell, WF. (2008). Effects of exposure to 0.06 ppm ozone on FEV1 in humans: A secondary analysis of existing data. Environ Health Perspect 116: 1023-1026. http://dx.doi.org/10.1289/ehp.11396

Brunekreef, B; Hoek, G; Breugelmans, O; Leentvaar, M. (1994). Respiratory effects of low-level photochemical air pollution in amateur cyclists. Am J Respir Crit Care Med 150: 962-966.

Buadong, D; Jinsart, W; Funatagawa, I; Karita, K; Yano, E. (2009). Association between PM10 and O3 levels and hospital visits for cardiovascular diseases in Bangkok, Thailand. J Epidemiol 19: 182-188. http://dx.doi.org/10.2188/jea.JE20080047

Buckley, RD; Hackney, JD; Clark, K; Posin, C. (1975). Ozone and human blood. Arch Environ Health 30: 40-43.

Burleson, GR; Keyes, LL; Stutzman, JD. (1989). Immunosuppression of pulmonary natural killer activity by exposure to ozone. Immunopharmacol Immunotoxicol 11: 715-735. http://dx.doi.org/10.3109/08923978909005397

Burnett, R; Raizenne, M; Krewski, D. (1990). Acute health effects of transported air pollution: A study of children attending a residential summer camp. Can J Stat 18: 367-373. http://dx.doi.org/10.2307/3315843

Burra, TA; Moineddin, R; Agha, MM; Glazier, RH. (2009). Social disadvantage, air pollution, and asthma physician visits in Toronto, Canada. Environ Res 109: 567-574. http://dx.doi.org/10.1016/j.envres.2009.03.004

Bush, ML; Asplund, PT; Miles, KA; Ben-Jebria, A; Ultman, JS. (1996). Longitudinal distribution of O3 absorption in the lung: gender differences and intersubject variability. J Appl Physiol 81: 1651-1657.

Cakmak, S; Dales, RE; Angelica Rubio, M; Blanco Vidal, C. (2011). The risk of dying on days of higher air pollution among the socially disadvantaged elderly. Environ Res 111: 388-393. http://dx.doi.org/10.1016/j.envres.2011.01.003

Cakmak, S; Dales, RE; Judek, S. (2006a). Do gender, education, and income modify the effect of air pollution gases on cardiac disease? J Occup Environ Med 48: 89-94. http://dx.doi.org/10.1097/01.jom.0000184878.11956.4b

Cakmak, S; Dales, RE; Judek, S. (2006b). Respiratory health effects of air pollution gases: Modification by education and income. Arch Environ Occup Health 61: 5-10. http://dx.doi.org/10.3200/AEOH.61.1.5-10

Calderón Guzmán, D; Barragan Mejia, G; Hernandez Garcia, E; Juarez Olguin, H. (2006). Effect of nutritional status and ozone exposure on some biomarkers of oxidative stress in rat brain regions. Nutr Cancer 55: 195-200. http://dx.doi.org/10.1207/s15327914nc5502_11

Calderón Guzmán, D; Hernández Islas, JL; Mejía, GB; Santamaría del Angel, D; Hernández García, E; Juárez Olguín, H. (2005). Effect of nutritional status and ozone exposure on Na+/K+ ATPpase and lipid peroxidation in rat brain. Proc West Pharmacol Soc 48: 118-121.

Carpagnano, GE; Foschino Barbaro, MP; Cagnazzo, M; Di Gioia, G; Giliberti, T; Di Matteo, C; Resta, O. (2005). Use of exhaled breath condensate in the study of airway inflammation after hypertonic saline solution challenge. Chest 128: 3159-3166. http://dx.doi.org/10.1378/chest.128.5.3159

Castillejos, M; Gold, DR; Damokosh, AI; Serrano, P; Allen, G; McDonnell, WF; Dockery, D; Velasco, SR; Hernandez, M; Hayes, C. (1995). Acute effects of ozone on the pulmonary function of exercising schoolchildren from Mexico City. Am J Respir Crit Care Med 152: 1501-1507.

Chan, CC; Chuang, KJ; Chien, LC; Chen, WJ; Chang, WT. (2006). Urban air pollution and emergency admissions for cerebrovascular diseases in Taipei, Taiwan. Eur Heart J 27: 1238-1244. http://dx.doi.org/10.1093/eurheartj/ehi835

Chan, CC; Chuang, KJ; Su, TC; Lin, LY. (2005a). Association between nitrogen dioxide and heart rate variability in a susceptible population. Eur J Cardiovasc Prev Rehabil 12: 580-586.

Chan, CC; Wu, TH. (2005). Effects of ambient ozone exposure on mail carriers' peak expiratory flow rates. Environ Health Perspect 113: 735-738. http://dx.doi.org/10.1289/ehp.7636

Chang, CC; Tsai, SS; Ho, SC; Yang, CY. (2005). Air pollution and hospital admissions for cardiovascular disease in Taipei, Taiwan. Environ Res 98: 114-119.

Chang, MM; Wu, R; Plopper, CG; Hyde, DM. (1998). IL-8 is one of the major chemokines produced by monkey airway epithelium after ozone-induced injury. Am J Physiol 275: L524-L532.

Chen, C; Arjomandi, M; Balmes, J; Tager, I; N, H. (2007a). Effects of chronic and acute ozone exposure on lipid peroxidation and antioxidant capacity in healthy young adults. Environ Health Perspect 115: 1732-1737. http://dx.doi.org/10.1289/ehp.10294

Chen, JC; Schwartz, J. (2009). Neurobehavioral effects of ambient air pollution on cognitive performance in US adults. Neurotoxicology 30: 231-239. http://dx.doi.org/10.1016/j.neuro.2008.12.011

Chen, L; Jennison, BL; Yang, W; Omaye, ST. (2000). Elementary school absenteeism and air pollution. Inhal Toxicol 12: 997-1016. http://dx.doi.org/10.1080/08958370050164626

Chen, PC; Lai, YM; Chan, CC; Hwang, JS; Yang, CY; Wang, JD. (1999). Short-term effect of ozone on the pulmonary function of children in primary school. Environ Health Perspect 107: 921-925. http://dx.doi.org/10.1289/ehp.99107921

Chen, XQ; Yang, J; Hu, SP; Nie, HX; Mao, GY; Chen, HB. (2006b). Increased expression of CD86 and reduced production of IL-12 and IL-10 by monocyte-derived dendritic cells from allergic asthmatics and their effects on Th1- and Th2-type cytokine balance. Respiration 73: 34-40. http://dx.doi.org/10.1159/000087457

Chhabra, SK; Yasir, A; Chaudhry, K; Shah, B. (2010). Effect of ozone on response to ovalbumin & its modulation by vitamins C & E in sensitized guinea pigs. Indian J Med Res 132: 87-93.

Chimenti, L; Morici, G; Paterno, A; Bonanno, A; Vultaggio, M; Bellia, V; Bonsignore, MR. (2009). Environmental conditions, air pollutants, and airway cells in runners: A longitudinal field study. J Sports Sci 27: 925-935. http://dx.doi.org/10.1080/02640410902946493

Chiu, HF; Cheng, MH; Yang, CY. (2009). Air pollution and hospital admissions for pneumonia in a subtropical city: Taipei, Taiwan. Inhal Toxicol 21: 32-37. http://dx.doi.org/10.1080/08958370802441198

Chiu, HF; Yang, CY. (2009). Air pollution and emergency room visits for arrhythmias: Are there potentially sensitive groups? J Toxicol Environ Health A 72: 817-823. http://dx.doi.org/10.1080/15287390902800405

Choi, JH; Xu, QS; Park, SY; Kim, JH; Hwang, SS; Lee, KH; Lee, HJ; Hong, YC. (2007). Seasonal variation of effect of air pollution on blood pressure. J Epidemiol Community Health 61: 314-318.

Christian, DL; Chen, LL; Scannell, CH; Ferrando, RE; Welch, BS; Balmes, JR. (1998). Ozone-induced inflammation is attenuated with multiday exposure. Am J Respir Crit Care Med 158: 532-537.

Chuang, GC; Yang, Z; Westbrook, DG; Pompilius, M; Ballinger, CA; White, RC; Krzywanski, DM; Postlethwait, EM; Ballinger, SW. (2009). Pulmonary ozone exposure induces vascular dysfunction, mitochondrial damage, and atherogenesis. Am J Physiol Lung Cell Mol Physiol 297: L209-L216. http://dx.doi.org/10.1152/ajplung.00102.2009

Chuang, KJ; Chan, CC; Su, TC; Lee, CT; Tang, CS. (2007a). The effect of urban air pollution on inflammation, oxidative stress, coagulation, and autonomic dysfunction in young adults. Am J Respir Crit Care Med 176: 370-376. http://dx.doi.org/10.1164/rccm.200611-1627OC

Chuang, KJ; Chan, CC; Su, TC; Lin, LY; Lee, CT. (2007b). Associations between particulate sulfate and organic carbon exposures and heart rate variability in patients with or at risk for cardiovascular diseases. J Occup Environ Med 49: 610-617. http://dx.doi.org/10.1097/JOM.0b013e318058205b

Chuang, KJ; Yan, YH; Cheng, TJ. (2010). Effect of air pollution on blood pressure, blood lipids, and blood sugar: A population-based approach. J Occup Environ Med 52: 258-262. http://dx.doi.org/10.1097/JOM.0b013e3181ceff7a

Clemons, GK; Garcia, JF. (1980). Changes in thyroid function after short-term ozone exposure in rats. J Environ Pathol Toxicol 4: 359-369.

Cockcroft, DW; Davis, BE; Todd, DC; Smycniuk, AJ. (2005). Methacholine challenge: Comparison of two methods. Chest 127: 839-844.

Coffin, DL; Blommer, EJ; Gardner, DE; Holzman, R. (1967). Effect of air pollution on alteration of susceptibility to pulmonary infection (pp. 71-80). (AMRL-TR-67-200). Cincinnati, OH: Aerospace Medical Research Laboratories.

Coffin, DL; Gardner, DE. (1972). Interaction of biological agents and chemical air pollutants. Ann Occup Hyg 15: 219-234.

Cohen, MD; Sisco, M; Baker, K; Li, Y; Lawrence, D; Van Loveren, H; Zelikoff, JT; Schlesinger, RB. (2002). Effects of inhaled ozone on pulmonary immune cells critical to antibacterial responses in situ. Inhal Toxicol 14: 599-619. http://dx.doi.org/10.1080/08958370290084520

Cole, MP; Freeman, BA. (2009). Promotion of cardiovascular disease by exposure to the air pollutant ozone [Review]. Am J Physiol Lung Cell Mol Physiol 297: L209-L216. http://dx.doi.org/10.1152/ajplung.00187.2009

Colín-Barenque, L; Dorado-Martinez, C; Rivas-Arancibia, S; Avila-Costa, MR; Fortoul, TI. (2005). Morphological recovery of the granule cells from the olfactory bulb after the cessation of acute ozone exposure. Int J Neurosci 115: 411-421. http://dx.doi.org/10.1080/00207450590521028

Corradi, M; Alinovi, R; Goldoni, M; Vettori, M; Folesani, G; Mozzoni, P; Cavazzini, S; Bergamaschi, E; Rossi, L; Mutti, A. (2002). Biomarkers of oxidative stress after controlled human exposure to ozone. Toxicol Lett 134: 219-225.

Corradi, M; Folesani, G; Andreoli, R; Manini, P; Bodini, A; Piacentini, G; Carraro, S; Zanconato, S; Baraldi, E. (2003). Aldehydes and glutathione in exhaled breath condensate of children with asthma exacerbation. Am J Respir Crit Care Med 167: 395-399. http://dx.doi.org/10.1164/rccm.200206-507OC

Crémillieux, Y; Servais, S; Berthezène, Y; Dupuich, D; Boussouar, A; Stupar, V; Pequignot, JM. (2008). Effects of ozone exposure in rat lungs investigated with hyperpolarized 3He MRI. J Magn Reson Imaging 27: 771-776. http://dx.doi.org/10.1002/jmri.21216

Curriero, FC; Heiner, KS; Samet, JM; Zeger, SL; Strug, L; Patz, JA. (2002). Temperature and mortality in 11 cities of the eastern United States. Am J Epidemiol 155: 80-87. http://dx.doi.org/10.1093/aje/155.1.80

Dales, R; Chen, L; Frescura, AM; Liu, L; Villeneuve, PJ. (2009). Acute effects of outdoor air pollution on forced expiratory volume in 1 s: A panel study of schoolchildren with asthma. Eur Respir J 34: 316-323. http://dx.doi.org/10.1183/09031936.00138908

Dales, RE; Cakmak, S; Doiron, MS. (2006). Gaseous air pollutants and hospitalization for respiratory disease in the neonatal period. Environ Health Perspect 114: 1751-1754. http://dx.doi.org/10.1289/ehp.9044

Damera, G; Jester William, F; Jiang, M; Zhao, H; Fogle Homer, W; Mittelman, M; Haczku, A; Murphy, E; Parikh, I; Panettieri Reynold, A. (2010). Inhibition of myristoylated alanine-rich C kinase substrate (MARCKS) protein inhibits ozone-induced airway neutrophilia and inflammation. Exp Lung Res 36: 75-84. http://dx.doi.org/10.3109/01902140903131200

Darrow, LA; Klein, M; Sarnat, JA; Mulholland, JA; Strickland, MJ; Sarnat, SE; Russell, AG; Tolbert, PE. (2011a). The use of alternative pollutant metrics in time-series studies of ambient air pollution and respiratory emergency department visits. J Expo Sci Environ Epidemiol 21: 10-19. http://dx.doi.org/10.1038/jes.2009.49

De Pablo, F; Lopez, A; Soriano, LR; Tomas, C; Diego, L; Gonzalez, M; Barrueco, M. (2006). Relationships of daily mortality and hospital admissions to air pollution in Castilla-Leon, Spain. Atmosfera 19: 23-39.

Delfino, RJ; Gillen, DL; Tjoa, T; Staimer, N; Polidori, A; Arhami, M; Sioutas, C; Longhurst, J. (2011). Electrocardiographic ST-segment depression and exposure to trafficrelated aerosols in elderly subjects with coronary artery disease. Environ Health Perspect 119: 196-202. http://dx.doi.org/10.1289/ehp.1002372

Delfino, RJ; Gone, H; Linn, WS; Pellizzari, ED; Hu, Y. (2003). Asthma symptoms in Hispanic children and daily ambient exposures to toxic and criteria air pollutants. Environ Health Perspect 111: 647-656. http://dx.doi.org/10.1289/ehp.5992

Delfino, RJ; Quintana, PJE; Floro, J; Gastanaga, VM; Samimi, BS; Kleinman, MT; Liu, LJS; Bufalino, C; Wu, CF; McLaren, CE. (2004). Association of FEV1 in asthmatic children with personal and microenvironmental exposure to airborne particulate matter. Environ Health Perspect 112: 932-941.

Delfino, RJ; Staimer, N; Tjoa, T; Arhami, M; Polidori, A; Gillen, DL; George, SC; Shafer, MM; Schauer, JJ; Sioutas, C. (2010a). Associations of primary and secondary organic aerosols with airway and systemic inflammation in an elderly panel cohort. Epidemiology 21: 892-902. http://dx.doi.org/10.1097/EDE.0b013e3181f20e6c

Delfino, RJ; Tjoa, T; Gillen, DL; Staimer, N; Polidori, A; Arhami, M; Jamner, L; Sioutas, C; Longhurst, J. (2010b). Traffic-related air pollution and blood pressure in elderly subjects with coronary artery disease. Epidemiology 21: 396-404. http://dx.doi.org/10.1097/EDE.0b013e3181d5e19b

Delfino, RJ; Zeiger, RS; Seltzer, JM; Street, DH; Matteucci, RM; Anderson, PR; Koutrakis, P. (1997). The effect of outdoor fungal spore concentrations on daily asthma severity. Environ Health Perspect 105: 622-635.

DeLucia, AJ; Adams, WC. (1977). Effects of O3 inhalation during exercise on pulmonary function and blood biochemistry. J Appl Physiol 43: 75-81.

Dennekamp, M; Akram, M; Abramson, MJ; Tonkin, A; Sim, MR; Fridman, M; Erbas, B. (2010). Outdoor air pollution as a trigger for out-of-hospital cardiac arrests. Epidemiology 21: 494-500. http://dx.doi.org/10.1097/EDE.0b013e3181e093db

Depuydt, P; Joos, GF; Pauwels, RA. (1999). Ambient ozone concentrations induce airway hyperresponsiveness in some rat strains. Eur Respir J 14: 125-131.

Devlin, RB; Duncan, KE; Jardim, M; Schmitt, MT; Rappold, AG; Diaz-Sanchez, D. (2012). Controlled exposure of healthy young volunteers to ozone causes cardiovascular effects. Circulation 126: 104-111. http://dx.doi.org/10.1161/CIRCULATIONAHA.112.094359

Devlin, RB; Folinsbee, LJ; Biscardi, F; Hatch, G; Becker, S; Madden, MC; Robbins, M; Koren, HS. (1997). Inflammation and cell damage induced by repeated exposure of humans to ozone. Inhal Toxicol 9: 211-235.

Devlin, RB; Mcdonnell, WF; Becker, S; Madden, MC; Mcgee, MP; Perez, R; Hatch, G; House, DE; Koren, HS. (1996). Time-dependent changes of inflammatory mediators in the lungs of humans exposed to 0.4 ppm ozone for 2 hr: A comparison of mediators found in bronchoalveolar lavage fluid 1 and 18 hr after exposure. Toxicol Appl Pharmacol 138: 176-185. http://dx.doi.org/10.1006/taap.1996.0111

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

Dimeo, MJ; Glenn, MG; Holtzman, MJ; Sheller, JR; Nadel, JA; Boushey, HA. (1981). Threshold concentration of ozone causing an increase in bronchial reactivity in humans and adaptation with repeated exposures. Am Rev Respir Dis 124: 245-248.

Dimitriadis, VK. (1992). Carbohydrate cytochemistry of bonnet monkey (Macaca radiata) nasal epithelium: Response to ambient levels of ozone. Histol Histopathol 7: 479-488.

Dockery, DW; Luttmann-Gibson, H; Rich, DQ; Link, MS; Mittleman, MA; Gold, DR; Koutrakis, P; Schwartz, JD; Verrier, RL. (2005). Association of air pollution with increased incidence of ventricular tachyarrhythmias recorded by implanted cardioverter defibrillators. Environ Health Perspect 113: 670-674.

Dohm, MR; Mautz, WJ; Andrade, JA; Gellert, KS; Salas-Ferguson, LJ; Nicolaisen, N; Fujie, N. (2005). Effects of ozone exposure on nonspecific phagocytic capacity of pulmonary macrophages from an amphibian, Bufo marinus. Environ Toxicol Chem 24: 205-210.

Dorado-Martinez, C; Parades-Carbajal, C; Mascher, D; Borgonio-Perez, G; Rivas-Arancibia, S. (2001). Effects of different ozone doses on memory, motor activity and lipid peroxidation levels, in rats. Int J Neurosci 108: 149-161.

Driscoll, KE; Vollmuth, TA; Schlesinger, RB. (1987). Acute and subchronic ozone inhalation in the rabbit: Response of alveolar macrophages. J Toxicol Environ Health 21: 27-43. http://dx.doi.org/10.1080/15287398709531000

Dryden, DM; Spooner, CH; Stickland, MK; Vandermeer, B; Tjosvold, L; Bialy, L; Wong, K; Rowe, BH. (2010). Exercise-induced bronchoconstriction and asthma. (AHRQ Publication No. 10-E001). Rockville, MD: Agency for Healthcare Research and Quality.

Dungworth, DL. (1976). Short-term effects of ozone on lungs of rats, mice, and monkeys. Environ Health Perspect 13: 179.

Dungworth, DL; Castleman, WL; Chow, CK; Mellick, PW; Mustafa, MG; Tarkington, B; Tyler, WS. (1975). Effect of ambient levels of ozone on monkeys. Fed Proc 34: 1670-1674.

Duramad, P; Tager, IB; Holland, NT. (2007). Cytokines and other immunological biomarkers in children's environmental health studies [Review]. Toxicol Lett 172: 48-59. http://dx.doi.org/10.1016/j.toxlet.2007.05.017

Eiswerth, ME; Shaw, WD; Yen, ST. (2005). Impacts of ozone on the activities of asthmatics: Revisiting the data. J Environ Manage 77: 56-63. http://dx.doi.org/10.1016/j.jenvman.2005.02.010

Escalante-Membrillo, C; Gonzalez-Maciel, A; Reynoso-Robles, R; Gonzalez-Pina, R. (2005). Brain thiobarbituric acid-reactive substances in rats after short periods of ozone exposure. Environ Res 99: 68-71. http://dx.doi.org/10.1016/j.envres.2005.02.006

Escamilla-Nuñez, MC; Barraza-Villarreal, A; Hernandez-Cadena, L; Moreno-Macias, H; Ramirez-Aguilar, M; Sienra-Monge, JJ; Cortez-Lugo, M; Texcalac, JL; del Rio-Navarro, B; Romieu, I. (2008). Traffic-related air pollution and respiratory symptoms among asthmatic children, resident in Mexico City: The EVA cohort study. Respir Res 9: 74. http://dx.doi.org/10.1186/1465-9921-9-74

Fakhri, AA; Ilic, LM; Wellenius, GA; Urch, B; Silverman, F; Gold, DR; Mittleman, MA. (2009). Autonomic effects of controlled fine particulate exposure in young healthy adults: Effect modification by ozone. Environ Health Perspect 117: 1287-1292. http://dx.doi.org/10.1289/ehp.0900541

Farraj, AK; Boykin, E; Ledbetter, A; Andrews, D; Gavett, SH. (2010). Increased lung resistance after diesel particulate and ozone co-exposure not associated with enhanced lung inflammation in allergic mice. Inhal Toxicol 22: 33-41. http://dx.doi.org/10.3109/08958370902862434

Feng, R; He, W; Ochi, H; Castranova, V. (2006). Ozone exposure impairs antigen-specific immunity but activates IL-7-induced proliferation of CD4-CD8- thymocytes in BALB/c mice. J Toxicol Environ Health A 69: 1511-1526. http://dx.doi.org/10.1080/15287390500468696

Feo Brito, F; Mur Gimeno, P; Martinez, C; Tobias, A; Suarez, L; Guerra, F; Borja, JM; Alonso, AM. (2007). Air pollution and seasonal asthma during the pollen season: A cohort study in Puertollano and Ciudad Real (Spain). Allergy 62: 1152-1157. http://dx.doi.org/10.1111/j.1398-9995.2007.01438.x

Ferdinands, JM; Crawford, CA; Greenwald, R; Van Sickle, D; Hunter, E; Teague, WG. (2008). Breath acidification in adolescent runners exposed to atmospheric pollution: A prospective, repeated measures observational study. Environ Health Global Access Sci Source 7: 11. http://dx.doi.org/10.1186/1476-069X-7-10

Folinsbee, LJ; Bedi, JF; Horvath, SM. (1980). Respiratory responses in humans repeatedly exposed to low concentrations of ozone. Am Rev Respir Dis 121: 431-439.

Folinsbee, LJ; Bedi, JF; Horvath, SM. (1984). Pulmonary function changes after 1 h continuous heavy exercise in 0.21 ppm ozone. J Appl Physiol 57: 984-988.

Folinsbee, LJ; Devlin, RB; Abdul-Salaam, S; Koren, HS. (1993). Repeated severe ozone exposure causes depressed baseline spirometry [Abstract]. Am Rev Respir Dis 147: A638.

Folinsbee, LJ; Drinkwater, BL; Bedi, JF; Horvath, SM. (1978). The influence of exercise on the pulmonary function changes due to exposure to low concentrations of ozone. In LJ Folinsbee; JA Wagner; JF Borgia; BL Drinkwater; JA Gliner; JF Bedi (Eds.), Environmental stress: Individual human adaptations (pp. 125-145). New York, NY: Academic Press.

Folinsbee, LJ; Hazucha, MJ. (1989). Persistence of ozone-induced changes in lung function and airway responsiveness. In Atmospheric ozone research and its policy implications. Amsterdam, The Netherlands: Elsevier.

Folinsbee, LJ; Hazucha, MJ. (2000). Time course of response to ozone exposure in healthy adult females. Inhal Toxicol 12: 151-167.

Folinsbee, LJ; Horstman, DH; Kehrl, HR; Harder, S; Abdul-Salaam, S; Ives, PJ. (1994). Respiratory responses to repeated prolonged exposure to 0.12 ppm ozone. Am J Respir Crit Care Med 149: 98-105.

Folinsbee, LJ; McDonnell, WF; Horstman, DH. (1988). Pulmonary function and symptom responses after 6.6-hour exposure to 0.12 ppm ozone with moderate exercise. J Air Waste Manag Assoc 38: 28-35.

Folinsbee, LJ; Silverman, F; Shephard, RJ. (1977). Decrease of maximum work performance following ozone exposure. J Appl Physiol 42: 531-536.

Folnsbee, LJ; Devlin, RB; Robbins, MK; Biscardi, FH; Abdul-Salaam, S; Koren, HS. (1995). Repeated exposure of humans to ozone: I. Pulmonary function and symptom responses. Available online

Fortino, V; Maioli, E; Torricelli, C; Davis, P; Valacchi, G. (2007). Cutaneous MMPs are differently modulated by environmental stressors in old and young mice. Toxicol Lett 173: 73-79. http://dx.doi.org/10.1016/j.toxlet.2007.06.004

Foster, WM; Silver, JA; Groth, ML. (1993). Exposure to ozone alters regional function and particle dosimetry in the human lung. J Appl Physiol 75: 1938-1945.

Foster, WM; Stetkiewicz, PT. (1996). Regional clearance of solute from the respiratory epithelia: 18-20 h postexposure to ozone. J Appl Physiol 81: 1143-1149.

Foster, WM; Weinmann, GG; Menkes, E; Macri, K. (1997). Acute exposure of humans to ozone impairs small airway function. Ann Occup Hyg 1: 659-666.

Foster, WM; Wills-Karp, M; Tankersley, CG; Chen, X; Paquette, NC. (1996). Bloodborne markers in humans during multiday exposure to ozone. J Appl Physiol 81: 794-800.

Fox, SD; Adams, WC; Brookes, KA; Lasley, BL. (1993). Enhanced response to ozone exposure during the follicular phase of the menstrual cycle. Environ Health Perspect 101: 242-244.

Foxcroft, WJ; Adams, WC. (1986). Effects of ozone exposure on four consecutive days on work performance and VO2max. J Appl Physiol 61: 960-966.

Frampton, MW; Morrow, PE; Torres, A; Cox, C; Voter, KZ; Utell, MJ; Gibb, FR; Speers, DM. (1997a). Ozone responsiveness in smokers and nonsmokers. Am J Respir Crit Care Med 155: 116-121.

Frank, R; Liu, MC; Spannhake, EW; Mlynarek, S; Macri, K; Weinmann, GG. (2001). Repetitive ozone exposure of young adults: Evidence of persistent small airway dysfunction. Am J Respir Crit Care Med 164: 1253-1260.

Franklin, M; Schwartz, J. (2008). The impact of secondary particles on the association between ambient ozone and mortality. Environ Health Perspect 116: 453-458. http://dx.doi.org/10.1289/ehp.10777

Franze, T; Weller, MG; Niessner, R; Pöschl, U. (2005). Protein nitration by polluted air. Environ Sci Technol 39: 1673-1678. http://dx.doi.org/10.1021/es0488737

Friedman, M; Gallo, JM; Nichols, HP; Bromberg, PA. (1983). Changes in inert gas rebreathing parameters after ozone exposure in dogs. Am Rev Respir Dis 128: 851-856.

Fung, KY; Luginaah, I; Gorey, KM; Webster, G. (2005). Air pollution and daily hospital admissions for cardiovascular diseases in Windsor, Ontario. Can J Public Health 96: 29-33.

Gao, X; Raghavamenon, AC; D'Auvergne, O; Uppu, RM. (2009b). Cholesterol secoaldehyde induces apoptosis in J774 macrophages via mitochondrial pathway but not involving reactive oxygen species as mediators. Biochem Biophys Res Commun 389: 382-387. http://dx.doi.org/10.1016/j.bbrc.2009.09.005

Garantziotis, S; Li, Z; Potts, EN; Lindsey, JY; Stober, VP; Polosukhin, VV; Blackwell, TS; Schwartz, DA; Foster, WM; Hollingsworth, JW. (2010). TLR4 is necessary for hyaluronan-mediated airway hyperresponsiveness after ozone inhalation. Am J Respir Crit Care Med 181: 666-675. http://dx.doi.org/10.1164/rccm.200903-0381OC

Gent, JF; Triche, EW; Holford, TR; Belanger, K; Bracken, MB; Beckett, WS; Leaderer, BP. (2003). Association of low-level ozone and fine particles with respiratory symptoms in children with asthma. JAMA 290: 1859-1867. http://dx.doi.org/10.1001/jama.290.14.1859

Gershwin, LJ; Osebold, JW; Zee, YC. (1981). Immunoglobulin E-containing cells in mouse lung following allergen inhalation and ozone exposure. International Arch Allergy Appl Immunol 65: 266-277.

Gielen, MH; Van Der Zee, SC; Van Wijnen, JH; Van Steen, CJ; Brunekreef, B. (1997). Acute effects of summer air pollution on respiratory health of asthmatic children. Am J Respir Crit Care Med 155: 2105-2108.

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

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

Girardot, SP; Ryan, PB; Smith, SM; Davis, WT; Hamilton, CB; Obenour, RA; Renfro, JR; Tromatore, KA; Reed, GD. (2006). Ozone and PM2.5 exposure and acute pulmonary health effects: A study of hikers in the Great Smoky Mountains National Park. Environ Health Perspect 113: 612-617. http://dx.doi.org/10.1289/ehp.8637

Gold, DR; Damokosh, AI; III, PC; Dockery, DW; McDonnell, WF; Serrano, P; Retama, A; Castillejos, M. (1999). Particulate and ozone pollutant effects on the respiratory function of children in southwest Mexico City. Epidemiology 10: 8-16.

Goldberg, MS; Giannetti, N; Burnett, RT; Mayo, NE; Valois, MF; Brophy, JM. (2008). A panel study in congestive heart failure to estimate the short-term effects from personal factors and environmental conditions on oxygen saturation and pulse rate. Occup Environ Med 65: 659-666. http://dx.doi.org/10.1136/oem.2007.034934

Gong, H, Jr; Bradley, PW; Simmons, MS; Tashkin, DP. (1986). Impaired exercise performance and pulmonary function in elite cyclists during low-level ozone exposure in a hot environment. Am J Respir Crit Care Med 134: 726-733.

Gong, H, Jr; McManus, MS; Linn, WS. (1997a). Attenuated response to repeated daily ozone exposures in asthmatic subjects. Arch Environ Occup Health 52: 34-41.

Gong, H, Jr; Shamoo, DA; Anderson, KR; Linn, WS. (1997b). Responses of older men with and without chronic obstructive pulmonary disease to prolonged ozone exposure. Arch Environ Occup Health 52: 18-25.

Gong, H, Jr; Wong, R; Sarma, RJ; Linn, WS; Sullivan, ED; Shamoo, DA; Anderson, KR; Prasad, SB. (1998). Cardiovascular effects of ozone exposure in human volunteers. Am J Respir Crit Care Med 158: 538-546.

Gonzalez-Pina, R; Escalante-Membrillo, C; Alfaro-Rodriguez, A; Gonzalez-Maciel, A. (2008). Prenatal exposure to ozone disrupts cerebellar monoamine contents in newborn rats. Neurochem Res 33: 912-918. http://dx.doi.org/10.1007/s11064-007-9534-3

Graham, JA; Menzel, DB; Miller, FJ; Illing, JW; Gardner, DE. (1981). Influence of ozone on pentobarbital-induced sleeping time in mice, rats, and hamsters. Toxicol Appl Pharmacol 61: 64-73. http://dx.doi.org/10.1016/0041-008X(81)90008-9

Gryparis, A; Forsberg, B; Katsouyanni, K; Analitis, A; Touloumi, G; Schwartz, J; Samoli, E; Medina, S; Anderson, HR; Niciu, EM; Wichmann, HE; Kriz, B; Kosnik, M; Skorkovsky, J; Vonk, JM; Dortbudak, Z. (2004). Acute effects of ozone on mortality from the "Air pollution and health: A European approach" project. Am J Respir Crit Care Med 170: 1080-1087. http://dx.doi.org/10.1164/rccm.200403-333OC

Guerrero, AL; Dorado-Martinez, C; Rodriguez, A; Pedroza-Rios, K; Borgonio-Perez, G; Rivas-Arancibia, S. (1999). Effects of vitamin E on ozone-induced memory deficits and lipid peroxidation in rats. Neuroreport 10: 1689-1692.

Guevara-Guzmán, R; Arriaga, V; Kendrick, KM; Bernal, C; Vega, X; Mercado-Gómez, OF; Rivas-Arancibia, S. (2009). Estradiol prevents ozone-induced increases in brain lipid peroxidation and impaired social recognition memory in female rats. Neuroscience 159: 940-950. http://dx.doi.org/10.1016/j.neuroscience.2009.01.047

Hackney, JD; Linn, WS; Buckley, RD; Hislop, HJ. (1976). Studies in adaptation to ambient oxidant air pollution: effects of ozone exposure in Los Angeles residents vs new arrivals. Environ Health Perspect 18: 141-146.

Hackney, JD; Linn, WS; Karuza, SK; Buckley, RD; Law, DC; Bates, DV; Hazucha, M; Pengelly, LD; Silverman, F. (1977a). Effects of ozone exposure in Canadians and southern Californians: evidence for adaptation? Arch Environ Occup Health 32: 110-116.

Hackney, JD; Linn, WS; Mohler, JG; Pedersen, EE; Breisacher, P; Russo, A. (1975). Experimental studies on human health effects of air pollutants: II. Four-hour exposure to ozone alone and in combination with other pollutant gases. Arch Environ Occup Health 30: 379-384.

Halonen, JI; Lanki, T; Tiittanen, P; Niemi, JV; Loh, M; Pekkanen, J. (2009). Ozone and cause-specific cardiorespiratory morbidity and mortality. J Epidemiol Community Health 64: 814-820. http://dx.doi.org/10.1136/jech.2009.087106

Hamade, AK; Misra, V; Rabold, R; Tankersley, CG. (2010). Age-related changes in cardiac and respiratory adaptation to acute ozone and carbon black exposures: Interstrain variation in mice. Inhal Toxicol 22: 84-94. http://dx.doi.org/10.3109/08958378.2010.503974

Hamade, AK; Rabold, R; Tankersley, CG. (2008). Adverse cardiovascular effects with acute particulate matter and ozone exposures: Interstrain variation in mice. Environ Health Perspect 116: 1033-1039. http://dx.doi.org/10.1289/ehp.10689

Hamade, AK; Tankersley, CG. (2009). Interstrain variation in cardiac and respiratory adaptation to repeated ozone and particulate matter exposures. Am J Physiol Regul Integr Comp Physiol 296: R1202-R1215. http://dx.doi.org/10.1152/ajpregu.90808.2008

Hampel, R; Breitner, S; Zareba, W; Kraus, U; Pitz, M; Geruschkat, U; Belcredi, P; Peters, A; Schneider, A; Group, ftCHRitRoAKS. (In Press) Immediate ozone effects on heart rate and repolarisation parameters in potentially susceptible individuals. Occup Environ Med. http://dx.doi.org/10.1136/oemed-2011-100179

Han, SG; Andrews, R; Gairola, CG; Bhalla, DK. (2008). Acute pulmonary effects of combined exposure to carbon nanotubes and ozone in mice. Inhal Toxicol 20: 391-398. http://dx.doi.org/10.1080/08958370801904014

Harkema, JR; Plopper, CG; Hyde, DM; St George, JA; Dungworth, DL. (1987a). Effects of an ambient level of ozone on primate nasal epithelial mucosubstances: quantitative histochemistry. Am J Pathol 127: 90-96.

Harkema, JR; Plopper, CG; Hyde, DM; St George, JA; Wilson, DW; Dungworth, DL. (1987b). Response of the macaque nasal epithelium to ambient levels of ozone: A morphologic and morphometric study of the transitional and respiratory epithelium. Am J Pathol 128: 29-44.

Harkema, JR; Plopper, CG; Hyde, DM; St George, JA; Wilson, DW; Dungworth, DL. (1993). Response of macaque bronchiolar epithelium to ambient concentrations of ozone. Am J Pathol 143: 857-866.

Hatch, GE; Slade, R; Harris, LP; Mcdonnell, WF; Devlin, RB; Koren, HS; Costa, DL; Mckee, J. (1994). Ozone dose and effect in humans and rats: A comparison using oxygen-18 labeling and bronchoalveolar lavage. Am J Respir Crit Care Med 150: 676-683.

Hazucha, MJ; Folinsbee, LJ; Bromberg, PA. (2003). Distribution and reproducibility of spirometric response to ozone by gender and age. J Appl Physiol 95: 1917-1925.

Hazucha, MJ; Folinsbee, LJ; Seal, E, Jr. (1992). Effects of steady-state and variable ozone concentration profiles on pulmonary function. Am J Respir Crit Care Med 146: 1487-1493.

Hazucha, MJ; Lefohn, AS. (2007). Nonlinearity in human health response to ozone: Experimental laboratory considerations. Atmos Environ 41: 4559-4570. http://dx.doi.org/10.1016/j.atmosenv.2007.03.052

HEI (Health Effects Institute). (2003). Revised analyses of time-series studies of air pollution and health: Revised analyses of the National Morbidity, Mortality, and Air Pollution Study (NMMAPS), Part II. Cambridge, MA. http://pubs.healtheffects.org/view.php?id=4

Heidenfelder, BL; Reif, DM; Harkema, J. R.; Cohen Hubal, EA; Hudgens, EE; Bramble, LA; Wagner, JG; Morishita, M; Keeler, GJ; Edwards, SW; Gallagher, JE. (2009). Comparative microarray analysis and pulmonary changes in brown Norway rats exposed to ovalbumin and concentrated air particulates. Toxicol Sci 108: 207-221. http://dx.doi.org/10.1093/toxsci/kfp005

Hemmingsen, A; Fryer, AA; Hepple, M; Strange, RC; Spiteri, MA. (2001). Simultaneous identification of GSTP1 Ile105->Val105 and Ala114->Val114 substitutions using an amplification refractory mutation system polymerase chain reaction assay: Studies in patients with asthma. Respir Res 2: 255-260. http://dx.doi.org/10.1186/rr64

Henderson, FW; Dubovi, EJ; Harder, S; Seal, E, Jr; Graham, D. (1988). Experimental rhinovirus infection in human volunteers exposed to ozone. Am J Respir Crit Care Med 137: 1124-1128.

Henrotin, JB; Besancenot, JP; Bejot, Y; Giroud, M. (2007). Short-term effects of ozone air pollution on ischaemic stroke occurrence: A case-crossover analysis from a 10-year population-based study in Dijon, France. Occup Environ Med 64: 439-445. http://dx.doi.org/10.1136/oem.2006.029306

Henrotin, JB; Zeller, M; Lorgis, L; Cottin, Y; Giroud, M; Béjot, Y. (2010). Evidence of the role of short-term exposure to ozone on ischaemic cerebral and cardiac events: The Dijon Vascular Project (DIVA). Heart 96: 1990-1996. http://dx.doi.org/10.1136/hrt.2010.200337

Hernández-Cadena, L; Holguin, F; Barraza-Villarreal, A; Del Río-Navarro, BE; Sienra-Monge, JJ; Romieu, I. (2009). Increased levels of outdoor air pollutants are associated with reduced bronchodilation in children with asthma. Chest 136: 1529-1536. http://dx.doi.org/10.1378/chest.08-1463

Hernandez, ML; Lay, JC; Harris, B; Esther, CR; Brickey, WJ; Bromberg, PA; Diaz-Sanchez, D; Devlin, RB; Kleeberger, SR; Alexis, NE; Peden, DB. (2010). Atopic asthmatic subjects but not atopic subjects without asthma have enhanced inflammatory response to ozone. J Allergy Clin Immunol 126: 537-544. http://dx.doi.org/10.1016/j.jaci.2010.06.043

Hicks, A; Goodnow, R, Jr; Cavallo, G; Tannu, SA; Ventre, JD; Lavelle, D; Lora, JM; Satjawatcharaphong, J; Brovarney, M; Dabbagh, K; Tare, NS; Oh, H; Lamb, M; Sidduri, A; Dominique, R; Qiao, Q; Lou, JP; Gillespie, P; Fotouhi, N; Kowalczyk, A; Kurylko, G; Hamid, R; Wright, MB; Pamidimukkala, A; Egan, T; Gubler, U; Hoffman, AF; Wei, X; Li, YL; O'Neil, J; Marcano, R; Pozzani, K; Molinaro, T; Santiago, J; Singer, L; Hargaden, M; Moore, D; Catala, AR; Chao, LC; Benson, J; March, T; Venkat, R; Mancebo, H; Renzetti, LM. (2010a). Effects of LTB4 receptor antagonism on pulmonary inflammation in rodents and non-human primates. Prostaglandins Other Lipid Mediat 92: 33-43. http://dx.doi.org/10.1016/j.prostaglandins.2010.02.003

Hicks, A; Kourteva, G; Hilton, H; Li, H; Lin, T; Liao, W; Li, Y; Wei, X; March, T; Benson, J; Renzetti, L. (2010b). Cellular and molecular characterization of ozone-induced pulmonary inflammation in the Cynomolgus monkey. Inflammation 33: 144-156. http://dx.doi.org/10.1007/s10753-009-9168-5

Higgins, ITT; D'Arcy, JB; Gibbons, DI; Avol, EL; Gross, KB. (1990). Effect of exposures to ambient ozone on ventilatory lung function in children. Am J Respir Crit Care Med 141: 1136-1146.

Hiltermann, JTN; Lapperre, TS; Van Bree, L; Steerenberg, PA; Brahim, JJ; Sont, JK; Sterk, PJ; Hiemstra, PS; Stolk, J. (1999). Ozone-induced inflammation assessed in sputum and bronchial lavage fluid from asthmatics: A new noninvasive tool in epidemiologic studies on air pollution and asthma. Free Radic Biol Med 27: 1448-1454.

Hiltermann, TJN; Peters, EA; Alberts, B; Kwikkers, K; Borggreven, PA; Hiemstra, PS; Dijkman, JH; van Bree, LA; Stolk, J. (1998). Ozone-induced airway hyperresponsiveness in patients with asthma: Role of neutrophil-derived serine proteinases. Free Radic Biol Med 24: 952-958.

Hiltermann, TJN; Stolk, J; Hiemstra, PS; Fokkens, PHB; Rombout, PJA; Sont, JK; Sterk, PJ; Dijkman, JH. (1995). Effect of ozone exposure on maximal airway narrowing in non-asthmatic and asthmatic subjects. Clin Sci (Lond) 89: 619-624.

Hinwood, AL; De Klerk, N; Rodriguez, C; Jacoby, P; Runnion, T; Rye, P; Landau, L; Murray, F; Feldwick, M; Spickett, J. (2006). The relationship between changes in daily air pollution and hospitalizations in Perth, Australia 1992-1998: A case-crossover study. Int J Environ Health Res 16: 27-46. http://dx.doi.org/10.1080/09603120500397680

Hoek, G; Brunekreef, B. (1995). Effect of photochemical air pollution on acute respiratory symptoms in children. Am J Respir Crit Care Med 151: 27-32.

Hoek, G; Brunekreef, B; Kosterink, P; Van den Berg, R; Hofschreuder, P. (1993). Effect of ambient ozone on peak expiratory flow of exercising children in the Netherlands. Arch Environ Occup Health 48: 27-32. http://dx.doi.org/10.1080/00039896.1993.9938390

Hollingsworth, JW; Cook, DN; Brass, DM; Walker, JKL; Morgan, DL; Foster, WM; Schwartz, DA. (2004). The role of Toll-like receptor 4 in environmental airway injury in mice. Am J Respir Crit Care Med 170: 126-132. http://dx.doi.org/10.1164/rccm.200311-1499OC

Hollingsworth, JW; Free, ME; Li, Z; Andrews, LN; Nakano, H; Cook, DN. (2010). Ozone activates pulmonary dendritic cells and promotes allergic sensitization through a Toll-like receptor 4-dependent mechanism [Letter]. J Allergy Clin Immunol 125: 1167-1170. http://dx.doi.org/10.1016/j.jaci.2010.03.001

Holz, O; Jorres, RA; Timm, P; Mucke, M; Richter, K; Koschyk, S; Magnussen, H. (1999). Ozone-induced airway inflammatory changes differ between individuals and are reproducible. Am J Respir Crit Care Med 159: 776-784.

Holz, O; Mucke, M; Paasch, K; Bohme, S; Timm, P; Richter, K; Magnussen, H; Jorres, RA. (2002). Repeated ozone exposures enhance bronchial allergen responses in subjects with rhinitis or asthma. Clin Exp Allergy 32: 681-689.

Holz, O; Tal-Singer, R; Kanniess, F; Simpson, KJ; Gibson, A; Vessey, RSJ; Janicki, S; Magnussen, H; Jorres, RA; Richter, K. (2005). Validation of the human ozone challenge model as a tool for assessing anti-inflammatory drugs in early development. J Clin Pharmacol 45: 498-503.

Hoppe, P; Peters, A; Rabe, G; Praml, G; Lindner, J; Jakobi, G; Fruhmann, G; Nowak, D. (2003). Environmental ozone effects in different population subgroups. Int J Hyg Environ Health 206: 505-516. http://dx.doi.org/10.1078/1438-4639-00250

Hoppe, P; Praml, G; Rabe, G; Lindner, J; Fruhmann, G; Kessel, R. (1995). Environmental ozone field study on pulmonary and subjective responses of assumed risk groups. Environ Res 71: 109-121.

Horstman, DH; Ball, BA; Brown, J; Gerrity, T; Folinsbee, LJ. (1995). Comparison of pulmonary responses of asthmatic and nonasthmatic subjects performing light exercise while exposed to a low level of ozone. Toxicol Ind Health 11: 369-385.

Horstman, DH; Folinsbee, LJ; Ives, PJ; Abdul-Salaam, S; McDonnell, WF. (1990). Ozone concentration and pulmonary response relationships for 6.6-hour exposures with five hours of moderate exercise to 0.08, 0.10, and 0.12 ppm. Am J Respir Crit Care Med 142: 1158-1163.

Horvath, SM; Gliner, JA; Folinsbee, LJ. (1981). Adaptation to ozone: Duration of effect. Am Rev Respir Dis 123: 496-499.

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

Hosseinpoor, AR; Forouzanfar, MH; Yunesian, M; Asghari, F; Naieni, KH; Farhood, D. (2005). Air pollution and hospitalization due to angina pectoris in Tehran, Iran: A time-series study. Environ Res 99: 126-131.

Housley, DG; Eccles, R; Richards, RJ. (1996). Gender difference in the concentration of the antioxidant uric acid in human nasal lavage. Acta Otolaryngol 116: 751-754.

Howarth, PH; Persson, CG; Meltzer, EO; Jacobson, MR; Durham, SR; Silkoff, PE. (2005). Objective monitoring of nasal airway inflammation in rhinitis [Review]. J Allergy Clin Immunol 115: S414-S441. http://dx.doi.org/10.1016/j.jaci.2004.12.1134

Hunt, J. (2002). Exhaled breath condensate: An evolving tool for noninvasive evaluation of lung disease [Review]. J Allergy Clin Immunol 110: 28-34. http://dx.doi.org/10.1067/mai.2002.124966

Hunt, JF; Fang, K; Malik, R; Snyder, A; Malhotra, N; Platts-Mills, TAE; Gaston, B. (2000). Endogenous airway acidification: Implications for asthma pathophysiology. Am J Respir Crit Care Med 161: 694-699.

Hurst, DJ; Gardner, DE; Coffin, DL. (1970). Effect of ozone on acid hydrolases of the pulmonary alveolar macrophage. J Reticuloendothel Soc 8: 288-300.

Hyde, DM; Hubbard, WC; Wong, V; Wu, R; Pinkerton, K; Plopper, CG. (1992). Ozone-induced acute tracheobronchial epithelial injury: relationship to granulocyte emigration in the lung. Am J Respir Cell Mol Biol 6: 481-497.

Inoue, K; Takano, H; Kaewamatawong, T; Shimada, A; Suzuki, J; Yanagisawa, R; Tasaka, S; Ishizaka, A; Satoh, M. (2008). Role of metallothionein in lung inflammation induced by ozone exposure in mice. Free Radic Biol Med 45: 1714-1722. http://dx.doi.org/10.1016/j.freeradbiomed.2008.09.008

Ito, K; De Leon, SF; Lippmann, M. (2005). Associations between ozone and daily mortality, analysis and meta-analysis. Epidemiology 16: 446-457.

Ito, K; Thurston, GD; Silverman, RA. (2007b). Characterization of PM2.5, gaseous pollutants, and meteorological interactions in the context of time-series health effects models. J Expo Sci Environ Epidemiol 17: S45-S60. http://dx.doi.org/10.1038/sj.jes.7500627

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

Jakab, GJ; Hmieleski, RR. (1988). Reduction of influenza virus pathogenesis by exposure to 0.5 ppm ozone. J Toxicol Environ Health 23: 455-472. http://dx.doi.org/10.1080/15287398809531128

Jalaludin, BB; Chey, T; O'Toole, BI; Smith, WT; Capon, AG; Leeder, SR. (2000). Acute effects of low levels of ambient ozone on peak expiratory flow rate in a cohort of Australian children. Int J Epidemiol 29: 549-557. http://dx.doi.org/10.1093/ije/29.3.549

Jalaludin, BB; O'Toole, BI; Leeder, S. R. (2004). Acute effects of urban ambient air pollution on respiratory symptoms, asthma medication use, and doctor visits for asthma in a cohort of Australian children. Environ Res 95: 32-42. http://dx.doi.org/10.1016/S0013-9351(03)00038-0

Janero, DR. (1990). Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic Biol Med 9: 515-540. http://dx.doi.org/10.1016/0891-5849(90)90131-2

Jang, AS; Choi, IS; Yang, SY; Kim, YG; Lee, JH; Park, SW; Park, CS. (2005). Antioxidant responsiveness in BALB/c mice exposed to ozone. Respiration 72: 79-84. http://dx.doi.org/10.1159/000083405

Johnston, RA; Mizgerd, JP; Flynt, L; Quinton, LJ; Williams, ES; Shore, SA. (2007). Type I interleukin-1 receptor is required for pulmonary responses to subacute ozone exposure in mice. Am J Respir Cell Mol Biol 37: 477-484. http://dx.doi.org/10.1165/rcmb.2006-0315OC

Johnston, RA; Schwartzman, IN; Flynt, L; Shore, SA. (2005b). Role of interleukin-6 in murine airway responses to ozone. Am J Physiol Lung Cell Mol Physiol 288: L390-L397. http://dx.doi.org/10.1152/ajplung.00007.2004

Jones, SL; Kittelson, J; Cowan, JO; Flannery, EM; Hancox, RJ; McLachlan, CR; Taylor, DR. (2001). The predictive value of exhaled nitric oxide measurements in assessing changes in asthma control. Am J Respir Crit Care Med 164: 738-743.

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

Jorres, RA; Holz, O; Zachgo, W; Timm, P; Koschyk, S; Muller, B; Grimminger, F; Seeger, W; Kelly, FJ; Dunster, C; Frischer, T; Lubec, G; Waschewski, M; Niendorf, A; Magnussen, H. (2000). The effect of repeated ozone exposures on inflammatory markers in bronchoalveolar lavage fluid and mucosal biopsies. Am J Respir Crit Care Med 161: 1855-1861.

Just, J; Segala, C; Sahraoui, F; Priol, G; Grimfeld, A; Neukirch, F. (2002). Short-term health effects of particulate and photochemical air pollution in asthmatic children. Eur Respir J 20: 899-906. http://dx.doi.org/10.1183/09031936.02.00236902

Katsouyanni, K; Samet, JM; Anderson, HR; Atkinson, R; Le Tertre, A; Medina, S; Samoli, E; Touloumi, G; Burnett, RT; Krewski, D; Ramsay, T; Dominici, F; Peng, RD; Schwartz, J; Zanobetti, A. (2009). Air pollution and health: A European and North American approach (APHENA). (Research Report 142). Boston, MA: Health Effects Institute. http://pubs.healtheffects.org/view.php?id=327

Katsouyanni, K; Touloumi, G; Samoli, E; Gryparis, A; Le Tertre, A; Monopolis, Y; Rossi, G; Zmirou, D; Ballester, F; Boumghar, A; Anderson, HR; Wojtyniak, B; Paldy, A; Braunstein, R; Pekkanen, J; Schindler, C; Schwartz, J. (2001). Confounding and effect modification in the short-term effects of ambient particles on total mortality: Results from 29 European cities within the APHEA2 project. Epidemiology 12: 521-531.

Kehrl, HR; Hazucha, MJ; Solic, JJ; Bromberg, PA. (1985). Responses of subjects with chronic obstructive pulmonary disease after exposures to 0.3 ppm ozone. Am Rev Respir Dis 131: 719-724.

Kehrl, HR; Peden, DB; Ball, BA; Folinsbee, LJ; Horstman, DH. (1999). Increased specific airway reactivity of persons with mild allergic asthma after 7.6 hours of exposure to 0.16 ppm ozone. J Allergy Clin Immunol 104: 1198-1204.

Kehrl, HR; Vincent, LM; Kowalsky, RJ; Horstman, DH; O'Neil, JJ; McCartney, WH; Bromberg, PA. (1987). Ozone exposure increases respiratory epithelial permeability in humans. Am Rev Respir Dis 135: 1124-1128.

Kharitonov, SA; Barnes, PJ. (2000). Clinical aspects of exhaled nitric oxide [Review]. Eur Respir J 16: 781-792.

Khatri, SB; Holguin, FC; Ryan, PB; Mannino, D; Erzurum, SC; Teague, WG. (2009). Association of ambient ozone exposure with airway inflammation and allergy in adults with asthma. J Asthma 46: 777-785. http://dx.doi.org/10.1080/02770900902779284

Kim, CS; Alexis, NE; Rappold, AG; Kehrl, H; Hazucha, MJ; Lay, JC; Schmitt, MT; Case, M; Devlin, RB; Peden, DB; Diaz-Sanchez, D. (2011). Lung function and inflammatory responses in healthy young adults exposed to 0.06 ppm ozone for 6.6 hours. Am J Respir Crit Care Med 183: 1215-1221. http://dx.doi.org/10.1164/rccm.201011-1813OC

Kinney, PL; Thurston, GD; Raizenne, M. (1996). The effects of ambient ozone on lung function in children: A reanalysis of six summer camp studies. Environ Health Perspect 104: 170-174.

Kleeberger, SR; Reddy, S; Zhang, LY; Jedlicka, AE. (2000). Genetic susceptibility to ozone-induced lung hyperpermeability: Role of toll-like receptor 4. Am J Respir Cell Mol Biol 22: 620-627.

Klestadt, D; Laval-Gilly, P; Foucaud, L; Falla, J. (2005). Influences of ozone exposure upon macrophage responsivity to N-formyl-methionyl-leucyl-phenylalanine: Mobility and metabolic changes. Toxicol In Vitro 19: 199-206. http://dx.doi.org/10.1016/j.tiv.2004.08.004

Kodavanti, UP; Thomas, R; Ledbetter, AD; Schladweiler, MC; Shannahan, JH; Wallenborn, JG; Lund, AK; Campen, MJ; Butler, EO; Gottipolu, RR; Nyska, A; Richards, JE; Andrews, D; Jaskot, RH; Mckee, J; Kotha, S. R.; Patel, RB; Parianandi, NL. (2011). Vascular and cardiac impairments in rats Inhaling ozone and diesel exhaust particles. Environ Health Perspect 119: 312-318. http://dx.doi.org/10.1289/ehp.1002386

Koren, HS; Devlin, RB; Graham, DE; Mann, R; Mcgee, MP; Horstman, DH; Kozumbo, WJ; Becker, S; House, DE; Mcdonnell, WF; Bromberg, PA. (1989). Ozone-induced inflammation in the lower airways of human subjects. Am J Respir Crit Care Med 139: 407-415.

Korrick, SA; Neas, LM; Dockery, DW; Gold, DR; Allen, GA; Hill, LB; Kimball, KD; Rosner, BA; Speizer, FE. (1998). Effects of ozone and other pollutants on the pulmonary function of adult hikers. Environ Health Perspect 106: 93-99. http://dx.doi.org/10.1289/ehp.9810693

Kostikas, K; Papatheodorou, G; Ganas, K; Psathakis, K; Panagou, P; Loukides, S. (2002). pH in expired breath condensate of patients with inflammatory airway diseases. Am J Respir Crit Care Med 165: 1364-1370.

Kreit, JW; Gross, KB; Moore, TB; Lorenzen, TJ; D'Arcy, J; Eschenbacher, WL. (1989). Ozone-induced changes in pulmonary function and bronchial responsiveness in asthmatics. J Appl Physiol 66: 217-222.

Kulle, TJ; Sauder, LR; Hebel, JR; Chatham, MD. (1985). Ozone response relationships in healthy nonsmokers. Am Rev Respir Dis 132: 36-41.

Kulle, TJ; Sauder, LR; Kerr, HD; Farrell, BP; Bermel, MS; Smith, DM. (1982). Duration of pulmonary function adaptation to ozone in humans. Am Ind Hyg Assoc J 43: 832-837.

Lagorio, S; Forastiere, F; Pistelli, R; Iavarone, I; Michelozzi, P; Fano, V; Marconi, A; Ziemacki, G; Ostro, BD. (2006). Air pollution and lung function among susceptible adult subjects: A panel study. Environ Health 5: 11. http://dx.doi.org/10.1186/1476-069X-5-11

Lanki, T; Pekkanen, J; Aalto, P; Elosua, R; Berglind, N; D'Ippoliti, D; Kulmala, M; Nyberg, F; Peters, A; Picciotto, S; Salomaa, V; Sunyer, J; Tiittanen, P; Von Klot, S; Forastiere, F. (2006). Associations of traffic-related air pollutants with hospitalisation for first acute myocardial infarction: The HEAPSS study. Occup Environ Med 63: 844-851. http://dx.doi.org/10.1136/oem.2005.023911

Larrieu, S; Jusot, JF; Blanchard, M; Prouvost, H; Declercq, C; Fabre, P; Pascal, L; Le Tertre, A; Wagner, V; Riviere, S; Chardon, B; Borelli, D; Cassadou, S; Eilstein, D; Lefranc, A. (2007). Short term effects of air pollution on hospitalizations for cardiovascular diseases in eight French cities: The PSAS program. Sci Total Environ 387: 105-112.

Larsen, ST; Matsubara, S; Mcconville, G; Poulsen, SS; Gelfand, EW. (2010). Ozone increases airway hyperreactivity and mucus hyperproduction in mice previously exposed to allergen. J Toxicol Environ Health A 73: 738-747. http://dx.doi.org/10.1080/15287391003614034

Laskin, DL; Heck, DE; Laskin, JD. (1998). Role of inflammatory cytokines and nitric oxide in hepatic and pulmonary toxicity. Toxicol Lett 102-103: 289-293.

Laskin, DL; Laskin, JD. (2001). Role of macrophages and inflammatory mediators in chemically induced toxicity [Review]. Toxicology 160: 111-118.

Laskin, DL; Pendino, KJ; Punjabi, CJ; del Valle, MR; Laskin, JD. (1994). Pulmonary and hepatic effects of inhaled ozone in rats. Environ Health Perspect 10: 61-64.

Laskin, JD; Heck, DE; Laskin, DL. (1996). Nitric oxide production in the lung and liver following inhalation of the pulmonary irritant ozone. Adv Exp Med Biol 387: 141-146.

Last, JA; Gohil, K; Mathrani, VC; Kenyon, NJ. (2005). Systemic responses to inhaled ozone in mice: cachexia and down-regulation of liver xenobiotic metabolizing genes. Toxicol Appl Pharmacol 208: 117-126. http://dx.doi.org/10.1016/j.taap.2005.02.001

Last, JA; Reiser, KM; Tyler, WS; Rucker, RB. (1984). Long-term consequences of exposure to ozone. I. Lung collagen content. Toxicol Appl Pharmacol 72: 111-118.

Lawrence, SO; Simpson-Haidaris, PJ. (2004). Regulated de novo biosynthesis of fibrinogen in extrahepatic epithelial cells in response to inflammation. Thromb Haemostasis 92: 234-243. http://dx.doi.org/10.1160/TH04-01-0024

Lay, JC; Alexis, NE; Kleeberger, SR; Roubey, RA; Harris, BD; Bromberg, PA; Hazucha, MJ; Devlin, RB; Peden, DB. (2007). Ozone enhances markers of innate immunity and antigen presentation on airway monocytes in healthy individuals [Letter]. J Allergy Clin Immunol 120: 719-722. http://dx.doi.org/10.1016/j.jaci.2007.05.005

Lee, IM; Tsai, SS; Ho, CK; Chiu, HF; Wu, TN; Yang, CY. (2008a). Air pollution and hospital admissions for congestive heart failure: Are there potentially sensitive groups? Environ Res 108: 348-353. http://dx.doi.org/10.1016/j.envres.2008.07.024

Lee, IM; Tsai, SS; Ho, CK; Chiu, HF; Yang, CY. (2007). Air pollution and hospital admissions for congestive heart failure in a tropical city: Kaohsiung, Taiwan. Inhal Toxicol 19: 899-904. http://dx.doi.org/10.1080/08958370701479406

Lee, JT; Kim, H; Cho, YS; Hong, YC; Ha, EH; Park, H. (2003b). Air pollution and hospital admissions for ischemic heart diseases among individuals 64+ years of age residing in Seoul, Korea. Arch Environ Health 58: 617-623.

Lefohn, AS; Hazucha, MJ; Shadwick, D; Adams, WC. (2010a). An alternative form and level of the human health ozone standard. Inhal Toxicol 22: 999-1011. http://dx.doi.org/10.3109/08958378.2010.505253

Leonard, RJ; Charpied, GL; Faddis, B. (1991). Effects of ambient inhaled ozone on vocal fold mucosa in Bonnet monkeys. J Voice 5: 304-309. http://dx.doi.org/10.1016/S0892-1997(05)80060-8

Levy, JI; Chemerynski, SM; Sarnat, JA. (2005). Ozone exposure and mortality, an empiric Bayes metaregression analysis. Epidemiology 16: 458-468.

Lewis, TC; Robins, TG; Dvonch, JT; Keeler, GJ; Yip, FY; Mentz, GB; Lin, X; Parker, EA; Israel, BA; Gonzalez, L; Hill, Y. (2005). Air pollution-associated changes in lung function among asthmatic children in Detroit. Environ Health Perspect 113: 1068-1075.

Liao, D; Duan, Y; Whitsel, EA; Zheng, ZJ; Heiss, G; Chinchilli, VM; Lin, HM. (2004a). Association of higher levels of ambient criteria pollutants with impaired cardiac autonomic control: a population-based study. Am J Epidemiol 159: 768-777.

Liao, D; Heiss, G; Chinchilli, VM; Duan, Y; Folsom, AR; Lin, HM; Salomaa, V. (2005). Association of criteria pollutants with plasma hemostatic/inflammatory markers: A population-based study. J Expo Sci Environ Epidemiol 15: 319-328.

Lim, Y; Phung, AD; Corbacho, AM; Aung, HH; Maioli, E; Reznick, AZ; Cross, CE; Davis, PA; Valacchi, G. (2006). Modulation of cutaneous wound healing by ozone: Differences between young and aged mice. Toxicol Lett 160: 127-134. http://dx.doi.org/10.1016/j.toxlet.2005.06.013

Lin, S; Bell, EM; Liu, W; Walker, RJ; Kim, NK; Hwang, SA. (2008a). Ambient ozone concentration and hospital admissions due to childhood respiratory diseases in New York State, 1991-2001. Environ Res 108: 42-47. http://dx.doi.org/10.1016/j.envres.2008.06.007

Linares, C; Diaz, J. (2010). Short-term effect of concentrations of fine particulate matter on hospital admissions due to cardiovascular and respiratory causes among the over-75 age group in Madrid, Spain. Public Health 124: 28-36. http://dx.doi.org/10.1016/j.puhe.2009.11.007

Linn, WS; Avol, EL; Shamoo, DA; Peng, RC; Valencia, LM; Little, DE; Hackney, JD. (1988). Repeated laboratory ozone exposures of volunteer Los Angeles residents: an apparent seasonal variation in response. Toxicol Ind Health 4: 505-520.

Linn, WS; Avol, EL; Shamoo, DA; Spier, CE; Valencia, LM; Venet, TG; Fischer, DA; Hackney, JD. (1986). A dose-response study of healthy, heavily exercising men exposed to ozone at concentrations near the ambient air quality standard. Toxicol Ind Health 2: 99-112.

Linn, WS; Fischer, DA; Medway, DA; Anzar, UT; Spier, CE; Valencia, LM; Venet, TG; Hackney, JD. (1982a). Short-term respiratory effects of 0.12 ppm ozone exposure in volunteers with chronic obstructive pulmonary disease. Am Rev Respir Dis 125: 658-663.

Linn, WS; Medway, DA; Anzar, UT; Valencia, LM; Spier, CE; FS-D, T; Fischer, DA; Hackney, JD. (1982b). Persistence of adaptation to ozone in volunteers exposed repeatedly for six weeks. Am Rev Respir Dis 125: 491-495.

Linn, WS; Shamoo, DA; Anderson, KR; Peng, RC; Avol, EL; Hackney, JD; Gong, H, Jr. (1996). Short-term air pollution exposures and responses in Los Angeles area schoolchildren. J Expo Sci Environ Epidemiol 6: 449-472.

Linn, WS; Shamoo, DA; Venet, TG; Spier, CE; Valencia, LM; Anzar, UT; Hackney, JD. (1983). Response to ozone in volunteers with chronic obstructive pulmonary disease. Arch Environ Occup Health 38: 278-283.

Linn, WS; Szlachcic, Y; Gong, H, Jr; Kinney, PL; Berhane, KT. (2000). Air pollution and daily hospital admissions in metropolitan Los Angeles. Environ Health Perspect 108: 427-434.

Lisabeth, LD; Escobar, JD; Dvonch, JT; Sanchez, BN; Majersik, JJ; Brown, DL; Smith, MA; Morgenstern, LB. (2008). Ambient air pollution and risk for ischemic stroke and transient ischemic attack. Ann Neurol 64: 53-59. http://dx.doi.org/10.1002/ana.21403

Liu, L; Leech, JA; Urch, RB; Poon, R; Zimmerman, B; Kubay, JM; Silverman, FS. (1999). A comparison of biomarkers of ozone exposure in human plasma, nasal lavage, and sputum. Inhal Toxicol 11: 657-674. http://dx.doi.org/10.1080/089583799196790

Liu, L; Leech, JA; Urch, RB; Silverman, FS. (1997). In vivo salicylate hyroxylation: A potential biomarker for assessing acute ozone exposure and effects in humans. Am J Respir Crit Care Med 156: 1405-1412.

Liu, L; Poon, R; Chen, L; Frescura, AM; Montuschi, P; Ciabattoni, G; Wheeler, A; Dales, R. (2009a). Acute effects of air pollution on pulmonary function, airway inflammation, and oxidative stress in asthmatic children. Environ Health Perspect 117: 668-674. http://dx.doi.org/10.1289/ehp11813

Mann, JK; Balmes, JR; Bruckner, TA; Mortimer, KM; Margolis, HG; Pratt, B; Hammond, SK; Lurmann, F; Tager, IB. (2010). Short-term effects of air pollution on wheeze in asthmatic children in Fresno, California. Environ Health Perspect 118: 1497-1502. http://dx.doi.org/10.1289/ehp.0901292

Mapp, CE; Fryer, AA; De Marzo, N; Pozzato, V; Padoan, M; Boschetto, P; Strange, RC; Hemmingsen, A; Spiteri, MA. (2002). Glutathione S-transferase GSTP1 is a susceptibility gene for occupational asthma induced by isocyanates. J Allergy Clin Immunol 109: 867-872. http://dx.doi.org/10.1067/mai.2002.123234

Mar, TF; Koenig, JQ. (2009). Relationship between visits to emergency departments for asthma and ozone exposure in greater Seattle, Washington. Ann Allergy Asthma Immunol 103: 474-479. http://dx.doi.org/10.1016/S1081-1206(10)60263-3

Martínez-Canabal, A; Angora-Perez, M. (2008). Effect of growth hormone on cyclooxygenase-2 expression in the hippocampus of rats chronically exposed to ozone. Int J Neurosci 118: 455-469. http://dx.doi.org/10.1080/00207450701593160

Martrette, JM; Thornton, SN; Trabalon, M. (2011). Prolonged ozone exposure effects behaviour, hormones and respiratory muscles in young female rats. Physiol Behav 103: 302-307. http://dx.doi.org/10.1016/j.physbeh.2011.02.024

McDonnell, WF. (1996). Individual variability in human lung function responses to ozone exposure. Environ Toxicol Pharmacol 2: 171-175.

McDonnell, WF; Chapman, RS; Horstman, DH; Leigh, MW; Abdul-Salaam, S. (1985a). A comparison of the responses of children and adults to acute ozone exposure.

McDonnell, WF; Horstman, DH; Hazucha, MJ; Seal, E, Jr; Haak, ED; Salaam, SA; House, DE. (1983). Pulmonary effects of ozone exposure during exercise: Dose-response characteristics. J Appl Physiol 54: 1345-1352.

McDonnell, WF, III; Chapman, RS; Leigh, MW; Strope, GL; Collier, AM. (1985b). Respiratory responses of vigorously exercising children to 0.12 ppm ozone exposure. Am Rev Respir Dis 132: 875-879.

McDonnell, WF, III; Horstman, DH; Abdul-Salaam, S; House, DE. (1985c). Reproducibility of individual responses to ozone exposure. Am Rev Respir Dis 131: 36-40.

McDonnell, WF; Kehrl, HR; Abdul-Salaam, S; Ives, PJ; Folinsbee, LJ; Devlin, RB; O'Neil, JJ; Horstman, DH. (1991). Respiratory response of humans exposed to low levels of ozone for 6.6 hours. Arch Environ Occup Health 46: 145-150.

McDonnell, WF; Stewart, PW; Andreoni, S; Seal, E, Jr; Kehrl, HR; Horstman, DH; Folinsbee, LJ; Smith, MV. (1997). Prediction of ozone-induced FEV1 changes: Effects of concentration, duration, and ventilation. Am J Respir Crit Care Med 156: 715-722.

Mcdonnell, WF; Stewart, PW; Smith, MV. (2007). The temporal dynamics of ozone-induced FEV1 changes in humans: An exposure-response model. Inhal Toxicol 19: 483-494.

McDonnell, WF; Stewart, PW; Smith, MV. (2010). Prediction of ozone-induced lung function responses in humans. Inhal Toxicol 22: 160-168. http://dx.doi.org/10.3109/08958370903089557

Mcdonnell, WF; Stewart, PW; Smith, MV; Kim, CS; Schelegle, ES. (2012). Prediction of lung function response for populations exposed to a wide range of ozone conditions. Inhal Toxicol 24: 619-633. http://dx.doi.org/10.3109/08958378.2012.705919

McDonnell, WF; Stewart, PW; Smith, MV; Pan, WK; Pan, J. (1999b). Ozone-induced respiratory symptoms: Exposure-response models and association with lung function. Eur Respir J 14: 845-853.

Medina-Ramón, M; Schwartz, J. (2008). Who is more vulnerable to die from ozone air pollution? Epidemiology 19: 672-679. http://dx.doi.org/10.1097/EDE.0b013e3181773476

Medina-Ramon, M; Zanobetti, A; Schwartz, J. (2006). The effect of ozone and PM10 on hospital admissions for pneumonia and chronic obstructive pulmonary disease: A national multicity study. Am J Epidemiol 163: 579-588. http://dx.doi.org/10.1093/aje/kwj078

Mellick, PW; Dungworth, DL; Schwartz, LW; Tyler, WS. (1977). Short term morphologic effects of high ambient levels of ozone on lungs of rhesus monkeys. Lab Invest 36: 82-90.

Messineo, TD; Adams, WC. (1990). Ozone inhalation effects in females varying widely in lung size: Comparison with males. J Appl Physiol 69: 96-103.

Metzger, KB; Klein, M; Flanders, WD; Peel, JL; Mulholland, JA; Langberg, JJ; Tolbert, PE. (2007). Ambient air pollution and cardiac arrhythmias in patients with implantable defibrillators. Epidemiology 18: 585-592. http://dx.doi.org/10.1097/EDE.0b013e318124ff0e

Metzger, KB; Tolbert, PE; Klein, M; Peel, JL; Flanders, WD; Todd, KH; Mulholland, JA; Ryan, PB; Frumkin, H. (2004). Ambient air pollution and cardiovascular emergency department visits. Epidemiology 15: 46-56.

Middleton, N; Yiallouros, P; Kleanthous, S; Kolokotroni, O; Schwartz, J; Dockery, DW; Demokritou, P; Koutrakis, P. (2008). A 10-year time-series analysis of respiratory and cardiovascular morbidity in Nicosia, Cyprus: The effect of short-term changes in air pollution and dust storms. Environ Health 7: 39.

Mikerov, AN; Umstead, TM; Gan, X; Huang, W; Guo, X; Wang, G; Phelps, DS; Floros, J. (2008c). Impact of ozone exposure on the phagocytic activity of human surfactant protein A (SP-A) and SP-A variants. Am J Physiol Lung Cell Mol Physiol 294: L121-L130. http://dx.doi.org/10.1152/ajplung.00288.2007

Miller, FJ; Illing, JW; Gardner, DE. (1978). Effect of urban ozone levels on laboratory-induced respiratory infections. Toxicol Lett 2: 163-169.

Mokoena, ML; Harvey, BH; Oliver, DW; Brink, CB. (2010). Ozone modulates the effects of imipramine on immobility in the forced swim test, and nonspecific parameters of hippocampal oxidative stress in the rat. Metab Brain Dis 25: 125-133. http://dx.doi.org/10.1007/s11011-010-9189-7

Molfino, NA; Wright, SC; Katz, I; Tarlo, S; Silverman, F; Mcclean, PA; Szalai, JP; Raizenne, M; Slutsky, AS; Zamel, N. (1991). Effect of low concentrations of ozone on inhaled allergen responses in asthmatic subjects. Lancet 338: 199-203.

Moon, JS; Kim, YS; Kim, JH; Son, BS; Kim, DS; Yang, W. (2009). Respiratory health effects among schoolchildren and their relationship to air pollutants in Korea. Int J Environ Health Res 19: 31-48. http://dx.doi.org/10.1080/09603120802272201

Morgan, G; Corbett, S; Wlodarczyk, J. (1998). Air pollution and hospital admissions in Sydney, Australia, 1990 to 1994. Am J Public Health 88: 1761-1766.

Morrison, D; Rahman, I; MacNee, W. (2006). Permeability, inflammation and oxidant status in airspace epithelium exposed to ozone. Respir Med 100: 2227-2234. http://dx.doi.org/10.1016/j.rmed.2005.10.005

Morrow, JD; Hill, KE; Burk, RF; Nammour, TM; Badr, KF; Roberts, LJ, 2nd. (1990). A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. PNAS 87: 9383-9387.

Mortimer, KM; Neas, LM; Dockery, DW; Redline, S; Tager, IB. (2002). The effect of air pollution on inner-city children with asthma. Eur Respir J 19: 699-705. http://dx.doi.org/10.1183/09031936.02.00247102

Mortimer, KM; Tager, IB; Dockery, DW; Neas, LM; Redline, S. (2000). The effect of ozone on inner-city children with asthma: Identification of susceptible subgroups. Am J Respir Crit Care Med 162: 1838-1845.

Mudway, IS; Blomberg, A; Frew, AJ; Holgate, ST; Sandstrom, T; Kelly, FJ. (1999a). Antioxidant consumption and repletion kinetics in nasal lavage fluid following exposure of healthy human volunteers to ozone. Eur Respir J 13: 1429-1438.

Mudway, IS; Kelly, FJ. (2004a). An investigation of inhaled ozone dose and the magnitude of airway inflammation in healthy adults. Am J Respir Crit Care Med 169: 1089-1095.

Mudway, IS; Stenfors, N; Blomberg, A; Helleday, R; Dunster, C; Marklund, SL; Frew, AJ; Sandstrom, T; Kelly, FJ. (2001). Differences in basal airway antioxidant concentrations are not predictive of individual responsiveness to ozone: A comparison of healthy and mild asthmatic subjects. Free Radic Biol Med 31: 962-974.

Murphy, SD; Ulrich, CE; Frankowitz, SH; Xintaras, C. (1964). Altered function in animals inhaling low concentrations of ozone and nitrogen dioxide. Am Ind Hyg Assoc J 25: 246-253.

Murugan, A; Prys-Picard, C; Calhoun, WJ. (2009). Biomarkers in asthma [Review]. Curr Opin Pulm Med 15: 12-18. http://dx.doi.org/10.1097/MCP.0b013e32831de235

Naeher, LP; Holford, TR; Beckett, WS; Belanger, K; Triche, EW; Bracken, MB; Leaderer, BP. (1999). Healthy women's PEF variations with ambient summer concentrations of PM10, PM2.5, SO42-, H+, and O3. Am J Respir Crit Care Med 160: 117-125.

Nakamura, K; Matsunaga, K. (1998). Susceptibility of natural killer (NK) cells to reactive oxygen species (ROS) and their restoration by the mimics of superoxide dismutase (SOD). Cancer Biother Radiopharm 13: 275-290.

Neas, LM; Dockery, DW; Koutrakis, P; Speizer, FE. (1999). Fine particles and peak flow in children: Acidity versus mass. Epidemiology 10: 550-553.

Neas, LM; Dockery, DW; Koutrakis, P; Tollerud, DJ; Speizer, FE. (1995). The association of ambient air pollution with twice daily peak expiratory flow rate measurements in children. Am J Epidemiol 141: 111-122.

Neidell, M. (2009). Information, avoidance behavior, and health: The effect of ozone on asthma hospitalizations. Journal of Human Resources 44: 450-478.

Neidell, M; Kinney, PL. (2010). Estimates of the association between ozone and asthma hospitalizations that account for behavioral responses to air quality information. Environ Sci Pol 13: 97-103. http://dx.doi.org/10.1016/j.envsci.2009.12.006

Neuberger, M; Schimek, MG; Horak, F, Jr; Moshammer, H; Kundi, M; Frischer, T; Gomiscek, B; Puxbaum, H; Hauck, H; AUPHEP-Team. (2004). Acute effects of particulate matter on respiratory diseases, symptoms and functions: Epidemiological results of the Austrian Projects on Health Effects of Particulate Matter (AUPHEP). Atmos Environ 38: 3971-3981.

Nickmilder, M; De Burbure, C; Sylviane, C; Xavier, D; Alfred, B; Alain, D. (2007). Increase of exhaled nitric oxide in children exposed to low levels of ambient ozone. J Toxicol Environ Health A 70: 270-274.

Nightingale, JA; Rogers, DF; Chung, KF; Barnes, PJ. (2000). No effect of inhaled budesonide on the response to inhaled ozone in normal subjects. Am J Respir Crit Care Med 161: 479-486.

O'Connor, GT; Neas, L; Vaughn, B; Kattan, M; Mitchell, H; Crain, EF; III, ER; Gruchalla, R; Morgan, W; Stout, J; Adams, GK; Lippmann, M. (2008). Acute respiratory health effects of air pollution on children with asthma in US inner cities. J Allergy Clin Immunol 121: 1133-1139. http://dx.doi.org/10.1016/j.jaci.2008.02.020

O'Neill, MS; Ramirez-Aguilar, M; Meneses-Gonzalez, F; Hernandez-Avila, M; Geyh, AS; Sienra-Monge, JJ; Romieu, I. (2003). Ozone exposure among Mexico City outdoor workers. J Air Waste Manag Assoc 53: 339-346.

Orazzo, F; Nespoli, L; Ito, K; Tassinari, D; Giardina, D; Funis, M; Cecchi, A; Trapani, C; Forgeschi, G; Vignini, M; Nosetti, L; Pigna, S; Zanobetti, A. (2009). Air pollution, aeroallergens, and emergency room visits for acute respiratory diseases and gastroenteric disorders among young children in six Italian cities. Environ Health Perspect 117: 1780-1785. http://dx.doi.org/10.1289/ehp.0900599

Ostro, B; Lipsett, M; Mann, J; Braxton-Owens, H; White, M. (2001). Air pollution and exacerbation of asthma in African-American children in Los Angeles. Epidemiology 12: 200-208.

Oudin, A; Stromberg, U; Jakobsson, K; Stroh, E; Bjork, J. (2010). Estimation of short-term effects of air pollution on stroke hospital admissions in southern Sweden. Neuroepidemiology 34: 131-142. http://dx.doi.org/10.1159/000274807

Oyarzún, M; Dussaubat, N; González, S. (2005). Effect of 0.25 ppm ozone exposure on pulmonary damage induced by bleomycin. Biol Res 38: 353-358.

Park, JW; Lim, YH; Kyung, SY; An, CH; Lee, SP; Jeong, SH; Ju, SY. (2005a). Effects of ambient particulate matter on peak expiratory flow rates and respiratory symptoms of asthmatics during Asian dust periods in Korea. Respirology 10: 470-476. http://dx.doi.org/10.1111/j.1440-1843.2005.00728.x

Park, SK; O'Neill, MS; Stunder, BJB; Vokonas, PS; Sparrow, D; Koutrakis, P; Schwartz, J. (2007). Source location of air pollution and cardiac autonomic function: Trajectory cluster analysis for exposure assessment. J Expo Sci Environ Epidemiol 17: 488-497. http://dx.doi.org/10.1038/sj.jes.7500552

Park, SK; O'Neill, MS; Vokonas, PS; Sparrow, D; Schwartz, J. (2005b). Effects of air pollution on heart rate variability: The VA Normative Aging Study. Environ Health Perspect 113: 304-309.

Park, SK; O'Neill, MS; Vokonas, PS; Sparrow, D; Wright, RO; Coull, B; Nie, H; Hu, H; Schwartz, J. (2008). Air pollution and heart rate variability: Effect modification by chronic lead exposure. Epidemiology 19: 111-120. http://dx.doi.org/10.1097/EDE.0b013e31815c408a

Passannante, AN; Hazucha, MJ; Bromberg, PA; Seal, E; Folinsbee, L; Koch, G. (1998). Nociceptive mechanisms modulate ozone-induced human lung function decrements. J Appl Physiol 85: 1863-1870.

Peacock, JL; Anderson, HR; Bremner, SA; Marston, L; Seemungal, TA; Strachan, DP; Wedzicha, JA. (2011). Outdoor air pollution and respiratory health in patients with COPD. Thorax 66: 591-596. http://dx.doi.org/10.1136/thx.2010.155358

Peden, DB. (2011). The role of oxidative stress and innate immunity in O3 and endotoxin-induced human allergic airway disease [Review]. Immunol Rev 242: 91-105. http://dx.doi.org/10.1111/j.1600-065X.2011.01035.x

Peden, DB; Boehlecke, B; Horstman, D; Devlin, R. (1997). Prolonged acute exposure to 0.16 ppm ozone induces eosinophilic airway inflammation in asthmatic subjects with allergies. J Allergy Clin Immunol 100: 802-808.

Peel, JL; Metzger, KB; Klein, M; Flanders, WD; Mulholland, JA; Tolbert, PE. (2007). Ambient air pollution and cardiovascular emergency department visits in potentially sensitive groups. Am J Epidemiol 165: 625-633. http://dx.doi.org/10.1093/aje/kwk051

Pellegrino, R; Viegi, G; Brusasco, V; Crapo, RO; Burgos, F; Casaburi, R; Coates, A; van der Grinten, CP; Gustafsson, P; Hankinson, J; Jensen, R; Johnson, DC; MacIntyre, N; McKay, R; Miller, MR; Navajas, D; Pedersen, OF; Wanger, J. (2005). Interpretative strategies for lung function tests. Eur Respir J 26: 948-968. http://dx.doi.org/10.1183/09031936.05.00035205

Peng, RD; Dominici, F; Pastor-Barriuso, R; Zeger, SL; Samet, JM. (2005). Seasonal analyses of air pollution and mortality in 100 US cities. 161: 585-594. http://dx.doi.org/10.1093/aje/kwi075

Perepu, RS; Garcia, C; Dostal, D; Sethi, R. (2010). Enhanced death signaling in ozone-exposed ischemic-reperfused hearts. Mol Cell Biochem 336: 55-64. http://dx.doi.org/10.1007/s11010-009-0265-4

Pereyra-Muñoz, N; Rugerio-Vargas, C; Angoa-Pérez, M; Borgonio-Pérez, G; Rivas-Arancibia, S. (2006). Oxidative damage in substantia nigra and striatum of rats chronically exposed to ozone. J Chem Neuroanat 31: 114-123. http://dx.doi.org/10.1016/j.jchemneu.2005.09.006

Peters, A; Dockery, DW; Muller, JE; Mittleman, MA. (2001). Increased particulate air pollution and the triggering of myocardial infarction. Circulation 103: 2810-2815. http://dx.doi.org/10.1161/01.CIR.103.23.2810

Peterson, DC; Andrews, HL. (1963). The role of ozone in radiation avoidance in the mouse. Radiat Res 19: 331-336.

Petroeschevsky, A; Simpson, RW; Thalib, L; Rutherford, S. (2001). Associations between outdoor air pollution and hospital admissions in Brisbane, Australia. Arch Environ Occup Health 56: 37-52.

Plopper, CG; Mango, GW; Hatch, GE; Wong, VJ; Toskala, E; Reynolds, SD; Tarkington, BK; Stripp, BR. (2006). Elevation of susceptibility to ozone-induced acute tracheobronchial injury in transgenic mice deficient in Clara cell secretory protein. Toxicol Appl Pharmacol 213: 74-85. http://dx.doi.org/10.1016/j.taap.2005.09.003

Poloniecki, JD; Atkinson, RW; Ponce de Leon, A; Anderson, HR. (1997). Daily time series for cardiovascular hospital admissions and previous day's air pollution in London, UK. Occup Environ Med 54: 535-540.

Power, K; Balmes, J; Solomon, C. (2008). Controlled exposure to combined particles and ozone decreases heart rate variability. J Occup Environ Med 50: 1253-1260. http://dx.doi.org/10.1097/JOM.0b013e3181814239

Prescott, GJ; Cohen, GR; Elton, RA; Fowkes, FGR; Agius, RM. (1998). Urban air pollution and cardiopulmonary ill health: A 145 year time series study. Occup Environ Med 55: 697-704.

Pulfer, MK; Murphy, RC. (2004). Formation of biologically active oxysterols during ozonolysis of cholesterol present in lung surfactant. J Biol Chem 279: 26331-26338. http://dx.doi.org/10.1074/jbc.M403581200

Pulfer, MK; Taube, C; Gelfand, E; Murphy, RC. (2005). Ozone exposure in vivo and formation of biologically active oxysterols in the lung. J Pharmacol Exp Ther 312: 256-264. http://dx.doi.org/10.1124/jpet.104.073437

Qian, Z; Lin, HM; Chinchilli, VM; Lehman, EB; Duan, Y; Craig, TJ; Wilson, WE; Liao, D; Lazarus, SC; Bascom, R. (2009). Interaction of ambient air pollution with asthma medication on exhaled nitric oxide among asthmatics. Arch Environ Occup Health 64: 168-176. http://dx.doi.org/10.1080/19338240903240616

Que, LG; Stiles, JV; Sundy, JS; Foster, WM. (2011). Pulmonary function, bronchial reactivity, and epithelial permeability are response phenotypes to ozone and develop differentially in healthy humans. J Appl Physiol 111: 679-687. http://dx.doi.org/10.1152/japplphysiol.00337.2011

Rabinovitch, N; Zhang, LN; Murphy, JR; Vedal, S; Dutton, SJ; Gelfand, EW. (2004). Effects of wintertime ambient air pollutants on asthma exacerbations in urban minority children with moderate to severe disease. J Allergy Clin Immunol 114: 1131-1137. http://dx.doi.org/10.1016/j.jaci.2004.08.026

Raizenne, ME; Burnett, RT; Stern, B; Franklin, CA; Spengler, JD. (1989). Acute lung function responses to ambient acid aerosol exposures in children. Environ Health Perspect 79: 179-185.

Ramírez-Aguilar, M; Barraza-Villarreal, A; Moreno-Macías, H; Winer, AM; Cicero-Fernández, P; Vélez-Márquez, MG; Cortez-Lugo, M; Sienra-Monge, JJ; Romieu, I. (2008). Assessment of personal exposure to ozone in asthmatic children residing in Mexico City. Salud Publica Mex 50: 67-75.

Ren, C; Williams, GM; Mengersen, K; Morawska, L; Tong, S. (2008). Does temperature modify short-term effects of ozone on total mortality in 60 large eastern US communities? An assessment using the NMMAPS data. Environ Int 34: 451-458.

Revis, NW; Major, T; Dalbey, WE. (1981). Cardiovascular effects of ozone and cadmium inhalation in the rat. In Proceedings of the research planning workshop on health effects of oxidants (pp. 171-179). (EPA-600/9-81-001). Research Triangle Park, NC: U.S. Environmental Protection Agency.

Rich, DQ; Kim, MH; Turner, JR; Mittleman, MA; Schwartz, J; Catalano, PJ; Dockery, DW. (2006a). Association of ventricular arrhythmias detected by implantable cardioverter defibrillator and ambient air pollutants in the St Louis, Missouri metropolitan area. Occup Environ Med 63: 591-596. http://dx.doi.org/10.1136/oem.2005.023457

Rich, DQ; Kipen, HM; Zhang, J; Kamat, L; Wilson, AC; Kostis, JB. (2010). Triggering of transmural infarctions, but not nontransmural infarctions, by ambient fine particles. Environ Health Perspect 118: 1229-1234. http://dx.doi.org/10.1289/ehp.0901624

Rich, DQ; Mittleman, MA; Link, MS; Schwartz, J; Luttmann-Gibson, H; Catalano, PJ; Speizer, FE; Gold, DR; Dockery, DW. (2006b). Increased risk of paroxysmal atrial fibrillation episodes associated with acute increases in ambient air pollution. Environ Health Perspect 114: 120-123. http://dx.doi.org/10.1289/ehp.8371

Rich, DQ; Schwartz, J; Mittleman, MA; Link, M; Luttmann-Gibson, H; Catalano, PJ; Speizer, FE; Dockery, DW. (2005). Association of short-term ambient air pollution concentrations and ventricular arrhythmias. Am J Epidemiol 161: 1123-1132.

Riediker, M; Monn, C; Koller, T; Stahel, WA; Wuthrich, B. (2001). Air pollutants enhance rhinoconjunctivitis symptoms in pollen-allergic individuals. Ann Allergy Asthma Immunol 87: 311-318. http://dx.doi.org/10.1016/S1081-1206(10)62246-6

Rivas-Arancibia, S; Dorado-Martinez, C; Borgonio-Perez, G; Hiriart-Urdanivia, M; Verdugo-Diaz, L; Duran-Vazquez, A; Colin-Baranque, L; Avila-Costa, MR. (2000). Effects of taurine on ozone-induced memory deficits and lipid peroxidation levels in brains of young, mature, and old rats. Environ Res 82: 7-17. http://dx.doi.org/10.1006/enrs.1999.3996

Rivas-Arancibia, S; Guevara-Guzmán, R; López-Vidal, Y; Rodríguez-Martínez, E; Gomes, MZ; Angoa-Pérez, M; Raisman-Vozari, R. (2010). Oxidative stress caused by ozone exposure induces loss of brain repair in the hippocampus of adult rats. Toxicol Sci 113: 187-197. http://dx.doi.org/10.1093/toxsci/kfp252

Rivas-Arancibia, S; Vazquez-Sandoval, R; Gonzalez-Kladiano, D; Schneider-Rivas, S; Lechuga-Guerrero, A. (1998). Effects of ozone exposure in rats on memory and levels of brain and pulmonary superoxide dismutase. Environ Res 76: 33-39.

Rodriguez, C; Tonkin, R; Heyworth, J; Kusel, M; De Klerk, N; Sly, PD; Franklin, P; Runnion, T; Blockley, A; Landau, L; Hinwood, AL. (2007). The relationship between outdoor air quality and respiratory symptoms in young children. Int J Environ Health Res 17: 351-360. http://dx.doi.org/10.1080/09603120701628669

Romieu, I; Barraza-Villarreal, A; Escamilla-Nunez, C; Almstrand, AC; Diaz-Sanchez, D; Sly, PD; Olin, AC. (2008). Exhaled breath malondialdehyde as a marker of effect of exposure to air pollution in children with asthma. J Allergy Clin Immunol 121: 903-909. http://dx.doi.org/10.1016/j.jaci.2007.12.004

Romieu, I; Barraza-Villarreal, A; Escamilla-Núñez, C; Texcalac-Sangrador, JL; Hernandez-Cadena, L; Díaz-Sánchez, D; De Batlle, J; Del Rio-Navarro, BE. (2009). Dietary intake, lung function and airway inflammation in Mexico City school children exposed to air pollutants. Respir Res 10: 122.

Romieu, I; Meneses, F; Ramirez, M; Ruiz, S; Padilla, RP; Sienra, JJ; Gerber, M; Grievink, L; Dekker, R; Walda, I; Brunekreef, B. (1998b). Antioxidant supplementation and respiratory functions among workers exposed to high levels of ozone. Am J Respir Crit Care Med 158: 226-232.

Romieu, I; Meneses, F; Ruiz, S; Huerta, J; Sienra, JJ; White, M; Etzel, R; Hernandez, M. (1997). Effects of intermittent ozone exposure on peak expiratory flow and respiratory symptoms among asthmatic children in Mexico City. Arch Environ Occup Health 52: 368-376.

Romieu, I; Meneses, F; Ruiz, S; Sienra, JJ; Huerta, J; White, MC; Etzel, RA. (1996). Effects of air pollution on the respiratory health of asthmatic children living in Mexico City. Am J Respir Crit Care Med 154: 300-307.

Romieu, I; Ramirez-Aguilar, M; Sienra-Monge, JJ; Moreno-Macias, H; Del Rio-Navarro, BE; David, G; Marzec, J; Hernandez-Avila, M; London, S. (2006). GSTM1 and GSTP1 and respiratory health in asthmatic children exposed to ozone. Eur Respir J 28: 953-959. http://dx.doi.org/10.1183/09031936.06.00114905

Romieu, I; Sienra-Monge, JJ; Ramirez-Aguilar, M; Moreno-Macias, H; Reyes-Ruiz, NI; Estela del Rio-Navarro, B; Hernandez-Avila, M; London, SJ. (2004b). Genetic polymorphism of GSTM1 and antioxidant supplementation influence lung function in relation to ozone exposure in asthmatic children in Mexico City. Thorax 59: 8-10.

Romieu, I; Sienra-Monge, JJ; Ramirez-Aguilar, M; Tellez-Rojo, MM; Moreno-Macias, H; Reyes-Ruiz, NI; Del Rio-Navarro, BE; Ruiz-Navarro, MX; Hatch, G; Slade, R; Hernandez-Avila, M. (2002). Antioxidant supplementation and lung functions among children with asthma exposed to high levels of air pollutants. Am J Respir Crit Care Med 166: 703-709.

Rosenfeld, MA; Leonova, VB; Konstantinova, ML; Razumovskii, SD. (2009). Self-assembly of fibrin monomers and fibrinogen aggregation during ozone oxidation. Biochemistry (Mosc) 74: 41-46. http://dx.doi.org/10.1134/S0006297909010064

Ross, MA; Persky, VW; Scheff, PA; Chung, J; Curtis, L; Ramakrishnan, V; Wadden, RA; Hryhorczuk, DO. (2002). Effect of ozone and aeroallergens on the respiratory health of asthmatics. Arch Environ Occup Health 57: 568-578. http://dx.doi.org/10.1080/00039890209602090

Rozenfeld, MA; Leonova, VB; Konstantinova, ML; Razumovskii, SD; Makarov, VA; Nevedrova, OE; Belozerskaja, GG. (2008). Disturbance of functional properties of fibrinogen under ozone oxidation. Dokl Biochem Biophys 422: 315-318. http://dx.doi.org/10.1134/S1607672908050165

Rudez, G; Janssen, NA; Kilinc, E; Leebeek, FW; Gerlofs-Nijland, ME; Spronk, HM; ten Cate, H; Cassee, FR; de Maat, MP. (2009). Effects of ambient air pollution on hemostasis and inflammation. Environ Health Perspect 117: 995-1001. http://dx.doi.org/10.1289/ehp.0800437

Ruidavets, JB; Cassadou, S; Cournot, M; Bataille, V; Meybeck, M; Ferrieres, J. (2005a). Increased resting heart rate with pollutants in a population based study. J Epidemiol Community Health 59: 685-693.

Ruidavets, JB; Cournot, M; Cassadou, S; Giroux, M; Meybeck, M; Ferrieres, J. (2005b). Ozone air pollution is associated with acute myocardial infarction. Circulation 111: 563-569.

Samet, JM; Hatch, GE; Horstman, D; Steck-Scott, S; Arab, L; Bromberg, PA; Levine, M; McDonnell, WF; Devlin, RB. (2001). Effect of antioxidant supplementation on ozone-induced lung injury in human subjects. Am J Respir Crit Care Med 164: 819-825.

Samet, JM; Zeger, SL; Dominici, F; Curriero, F; Coursac, I; Dockery, DW; Schwartz, J; Zanobetti, A. (2000). The national morbidity, mortality, and air pollution study. Part II: Morbidity, mortality, and air pollution in the United States. Cambridge, MA: Health Effects Institute.

Samoli, E; Zanobetti, A; Schwartz, J; Atkinson, R; Le Tertre, A; Schindler, C; Pérez, L; Cadum, E; Pekkanen, J; Paldy, A; Touloumi, G; Katsouyanni, K. (2009). The temporal pattern of mortality responses to ambient ozone in the APHEA project. J Epidemiol Community Health 63: 960-966. http://dx.doi.org/10.1136/jech.2008.084012

Santiago-López, D; Bautista-Martínez, JA; Reyes-Hernandez, CI; Aguilar-Martínez, M; Rivas-Arancibia, S. (2010). Oxidative stress, progressive damage in the substantia nigra and plasma dopamine oxidation, in rats chronically exposed to ozone. Toxicol Lett 197: 193-200. http://dx.doi.org/10.1016/j.toxlet.2010.05.020

Santucci, D; Sorace, A; Francia, N; Aloe, L; Alleva, E. (2006). Prolonged prenatal exposure to low-level ozone affects aggressive behaviour as well as NGF and BDNF levels in the central nervous system of CD-1 mice. Behav Brain Res 166: 124-130. http://dx.doi.org/10.1016/j.bbr.2005.07.032

Sarnat, SE; Coull, BA; Schwartz, J; Gold, DR; Suh, HH. (2006a). Factors affecting the association between ambient concentrations and personal exposures to particles and gases. Environ Health Perspect 114: 649-654.

Sarnat, SE; Suh, HH; Coull, BA; Schwartz, J; Stone, PH; Gold, DR. (2006b). Ambient particulate air pollution and cardiac arrhythmia in a panel of older adults in Steubenville, Ohio. Occup Environ Med 63: 700-706. http://dx.doi.org/10.1136/oem.2006.027292

Sathishkumar, K; Gao, X; Raghavamenon, AC; Parinandi, N; Pryor, WA; Uppu, RM. (2009). Cholesterol secoaldehyde induces apoptosis in H9c2 cardiomyoblasts through reactive oxygen species involving mitochondrial and death receptor pathways. Free Radic Biol Med 47: 548-558. http://dx.doi.org/10.1016/j.freeradbiomed.2009.05.020

Sathishkumar, K; Haque, M; Perumal, TE; Francis, J; Uppu, RM. (2005). A major ozonation product of cholesterol, 3beta-hydroxy-5-oxo-5,6-secocholestan-6-al, induces apoptosis in H9c2 cardiomyoblasts. FEBS Lett 579: 6444-6450. http://dx.doi.org/10.1016/j.febslet.2005.10.044

Scannell, C; Chen, L; Aris, RM; Tager, I; Christian, D; Ferrando, R; Welch, B; Kelly, T; Balmes, JR. (1996). Greater ozone-induced inflammatory responses in subjects with asthma. Am J Respir Crit Care Med 154: 24-29.

Scarlett, JF; Abbott, KJ; Peacock, JL; Strachan, DP; Anderson, HR. (1996). Acute effects of summer air pollution on respiratory function in primary school children in southern England. Thorax 51: 1109-1114.

Schelegle, ES; Adams, WC. (1986). Reduced exercise time in competitive simulations consequent to low level ozone exposure. Med Sci Sports Exerc 18: 408-414.

Schelegle, ES; Adams, WC; Walby, WF; Marion, MS. (2012). Modelling of individual subject ozone exposure response kinetics. Inhal Toxicol 24: 401-415. http://dx.doi.org/10.3109/08958378.2012.683891

Schelegle, ES; Morales, CA; Walby, WF; Marion, S; Allen, RP. (2009). 6.6-hour inhalation of ozone concentrations from 60 to 87 parts per billion in healthy humans. Am J Respir Crit Care Med 180: 265-272. http://dx.doi.org/10.1164/rccm.200809-1484OC

Schelegle, ES; Siefkin, AD; McDonald, RJ. (1991). Time course of ozone-induced neutrophilia in normal humans. Am J Respir Crit Care Med 143: 1353-1358.

Schildcrout, JS; Sheppard, L; Lumley, T; Slaughter, JC; Koenig, JQ; Shapiro, GG. (2006). Ambient air pollution and asthma exacerbations in children: An eight-city analysis. Am J Epidemiol 164: 505-517. http://dx.doi.org/10.1093/aje/kwj225

Schmekel, B; Ahlner, J; Malmström, M; Venge, P. (2001). Eosinophil cationic protein (ECP) in saliva: A new marker of disease activity in bronchial asthma. Respir Med 98: 670-675. http://dx.doi.org/10.1053/rmed.2001.1123

Schwartz, J. (2005a). How sensitive is the association between ozone and daily deaths to control for temperature? Am J Respir Crit Care Med 171: 627-631. http://dx.doi.org/10.1164/rccm.200407-933OC

Schwartz, J. (2005b). Who is sensitive to extremes of temperature? A case-only analysis. Epidemiology 16: 67-72. http://dx.doi.org/10.1097/01.ede.0000147114.25957.71

Schwartz, J; Litonjua, A; Suh, H; Verrier, M; Zanobetti, A; Syring, M; Nearing, B; Verrier, R; Stone, P; Maccallum, G; Speizer, FE; Gold, DR. (2005). Traffic related pollution and heart rate variability in a panel of elderly subjects. Thorax 60: 455-461. http://dx.doi.org/10.1136/thx.2004.024836

Schwartz, LW. (1976). Comparison of the effects of ozone and oxygen on lungs of rats. Environ Health Perspect 16: 179-180.

Schwartz, LW; Dungworth, DL; Mustafa, MG; Tarkington, BK; Tyler, WS. (1976). Pulmonary responses of rats to ambient levels of ozone: effects of 7-day intermittent or continuous exposure. Lab Invest 34: 565-578.

Seal, E, Jr; McDonnell, WF; House, DE. (1996). Effects of age, socioeconomic status, and menstrual cycle on pulmonary response to ozone. Arch Environ Occup Health 51: 132-137.

Seal, E, Jr; McDonnell, WF; House, DE; Salaam, SA; Dewitt, PJ; Butler, SO; Green, J; Raggio, L. (1993). The pulmonary response of white and black adults to six concentrations of ozone. Am J Respir Crit Care Med 147: 804-810.

Selgrade, MK; Daniels, MJ; Grose, EC. (1990). Acute, subchronic, and chronic exposure to a simulated urban profile of ozone: Effects on extrapulmonary natural killer cell activity and lymphocyte mitogenic responses. Inhal Toxicol 2: 375-389.

Selwyn, BJ; Stock, TH; Hardy, RJ; Chan, FA; Jenkins, DE; Kotchmar, DJ; Chapman, RS. (1985). Health effects of ambient ozone exposure in vigorously exercising adults. In Evaluation of the scientific basis for ozone/oxidants standards: Proceedings of an apca international specialty conference. Pittsburgh, PA: Air Pollution Control Association.

Sharkhuu, T; Doerfler, DL; Copeland, C; Luebke, RW; Gilmour, MI. (2011). Effect of maternal exposure to ozone on reproductive outcome and immune, inflammatory, and allergic responses in the offspring. J Immunotoxicol 8: 183-194. http://dx.doi.org/10.3109/1547691X.2011.568978

Sienra-Monge, JJ; Ramirez-Aguilar, M; Moreno-Macias, H; Reyes-Ruiz, NI; Del Rio-Navarro, BE; Ruiz-Navarro, MX; Hatch, G; Crissman, K; Slade, R; Devlin, RB; Romieu, I. (2004). Antioxidant supplementation and nasal inflammatory responses among young asthmatics exposed to high levels of ozone. Clin Exp Immunol 138: 317-322. http://dx.doi.org/10.1111/j.1365-2249.2004.02606.x

Silverman, RA; Ito, K. (2010). Age-related association of fine particles and ozone with severe acute asthma in New York City. J Allergy Clin Immunol 125: 367-373. http://dx.doi.org/10.1016/j.jaci.2009.10.061

Silverman, RA; Ito, K; Freese, J; Kaufman, BJ; De Claro, D; Braun, J; Prezant, DJ. (2010). Association of ambient fine particles with out-of-hospital cardiac arrests in New York City. Am J Epidemiol 172: 917-923. http://dx.doi.org/10.1093/aje/kwq217

Simonian, NA; Coyle, JT. (1996). Oxidative stress in neurodegenerative diseases [Review]. Annu Rev Pharmacol Toxicol 36: 83-106. http://dx.doi.org/10.1146/annurev.pa.36.040196.000503

Simpson, R; Williams, G; Petroeschevsky, A; Best, T; Morgan, G; Denison, L; Hinwood, A; Neville, G. (2005). The short-term effects of air pollution on hospital admissions in four Australian cities. Aust N Z J Public Health 29: 213-221.

Sinclair, AH; Edgerton, ES; Wyzga, R; Tolsma, D. (2010). A two-time-period comparison of the effects of ambient air pollution on outpatient visits for acute respiratory illnesses. J Air Waste Manag Assoc 60: 163-175. http://dx.doi.org/10.3155/1047-3289.60.2.163

Sinclair, AH; Tolsma, D. (2004). Associations and lags between air pollution and acute respiratory visits in an ambulatory care setting: 25-month results from the aerosol research and inhalation epidemiological study. J Air Waste Manag Assoc 54: 1212-1218.

Sivagangabalan, G; Spears, D; Masse, S; Urch, B; Brook, RD; Silverman, F; Gold, DR; Lukic, KZ; Speck, M; Kusha, M; Farid, T; Poku, K; Shi, E; Floras, J; Nanthakumar, K. (2011). The effect of air pollution on spatial dispersion of myocardial repolarization in healthy human volunteers. J Am Coll Cardiol 57: 198-206. http://dx.doi.org/10.1016/j.jacc.2010.08.625

Smith, RL; Xu, B; Switzer, P. (2009b). Reassessing the relationship between ozone and short-term mortality in U.S. urban communities. Inhal Toxicol 21: 37-61. http://dx.doi.org/10.1080/08958370903161612

Solic, JJ; Hazucha, MJ; Bromberg, PA. (1982). The acute effects of 0.2 ppm ozone in patients with chronic obstructive pulmonary disease. Am Rev Respir Dis 125: 664-669.

Son, JY; Bell, ML; Lee, JT. (2010). Individual exposure to air pollution and lung function in Korea: Spatial analysis using multiple exposure approaches. Environ Res 110: 739-749. http://dx.doi.org/10.1016/j.envres.2010.08.003

Soulage, C; Perrin, D; Cottet-Emard, JM; Pequignot, J; Dalmaz, Y; Pequignot, JM. (2004). Central and peripheral changes in catecholamine biosynthesis and turnover in rats after a short period of ozone exposure. Neurochem Int 45: 979-986. http://dx.doi.org/10.1016/j.neuint.2004.06.015

Spannhake, EW; Reddy, SPM; Jacoby, DB; Yu, XY; Saatian, B; Tian, J. (2002). Synergism between rhinovirus infection and oxidant pollutant exposure enhances airway epithelial cell cytokine production. Environ Health Perspect 110: 665-670.

Spektor, DM; Lippmann, M. (1991). Health effects of ambient ozone on healthy children at a summer camp. In RL Berglund; DR Lawson; DJ McKee (Eds.), Tropospheric Ozone and the Environment: Papers from an International Conference; March 1990; Los Angeles, CA (pp. 83-89). Pittsburgh, PA: Air & Waste Management Association.

Spektor, DM; Lippmann, M; Lioy, PJ; Thurston, GD; Citak, K; James, DJ; Bock, N; Speizer, FE; Hayes, C. (1988a). Effects of ambient ozone on respiratory function in active, normal children. Am Rev Respir Dis 137: 313-320.

Spektor, DM; Lippmann, M; Thurston, GD; Lioy, PJ; Stecko, J; O'Connor, G; Garshick, E; Speizer, FE; Hayes, C. (1988b). Effects of ambient ozone on respiratory function in healthy adults exercising outdoors. Am Rev Respir Dis 138: 821-828.

Stafoggia, M; Forastiere, F; Faustini, A; Biggeri, A; Bisanti, L; Cadum, E; Cernigliaro, A; Mallone, S; Pandolfi, P; Serinelli, M; Tessari, R; Vigotti, MA; Perucci, CA. (2010). Susceptibility factors to ozone-related mortality: A population-based case-crossover analysis. Am J Respir Crit Care Med 182: 376-384. http://dx.doi.org/10.1164/rccm.200908-1269OC

Steinvil, A; Fireman, E; Kordova-Biezuner, L; Cohen, M; Shapira, I; Berliner, S; Rogowski, O. (2009). Environmental air pollution has decremental effects on pulmonary function test parameters up to one week after exposure. Am J Med Sci 338: 273-279. http://dx.doi.org/10.1097/MAJ.0b013e3181adb3ed

Steinvil, A; Kordova-Biezuner, L; Shapira, I; Berliner, S; Rogowski, O. (2008). Short-term exposure to air pollution and inflammation-sensitive biomarkers. Environ Res 106: 51-61. http://dx.doi.org/10.1016/j.envres.2007.08.006

Stenfors, N; Bosson, J; Helleday, R; Behndig, AF; Pourazar, J; Tornqvist, H; Kelly, FJ; Frew, AJ; Sandstrom, T; Mudway, IS; Blomber, A. (2010). Ozone exposure enhances mast-cell inflammation in asthmatic airways despite inhaled corticosteroid therapy. Inhal Toxicol 22: 133-139. http://dx.doi.org/10.3109/08958370903005736

Stenfors, N; Pourazar, J; Blomberg, A; Krishna, MT; Mudway, I; Helleday, R; Kelly, FJ; Frew, AJ; Sandstrom, T. (2002). Effect of ozone on bronchial mucosal inflammation in asthmatic and healthy subjects. Respir Med 96: 352-358.

Stieb, DM; Szyszkowicz, M; Rowe, BH; Leech, JA. (2009). Air pollution and emergency department visits for cardiac and respiratory conditions: A multi-city time-series analysis. Environ Health Global Access Sci Source 8: 25. http://dx.doi.org/10.1186/1476-069X-8-25

Strickland, MJ; Darrow, LA; Klein, M; Flanders, WD; Sarnat, JA; Waller, LA; Sarnat, SE; Mulholland, JA; Tolbert, PE. (2010). Short-term associations between ambient air pollutants and pediatric asthma emergency department visits. Am J Respir Crit Care Med 182: 307-316. http://dx.doi.org/10.1164/rccm.200908-1201OC

Strickland, MJ; Darrow, LA; Mulholland, JA; Klein, M; Flanders, WD; Winquist, A; Tolbert, PE. (2011). Implications of different approaches for characterizing ambient air pollutant concentrations within the urban airshed for time-series studies and health benefits analyses. Environ Health Global Access Sci Source 10: 36. http://dx.doi.org/10.1186/1476-069X-10-36

Stylianou, M; Nicolich, MJ. (2009). Cumulative effects and threshold levels in air pollution mortality: Data analysis of nine large US cities using the NMMAPS dataset. Environ Pollut 157: 2216-2223. http://dx.doi.org/10.1016/j.envpol.2009.04.011

Symons, JM; Wang, L; Guallar, E; Howell, E; Dominici, F; Schwab, M; Ange, BA; Samet, J; Ondov, J; Harrison, D; Geyh, A. (2006). A case-crossover study of fine particulate matter air pollution and onset of congestive heart failure symptom exacerbation leading to hospitalization. Am J Epidemiol 164: 421-433. http://dx.doi.org/10.1093/aje/kwj206

Szyszkowicz, M. (2008). Ambient air pollution and daily emergency department visits for ischemic stroke in Edmonton, Canada. Int J Occup Med Environ Health 21: 295-300. http://dx.doi.org/10.2478/v10001-008-0029-5

Takeuchi, C; Galve, R; Nieva, J; Witter, DP; Wentworth, AD; Troseth, RP; Lerner, RA; Wentworth, P, Jr. (2006). Proatherogenic effects of the cholesterol ozonolysis products, atheronal-A and atheronal-B. Biochemistry 45: 7162-7170. http://dx.doi.org/10.1021/bi0604330

Tamer, L; Calikoglu, M; Ates, NA; Yildirim, H; Ercan, B; Saritas, E; Unlu, A; Atik, U. (2004). Glutathione-S-transferase gene polymorphisms (GSTT1, GSTM1, GSTP1) as increased risk factors for asthma. Respirology 9: 493-498. http://dx.doi.org/10.1111/j.1440-1843.2004.00657.x

Tankersley, CG; Peng, RD; Bedga, D; Gabrielson, K; Champion, HC. (2010). Variation in echocardiographic and cardiac hemodynamic effects of PM and ozone inhalation exposure in strains related to Nppa and Npr1 gene knock-out mice. Inhal Toxicol 22: 695-707. http://dx.doi.org/10.3109/08958378.2010.487549

Tepper, JL; Weiss, B; Cox, C. (1982). Microanalysis of ozone depression of motor activity. Toxicol Appl Pharmacol 64: 317-326.

Tepper, JL; Weiss, B; Wood, RW. (1983). Behavioral indices of ozone exposure. In SD Lee; MG Mustafa; MA Mehlman (Eds.), International symposium on the biomedical effects of ozone and related photochemical oxidants (pp. 515526). Princeton NJ: Princeton Scientific.

Tepper, JS; Costa, DL; Lehmann, JR; Weber, MF; Hatch, GE. (1989). Unattenuated structural and biochemical alterations in the rat lung during functional adaptation to ozone. Am J Respir Crit Care Med 140: 493-501.

Tepper, JS; Weiss, B; Wood, RW. (1985). Alterations in behavior produced by inhaled ozone or ammonia. Toxicol Sci 5: 1110-1118.

Thaller, EI; Petronella, SA; Hochman, D; Howard, S; Chhikara, RS; Brooks, EG. (2008). Moderate increases in ambient PM2.5 and ozone are associated with lung function decreases in beach lifeguards. J Occup Environ Med 50: 202-211. http://dx.doi.org/10.1097/JOM.0b013e31816386b4

Thompson, AM; Zanobetti, A; Silverman, F; Schwartz, J; Coull, B; Urch, B; Speck, M; Brook, JR; Manno, M; Gold, DR. (2010). Baseline Repeated Measures from Controlled Human Exposure Studies: Associations between Ambient Air Pollution Exposure and the Systemic Inflammatory Biomarkers IL-6 and Fibrinogen. Environ Health Perspect 118: 120-124. http://dx.doi.org/10.1289/ehp.0900550

Thomson, E; Kumarathasan, P; Goegan, P; Aubin, RA; Vincent, R. (2005). Differential regulation of the lung endothelin system by urban particulate matter and ozone. Toxicol Sci 88: 103-113. http://dx.doi.org/10.1093/toxsci/kfi272

Thomson, E; Kumarathasan, P; Vincent, R. (2006). Pulmonary expression of preproET-1 and preproET-3 mRNAs is altered reciprocally in rats after inhalation of air pollutants. Exp Biol Med 231: 979-984.

Thomson, EM; Kumarathasan, P; Calderon-Garciduenas, L; Vincent, R. (2007). Air pollution alters brain and pituitary endothelin-1 and inducible nitric oxide synthase gene expression. Environ Res 105: 224-233. http://dx.doi.org/10.1016/j.envres.2007.06.005

Thurston, GD; Lippmann, M; Scott, MB; Fine, JM. (1997). Summertime haze air pollution and children with asthma. Am J Respir Crit Care Med 155: 654-660.

Tolbert, PE; Klein, M; Peel, JL; Sarnat, SE; Sarnat, JA. (2007). Multipollutant modeling issues in a study of ambient air quality and emergency department visits in Atlanta. J Expo Sci Environ Epidemiol 17: S29-S35. http://dx.doi.org/10.1038/sj.jes.7500625

Torres, A; Utell, MJ; Morow, PE; Voter, KZ; Whitin, JC; Cox, C; Looney, RJ; Speers, DM; Tsai, Y; Frampton, MW. (1997). Airway inflammation in smokers and nonsmokers with varying responsiveness to ozone. Am J Respir Crit Care Med 156: 728-736.

Trenga, CA; Koenig, JQ; Williams, PV. (2001). Dietary antioxidants and ozone-induced bronchial hyperresponsiveness in adults with asthma. Arch Environ Occup Health 56: 242-249.

Triche, EW; Gent, JF; Holford, TR; Belanger, K; Bracken, MB; Beckett, WS; Naeher, L; McSharry, JE; Leaderer, BP. (2006). Low-level ozone exposure and respiratory symptoms in infants. Environ Health Perspect 114: 911-916. http://dx.doi.org/10.1289/ehp.8559

Turner, RM; Muscatello, DJ; Zheng, W; Willmore, A; Arendts, G. (2007). An outbreak of cardiovascular syndromes requiring urgent medical treatment and its association with environmental factors: an ecological study. Environ Health 6: 37. http://dx.doi.org/10.1186/1476-069X-6-37

Tyler, WS; Tyler, NK; Last, JA; Gillespie, MJ; Barstow, TJ. (1988). Comparison of daily and seasonal exposures of young monkeys to ozone. Toxicology 50: 131-144.

U.S. EPA (U.S. Environmental Protection Agency). (1986). Air quality criteria for ozone and other photochemical oxidants [EPA Report]. (EPA-600/8-84-020aF - EPA-600/8-84-020eF). Research Triangle Park, NC. http://www.ntis.gov/search/product.aspx?ABBR=PB87142949

U.S. EPA (U.S. Environmental Protection Agency). (1996a). Air quality criteria for ozone and related photochemical oxidants [EPA Report]. (EPA/600/P-93/004AF). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (1996f). Table 6-5. Lung inflammation and permeability changes associated with ozone exposure [EPA Report]. In Air quality criteria for ozone and related photochemical oxidants (pp. 22-27). (EPA/600/P-93/004AF). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (1996g). Table 6-6. Effects of ozone on host defense mechanisms: Physical clearance [EPA Report]. In Air quality criteria for ozone and related photochemical oxidants (pp. 38). (EPA/600/P-93/004AF). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (1996h). Table 6-7. Effects of ozone on host defense mechanisms: Macrophage alterations [EPA Report]. In Air quality criteria for ozone and related photochemical oxidants (pp. 40-41). (EPA/600/P-93/004AF). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (1996i). Table 6-8. Effects of ozone on host defense mechanisms: Immunology [EPA Report]. In Air quality criteria for ozone and related photochemical oxidants (pp. 45-46). (EPA/600/P-93/004AF). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (1996j). Table 6-9. Effects of ozone on host defense mechanisms: Interactions with infectious agents [EPA Report]. In Air quality criteria for ozone and related photochemical oxidants (pp. 50). (EPA/600/P-93/004AF). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (1996k). Table 6-10. Effects of ozone on conducting airways [EPA Report]. In Air quality criteria for ozone and related photochemical oxidants (pp. 58-60). (EPA/600/P-93/004AF). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (1996l). Table 6-11. Effects of ozone on lung structure: Short-term exposures (<2 weeks) [EPA Report]. In Air quality criteria for ozone and related photochemical oxidants (pp. 61-63). (EPA/600/P-93/004AF). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (1996m). Table 6-13. Effects of ozone on pulmonary function [EPA Report]. In Air quality criteria for ozone and related photochemical oxidants (pp. 86-87). (EPA/600/P-93/004AF). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (1996n). Table 6-14. Effects of ozone on airway reactivity [EPA Report]. In Air quality criteria for ozone and related photochemical oxidants (pp. 88-90). (EPA/600/P-93/004AF). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (2006b). Air quality criteria for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-05/004AF). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=149923

U.S. EPA (U.S. Environmental Protection Agency). (2006d). Table AX5-7. Effects of ozone on lung host defenses [EPA Report]. In Air quality criteria for ozone and related photochemical oxidants (pp. 8-16). (EPA/600/R-05/004AF). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=149923

U.S. EPA (U.S. Environmental Protection Agency). (2006e). Table AX5-8. Effects of ozone on lung permeability and inflammation [EPA Report]. In Air quality criteria for ozone and related photochemical oxidants (pp. 17-26). (EPA/600/R-05/004AF). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=149923

U.S. EPA (U.S. Environmental Protection Agency). (2006f). Table AX5-9. Effects of ozone on lung structure: Acute and subchronic exposures [EPA Report]. In Air quality criteria for ozone and related photochemical oxidants (pp. 27-30). (EPA/600/R-05/004AF). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=149923

U.S. EPA (U.S. Environmental Protection Agency). (2006g). Table AX5-11. Effects of ozone on pulmonary function [EPA Report]. In Air quality criteria for ozone and related photochemical oxidants (pp. 34-35). (EPA/600/R-05/004AF). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=149923

U.S. EPA (U.S. Environmental Protection Agency). (2006h). Table AX5-12. Effects of ozone on airway responsiveness [EPA Report]. In Air quality criteria for ozone and related photochemical oxidants (pp. 36-42). (EPA/600/R-05/004AF). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=149923

Uchiyama, I; Simomura, Y; Yokoyama, E. (1986). Effects of acute exposure to ozone on heart rate and blood pressure of the conscious rat. Environ Res 41: 529-537.

Uchiyama, I; Yokoyama, E. (1989). Effects of short- and long-term exposure to ozone on heart rate and blood pressure of emphysematous rats. Environ Res 48: 76-86. http://dx.doi.org/10.1016/S0013-9351(89)80087-8

Ulmer, C; Kopp, M; Ihorst, G; Frischer, T; Forster, J; Kuehr, J. (1997). Effects of ambient ozone exposures during the spring and summer of 1994 on pulmonary function of schoolchildren. Pediatr Pulmonol 23: 344-353. http://dx.doi.org/10.1002/(SICI)1099-0496(199705)23:5<344::AID-PPUL6>3.0.CO;2-K

Ultman, JS; Ben-Jebria, A; Arnold, SF. (2004). Uptake distribution of ozone in human lungs: Intersubject variability in physiologic response (pp. 1-23; discussion 25-30). (ISSN 1041-5505

HEI Research Report 125). Boston, MA: Health Effects Institute. http://pubs.healtheffects.org/view.php?id=70

Urch, B; Silverman, F; Corey, P; Brook, JR; Lukic, KZ; Rajagopalan, S; Brook, RD. (2005). Acute blood pressure responses in healthy adults during controlled air pollution exposures. Environ Health Perspect 113: 1052-1055.

Urch, B; Speck, M; Corey, P; Wasserstein, D; Manno, M; Lukic, KZ; Brook, JR; Liu, L; Coull, B; Schwartz, J; Gold, DR; Silverman, F. (2010). Concentrated ambient fine particles and not ozone induce a systemic interleukin-6 response in humans. Inhal Toxicol 22: 210-218. http://dx.doi.org/10.3109/08958370903173666

Vagaggini, B; Bartoli, MLE; Cianchetti, S; Costa, F; Bacci, E; Dente, FL; Di Franco, A; Malagrino, L; Paggiaro, P. (2010). Increase in markers of airway inflammation after ozone exposure can be observed also in stable treated asthmatics with minimal functional response to ozone. Respir Res 11: 5. http://dx.doi.org/10.1186/1465-9921-11-5

Vagaggini, B; Cianchetti, S; Bartoli, M; Ricci, M; Bacci, E; Dente, FL; Di Franco, A; Paggiaro, P. (2007). Prednisone blunts airway neutrophilic inflammatory response due to ozone exposure in asthmatic subjects. Respiration 74: 61-58. http://dx.doi.org/10.1159/000096078

Vagaggini, B; Taccola, M; Clanchetti, S; Carnevali, S; Bartoli, ML; Bacci, E; Dente, FL; Di Franco, A; Giannini, D; Paggiaro, PL. (2002). Ozone exposure increases eosinophilic airway response induced by previous allergen challenge. Am J Respir Crit Care Med 166: 1073-1077.

Vagaggini, B; Taccola, M; Conti, I; Carnevali, S; Cianchetti, S; Bartoli, ML; Bacci, E; Dente, FL; Di Franco, A; Giannini, D; Paggiaro, PL. (2001). Budesonide reduces neutrophilic but not functional airway response to ozone in mild asthmatics. Am J Respir Crit Care Med 164: 2172-2176.

Valacchi, G; Pecorelli, A; Mencarelli, M; Maioli, E; Davis, PA. (2009). Beta-carotene prevents ozone-induced proinflammatory markers in murine skin. Toxicol Ind Health 25: 241-247. http://dx.doi.org/10.1177/0748233709103030

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

Van Loveren, H; Rombout, PJA; Wagenaar, SS; Walvoort, HC; Vos, JG. (1988). Effects of ozone on the defense to a respiratory Listeria monocytogenes infection in the rat: Suppression of macrophage function and cellular immunity and aggravation of histopathology in lung and liver during infection. Toxicol Appl Pharmacol 94: 374-393.

Vanguilder, HD; Freeman, WM. (2011). The hippocampal neuroproteome with aging and cognitive decline: Past progress and future directions. Front Aging Neurosci 3: 8. http://dx.doi.org/10.3389/fnagi.2011.00008

Vesely, DL; Giordano, AT; Raska-Emery, P; Montgomery, MR. (1994a). Increase in atrial natriuretic factor in the lungs, heart, and circulatory system owing to ozone. Chest 105: 1551-1554.

Vesely, DL; Giordano, AT; Raska-Emery, P; Montgomery, MR. (1994b). Ozone increases amino- and carboxy-terminal atrial natriuretic factor prohormone peptides in lung, heart, and circulation. J Biochem Mol Toxicol 9: 107-112. http://dx.doi.org/10.1002/jbt.2570090208

Vesely, DL; Giordano, AT; Raska-Emery, P; Montgomery, MR. (1994c). Ozone increases atrial natriuretic peptides in heart, lung and circulation of aged vs adult animals. Gerontology 40: 227-236. http://dx.doi.org/10.1159/000213590

Villeneuve, PJ; Chen, L; Rowe, BH; Coates, F. (2007). Outdoor air pollution and emergency department visits for asthma among children and adults: A case-crossover study in northern Alberta, Canada. Environ Health Global Access Sci Source 6: 40. http://dx.doi.org/10.1186/1476-069X-6-40

Villeneuve, PJ; Chen, L; Stieb, D; Rowe, BH. (2006a). Associations between outdoor air pollution and emergency department visits for stroke in Edmonton, Canada. Eur J Epidemiol 21: 689-700. http://dx.doi.org/10.1007/s10654-006-9050-9

Villeneuve, PJ; Doiron, MS; Stieb, D; Dales, R; Burnett, RT; Dugandzic, R. (2006b). Is outdoor air pollution associated with physician visits for allergic rhinitis among the elderly in Toronto, Canada? Allergy 61: 750-758. http://dx.doi.org/10.1111/j.1398-9995.2006.01070.x

Vincent, R; Janzen, EG; Chen, G; Kumarathasan, P; Haire, DL; Guenette, J; Chen, JZ; Bray, TM. (1996a). Spin trapping study in the lungs and liver of F344 rats after exposure to ozone. Free Radic Res 25: 475-488.

Von Klot, S; Peters, A; Aalto, P; Bellander, T; Berglind, N; D'Ippoliti, D; Elosua, R; Hormann, A; Kulmala, M; Lanki, T; Lowel, H; Pekkanen, J; Picciotto, S; Sunyer, J; Forastiere, F; Group, HEoPoSSHS. (2005). Ambient air pollution is associated with increased risk of hospital cardiac readmissions of myocardial infarction survivors in five European cities. Circulation 112: 3073-3079. http://dx.doi.org/10.1161/CIRCULATIONAHA.105.548743

Voynow, JA; Fischer, BM; Zheng, S; Potts, EN; Grover, AR; Jaiswal, AK; Ghio, AJ; Foster, WM. (2009). NAD(P)H quinone oxidoreductase 1 is essential for ozone-induced oxidative stress in mice and humans. Am J Respir Cell Mol Biol 41: 107-113. http://dx.doi.org/10.1165/rcmb.2008-0381OC

Wagner, JG; Harkema, JR; Jiang, Q; Illek, B; Ames, BN; Peden, DB. (2009). Gamma-tocopherol attenuates ozone-induced exacerbation of allergic rhinosinusitis in rats. Toxicol Pathol 37: 481-491. http://dx.doi.org/10.1177/0192623309335630

Wagner, JG; Jiang, Q; Harkema, JR; Illek, B; Patel, DD; Ames, BN; Peden, DB. (2007). Ozone enhancement of lower airway allergic inflammation is prevented by gamma-tocopherol. Free Radic Biol Med 43: 1176-1188. http://dx.doi.org/10.1016/j.freeradbiomed.2007.07.013

Ward, DJ; Roberts, KT; Jones, N; Harrison, RM; Ayres, JG; Hussain, S; Walters, S. (2002). Effects of daily variation in outdoor particulates and ambient acid species in normal and asthmatic children. Thorax 57: 489-502. http://dx.doi.org/10.1136/thorax.57.6.489

Watkinson, WP; Aileru, AA; Dowd, SM; Doerfler, DL; Tepper, JS; Costa, DL. (1993). Acute effects of ozone on heart rate and body temperature in the unanesthetized, unrestrained rat maintained at different ambient temperatures. Inhal Toxicol 5: 129-147.

Watkinson, WP; Campen, MJ; Wichers, LB; Nolan, JP; Costa, DL. (2003). Cardiac and thermoregulatory responses to inhaled pollutants in healthy and compromised rodents: Modulation via interaction with environmental factors. Environ Res 92: 35-47.

Weinmann, GG; Bowes, SM; Gerbase, MW; Kimball, AW; Frank, R. (1995a). Response to acute ozone exposure in healthy men. Results of a screening procedure. Am J Respir Crit Care Med 151: 33-40.

Weinmann, GG; Weidenbach-Gerbase, M; Foster, WM; Zacur, H; Frank, R. (1995c). Evidence for ozone-induced small-airway dysfunction: Lack of menstrual-cycle and gender effects. Am J Respir Crit Care Med 152: 988-996.

Wellenius, GA; Bateson, TF; Mittleman, MA; Schwartz, J. (2005). Particulate air pollution and the rate of hospitalization for congestive heart failure among medicare beneficiaries in Pittsburgh, Pennsylvania. Am J Epidemiol 161: 1030-1036.

Wellenius, GA; Yeh, GY; Coull, BA; Suh, HH; Phillips, RS; Mittleman, MA. (2007). Effects of ambient air pollution on functional status in patients with chronic congestive heart failure: A repeated-measures study. Environ Health 6: 1-7. http://dx.doi.org/10.1186/1476-069X-6-26

Wentworth, P, Jr; Nieva, J; Takeuchi, C; Galve, R; Wentworth, AD; Dilley, RB; Delaria, GA; Saven, A; Babior, BM; Janda, KD; Eschenmoser, A; Lerner, RA. (2003). Evidence for ozone formation in human atherosclerotic arteries. Science 302: 1053-1056. http://dx.doi.org/10.1126/science.1089525

Wheeler, A; Zanobetti, A; Gold, DR; Schwartz, J; Stone, P; Suh, HH. (2006). The relationship between ambient air pollution and heart rate variability differs for individuals with heart and pulmonary disease. Environ Health Perspect 114: 560-566.

Wiester, MJ; Watkinson, WP; Costa, DL; Crissman, KM; Richards, JH; Winsett, DW; Highfill, JW. (1996c). Ozone toxicity in the rat. III. Effect of changes in ambient temperature on pulmonary parameters. J Appl Physiol 81: 1691-1700.

Wiwatanadate, P; Liwsrisakun, C. (2011). Acute effects of air pollution on peak expiratory flow rates and symptoms among asthmatic patients in Chiang Mai, Thailand. Int J Hyg Environ Health 214: 251-257. http://dx.doi.org/10.1016/j.ijheh.2011.03.003

Wiwatanadate, P; Trakultivakorn, M. (2010). Air pollution-related peak expiratory flow rates among asthmatic children in Chiang Mai, Thailand. Inhal Toxicol 22: 301-308. http://dx.doi.org/10.3109/08958370903300327

Wong, CM; Ma, S; AJ, H; Lam, TH. (1999a). Does ozone have any effect on daily hospital admissions for circulatory diseases? J Epidemiol Community Health 53: 580-581.

Wong, CM; Vichit-Vadakan, N; Vajanapoom, N; Ostro, B; Thach, TQ; Chau, PY; Chan, EK; Chung, RY; Ou, CQ; Yang, L; Peiris, JS; Thomas, GN; Lam, TH; Wong, TW; Hedley, AJ; Kan, H; Chen, B; Zhao, N; London, SJ; Song, G; Chen, G; Zhang, Y; Jiang, L; Qian, Z; He, Q; Lin, HM; Kong, L; Zhou, D; Liang, S; Zhu, Z; Liao, D; Liu, W; Bentley, CM; Dan, J; Wang, B; Yang, N; Xu, S; Gong, J; Wei, H; Sun, H; Qin, Z. (2010). Part 5. Public health and air pollution in Asia (PAPA): A combined analysis of four studies of air pollution and mortality. In Public Health and Air Pollution in Asia (PAPA): Coordinated Studies of Short-Term Exposure to Air Pollution and Daily Mortality in Four Cities (pp. 377-418). Boston, MA: Health Effects Institute. http://pubs.healtheffects.org/view.php?id=348

Wong, CM; Yang, L; Thach, TQ; Chau, PY; Chan, KP; Thomas, GN; Lam, TH; Wong, TW; Hedley, AJ; Peiris, JS. (2009). Modification by influenza on health effects of air pollution in Hong Kong. Environ Health Perspect 117: 248-253. http://dx.doi.org/10.1289/ehp.11605

Wong, TW; Lau, TS; Yu, TS; Neller, A; Wong, SL; Tam, W; Pang, SW. (1999b). Air pollution and hospital admissions for respiratory and cardiovascular diseases in Hong Kong. Occup Environ Med 56: 679-683.

Wu, CF; Kuo, IC; Su, TC; Li, YR; Lin, LY; Chan, CC; Hsu, SC. (2010). Effects of personal exposure to particulate matter and ozone on arterial stiffness and heart rate variability in healthy adults. Am J Epidemiol 171: 1299-1309. http://dx.doi.org/10.1093/aje/kwq060

Xia, Y; Tong, H. (2006). Cumulative effects of air pollution on public health. Stat Med 25: 3548-3559. http://dx.doi.org/10.1002/sim.2446

Yang, CY. (2008). Air pollution and hospital admissions for congestive heart failure in a subtropical city: Taipei, Taiwan. J Toxicol Environ Health A 71: 1085-1090. http://dx.doi.org/10.1080/15287390802114428

Yang, CY; Chen, YS; Yang, CH; Ho, SC. (2004). Relationship between ambient air pollution and hospital admissions for cardiovascular diseases in Kaohsiung, Taiwan. J Toxicol Environ Health A 67: 483-493.

Yang, Q; Chen, Y; Krewski, D; Burnett, RT; Shi, Y; Mcgrail, KM. (2005b). Effect of short-term exposure to low levels of gaseous pollutants on chronic obstructive pulmonary disease hospitalizations. Environ Res 99: 99-105. http://dx.doi.org/10.1016/j.envres.2004.09.014

Yokoyama, E; Uchiyama, I; Arito, H. (1989). Extrapulmonary effects of low level ozone exposure. In T Schneider; SD Lee; GJR Wolters; LD Grant (Eds.), Atmospheric ozone research and its policy implications: proceedings of the 3rd US-Dutch international symposium; May 1988; Nijmegen, The Netherlands (pp. 301-309). Amsterdam, The Netherlands: Elsevier.

Yoon, HK; Cho, HY; Kleeberger, SR. (2007). Protective role of matrix metalloproteinase-9 in ozone-induced airway inflammation. Environ Health Perspect 115: 1557-1563. http://dx.doi.org/10.1289/ehp.10289

Zanobetti, A; Canner, MJ; Stone, PH; Schwartz, J; Sher, D; Eagan-Bengston, E; Gates, KA; Hartley, LH; Suh, H; Gold, DR. (2004). Ambient pollution and blood pressure in cardiac rehabilitation patients. Circulation 110: 2184-2189. http://dx.doi.org/10.1161/01.cir.0000143831.33243.d8

Zanobetti, A; Gold, DR; Stone, PH; Suh, HH; Schwartz, J; Coull, BA; Speizer, FE. (2010). Reduction in heart rate variability with traffic and air pollution in patients with coronary artery disease. Environ Health Perspect 118: 324-330. http://dx.doi.org/10.1289/ehp.0901003

Zanobetti, A; Schwartz, J. (2006). Air pollution and emergency admissions in Boston, MA. J Epidemiol Community Health 60: 890-895. http://dx.doi.org/10.1136/jech.2005.039834

Zanobetti, A; Schwartz, J. (2008a). Is there adaptation in the ozone mortality relationship: A multi-city case-crossover analysis. Environ Health 7: 22. http://dx.doi.org/10.1186/1476-069X-7-22

Zanobetti, A; Schwartz, J. (2008b). Mortality displacement in the association of ozone with mortality: An analysis of 48 cities in the United States. Am J Respir Crit Care Med 177: 184-189. http://dx.doi.org/10.1164/rccm.200706-823OC

# 7 INTEGRATED HEALTH EFFECTS OF LONG-TERM OZONE EXPOSURE

## 7.1 Introduction

This chapter reviews, summarizes, and integrates the evidence on relationships between health effects and long-term exposures to $O_3$. Both epidemiologic and toxicological studies provide a basis for examining long-term $O_3$ exposure health effects for respiratory effects, cardiovascular effects, reproductive and developmental effects, central nervous system effects, cancer outcomes, and mortality. Long-term exposure has been defined as a duration of approximately 30 days (1 month) or longer[1]. However, in order to characterize the weight of evidence for the effects of $O_3$ on reproductive and developmental effects in a consistent, cohesive and integrated manner, results from both short-term and long-term exposure periods are included in that section, and are identified accordingly in the text and tables.

Conclusions from the 2006 $O_3$ AQCD (U.S. EPA, 2006b) are summarized briefly at the beginning of each section, and the evaluation of evidence from recent studies builds upon what was available during the previous review. For each health outcome (e.g., respiratory disease, lung function), results are summarized for studies from the specific scientific discipline, i.e., epidemiologic and toxicological studies. The major sections (i.e., respiratory, cardiovascular, mortality, reproductive/developmental, cancer) conclude with summaries of the evidence for the various health outcomes within that category and integration of the findings that lead to conclusions regarding causality based upon the framework described in the Preamble to this ISA. Determination of causality is made for the overall health effect category, such as respiratory effects, with coherence and plausibility being based on evidence from across disciplines and also across the suite of related health outcomes, including cause-specific mortality.

As mentioned in Chapter 2 (Section 2.3), epidemiologic studies generally present $O_3$-related effect estimates for mortality and morbidity health outcomes based on an incremental change in exposure. Studies traditionally present the relative risk per an incremental change equal to the interquartile range in $O_3$ concentrations or some other arbitrary value (e.g., 10 ppb). Additionally, various exposure metrics are used in $O_3$ epidemiologic studies, with the three most common being the maximum 1-h average within a 24-hour period (1-h max), the maximum 8-h average within a 24-hour period (8-h max), and 24-h average (24-h avg). For the purpose of presenting results from studies that use different exposure metrics, EPA consistently applies the same $O_3$ increments to facilitate comparisons between the results of various studies that may present results for different incremental changes. Differences due to the use of varying exposure metrics (e.g., 1-h max, 24-h avg)

[1] Unless otherwise specified, the term “chronic” generally refers to an annual exposure duration for epidemiology studies and a duration of greater than 10% of the lifespan of the animal in toxicological studies.

become less apparent when averaged across longer exposure periods, because levels are typically lower and less variable. As such, throughout this chapter an increment of 10 ppb was consistently applied across studies, regardless of exposure metric, to facilitate comparisons between the results from these studies.

## 7.2 Respiratory Effects

Studies reviewed in the 2006 $O_3$ AQCD examined evidence for relationships between long-term $O_3$ exposure (several months to yearly) and effects on respiratory health outcomes including declines in lung function, increases in inflammation, and development of asthma in children and adults. Animal toxicology data provided a clearer picture indicating that long-term $O_3$ exposure may have lasting effects. Chronic exposure studies in animals have reported biochemical and morphological changes suggestive of irreversible long-term $O_3$ impacts on the lung. In contrast to supportive evidence from chronic animal studies, the epidemiologic studies on longer-term (annual) lung function declines, inflammation, and new asthma development remained inconclusive.

Several studies reviewed in the 2006 $O_3$ AQCD (Horak et al., 2002; Frischer et al., 1999) collectively indicated that $O_3$ exposure averaged over several summer months was associated with smaller increases in lung function growth in children. For longer averaging periods (annual), the definitive analysis in the Children's Health Study (CHS) reported by Gauderman et al. (2004) provided little evidence that such long-term exposure to ambient $O_3$ was associated with significant deficits in the growth rate of lung function in children in contrast to the effects observed with other pollutants such as acid vapor, $NO_2$, and $PM_{2.5}$. Limited epidemiologic research examined the relationship between long-term $O_3$ exposures and inflammation. Consistent with evidence of inflammation and allergic responses reported in experimental studies, an association between 30-day average $O_3$ and increased eosinophil levels was observed in an Austrian study (Frischer et al., 2001).
The cross-sectional studies available for the 2006 $O_3$ AQCD detected no associations between long-term $O_3$ exposures and asthma prevalence, asthma-related symptoms or allergy to common aeroallergens in children after controlling for covariates. However, longitudinal studies provided evidence that long-term $O_3$ exposure influences the risk of asthma development in children (McConnell et al., 2002) and adults (McDonnell et al., 1999a; Greer et al., 1993).

New evidence presented below reports interactions between genetic variants and long-term $O_3$ exposure in effects on new onset asthma in U.S. cohorts in multi-community studies where protection by specific oxidant gene variants was restricted to children living in low $O_3$ communities. Related studies report coherent relationships between respiratory symptoms among asthmatics and long-term $O_3$ exposure. This evidence for respiratory effects associated with long-term $O_3$ exposure is supported by a large evidence base indicating associations of short-term exposure to $O_3$ with increases in respiratory symptoms and asthma medication use in

children with asthma (Section 6.2.4.1) and asthma hospitalizations in children (Section 6.2.7.2). A new line of evidence reports a positive concentration-response relationship between first asthma hospitalization and long-term $O_3$ exposure. Results from recent studies examining pulmonary function, inflammation, and allergic responses are also presented.

## 7.2.1 Asthma

### 7.2.1.1 New Onset Asthma

Asthma is a heterogeneous disease with a high degree of temporal variability. Its progression and symptoms can vary within an individual's experience over time. The course of asthma may vary markedly between young children, older children and adolescents, and adults. This variation is probably more dependent on age than on symptoms (NHLBI, 2007). Longitudinal cohort studies have examined associations between long-term $O_3$ exposures and the onset of asthma in adults and children (McConnell et al., 2002; McDonnell et al., 1999a; Greer et al., 1993), with results indicating a direct effect of long-term $O_3$ exposure on asthma risk in adults and effect modification by $O_3$ in children.

Associations between long-term $O_3$ exposure and new cases of asthma were reported in a cohort of nonsmoking adults in California (McDonnell et al., 1999a; Greer et al., 1993). The Adventist Health and Smog (AHSMOG) study cohort of 3,914 (age 27 to 87 years, 36% male) was drawn from nonsmoking, non-Hispanic white California Seventh Day Adventists, who were surveyed in 1977, 1987, and 1992. To be eligible, subjects had to have lived 10 or more years within 5 miles of their current residence in 1977. Residences from 1977 onward were followed and linked in time and space to interpolate concentrations of $O_3$, $PM_{10}$, $SO_4^{2-}$, $SO_2$, and $NO_2$. New asthma cases were defined as self-reported doctor-diagnosed asthma at either the 1987 or 1992 follow-up questionnaire among those who had not reported having asthma upon enrollment in 1977. During the 10-year follow-up (1977 to 1987), the incidence of new asthma was 2.1% for males and 2.2% for females (Greer et al., 1993). Ozone concentration data were not provided. A relative risk of 3.12 (95% CI: 1.16, 5.85) per 10-ppb increase in annual mean $O_3$ (exposure metric not stated) was observed in males, compared to a relative risk of 0.94 (95% CI: 0.65, 1.34) in females.

In the 15-year follow-up study (1977-1992), 3.2% of the eligible males and a slightly greater 4.3% of the eligible females developed adult asthma (McDonnell et al., 1999a). The mean 20-year average (1973-1992) for 8-h avg $O_3$ (9 a.m. to 5 p.m.) was 46.5 ppb (SD 15.3). For males, the relative risk of developing asthma was 1.31 (95% CI: 1.01, 1.71) per 10-ppb increase in 8-h avg $O_3$. Once again, there was no evidence of a positive association between $O_3$ and new-onset asthma in females (relative risk of 0.94 [95% CI: 0.87, 1.02]). The lack of an association does not necessarily indicate no effect of $O_3$ on the development of asthma among females.

For example, differences between females and males in time-activity patterns may influence relative exposures to ambient $O_3$. During summer 1992, the mean (SD) hours per week spent outdoors for male and female asthma cases were 13.8 (10.6) and 11.4 (10.9), respectively, indicating potential greater misclassification of exposure in females. None of the other pollutants ($PM_{10}$, $SO_4^{2-}$, $SO_2$, and $NO_2$) were associated with development of asthma in either males or females. Adjusting for copollutants did not diminish the association between $O_3$ and asthma incidence for males. In no case was the $O_3$ coefficient reduced by more than 10% in the two-pollutant models compared to the model containing $O_3$ alone. The consistency of the results in the two studies with different follow-up times, as well as the independent and robust association between annual mean $O_3$ concentrations and asthma incidence, provide supportive evidence that long-term $O_3$ exposure may be associated with the development of asthma in adult males. However, because the AHSMOG cohort was drawn from a narrow subject definition, the representativeness of this cohort to the general U.S. population may be limited.

In children, the relationship between long-term $O_3$ exposure and new onset asthma has been extensively investigated in the CHS. In this cohort, evidence provides stronger support for long-term $O_3$ exposure modifying the risk of new onset asthma associated with other potential risk factors than having a main effect on new onset asthma. Initiated in the early 1990s, the CHS was originally designed to examine whether long-term exposure to ambient pollutants was related to chronic respiratory outcomes in children in 12 communities in southern California (Peters et al., 1999b; Peters et al., 1999a). New-onset asthma was classified as having no prior history of asthma at study entry with subsequent report of physician-diagnosed asthma at follow-up with the date of onset assigned to be the midpoint of the interval between the interview date when asthma diagnosis was first reported and the previous interview date. In a cohort recruited during 2002-2003 and followed for three years beginning in kindergarten or first grade, McConnell et al. (2010) reported a hazard ratio for new onset asthma of 0.76 (95% CI: 0.38, 1.54) comparing the communities with the highest (59.8 ppb) and lowest (29.5 ppb) annual average of 8-h avg (10 a.m.-6 p.m.) $O_3$. With adjustment for school and residential modeled non-freeway traffic-related exposure, the estimated HR for $O_3$ was 1.01 (95% CI: 0.49, 2.11).

Similarly in a cohort recruited in 1993, asthma risk was not higher for residents of the six high-$O_3$ communities versus residents of the six low-$O_3$ communities (McConnell et al., 2002). In this study, 3,535 initially nonasthmatic children (ages 9 to 16 years at enrollment) were followed for up to 5 years, during which 265 cases of new-onset asthma were identified. Communities were stratified by 4-year average 1-h max $O_3$ levels, with six high-$O_3$ communities (mean 75.4 ppb [SD 6.8]) and six low-$O_3$ communities (mean 50.1 ppb [SD 11.0]). Within the high-$O_3$ communities, asthma risk was 3.3 (95% CI: 1.9, 5.8) times greater for children who played three or more sports as compared with children who played no sports. None of the children who lived in high-$O_3$ communities and played three or more sports had a family history of asthma. In models with individual sports entered as dummy variables, only tennis was significantly associated with asthma and only in the high $O_3$ communities. This association was absent in the low-$O_3$ communities (relative risk of 0.8 [95% CI:

0.4, 1.6]). The overall observed pattern of effects of sports participation on asthma risk was robust to adjustment for SES, history of allergy, family history of asthma, insurance, maternal smoking, and BMI.

Analyses aimed at distinguishing the effects of $O_3$ from effects of other pollutants indicated that in communities with high $O_3$ and low levels of other pollutants there was a 4.2-fold (95% CI: 1.6, 10.7) increased risk of asthma in children playing three or more sports, compared to children who played no sports. The relative risk in children playing three or more sports was slightly lower (3.3 [95% CI: 1.6, 6.9]) in communities with a combination of high levels of $O_3$ and other pollutants. Ozone concentrations were not strongly correlated with $PM_{10}$, $PM_{2.5}$, $NO_2$, or inorganic acid vapors, and no associations with asthma were found for these other pollutants. These results provide additional support that the effects of physical activity on asthma are modified by long-term $O_3$ exposure. Overall, the results from McConnell et al. (2002) suggest that playing sports may indicate greater outdoor activity when $O_3$ levels are higher and an increased ventilation rate, which may lead to increased $O_3$ exposure. It should be noted, however, that these findings were based on a small number of new asthma cases (n = 29 among children who played three or more sports) and were not based on a priori hypotheses.

Recent studies from the CHS provide evidence for gene-environment interactions in effects on new-onset asthma by indicating that the lower risks associated with specific genetic variants are found in children who live in lower $O_3$ communities (Islam et al., 2009; Islam et al., 2008; Oryszczyn et al., 2007; Lee et al., 2004b; Gilliland et al., 2002). Risk for new-onset asthma is related in part to genetic susceptibility, behavioral factors and environmental exposure (Gilliland et al., 1999). Gene-environment interactions in asthma have been well discussed in the literature (von Mutius, 2009; Holgate et al., 2007; Martinez, 2007a, b; Rahman et al., 2006; Hoffjan et al., 2005; Kleeberger and Peden, 2005; Ober, 2005). Complex chronic diseases, such as asthma, are partially the result of a sequence of biochemical reactions involving exposures to various environmental agents metabolized by a number of different genes (Conti et al., 2003). Oxidative stress has been proposed to underlie these mechanistic hypotheses (Gilliland et al., 1999). Genetic variants may impact disease risk directly or modify disease risk by affecting internal dose of pollutants and other environmental agents and/or their reaction products or by altering cellular and molecular modes of action. Understanding the relation between genetic polymorphisms and environmental exposure can help identify high-risk subgroups in the population and provide better insight into pathway mechanisms for these complex diseases.

CHS analyses have found that asthma risk is related to interactions between $O_3$ and variants in genes for enzymes such as heme-oxygenase (HO-1), arginases (ARG1 and 2), and glutathione S transferase P1 (GSTP1) (Himes et al., 2009; Islam et al., 2008; Li et al., 2008; Hanene et al., 2007; Ercan et al., 2006; Li et al., 2006a; Tamer et al., 2004; Gilliland et al., 2002). Biological plausibility for these findings is provided by evidence that these enzymes have antioxidant and/or anti-inflammatory activity and participate in well recognized modes of action in asthma pathogenesis.

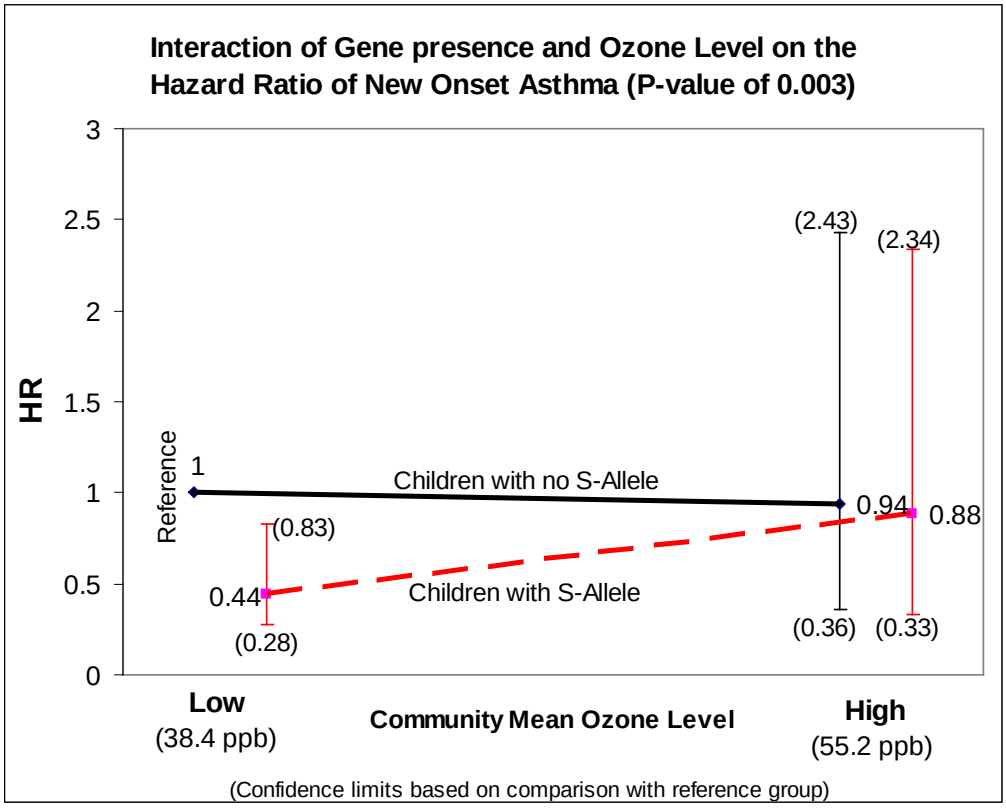
Further, several lines of evidence demonstrate that secondary oxidation products of $O_3$ initiate the key modes of action that mediate downstream health effects (Section 5.3.2). For example, HO-1 has been found to respond rapidly to oxidants, have anti-inflammatory and anti-oxidant effects (Exner et al., 2004), relax airway smooth muscle, and be induced in airways during asthma (Carter et al., 2004). The GSTP1 Val/Val genotype has been associated with increased risk of having atopic asthma (Tamer et al., 2004). Gene-environment interactions are discussed in greater detail in Section 5.4.2.1.

Islam et al. (2008) found that functional polymorphisms of the heme oxygenase-1 gene (HMOX-1, [(GT)n repeat]) influenced the risk of new-onset asthma, depending on ethnicity and long-term community $O_3$ concentrations. Ozone-gene interactions were not found for variants in other antioxidant genes: catalase (CAT [-262C >T -844C >T0]) or and manganese superoxide dismutase (MNSOD, [Ala-9Val]). Analyses were restricted to children of Hispanic (n = 576) or non-Hispanic white ethnicity (n = 1,125) and were conducted with long-term pollutant levels averaged from 1994 to 2003. The effect of ambient air pollution on the relationship between genetic polymorphism and new-onset asthma was assessed using Cox proportional hazard regression models where the community specific average air pollution levels were fitted as a continuous variable together with the appropriate interaction terms for genes and air pollutants and a random effect of community (Berhane et al., 2004).

Over the follow-up period, 160 new cases of asthma were diagnosed (Islam et al., 2008). For HMOX-1, the interaction ($p = 0.003$) indicated a greater protective effect of the S-allele (short, <23 (GT)n repeats) compared to the L-allele (long, >23 repeats) among non-Hispanic white children who lived in the low $O_3$ community (nonparallelism presented in Figure 7-1). Among children residing in low-$O_3$ communities, the hazard ratio (HR) of new onset asthma associated with the S-allele was 0.44 (95% CI: 0.23, 0.83) compared to non-Hispanic white children who lived in low $O_3$ communities and had no S-alleles. Biological plausibility for these results is provided by evidence that the S-allele variant of HMOX-1 is more readily induced than those with more numerous repeats. The S-allele was found to have a less protective effect in non-Hispanic white children who resided in high $O_3$ communities (HR = 0.88; [95% CI: 0.33, 2.34] compared to non-Hispanic white children in low $O_3$ communities with no S-allele). Because HMOX-1 variants were not associated with asthma risk in Hispanic children, effect modification by $O_3$ was not investigated. No significant interactions were observed between $PM_{10}$ or other pollutants and the HMOX-1 gene; quantitative results were not presented. Average $O_3$ levels showed low correlation with the other monitored pollutants. The authors did not consider the lack of adjustment for multiple testing to be a concern in this analysis because the selection of the genes was based on a priori hypotheses defined by a well-studied biological pathway, in which oxidative stress serves as the link among $O_3$ exposure, enzyme activity, and asthma.

Collectively, results from Islam et al. (2008) indicate that a variant in HMOX-1 that produces a more readily inducible enzyme is associated with lower risk of new-onset asthma in children who live in low $O_3$ communities. Results were not presented for

the main effects relating new-onset asthma to $O_3$ exposure. However, they do indicate that that in environments of low ambient $O_3$, enzymes with greater antioxidative activity may have the capacity to counter any temporary imbalance in an oxidant-antioxidant relationship. However, in the presence of high background $O_3$, the protective effect may be attenuated because with higher exposure to oxidants, the antioxidant genes may be at their maximal level of inducibility, and variation in promoters no longer affects levels of expression. Supporting evidence is provided by Schroer et al. (2009), who found that infants with multiple environmental exposures were at increased risk of wheeze regardless of variant in GSTP1, which encodes a gene with antioxidant activity.




Note: An interaction p-value of 0.003 was obtained from the hierarchical two stage Cox proportional hazard model fitting the community specific $O_3$ and controlling for random effect of the communities. The interaction indicates there is a greater protective effect of having a heme-oxygenase S-allele compared to having the L-allele among children living in communities with lower long-term ambient $O_3$ concentrations. The HRs are off-set as opposed to overlapping in the figure to allow clearer presentation of the results.

Source: Developed by EPA with data from Islam et al. (2008) (data used with permission of American Thoracic Society).

**Figure 7-1 Interaction of heme-oxygenase genetic variants and $O_3$ level on the Hazard Ratio (HR) of new-onset asthma in the 12 Children's Health Study communities.**

Expanding on the results of McConnell et al. (2002), Islam et al. (2009) provided evidence that variants in GSTM1 and GSTP1 may influence associations between outdoor exercise and new onset asthma. A primary conclusion that the authors (Islam et al., 2009) reported was that the GSTP1 Ile/Ile and GSTM1 null genotypes increased risk of new onset asthma during adolescence. The highest risk was found for participation in three or more team sports (compared to no sports) among children with GSTP1 Ile/Ile genotype living in high-$O_3$ communities (HR: 6.15, [95% CI: 2.2, 7.4]). No three-way interaction was found for GSTM1. These results demonstrate the potential importance of a combination of genetic variability, $O_3$ exposure, and outdoor activity on asthma risk. It is important to note that while some studies have found a modifying role of air pollution on the association between GSTP1 Ile/Ile and asthma in children (Lee et al., 2004b), others have found that the GSTP1 Val/Val variant to be associated with greater asthma prevalence and increased risk of $O_3$-associated respiratory morbidity (see discussion in Section 6.2.4.1).

The CHS also provided evidence of interactions between $O_3$ exposure and variants in genes for arginase (Salam et al., 2009). Arginase catalyzes the conversion of L-arginine. Because L-arginine is a precursor of NO, higher arginase activity can limit production of NO and subsequent nitrosative stress. Epidemiologic evidence of associations of arginase variants with asthma are limited (Li et al., 2006a); however, asthmatic subjects have been found to have higher arginase activity than non-asthmatic subjects (Morris et al., 2004). The modifying effect of $O_3$ and atopy on the association between ARG1 and ARG2 haplotypes and asthma were evaluated using likelihood ratio tests with appropriate interaction terms. Having more copies of the ARG1h4 haplotype (compared to having zero copies) was associated with lower odds of asthma, particularly among children with atopic asthma living in high $O_3$ communities (OR: 0.12; [95% CI: 0.04, 0.43]). Having more copies of the ARG2h3 haplotype (compared to having zero copies) was associated with increased risk of childhood-onset asthma among children in both low and high $O_3$ communities. The implications of findings are somewhat limited because the functional relevance of the ARG1 and ARG2 variants is not clear.

### 7.2.1.2 Prevalence of Asthma and Asthma Symptoms

Some cross-sectional studies reviewed in the 2006 $O_3$ AQCD observed positive relationships between chronic exposure to $O_3$ and prevalence of asthma and asthmatic symptoms in school children (Ramadour et al., 2000; Wang et al., 1999) while others (Kuo et al., 2002; Charpin et al., 1999) did not. Recent studies provide additional evidence.

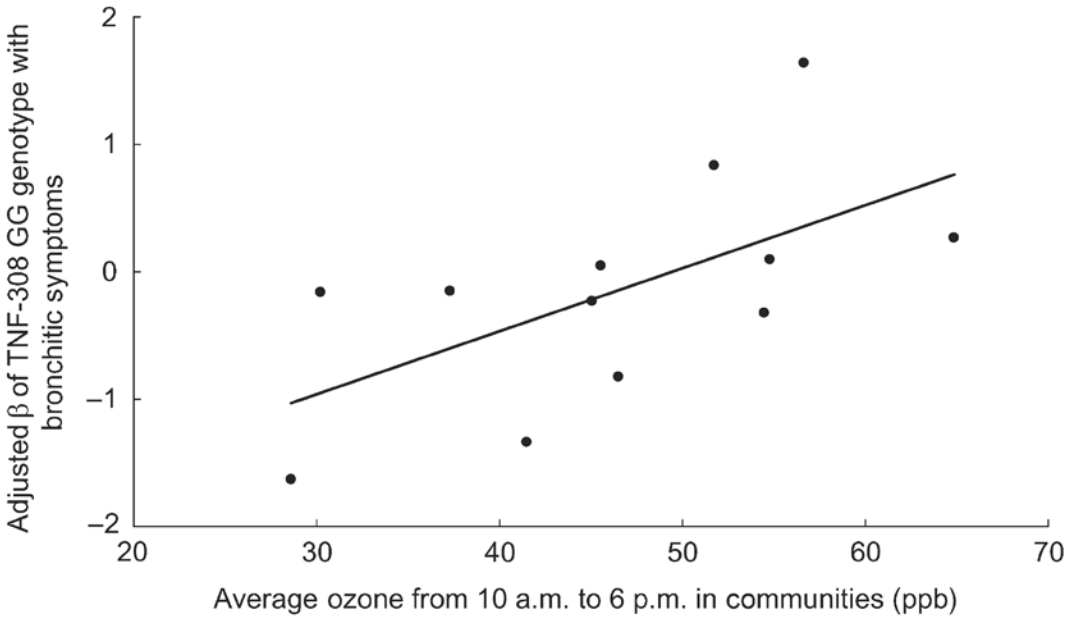
In a cross-sectional nationwide study of 32,672 Taiwanese school children, Hwang et al. (2005) assessed the effects of air pollutants on the risk of asthma. The study population was recruited from elementary and middle schools within 1 km of air monitoring stations. The risk of asthma was related to $O_3$ in the one-pollutant model. The addition of other pollutants ($NO_X$, CO, $SO_2$, and $PM_{10}$), in two-pollutant and

three-pollutant models, increased the $O_3$ risk estimates. The prevalence of childhood asthma was assessed in Portugal by contrasting the risk of asthma between a high $O_3$ rural area and an area with low $O_3$ levels (Sousa et al., 2011; Sousa et al., 2009; Sousa et al., 2008). The locations were selected to provide a difference in $O_3$ levels without the confounding effects of other pollutants. Both evaluation for asthma symptoms and $FEV_1$ suggested that $O_3$ increased asthma prevalence. Clark et al. (2010) investigated the effect of exposure to ambient air pollution in utero and during the first year of life on risk of subsequent incidence asthma diagnosis up to 3-4 years of age in a population-based nested case-control study for all children born in southwestern British Columbia in 1999 and 2000 (n = 37,401; including 3,482 [9.3%] with asthma). Air pollution exposure for each subject was estimated based on their residential address history using regulatory monitoring data, land use regression modeling, and proximity to stationary pollutant sources. Daily values from the three closest monitors within 50 km were used to calculate exposures. Traffic-related pollutants were associated with the highest risk. Ozone was inversely correlated with the primary traffic-related pollutants (r = -0.7 to -0.9). The low reliability of asthma diagnosis in infants makes this study difficult to interpret (Martinez et al., 1995). In a cross-sectional analysis, Akinbami et al. (2010) examined the association between chronic exposure to outdoor pollutants (12-month avg levels by county) and asthma outcomes in a national sample of children ages 3-17 years living in U.S. metropolitan areas (National Health Interview Survey, N = 34,073). A 5-ppb increase in estimated 8-h max $O_3$ concentration (annual average) yielded a positive association for both currently having asthma and for having at least 1 asthma attack in the previous year, while the adjusted odds ratios for other pollutants were not statistically significant. Models in which pollutant value ranges were divided into quartiles produced comparable results. Multipollutant models ($SO_2$ and PM) produced similar results. The median value for 12-month avg $O_3$ levels was 39.5 ppb and the IQR was 35.9-43.7 ppb. The adjusted odds for current asthma for the highest quartile (49.9-59.5 ppb) of estimated $O_3$ exposure was 1.56 (95% CI: 1.15, 2.10) with a positive concentration-response relationship apparent from the lowest quartile to the highest. Thus, this cross-sectional analysis and Hwang et al. (2005) provided further evidence relating $O_3$ exposure and the risk of asthma.

Relationships between long-term exposure and respiratory symptoms in asthmatic children also were examined in the CHS. McConnell et al. (1999) examined the association between $O_3$ levels and the prevalence of chronic lower respiratory tract symptoms in 3,676 cohort children with asthma. In this cross-sectional study, bronchitis, phlegm, and cough were not associated with annual mean 1-h max $O_3$ concentrations in children with asthma or wheeze. All other pollutants examined ($PM_{10}$, $PM_{2.5}$, $NO_2$, and gaseous acid) were associated with an increase in phlegm but not cough. The mean annual average 1-h max $O_3$ concentration was 65.6 ppb (range 35.5 to 97.5) across the 12 communities. In another CHS analysis, McConnell et al. (2003) evaluated relationships between air pollutants and bronchitic symptoms among 475 children with asthma. The mean 4-year average 8-h avg $O_3$ (10 a.m.-6 p.m.) concentration was 47.2 ppb (range 28.3 to 65.8) across the 12 communities. For a 10-ppb increase in 8-h avg $O_3$ averaged over 4 years, the between-community odds ratio was 0.90 (95% CI: 0.82, 1.00) whereas the within-community

(i.e., difference between one- and four-year average) odds ratio was larger, i.e., 1.79 (95% CI: 1.00, 3.21). The authors commented that if the larger within-community effect estimates were correct, then other cross-sectional (between-community) studies might have underestimated the true effect of air pollution on bronchitic symptoms in children. These differences might be attributable to confounding by poorly measured or unmeasured risk factors that vary between communities. Within community effects may more accurately represent risk associated with pollutant exposure because the analyses characterize health effects associated with changing pollutant concentrations within a community, thereby minimizing potential confounding by factors that are constant over time within a community. $PM_{2.5}$, $NO_2$, and organic carbon also were associated with bronchitic symptoms. In two-pollutant models, the within-community effect estimates for $O_3$ were markedly reduced and no longer statistically significant in some cases.

CHS also examined interactions between TNF-α 308 genotype and long-term $O_3$ exposure in the occurrence of bronchitic symptoms among children with asthma (Lee et al., 2009b). Increased airway levels of the cytokine TNF-α has been related to inflammation, and the GG genotype has been linked to lower expression of TNF-α. Asthmatic children with the GG genotype had a lower prevalence of bronchitic symptoms compared with children carrying at least one A-allele (e.g., GA or AA genotype). Low-versus high-$O_3$ strata were defined as less than or greater than 50-ppb $O_3$ avg. Asthmatic children with TNF-308 GG genotype had a significantly reduced risk of bronchitic symptoms with low-$O_3$ exposure (OR: 0.53 [95% CI: 0.31, 0.91]). The risk was not reduced in children living in high-$O_3$ communities (OR: 1.42 [95% CI: 0.75, 2.70]). The difference in genotypic effects between low- and high-$O_3$ environments was statistically significant among asthmatics (P for interaction = 0.01), but not significant among non-asthmatic children. Figure 7-2 presents adjusted $O_3$ community-specific regression coefficients plotted against ambient $O_3$ concentration, using weights proportional to the inverse variance. Investigators further reported no substantial differences in the effect of the GG genotype on bronchitic symptoms by long-term exposure to $PM_{10}$, $PM_{2.5}$, $NO_2$, acid vapor, or second-hand smoke.




Note: Using indicator variables for each category of genotype and $O_3$ exposure, investigators calculated effect estimates for TNF-α GG genotype on the occurrence of bronchitic symptoms among children with asthma.

Source: Reprinted with permission of John Wiley & Sons, (Lee et al., 2009b).

**Figure 7-2 Ozone modifies the effect of TNF GG genotype on bronchitic symptoms among children with asthma in the CHS.**

Another CHS analyses reported interrelationships between variants in CAT and myeloperoxidase (MPO) genes, ambient pollutants, and respiratory-related school absences for 1,136 Hispanic and non-Hispanic white cohort children (Wenten et al., 2009). A related study (Gilliland et al., 2001), found increased $O_3$ exposure to be related to greater school absenteeism due to respiratory illness but did not consider genetic variants. Wenten et al. (2009) hypothesized that variation in the level or function of antioxidant enzymes would modulate respiratory illness risk, especially under high levels of oxidative stress expected from high ambient $O_3$ exposure. The joint effect of variants in these two genes (genetic epistasis) on respiratory illness was examined because the enzyme products operate on the same substrate within the same biological pathway. Risk of respiratory-related school absences was elevated for children with CAT GG plus MPO GA or AA genotypes (RR: 1.35 [95% CI: 1.03, 1.77] compared to GG for both genes) and reduced for children with CAT GA or AA plus MPO GA or AA (RR: 0.81 [95% CI: 0.55, 1.19] compared to GG for both genes). Both CAT GG and MPO GA (or AA) genotypes produced a less activity enzyme. In analyses that stratified communities into high and low $O_3$ exposure groups by median levels (46.9 ppb), the protective effect of CAT GA or AA plus MPO GA or AA genotype was largely limited to children living in communities with high ambient $O_3$ levels (RR: 0.42 [95% CI: 0.20, 0.89]). The association of respiratory-illness absences with functional variants in CAT and

MPO that differed by air pollution levels illustrates the need to consider genetic epistasis in assessing gene-environment interactions.

Collective evidence from CHS provides an important demonstration of gene-environment interactions. In the complex gene-environment setting a modifying effect might not be reflected in an exposure main effect. The simultaneous occurrence of main effect and interaction effect can occur. The study of gene-environment interactions helps to dissect disease mechanisms in humans by using information on susceptibility genes to focus on the biological pathways that are most relevant to that disease (Hunter, 2005).

The French Epidemiology study on Genetics and Environment of Asthma (EGEA) investigated the relationship between ambient air pollution and asthma severity in a cohort in five French cities (Paris, Lyon, Marseille, Montpellier, and Grenoble) (Rage et al., 2009b). In this cross-sectional study, asthma severity over the past 12 months was assessed among 328 adult asthmatics using two methods: (1) a four-class severity score that integrated clinical events and type of treatment; and (2) a five-level asthma score based only on symptoms. Two measures of exposure were also assessed: (1) [first method] closest monitor data from 1991 to 1995 where a total of 93% of the subjects lived within 10 km of a monitor, but where 70% of the $O_3$ concentrations were back-extrapolated values; and (2) [second method] a validated spatial model that used geostatistical interpolations and then assigned air pollutants to the geocoded residential addresses of all participants and individually assigned exposure to ambient air pollution estimates. Higher asthma severity scores were significantly related to both the 8-h avg $O_3$ during April-September and the number of days with 8-h $O_3$ averages above 55 ppb. Both exposure assessment methods and severity score methods resulted in very similar findings. Effect estimates of $O_3$ were similar in three-pollutant models. No PM data were available. Since these estimates were not sensitive to the inclusion of ambient $NO_2$ in the three-pollutant models, the authors viewed the findings not to be explained by particles which usually have substantial correlations between PM and $NO_2$. Effect estimates for $O_3$ in three-pollutant models including $O_3$, $SO_2$, and $NO_2$ yielded OR for $O_3$-days of 2.74 (95% CI: 1.68, 4.48) per IQR days of 10-28 (+18) ppb. The effect estimates for $SO_2$ and $NO_2$ in the three-pollutant model were 1.33 (95% CI: 0.85, 2.11) and 0.94 (95% CI: 0.68, 1.29), respectively. Taking into account duration of residence did not change the result. This study suggests that a higher asthma severity score is related to long-term $O_3$ exposure.

An EGEA follow-up study (Jacquemin et al., 2012), examines the relationship between asthma and $O_3$, $NO_2$, and $PM_{10}$. New aspects considered include: (1) examination of three domains of asthma control (symptoms, exacerbations, and lung function); (2) levels of asthma control (controlled, partially controlled, and uncontrolled asthma); and (3) $PM_{10}$ and multipollutant analysis. In this cross-sectional analysis, EGEA2 studied 481 adult subjects with current asthma from 2003 to 2007. The IQRs were 11 (41-52) $\mu g/m^3$ for annual $O_3$ and 13 (25-38) $\mu g/m^3$ for summer (April-September) $O_3$. The association between asthma control and air pollutants was expressed by ORs (reported for one IQR of the pollutant), derived

from multinomial logistic regression. For each factor, the simultaneous assessment of the risk for uncontrolled asthma and for partly controlled asthma was compared with controlled asthma using a composite of the three domains. In crude and adjusted models, $O_3$-sum and $PM_{10}$ were positively associated with partly controlled and uncontrolled asthma, with a clear gradient from controlled, partly controlled (OR = 1.53, 95% CI: 1.01, 2.33) and uncontrolled (OR = 2.14, 95% CI: 1.34, 3.43) (from the multinomial logistic regression).

Separately, they used a composite asthma control classification that used the ordinal logistic regression for risk comparing controlled to partly controlled asthma and comparing partly controlled to uncontrolled asthma. For these two pollutants, the ORs assessed using the ordinal logistic regression were significant (ORs were 1.69 (95% CI: 1.22, 2.34) and 1.35 (95% CI: 1.13, 1.64) for $O_3$-sum and $PM_{10}$, respectively). For two pollutant models using the ordinal logistic regression, the adjusted ORs for $O_3$-sum and $PM_{10}$ included simultaneously in a unique model were 1.50 (95% CI: 1.07, 2.11) for $O_3$-sum and 1.28 (95% CI: 1.06, 1.55) for $PM_{10}$, respectively. This result suggests that the effects of both pollutants are independent.

The analysis of the associations between air pollution for all asthma subjects and each one of the three asthma control domains showed the following: (1) for lung function defined dichotomously as percent predicted $FEV_1$ value <or >=80 (OR = 1.35, 95% CI: 0.80, 2.28 for adjusted $O_3$-sum); (2) for symptoms defined as asthma attacks or dyspnea or woken by asthma attack or shortness of breath in the past three months (OR = 1.59, 95% CI: 1.10, 2.30 for adjusted $O_3$-sum); and (3) for exacerbations defined at least one hospitalizations or ER visits in the last year or oral corticosteroids in the past three months (OR = 1.58, 95% CI: 0.97, 2.59 for adjusted $O_3$-sum). Since the estimates for both pollutants were more stable and significant when using the integrated measure of asthma control, this indicates that the results are not driven by one domain. These results support an effect of long-term exposure to $O_3$ on asthma control in adulthood in subjects with pre-existing asthma.

Goss et al. (2004) investigated the effect of $O_3$ on pulmonary exacerbations and lung function in individuals over the age of 6 years with cystic fibrosis (n = 11,484). The study included patients enrolled in the Cystic Fibrosis Foundation National Patient Registry, which contains demographic and clinical data collected annually at accredited centers for cystic fibrosis. For 1999 through 2000, the annual mean $O_3$ concentration, calculated from 1-h averages from 616 monitors in the U.S. EPA Aerometric Information Retrieval System (AIRS), was 51.0 ppb (SD 7.3). Exposure was assessed by linking air pollution values from AIRS with the patient's home ZIP code. No clear association was found between annual mean $O_3$ and lung function parameters. However, a 10 ppb increase in annual mean $O_3$ was associated with a 10% (95% CI: 3, 17) increase in the odds of two or more pulmonary exacerbations. Significant excess odds of pulmonary exacerbations also were observed with increased annual mean $PM_{10}$ and $PM_{2.5}$ concentrations. The $O_3$ effect was robust to adjustment for $PM_{10}$ and $PM_{2.5}$, 8% (95% CI: 1, 15) increase in odds of two or more pulmonary exacerbations per 10 ppb increase in annual mean $O_3$.

### 7.2.2 Asthma Hospital Admissions and ED Visits

The studies on $O_3$-related hospital discharges and emergency department (ED) visits for asthma and respiratory disease that were available in the 2006 $O_3$ AQCD mainly looked at the daily time metric. Collectively the short-term $O_3$ studies presented earlier in Section 6.2.7.5 indicate that there is evidence for increases in both hospital admissions and ED visits related to all respiratory outcomes including asthma with stronger associations in the warm months. New studies evaluated long-term $O_3$ exposure metrics, providing a new line of evidence that suggests a positive exposure-response relationship between first asthma hospital admission and long-term $O_3$ exposure.

An ecologic study (Moore et al., 2008) evaluated time trends in associations between declining warm-season $O_3$ concentrations and hospitalization for asthma in children in California's South Coast Air Basin who ranged in age from birth to 19 years. Quarterly average concentrations from 195 spatial grids, 10×10 km, were used. Ozone was the only pollutant associated with increased hospital admissions over the study period. A linear relation was observed for asthma hospital discharges (Moore et al., 2008). A matched case-control study (Karr et al., 2007) was conducted on infant bronchiolitis (ICD 9, code 466.1) hospitalization and two measures of long-term pollutant exposure (the month prior to hospitalization and the lifetime average) for $O_3$ in the South Coast Air Basin of southern California among 18,595 infants born between 1995 and 2000. Ozone was associated with reduced risk in the single-pollutant model, but this relation did not persist in multipollutant models (CO, $NO_2$, and $PM_{2.5}$).

In a cross-sectional study, Meng et al. (2010) examined associations between air pollution and asthma morbidity in the San Joaquin Valley in California by using the 2001 California Health Interview Survey data from subjects ages 1 to 65+ who reported physician-diagnosed asthma (n = 1,502). Subjects were assigned annual average concentrations for $O_3$ based on residential ZIP code and the closet air monitoring station within 8 km but did not have data on duration of residence. Multipollutant models for $O_3$ and PM did not differ substantially from single-pollutant estimates, indicating that pollutant multi-collinearity is not a problem in these analyses. The authors reported increased asthma-related ED visits or hospitalizations for $O_3$ (OR = 1.49; [95% CI: 1.05, 2.11] per 10 ppb) for all ages. Positive associations were obtained for symptoms, but 95% confidence intervals included null values. Associations for symptoms for adults (ages 18+) were observed (OR = 1.40; [95% CI: 1.02, 1.91] per 10 ppb).

Associations between air pollution and poorly controlled asthma among adults in Los Angeles and San Diego Counties were investigated using the California Health Interview Survey data collected between November 2000 and September 2001 (Meng et al., 2007). Each respondent was assigned an annual average concentration measured at the nearest station within 5 miles of the residential cross-street intersection. Poorly controlled asthma was defined as having daily or weekly asthma symptoms or at least one ED visit or hospitalization because of asthma during the

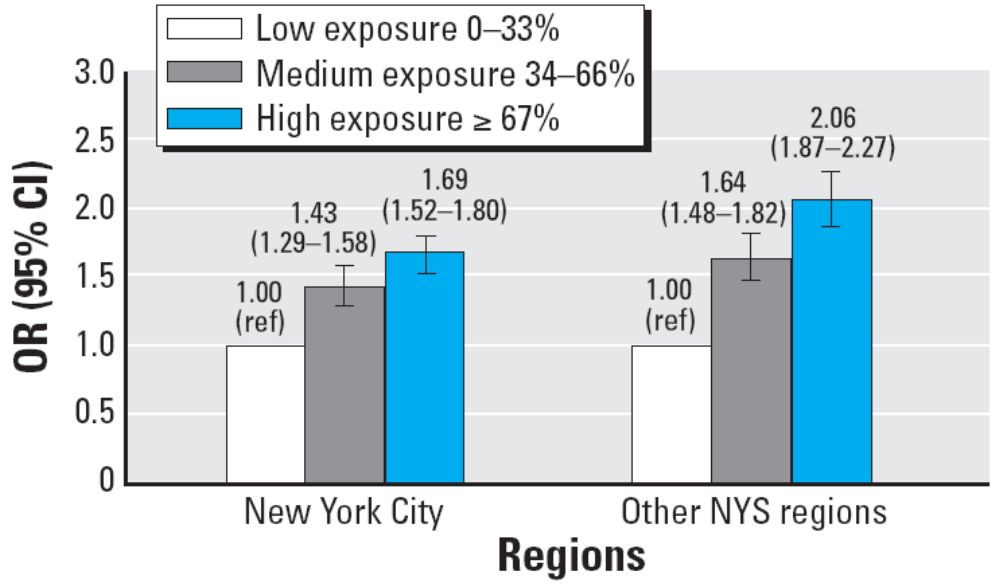
past 12 months. This cross-sectional study reports an OR of 3.34 (95% CI: 1.01, 11.09) for poorly controlled asthma when comparing those 65 years of age and older above the 90th percentile (28.7 ppb) level to those below that level. Copollutant (PM) analysis produced similar results.

Evidence associating long-term $O_3$ exposure to first asthma hospital admission in a concentration-response relationship is provided in a retrospective cohort study (Lin et al., 2008b). This study investigated the association between chronic exposure to $O_3$ and childhood asthma admissions (defined as a principal diagnosis of ICD9, code 493) by following a birth cohort of 1,204,396 eligible births born in New York State during 1995-1999 to first asthma admission or until 31 December 2000. There were 10,429 (0.87%) children admitted to the hospital for asthma between 1 and 6 years of age. The asthma hospitalization rate in New York State in 1993 was 2.87 per 1,000 (Lin et al., 1999). Three annual indicators (all 8-h max from 10:00 a.m. to 6:00 p.m.) were used to define chronic $O_3$ exposure: (1) mean concentration during the follow-up period (41.06 ppb); (2) mean concentration during the $O_3$ season (50.62 ppb); and (3) proportion of follow-up days with $O_3$ levels >70 ppb. In this study the authors aimed to predict the risk of having asthma admissions in a birth cohort, but the time to the first admission in children that is usually analyzed in survival models was not their primary interest. The effects of copollutants were assessed and controlled for using the Air Quality Index (AQI). Interaction terms were used to assess potential effect modifications. A positive association between chronic exposure to $O_3$ and childhood asthma hospital admissions was observed indicating that children exposed to high $O_3$ levels over time are more likely to develop asthma severe enough to be admitted to the hospital. The various factors were examined and differences were found for younger children (1-2 years), poor neighborhoods, Medicaid/self-paid births, geographic region and others. As shown in Figure 7-3, positive concentration-response relationships were observed. Asthma admissions were significantly associated with increased $O_3$ levels for all chronic exposure indicators (ORs, 1.16-1.68). When estimating the $O_3$ effect using the exceedance proportion, an increase was observed (OR = 1.68; [95% CI: 1.64, 1.73]) in hospital admissions with an IQR (2.51%) increase in $O_3$. A proportional hazards model for the New York City data was run as a sensitivity analysis and it yielded similar results between asthma admissions and chronic exposure to $O_3$ (Cox model: HR = 1.14, [95% CI: 1.124, 1.155] is similar to logistic model results: OR = 1.16 [95% CI: 1.15, 1.17]) (Lin, 2010). Thus, this study provides evidence associating long-term $O_3$ exposure to first asthma hospital admission in a concentration-response relationship.

In considering relationships between long-term pollutant exposure and chronic disease health endpoints, Künzli (2012) offers two hypotheses relevant to research on air pollution and chronic disease where chronic pathologies are found with acute expressions of the chronic disease: “H1: Exposure provides a basis for the development of the underlying chronic pathology, which increases the pool of people with chronic conditions prone to exacerbations; H2: Exposure triggers an acute event (or a state of frailty that results in an event with a delay of a few days or weeks) among those with the disease.” Künzli (2012) states if associations of pollution with events are much larger in the long-term studies, it provides some indirect evidence in

support of H1. If air pollution increases the pool of subjects with the chronic pathology (H1), more acute events are expected to be seen for higher exposures since events due to various causes are part of the chronic disease pathway.

Künzli (2012) makes such a comparison noting larger associations with long-term $NO_2$ exposures for adult asthma hospital admissions (Andersen et al., 2012) as compared to short-term $NO_2$ exposures for asthma hospital admissions (Peel et al., 2005). In a further example, Pope (2007) makes similar conclusions comparing long-term PM mortality study results to short-term PM mortality studies. The results of Lin et al. (2008b) for first asthma hospital admission, presented below, show effect estimates that are larger than those reported in a study of asthma hospital admissions in New York State by Silverman and Ito (2010), discussed in Chapter 6 (both studies are for young children). This provides some support for the hypothesis that $O_3$ exposure may not only have triggered the events but also increased the pool of asthmatics. However, caution is warranted in attributing associations in the Lin et al. (2008b) study to long-term exposures since there is potential for short-term exposures to contribute to the observed associations.




Note: Adjusted for child's sex, age, birth weight, and gestational age; maternal race, ethnicity, age, education, insurance, and smoking status during pregnancy; and regional poverty level and temperature. ORs by low, medium, and high exposure are shown for New York City (NYC: low [37.3 ppb], medium [37.3-38.11 ppb], high [38.11+ ppb] and other New York State regions (Other NYS regions: low [42.58 ppb], medium [42.58-45.06 ppb], high [45.06+ ppb]) for first asthma hospital admission.

Source: Lin (2010); Lin et al. (2008b)

**Figure 7-3 Ozone-asthma concentration-response relationship using the mean concentration during the entire follow-up period for first asthma hospital admission.**

### 7.2.3 Pulmonary Structure and Function

#### 7.2.3.1 Pulmonary Structure and Function: Evidence from Epidemiology Studies

The definitive 8-year follow-up analysis of the first cohort of the CHS, which is discussed in Section 7.2 (Gauderman et al., 2004), provided little evidence that long-term exposure to ambient $O_3$ was associated with significant deficits in the growth rate of lung function in children. A later CHS study (Islam et al., 2007) examined relationships between air pollution, lung function, and new onset asthma and reported no substantial differences in the effect of $O_3$ on lung function. Ozone concentrations from the least to most polluted communities (mean annual average of 8-h avg $O_3$) ranged from 30 to 65 ppb, as compared to the ranges observed for the other pollutants, which had 4-fold- to 8-fold differences in concentrations. In a more recent CHS study, Breton et al. (2011) hypothesized that genetic variation in genes on the glutathione metabolic pathway may influence the association between ambient air pollutant exposures and lung function growth in children. They investigated whether genetic variation in glutathione genes GSS, GSR, GCLC, and GCLM was associated with lung function growth in healthy children using data collected on 2,106 children over an 8-year time-period as part of the Children's Health Study. Breton et al. (2011) found that variation in the GSS locus was associated with differences in risk of children for lung function growth deficits associated with $NO_2$, $PM_{10}$, $PM_{2.5}$, elemental carbon, organic carbon, and $O_3$. The negative effects of air pollutants were largely observed within participants who had a particular GSS haplotype. The effects ranged from -124.2 to -149.1 mL for $FEV_1$, -92.9 to -126.7 mL for FVC and -193.9 to -277.9 mL/sec for MMEF for all pollutants except $O_3$, for which some positive associations were reported: 25.9 mL for $FEV_1$; 0.1 mL for FVC, and 166.5 mL/sec for MMEF. Ozone was associated with larger decreases in lung function in children without this haplotype, when compared to the other pollutants with values of -76.6 mL for $FEV_1$, -17.2 mL for FVC, and -200.3 mL/sec for MMEF, but only the association with MMEF was statistically significant.

As discussed in the 2006 $O_3$ AQCD, a study of freshman students at the University of California, Berkeley reported an interaction between lifetime exposure to $O_3$ and baseline $FEF_{25-75}$/FVC ratio, a measure of intrinsic airway size for decreased measures of small airways (<2 mm) function ($FEF_{75}$ and $FEF_{25-75}$) (Tager et al., 2005). Subjects with a small ratio (indicating an increased airway size relative to their lung volume) had decreases in $FEF_{75}$ and $FEF_{25-75}$ for increases in lifetime exposure to $O_3$. Kinney and Lippmann (2000) examined 72 nonsmoking adults (mean age 20 years) from the second-year class of students at the U.S. Military Academy in West Point, NY, and reported results that appear to be consistent with a decline in lung function that may in part be due to $O_3$ exposures over a period of several summer months. Ihorst et al. (2004) examined 2,153 children with a median age of 7.6 years and reported pulmonary function results which indicated that significantly lower FVC and $FEV_1$ increases were associated with higher $O_3$

exposures over the medium-term of several summer months, but not over several months in the winter. Semi-annual mean $O_3$ concentrations ranged from 22 to 54 ppb during the summer months and 4 to 36 ppb during the winter months. Further, over the longer-term 3.5-year period Ihorst et al. (2004) found that higher mean summer months $O_3$ levels were not associated with growth rates in lung function and for FVC and $FEV_1$, in contrast to the significant medium-term effects. Frischer et al. (1999) found that higher $O_3$ over one summer season, one winter season, and greater increases from one summer to the next over a three-year period were associated with smaller increases in lung function growth, indicating both medium and longer-term effects.

Mortimer et al. (2008a, b) examined the association of prenatal and lifetime exposures to air pollutants with pulmonary function and allergen sensitization in a subset of asthmatic children (ages 6-11) included in the Fresno Asthmatic Children's Environment Study (FACES). Monthly means of pollutant levels for the years 1989-2000 were created and averaged separately across several important developmental time-periods, including: the entire pregnancy, each trimester, the first 3 years of life, the first 6 years of life, and the entire lifetime. In the first analysis (Mortimer et al., 2008a), negative effects on pulmonary function were found for exposure to $PM_{10}$, $NO_2$, and CO during key neonatal and early life developmental periods. The authors did not find a negative effect of exposure to $O_3$ within this cohort. In the second analysis (Mortimer et al., 2008b), sensitization to at least one allergen was associated, in general, with higher levels of CO and $PM_{10}$ during the entire pregnancy and second trimester, and higher $PM_{10}$ during the first 2 years of life. Lower exposure to $O_3$ during the entire pregnancy or second trimester was associated with an increased risk of allergen sensitization. Although the pollutant metrics across time periods were correlated, the strongest associations with the outcomes were observed for prenatal exposures. Though it may be difficult to disentangle the effect of prenatal and postnatal exposures, the models from this group of studies suggest that each time period of exposure may contribute independently to different dimensions of school-aged children's pulmonary function. For 4 of the 8 pulmonary-function measures (FVC, $FEV_1$, PEF, $FEF_{25-75}$), prenatal exposures were more influential on pulmonary function than early-lifetime metrics, while, in contrast, the ratio of measures ($FEV_1$/FVC and $FEF_{25-75}$/FVC) were most influenced by postnatal exposures. When lifetime metrics were considered alone, or in combination with the prenatal metrics, the lifetime measures were not associated with any of the outcomes. This suggests that the timing of the $O_3$ exposure may be more important than the overall dose, and prenatal exposures are not just markers for lifetime or current exposures.

Latzin et al. (2009) examined whether prenatal exposure to air pollution was associated with lung function changes in the newborn. Tidal breathing, lung volume, ventilation inhomogeneity and eNO were measured in 241 unsedated, sleeping neonates (age = 5 weeks). Consistent with the previous studies, no association was found for prenatal exposure to $O_3$ and lung function.

In a cross-sectional study of adults, Qian et al. (2005) examined the association of long-term exposure to $O_3$ and $PM_{10}$ with pulmonary function from data of 10,240 middle-aged subjects who participated in the Atherosclerosis Risk in Communities (ARIC) study in four U.S. communities. A surrogate for long-term $O_3$ exposure from daily data was determined at the individual level. Ozone was significantly and negatively associated with measures of pulmonary function.

To determine the extent to which long-term exposure to outdoor air pollution accelerates adult decline in lung function, Forbes et al. (2009b) studied the association between chronic exposure to outdoor air pollution and lung function in approximately 42,000 adults aged 16 and older who were representatively sampled cross-sectionally from participants in the Health Survey for England (1995, 1996, 1997, and 2001). $FEV_1$ was not associated with $O_3$ concentrations. In contrast to the results for $PM_{10}$, $NO_2$, and $SO_2$; combining the results of all the survey years showed that a 5-ppb difference in $O_3$ was counter-intuitively associated with a higher $FEV_1$ by 22 mL.

In a prospective cohort study consisting of school-age, non-asthmatic children in Mexico City (n = 3,170) who were 8 years of age at the beginning of the study, Rojas-Martinez et al. (2007) evaluated the association between long-term exposure to $O_3$, $PM_{10}$ and $NO_2$ and lung function growth every 6 months from April 1996 through May 1999. Exposure data were provided by 10 air quality monitor stations located within 2 km of each child's school. Over the study period, 8-h $O_3$ concentrations ranged from 60 ppb (SD, ± 25) in the northeast area of Mexico City to 90 ppb (SD, ± 34) in the southwest, with an overall mean of 69.8 ppb.
In multipollutant models, an IQR increase in mean $O_3$ concentration of 11.3 ppb was associated with an annual deficit in $FEV_1$ of 12 mL in girls and 4 mL in boys. Single-pollutant models showed an association between ambient pollutants ($O_3$, $PM_{10}$, and $NO_2$) and deficits in lung function growth. While the estimates from copollutant models were not substantially different than single pollutant models, independent effects for pollutants could not be estimated accurately because the traffic-related pollutants were correlated. To reduce exposure misclassification, microenvironmental and personal exposure assessments were conducted in a randomly selected subsample of 60 children using passive $O_3$ samplers. Personal $O_3$ concentrations were correlated ($p < 0.05$) with the measurements obtained from the fixed-site air monitoring stations.

In the 2006 $O_3$ AQCD, few studies had investigated the effect of chronic $O_3$ exposure on pulmonary function. The strongest evidence was for medium-term effects of extended $O_3$ exposures over several summer months on lung function ($FEV_1$) in children, i.e., reduced lung function growth being associated with higher ambient $O_3$ levels. Longer-term studies (annual), investigating the association of chronic $O_3$ exposure on lung function ($FEV_1$) such as the definitive 8-year follow-up analysis of the first cohort (Gauderman et al., 2004) provide little evidence that long-term exposure to ambient $O_3$ at current levels is associated with significant deficits in the growth rate of lung function in children. Analyses indicated that there was no evidence that either 8-h avg $O_3$ (10 a.m. to 6 p.m.) or 24-h avg $O_3$ was associated

with any measure of lung function growth over a 4-year (age 10 to 14 years; (Gauderman et al., 2000)) or 8-year (age 10 to 18 years; (Gauderman et al., 2004)) period. However, most of the other pollutants examined (including $PM_{2.5}$, $NO_2$, acid vapor, and elemental carbon) were found to be significantly associated with reduced growth in lung function. In addition, there was only about a 2- to 2.5-fold difference in $O_3$ concentrations from the least to most polluted communities (mean annual average of 8-h avg $O_3$ ranged from 30 to 65 ppb), versus the ranges observed for the other pollutants (which had 4- to 8-fold differences in concentrations).

Short-term $O_3$ exposure studies presented in Section 6.2.1.2 provide a cumulative body of epidemiologic evidence that strongly supports associations between ambient $O_3$ exposure and decrements in lung function among children. For new studies of long-term $O_3$ exposure relationship to pulmonary function, one study, where $O_3$ and other pollutant levels were higher (90 ppb at high end of the range) than those in the CHS, observes a relationship between $O_3$ concentration and pulmonary function declines in school-aged children. Two studies of adult cohorts provide mixed results where long- term exposures were at the high end of the range with levels of 49.5 ppb in one study and 27 ppb IQR in the other. Toxicological studies examining monkeys have provided data for airway resistance in an asthma model but this is difficult to compare to $FEV_1$ results. Thus there is little new evidence to build upon the very limited studies of pulmonary function ($FEV_1$) from the 2006 $O_3$ AQCD.

#### 7.2.3.2 Pulmonary Structure and Function: Evidence from Toxicological Studies and Nonhuman Primate Asthma Models

Long-term studies in animals allow for greater insight into the potential effects of prolonged exposure to $O_3$, that may not be easily measured in humans, such as structural changes in the respiratory tract. As reviewed in the 1996 and 2006 $O_3$ AQCDs and Chapter 5 of this ISA, there are both qualitative and quantitative uncertainties in the extrapolation of data generated by rodent toxicology studies to the understanding of health effects in humans. Despite these uncertainties, epidemiologic studies observing functional changes in humans can attain biological plausibility, in conjunction with long-term toxicological studies, particularly $O_3$-inhalation studies performed in non-human primates whose respiratory system most closely resembles that of the human. An important series of studies have used nonhuman primates to examine the effect of $O_3$ alone or in combination with an inhaled allergen, house dust mite antigen, on morphology and lung function. These animals exhibit the hallmarks of allergic asthma defined for humans, including: a positive skin test for HDMA with elevated levels of IgE in serum and IgE-positive cells within the tracheobronchial airway walls; impaired airflow which is reversible by treatment with aerosolized albuterol; increased abundance of immune cells, especially eosinophils, in airway exudates and bronchial lavage; and development of nonspecific airway responsiveness (NHLBI, 2007). Hyde et al. (2006) compared asthma models of rodents (mice) and the nonhuman primate model to responses in

humans and concluded that the unique responses to inhaled allergen shown in the rhesus monkeys make it the most appropriate animal model of human asthma. These studies and others have demonstrated changes in pulmonary function and airway morphology in adult and infant nonhuman primates repeatedly exposed to environmentally relevant concentrations of $O_3$ (Joad et al., 2008; Carey et al., 2007; Plopper et al., 2007; Fanucchi et al., 2006; Joad et al., 2006; Evans et al., 2004; Larson et al., 2004; Tran et al., 2004; Evans et al., 2003; Schelegle et al., 2003; Fanucchi et al., 2000; Hyde et al., 1989; Harkema et al., 1987a; Harkema et al., 1987b; Fujinaka et al., 1985). Many of the observations found in adult monkeys have also been noted in infant rhesus monkeys, although a direct comparison of the degree of effects between adult and infant monkeys has not been reported. The findings of these nonhuman primate studies have also been observed in rodent studies discussed at the end of this section and included in Table 7-1.

The initial observations in adult nonhuman primates have been expanded in a series of experiments using infant rhesus monkeys repeatedly exposed to 0.5 ppm $O_3$ starting at 1 month of age[1] (Plopper et al., 2007). The purpose of these studies, designed by Plopper and colleagues, was to determine if a cyclic regimen of $O_3$ inhalation would amplify the allergic responses and structural remodeling associated with allergic sensitization and inhalation in the infant rhesus monkey. In terms of pulmonary function changes, after several episodic exposures of infant monkeys to $O_3$, they observed a significant increase in the baseline airway resistance, which was accompanied by a small increase in airway responsiveness to inhaled histamine (Schelegle et al., 2003), although neither measurement was statistically different from filtered air control values. Exposure of animals to inhaled house dust mite antigen alone also produced small but not statistically significant changes in baseline airway resistance and airway responsiveness, whereas the combined exposure to both ($O_3$ + antigen) produced statistically significant and greater than additive changes in both functional measurements. This nonhuman primate evidence of an $O_3$-induced change in airway resistance and responsiveness supports the biologic plausibility of long-term exposure to $O_3$ contributing to the effects of asthma in children.
To understand which conducting airways and inflammatory mechanisms are involved in $O_3$-induced airway hyperresponsiveness in the infant rhesus monkey, a follow-up study examined airway responsiveness ex vivo in lung slices (Joad et al., 2006). Using video microscopy to morphometrically evaluate the response of bronchi and respiratory bronchioles to methacholine, (a bronchoconstricting agent commonly used to evaluate airway responsiveness in asthmatics), the investigators observed differential effects for the two airway sizes. While episodic exposure to $O_3$ alone (0.5 ppm) had little effect on ex vivo airway responsiveness in bronchi and respiratory bronchioles, exposure to dust mite antigen alone produced airway hyperresponsiveness in the large bronchi, whereas $O_3$ + antigen produced significant increases in airway hyperresponsiveness only in the respiratory bronchioles. These

[1] Schelegle et al. (2003) used a two-by-two block design. Twenty-four infant rhesus monkeys (30 days old) were exposed to 11 episodes (total of 6-months exposure period) of filtered air (FA), house dust mite allergen (HDMA), $O_3$ (5 days each followed by 9 days of FA). Ozone was delivered for 8h/day at 0.5 ppm. Twelve of the monkeys (HDMA, and HDMA + $O_3$ groups) were sensitized to house dust mite allergen (HDMA, confirmed by skin testing). To evaluate the potential for recovery, the 5 months of exposure were followed by another 6 months in FA until the monkeys were reevaluated at 12 months of age.

results suggest that effect of $O_3$ on airway responsiveness occurs predominantly in the smaller bronchioles, where dosimetric models indicate the dose would be higher.

The functional changes in the conducting airways of infant rhesus monkeys exposed to either $O_3$ alone or $O_3$ + antigen were accompanied by a number of cellular and morphological changes, including a significant 4-fold increase in eosinophils, (a cell type important in allergic asthma), in the bronchoalveolar lavage of infant monkeys exposed to $O_3$ alone. Thus, these studies demonstrate both functional and cellular changes in the lung of infant monkeys after cyclic exposure to 0.5 ppm $O_3$. This concentration, provides relevant information to understanding the potentially damaging effects of ambient $O_3$ exposure on the respiratory tract of humans. No concentration-response data, however, are available from these nonhuman primate studies.

In addition to these functional and cellular changes, significant structural changes in the respiratory tract have been observed in infant rhesus monkeys exposed to $O_3$. During normal respiratory tract development, conducting airways increase in diameter and length in the infant rhesus monkey. Exposure to $O_3$ alone (5 days of 0.5 ppm $O_3$ at 8 h/day, followed by 9 days of filtered air exposures for 11 cycles), however, markedly affected the growth pattern of distal conducting airways (Fanucchi et al., 2006). Whereas the first alveolar outpocketing occurred at airway generation 13 or 14 in filtered air-control infant monkeys, the most proximal alveolarized airways occurred at an average of 10 airway generations in $O_3$-exposed monkeys. Similarly, the diameter and length of the terminal and respiratory bronchioles were significantly decreased in $O_3$-exposed monkeys. Importantly, the $O_3$-induced structural pathway changes persisted after recovery in filtered air for 6 months after cessation of the $O_3$ exposures. These structural effects were accompanied by significant increases in mucus goblet cell mass, alterations in smooth muscle orientation in the respiratory bronchioles, epithelial nerve fiber distribution, and basement membrane zone morphometry. These latter effects are noteworthy because of their potential contribution to airway obstruction and airway hyperresponsiveness which are central features of asthma.

Because many cellular and biochemical factors are known to contribute to allergic asthma, the effect of exposure to $O_3$ alone or $O_3$ + antigen on immune system parameters was also examined in infant rhesus monkeys. Mast cells, which contribute to asthma via the release of potent proteases, were elevated in animals exposed to antigen alone but $O_3$ alone had little effect on mast cell numbers and the response of animals exposed to $O_3$ + antigen was not different from that of animals exposed to antigen alone; thus suggesting that mast cells played little role in the interaction between $O_3$ and antigen in this model of allergic asthma (Van Winkle et al., 2010). Increases in CD4+ and CD8+ lymphocytes were observed at 6 months of age in the blood and bronchoalveolar lavage fluid of infant rhesus monkeys exposed to $O_3$ + antigen but not in monkeys exposed to either agent alone (Miller et al., 2009). Activated lymphocytes (i.e., CD25+ cells) were morphometrically evaluated in the airway mucosa and significantly increased in infant monkeys exposed to antigen alone or $O_3$ + antigen. Although $O_3$ alone had no effect on CD25+ cells, it

did alter the anatomic distribution of CD25+ cells within the airways. Ozone had only a small effect on these sets of immune cells and did not produce a strong interaction with an inhaled allergen in this nonhuman primate model.

In addition to alterations in the immune system, nervous system interactions with epithelial cells are thought to play a contributing role to airway hyperresponsiveness. A critical aspect of postnatal lung development is the laying of nerve axons with specific connections serving to maintain lung homeostasis. Aberrant innervation patterns may underlie allergic airways disease pathology and long-term decrements in airway function. As noted in the 2006 $O_3$ AQCD, exposure of infant rhesus monkeys altered the normal development of neural innervation in the epithelium of the conducting airways (Larson et al., 2004). Significant mean reductions in nerve fiber density were observed in the midlevel airways of animals exposed to $O_3$ alone (49% reduction), and $O_3$ + antigen (55% reduction). Moreover, the morphology of nerve bundles was altered. The persistence of these effects was examined after a 6-month recovery period, and although nerve distribution remained atypical, there was a dramatic increase in airway nerve density (hyperinnervation) (Kajekar et al., 2007). Thus, in addition to structural, immune, and inflammatory effects, exposure to $O_3$ produces alterations in airway innervation which may contribute to $O_3$-induced exacerbation of asthma. Evaluation of the pathobiology of airway remodeling in growing lungs of neonates using an animal model where exposure to allergen generates reactive airway disease with all the hallmarks of asthma in humans illustrates that exposure to $O_3$ and allergen early in life produces a large number of disruptions of fundamental growth and differentiation processes.

A number of studies in both nonhuman primates and rodents demonstrate that $O_3$ exposure can increase collagen synthesis and deposition, inducing fibrotic-like changes in the lung (Last et al., 1994; Chang et al., 1992; Moffatt et al., 1987; Reiser et al., 1987; Last et al., 1984). Increased collagen content is often associated with elevated abnormal cross links that appear to be irreversible (Reiser et al., 1987). Generally changes in collagen content have been observed in rats exposed to 0.5 ppm $O_3$ or higher, although extracellular matrix thickening has been observed in the lungs of rats exposed to an urban pattern of $O_3$ with daily peaks of 0.25 ppm for 38 weeks (Chang et al., 1992; Chang et al., 1991). A more recent study using an urban pattern of exposure to 0.5 ppm $O_3$ demonstrated that $O_3$-induced collagen deposition in mice is dependent on the activity of TGF-β (Katre et al., 2011). Sex differences have been observed with respect to increased centriacinar collagen deposition and crosslinking, which was observed in female but not male rats exposed to 0.5 and 1.0 ppm $O_3$ for 20 months (Last et al., 1994). Few other long-term exposure morphological studies have presented sex differences and most only evaluated males.

As described in the 1996 and 2006 $O_3$ AQCDs, perhaps the largest chronic $O_3$ study was an NIEHS-NTP/HEI funded rodent study conducted by multiple investigators studying a number of different respiratory tract endpoints (Catalano et al., 1995b). Rats were exposed to 0.12, 0.5, or 1.0 ppm $O_3$ for 6 h/day and 5 d/week for 20 months. The most prominent changes were observed in the nasal cavity where a large fraction of $O_3$ is absorbed. Alterations in nasal function (increased mucous flow) and

structure (goblet cell metaplasia) were observed at 0.5 and 1.0 ppm but not 0.12 ppm $O_3$. In the lung, the centriacinar region (CAR) was the anatomical site most affected by $O_3$. The epithelial cell lining was changed to resemble that seen in respiratory bronchioles and the interstitial volume was increased. Biochemical analyses demonstrated increased collagen and glycoaminoglycans, an observation that supported the structural changes. As in the nose, these changes were observed only at the two highest exposure concentrations. Importantly, despite these morphologic and biochemical changes after 20 months of exposure, detailed pulmonary function testing revealed little to no measurable change in function. Thus, minor respiratory tract changes were observed after chronic exposure to $O_3$ up to 1.0 ppm in the F344 rat model.

It is unclear what the long-term impact of $O_3$-induced structural changes may be. Simulated seasonal (episodic) exposure studies suggest that such exposures might have cumulative impacts, and a number of studies indicate that structural changes in the respiratory system are persistent or irreversible. For example, $O_3$-induced hyperplasia was still evident in the nasal epithelia of rats 13 weeks after recovery from 0.5 ppm $O_3$ exposure (Harkema et al., 1999). In a study of episodic exposure to 0.25 ppm $O_3$, Chang et al. (1992) observed no reversal of basement membrane thickening in rat lungs up to 17 weeks post-exposure. Thickening of the sub-basement membrane is one of the persistent structural features observed in human asthmatics (NHLBI, 2007). Episodic exposure (0.25 ppm $O_3$, every other month) of young monkeys induced equivalent morphological changes compared to continuously exposed animals, even though they were exposed for half the time and evaluation occurred a month after exposure ceased as opposed to immediately (Tyler et al., 1988). Notably, episodic $O_3$ exposure increased total lung collagen content, chest wall compliance, and inspiratory capacity, suggesting a delay in lung maturation in episodically-exposed animals. These changes were in contrast to the continuously exposed group, which did not differ from the air exposed group in these particular parameters but did exhibit greater bronchiolitis than the episodically exposed animals. In a study by Harkema and colleagues (Harkema et al., 1993, 1987b), monkeys (both males and females) were acutely exposed for 8 h/day to 0.15 ppm $O_3$ (6 days) or chronically to 0.15 ppm or 0.3 ppm $O_3$ (90 days). For most endpoints in the nasal cavity, the observed morphologic changes and inflammation were greater in the monkeys exposed for 6 days compared to 90 days, whereas in the respiratory bronchioles of the same animals, there were no significant time or concentration dependent differences (increased epithelial thickness and proportion of cuboidal cells) between the 6 and 90 day exposure groups.

Stokinger (1962) reported that chronic bronchitis, bronchiolitis, and emphysematous and fibrotic changes develop in the lung tissues of mice, rats, hamsters, and guinea pigs exposed 6 h/day, 5 days/week for 14.5 months to a concentration slightly above 1 ppm $O_3$. Rats continuously exposed for 3 to 5 months to 0.8 ppm $O_3$ develop a disease that resembles emphysema, and they finally die of respiratory failure (Stephens et al., 1976). Ozone results in a greater response of fibroblasts in the lesion, thickening of the alveolar septae, and an increase in number of alveolar macrophages in the proximal alveoli.

## Table 7-1 Respiratory effects in nonhuman primates and rodents resulting from long-term $O_3$ exposure.

| Study | Model | $O_3$ (ppm) | Exposure Duration | Effects |
|---|---|---|---|---|
| Pinkerton et al. (1998); Harkema et al. (1997a); Harkema et al. (1997b); Catalano et al. (1995b); Catalano et al. (1995a); Chang et al. (1995); Pinkerton et al. (1995); Stockstill et al. (1995); Harkema et al. (1994); Last et al. (1994); Plopper et al. (1994) | Rat, male and female, Fischer F344, 6-8 weeks old | 0.12<br>0.5<br>1.0 | 6 h/day, 5 days/week for 20 months | Effects similar to (or a model of) early fibrotic human disease were greater in the periacinar region than in terminal bronchioles. Thickened alveolar septa observed at 0.12 ppm $O_3$. Other effects (e.g., mucous cell metaplasia in the nose, mild fibrotic response in the parenchyma, and increased collagen in CAR of females) observed at 0.5 to 1.0 ppm. Some morphometric changes (epithelial thickening and bronchiolarization) occurred after 2 or 3 months of exposure to 1.0 ppm. |
| Herbert et al. (1996) | Mice, male and female, B6C3F1, 6-7 weeks old, | 0.12<br>0.50<br>1.0 | 6 h/day, 5 days/week for 24 and 30 months | Similar to the response of rats in the same study (see rat above). Effects were seen in the nose and centriacinar region of the lung at 0.5 and 1.0 ppm. |
| Chang et al. (1991) | Rat, male, F344, 6 weeks old | Continuous: 0.12 or 0.25<br>Episodic/urban: baseline 0.06; peak 0.25 | Continuous: 12 h/day for 6 weeks<br>Simulated urban pattern; slow rise to peak 9 h/day, 5 days/week, 13 weeks | Increased Type 1 and 2 epithelial volume assessed by TEM. Linear relationship observed between increases in Type 1 epithelial cell volume and concentration x time product. Degree of injury not related to pattern of exposure (continuous or episodic). |
| Chang et al. (1992) | Rat, male, F344, 6 weeks old | baseline 0.06; peak 0.25 | Slow rise to peak 9 h/day, 5 days/week, 13 and 78 weeks<br>Recovery in filtered air for 6 or 17 weeks | Progressive epithelial hyperplasia, fibroblast proliferation, and interstitial matrix accumulation observed using TEM. Interstitial matrix thickening due to deposition of basement membrane and collagen fibers. Partial recovery of interstitial matrix during follow-up periods in air; but no resolution of basement membrane thickening. |
| Barry et al. (1985, 1983) | Rat, male, 1 day old or 6 weeks old | 0.12 (adults only)<br>0.25 | 12 h/day for 6 weeks | Lung and alveolar development not significantly affected. Increased Type 1 and 2 epithelial cells and AM in CAR alveoli, thickened Type 1 cells with smaller volume and less surface coverage as assessed by TEM (adults and juveniles). In adults, smaller but statistically significant similar changes at 0.12 ppm, suggesting linear concentration-response relationship. No statistically significant age-related effects observed. |
| Tyler et al. (1988) | Monkey; male, *Macaca fascicularis*, 7 mo old | 0.25 | 8 h/day, 7 days/week, Daily for 18 mo or episodically every other month for 18 mo<br>Episodic group evaluated 1 mo postexposure | Increased collagen content, chest wall compliance, and inspiratory capacity in episodic group only. Respiratory bronchiolitis in both groups. Episodically exposed group incurred greater alterations in physiology and biochemistry and equivalent changes in morphometry even though exposed for half the time as the daily exposure group. |
| Harkema et al. | Rat, male, Fischer | 0.25 | 8 h/day, 7 days/week | Mucous cell hyperplasia in nasal |

| Study | Model | $O_3$ (ppm) | Exposure Duration | Effects |
|---|---|---|---|---|
| (1999) | F344/N HSD, 10-14 weeks old | 0.5 | for 13 weeks | epithelium after exposure to 0.25 and 0.5 ppm $O_3$; still evident after 13 weeks recovery from 0.5 ppm $O_3$ exposure. |
| Van Bree et al. (2002) | Rat, male, Wistar, 7 weeks old, n = 5/group | 0.4 | 23.5 h/day for 1, 3, 7, 28,or 56 days | Acute inflammatory response in BALF reached a maximum at day 1 and resolved within 6 days during exposure. Centriacinar region inflammatory responses throughout $O_3$ exposure with increased collagen and bronchiolization still present after a recovery period. |
| Katre et al. (2011) | Mice; male, C57BL/6, 6-8 weeks old | 0.5 | 8 h/day, [5 days/week $O_3$, and 2 days filtered air] for 5 or 10 cycles | Sustained elevation in TGF-β and PAI-1 in lung (5 or 10 cycles); elevated α-SMA and increased collagen deposition in airway walls (after 10 cycles). Collagen increase shown to depend on TGF-β. |
| Schelegle et al. (2003); | Monkey; Rhesus, 30 days old[a] | 0.5 | 8 h/day for 5 days, every 5 days for a total of 11 episodes | Goblet cell metaplasia, increased AHR, and increased markers of allergic asthma (e.g., eosinophilia) were observed, suggesting that episodic exposure to $O_3$ alters postnatal morphogenesis and epithelial differentiation and enhances the allergic effects of house dust mite allergen in the lungs of infant primates. |
| Harkema et al. (1993, 1987b) | Monkey; *Macaca radiata*, M, F<br>2-6 years old | 0.15<br>0.3 | 8 h/day for 90 days | Significant increase in epithelial thickness in respiratory bronchioles which was accompanied by increase in cuboidal cells; nasal lesions consisted of ciliated cell necrosis and secretory cell hyperplasia; no concentration response effects |
| Larson et al. (2004) | Monkey; *Macaca mulatta*, 30 days old[a] | 0.5 | 11 episodes of 5 days each, 8 h/day followed by 9 days of recovery | $O_3$ or $O_3$ + house dust mite antigen caused changes in density and number of airway epithelial nerves in small conducting airways. Suggests episodic $O_3$ alters pattern of neural innervation in epithelial compartment of developing lungs. |
| Plopper et al. (2007) | Monkey; Rhesus, 30 days old[a] | 0.5 | 5 months of episodic exposure; 5 days $O_3$ followed by 9 days of filtered air, 8h/day. | Non-significant increases airway resistance and airway responsiveness with $O_3$ or inhaled allergen alone. Allergen + $O_3$ produced additive changes in both measures. |
| Fanucchi et al. (2006) | Monkey; male Rhesus,30 days old | 0.5 | 5 months of episodic exposure; 5 days $O_3$ followed by 9 days of filtered air, 8h/day. | Cellular changes and significant structural changes in the distal respiratory tract in infant rhesus monkeys exposed to $O_3$ postnatally. |
| Reiser et al. (1987) | Monkey; male and female Cynomolgus 6-7 mo old | 0.61 | 8 h/day for 1 year | Increased lung collagen content associated with elevated abnormal cross links that were irreversibly deposited. |

[a]sex not reported

Collectively, evidence from animal studies strongly suggests that chronic $O_3$ exposure is capable of damaging the distal airways and proximal alveoli, resulting in lung tissue remodeling and leading to apparent irreversible changes. Potentially, persistent inflammation and interstitial remodeling play an important role in the

progression and development of chronic lung disease. Further discussion of the modes of action that lead to $O_3$-induced morphological changes can be found in Section 5.3.7. The findings reported in chronic exposure animal studies offer insight into potential biological mechanisms for the suggested association between seasonal $O_3$ exposure and reduced lung function development in children as observed in epidemiologic studies (see Section 7.2.3). Discussion of mechanisms involved in lifestage susceptibility and developmental effects can be found in Section 5.4.2.4.

### 7.2.4 Pulmonary Inflammation, Injury, and Oxidative Stress

The 2006 $O_3$ AQCD stated that the extensive human clinical and animal toxicological evidence, together with the limited epidemiologic evidence available, suggests a causal role for $O_3$ in inflammatory responses in the airways. Short-term exposure epidemiologic studies discussed earlier in Section 6.2.3.2 show consistent associations of $O_3$ exposure and increased airway inflammation and oxidative stress. Further discussion of the mechanisms underlying inflammation and oxidative stress responses can be found in Section 5.3.3. Though the majority of recent studies focus on short-term exposures, several epidemiologic and toxicology studies of long-term exposure add to observations of $O_3$-induced inflammation and injury.

Inflammatory markers and peak expiratory pulmonary function were examined in 37 allergic children with physician-diagnosed mild persistent asthma in a highly polluted urban area in Italy and then again 7 days after relocation to a rural location with significantly lower pollutant levels (Renzetti et al., 2009). The authors observed a 4-fold decrease in nasal eosinophils and a statistically significant decrease in fractional exhaled nitric oxide along with an improvement in lower airway function. Several pollutants were examined, including $PM_{10}$, $NO_2$, and $O_3$, though pollutant-specific results were not presented. These results are consistent with studies showing that traffic-related exposures are associated with increased airway inflammation and reduced lung function in children with asthma and contribute to the notion that this negative influence may be rapidly reversible. Exhaled NO (eNO) has been shown to be a useful biomarker for airway inflammation in large population-based studies (Linn et al., 2009). Thus, while the time scale of 7 days between examinations for eNO needs to be evaluated for appropriateness, the results suggest that inflammatory responses are reduced when $O_3$ levels are decreased.

Chest radiographs (CXR) of 249 children in Mexico City who were chronically exposed to $O_3$ and $PM_{2.5}$ were analyzed by Calderón-Garcidueñas et al. (2006). They reported an association between chronic exposures to $O_3$ and other pollutants and a significant increase in abnormal CXR's and lung CTs suggestive of a bronchiolar, peribronchiolar, and/or alveolar duct inflammatory process, in clinically healthy children with no risk factors for lung disease. These CXR and CT results should be viewed with caution because it is difficult to attribute effects to $O_3$ exposure.

In a cross-sectional study, Wood et al. (2009) examined the association of outdoor air pollution with respiratory phenotype (PiZZ type) in alpha 1-antitrypsin deficiency

(α-ATD) from the U.K. α-ATD registry. This deficiency leads to exacerbated responses to inflammatory stimuli. In total, 304 PiZZ subjects underwent full lung function testing and quantitative high-resolution computed tomography to identify the presence and severity of COPD – emphysema. Mean annual air pollution data for 2006 was matched to the location of patients' houses and used in regression models to identify phenotypic associations with pollution controlling for covariates. Relative trends in $O_3$ levels were assessed to validate use of a single year's data to indicate long-term exposure and validation; data showed good correlations between modeled and measured data (Stedman and Kent, 2008). Regression models showed that estimated higher exposure to $O_3$ exposure was associated with worse gas transfer and more severe emphysema, albeit accounting for only a small proportion of the lung function variability. This suggests that a gene-specific group demonstrates a long-term $O_3$ exposure effect.

The similarities of nonhuman primates to humans make them attractive models in which to study the effects of $O_3$ on the respiratory tract. The nasal mucous membranes, which protect the more distal regions of the respiratory tract, are susceptible to injury from $O_3$. Carey et al. (2007) conducted a study of $O_3$ exposure in infant rhesus macaques, whose nasal airways closely resemble that of humans. Monkeys were exposed either acutely for 5 days (8 h/day) to 0.5 ppm $O_3$, or episodically for several biweekly cycles alternating 5 days of 0.5 ppm $O_3$ with 9 days of filtered air (0 ppm $O_3$), designed to mimic human exposure (70 days total). All monkeys acutely exposed to $O_3$ had moderate to marked necrotizing rhinitis, with focal regions of epitheliar exfoliation, numerous infiltrating neutrophils, and some eosinophils. The distribution, character, and severity of lesions in episodically exposed monkeys were similar to that of acutely exposed animals. Neither group exhibited the mucous cell metaplasia proximal to the lesions, observed in adult monkeys exposed continuously to 0.3 ppm $O_3$ in another study (Harkema et al., 1987a). Adult monkeys also exhibited attenuation of inflammatory responses with continued daily exposure (Harkema et al., 1987a), but inflammation did not resolve over time in young episodically exposed monkeys (Carey et al., 2011). Inflammation in conducting airways has also been observed in rats chronically exposed to $O_3$. Using an agar-based technique to fill the alveoli so that only the rat bronchi are lavaged, a 90-day exposure of rats to 0.8 ppm $O_3$ (8 h/day) elicited significantly elevated pro-inflammatory eicosanoids $PGE_2$ and 12-HETE in the conducting airway compared to filtered air-exposed rats (Schmelzer et al., 2006).

Persistent inflammation and injury leading to interstitial remodeling may play an important role in the progression and development of chronic lung disease. Chronic airway inflammation is an important component of both asthma and COPD.
The epidemiological evidence supporting an association between long-term exposure to $O_3$ and inflammation or injury is limited. However, animal studies clearly demonstrate $O_3$-induced inflammation and injury, which may or may not attenuate with chronic exposure depending on the model. Further discussion of how $O_3$ initiates inflammation can be found in Section 5.3.3.

### 7.2.5 Allergic Responses

The association of air pollutants with childhood respiratory allergies was examined in the U.S. using the 1999-2005 National Health Interview Survey of approximately 70,000 children, and ambient air pollution data from the U.S. EPA, with monitors within 20 miles of each child's residential block (Parker et al., 2009). The authors examined the associations between the reporting of respiratory allergy or hay fever and medium-term exposure to $O_3$ over several summer months, controlling for demographic and geographic factors. Increased respiratory allergy/hay fever was associated with increased $O_3$ levels (adjusted OR per 10 ppb = 1.20; [95% CI: 1.15, 1.26]). These associations persisted after stratification by urban-rural status, inclusion of multiple pollutants ($O_3$, $SO_2$, $NO_2$, PM), and definition of exposure by differing exposure radii; smaller samples within 5 miles of monitors were remarkably similar to the primary results. No associations between the other pollutants and the reporting of respiratory allergy/hay fever were apparent. Ramadour et al. (2000) reported no relationship between $O_3$ levels and rhinitis symptoms and hay fever. Hwang et al. (2006) report the prevalence of allergic rhinitis (adjusted OR per 10 ppb = 1.05; [95% CI: 0.98, 1.12]) in a large cross-sectional study in Taiwan. In a large cross-sectional study in France, Penard-Morand et al. (2005) reported a positive relationship between lifetime allergic rhinitis and $O_3$ exposure in a two-pollutant model with $NO_2$. These studies related positive outcomes of allergic response and $O_3$ exposure but with variable strength for the effect estimates. A toxicological study reported that five weeks of continuous exposure to 0.4 ppm $O_3$ (but not 0.1 or 0.2 ppm $O_3$) augmented sneezing and nasal secretions in a guinea pig model of nasal allergy (Iijima and Kobayashi, 2004). Nasal eosinophils, which participate in allergic disease and inflammation, and allergic antibody levels in serum were also elevated by exposure to concentrations as low as 0.2 ppm (Iijima and Kobayashi, 2004).

Nasal eosinophils were observed to decrease by 4-fold in 37 atopic, mildly asthmatic children 7 days after relocation from a highly polluted urban area in Italy to a rural location with significantly lower pollutant levels (Renzetti et al., 2009). Inflammatory and allergic effects of $O_3$ exposure (30 day mean) such as increased eosinophil levels were observed in children in an Austrian study (Frischer et al., 2001). Episodic exposure of infant rhesus monkeys to 0.5 ppm $O_3$ for 5 months appears to significantly increase the number and proportion of eosinophils in the blood and airways (lavage) [protocol described above in Section 7.2.3.2 for Fanucchi et al. (2006)] (Maniar-Hew et al., 2011). These changes were not evident at 1 year of age (6 months after $O_3$ exposure ceased). Increased eosinophils levels have also been observed after acute or prolonged exposures to $O_3$ in adult bonnet and rhesus monkeys (Hyde et al., 1992; Eustis et al., 1981).

Total IgE levels were related to air pollution levels in 369 adult asthmatics in five French centers using generalized estimated equations (GEE) as part of the EGEA study described earlier (Rage et al., 2009a). Geostatistical models were performed on 4×4 km grids to assess individual outdoor air pollution exposure that was assigned to subject's home address. Ozone concentrations were positively related to total IgE levels and an increase of 5 ppb of $O_3$ resulted in an increase of 20.4% (95% CI: 3.0,

40.7) in total IgE levels. Nearly 75% of the subjects were atopic. In two-pollutant models including $O_3$ and $NO_2$, the $O_3$ effect estimate was decreased by 25% while the $NO_2$ effect estimate was decreased by 57%. Associations were not sensitive to adjustment for covariates or the season of IgE measurements. These cross-sectional results suggest that exposure to $O_3$ may increase total IgE in adult asthmatics.

Although very few toxicological studies of long-term exposure examining allergy are available, short-term exposure studies in rodents and nonhuman primates demonstrate allergic skewing of immune responses and enhanced IgE production. Due to the persistent nature of these responses, the short-term toxicological evidence lends biological plausibility to the limited epidemiologic findings of an association between long-term $O_3$ exposure and allergic outcomes.

### 7.2.6 Host Defense

Short-term exposures to $O_3$ have been shown to cause decreases in host defenses against infectious lung disease in animal models. Acute $O_3$-induced suppression of alveolar phagocytosis and immune functions observed in animals appears to be transient and attenuated with continuous or repeated exposures, although chronic exposure (weeks, months) has been shown to slow alveolar clearance. In an important study investigating 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). After 30 days, the lungs of the $O_3$-exposed animals had twice the number and mass of asbestos fibers as the air-exposed rats. However, chronic exposures of 0.1 ppm do not cause greater effects on infectivity than short exposures, due to defense parameters becoming reestablished with prolonged exposures. No detrimental effects were seen with 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, $O_3$ was shown to increase the severity of postinfluenzal alveolitis and lung parenchymal changes (Jakab and Bassett, 1990). A recent study by Maniar-Hew et al. (2011) demonstrated that the immune system of infant rhesus monkeys episodically exposed to 0.5 ppm $O_3$ for 5 months[1] appeared to be altered in ways that could diminish host defenses. Reduced numbers of circulating leukocytes were observed, particularly polymorphonuclear leukocytes (PMNs) and lymphocytes, which were decreased in the blood and airways (bronchoalveolar lavage). These changes did not persist at 1 year of age (6 months postexposure); rather, increased numbers of monocytes were observed at that time point. Challenge with LPS, a bacterial ligand that activates monocytes and other innate immune cells, elicited lower responses in $O_3$-exposed animals even though the relevant reactive cell population was increased. This was observed in both an in vivo inhalation challenge

[1] Exposure protocol is described above in Section 7.2.3.2 for Fanucchi et al. (2006).

and an ex vivo challenge of peripheral blood mononuclear cells. Thus a decreased ability to respond to pathogenic signals was observed six months after $O_3$ exposure ceased, in both the lungs and periphery.

### 7.2.7 Respiratory Mortality

A limited number of epidemiologic studies have assessed the relationship between long-term exposure to $O_3$ and mortality. The 2006 $O_3$ AQCD concluded that an insufficient amount of evidence existed "to suggest a causal relationship between chronic $O_3$ exposure and increased risk for mortality in humans" (U.S. EPA, 2006b). Though total and cardio-pulmonary mortality were considered in these studies, respiratory mortality was not specifically considered. In the most recent follow-up analysis of the ACS cohort (Jerrett et al., 2009), cardiopulmonary deaths were subdivided into respiratory and cardiovascular, separately, as opposed to combined in the Pope et al. (2002) work. A 10-ppb increment in exposure to $O_3$ elevated the risk of death from respiratory causes and this effect was robust to the inclusion of $PM_{2.5}$. The association between increased $O_3$ concentrations and increased risk of death from respiratory causes was insensitive to the use of a random-effects survival model allowing for spatial clustering within the metropolitan area and state of residence, and to adjustment for several ecologic variables considered individually. Additionally, a recent study (Zanobetti and Schwartz, 2011) observed an association between long-term exposure to $O_3$ and elevated risk of mortality among Medicare enrollees that had previously experienced an emergency hospital admission due to COPD.

### 7.2.8 Summary and Causal Determination

The epidemiologic studies reviewed in the 2006 $O_3$ AQCD detected no associations between long-term (annual) $O_3$ exposures and asthma-related symptoms, asthma prevalence, or allergy to common aeroallergens among children after controlling for covariates. Little evidence was available to relate long-term exposure to ambient $O_3$ concentrations with deficits in the growth rate of lung function in children. Additionally, limited evidence was available evaluating the relationship between long-term $O_3$ concentrations and pulmonary inflammation and other endpoints. From toxicological studies, it appeared that $O_3$-induced inflammation tapered off during long-term exposures, but that hyperplastic and fibrotic changes remained elevated and in some cases even worsened after a postexposure period in clean air. Episodic exposures were also known to cause more severe pulmonary morphologic changes than continuous exposure (U.S. EPA, 2006b).

The recent epidemiologic evidence base consists of studies using a variety of designs and analysis methods evaluating the relationship between long-term exposure to ambient $O_3$ concentrations and measures of respiratory health effects and mortality conducted by different research groups in different locations. See Table 7-2 for $O_3$

concentrations associated with selected studies. Table 7-2 is organized by longitudinal and cross-sectional studies both presented alphabetically. The positive results from various designs and locations support a relationship between long-term exposure to ambient $O_3$ concentrations and respiratory health effects and mortality.

Earlier studies reported associations of new-onset asthma and $O_3$ in an adult cohort in California (McDonnell et al., 1999a; Greer et al., 1993) but only in males. In the CHS cohort of children in 12 Southern California communities, long-term exposure to $O_3$ concentrations was not associated with increased risk of developing asthma (McConnell et al., 2010); however, greater outdoor exercise was associated with development of asthma in children living in communities with higher ambient $O_3$ concentrations (McConnell et al., 2002). Recent CHS studies examined interactions among genetic variants, long-term $O_3$ exposure, and new onset asthma in children. These prospective cohort studies are methodologically rigorous epidemiology studies, and evidence indicates gene-$O_3$ interactions. These studies have provided data supporting decreased risk of certain different genetic variants on new onset asthma (e.g., HMOX-1, ARG) that is limited to children either in low (Islam et al., 2008) or high (Salam et al., 2009) $O_3$ communities. Gene-environment interaction also was demonstrated with findings that greater outdoor exercise increased risk of asthma in GSTP1 Ile/Ile children living in high $O_3$ communities (Islam et al., 2009). Biological plausibility for these these gene-$O_3$ environment interactions is provided by evidence that these enzymes have antioxidant and/or anti-inflammatory activity and participate in well recognized modes of action in asthma pathogenesis. As $O_3$ is a source of oxidants in the airways, oxidative stress serves as the link among $O_3$ exposure, enzyme activity, and asthma.

Studies using a cross-sectional design provide support for a relationship between long-term $O_3$ exposure and health effects in asthmatics. A long-term $O_3$ exposure study relates bronchitic symptoms to TNF-308 genotype asthmatic children with ambient $O_3$ exposure in the CHS (Lee et al., 2009b). A study in five French cities reports effects on asthma severity related to long-term $O_3$ exposure (Rage et al., 2009b). A follow-up study of this cohort (Jacquemin et al., 2012) supports an effect of cumulative long-term $O_3$ exposure on asthma control in adulthood in subjects with pre-existing asthma. Akinbami et al. (2010) and Hwang et al. (2005) provide further evidence relating $O_3$ exposures and the risk of asthma. For the respiratory health of a cohort based on the general U.S. population, risk of respiratory-related school absences was elevated for children with the CAT and MPO variant genes related to communities with high ambient $O_3$ levels (Wenten et al., 2009).

**Table 7-2 Summary of selected key new studies examining annual $O_3$ exposure and respiratory health effects.**

| Study; Health Effect; Location | Annual Mean $O_3$ Concentration (ppb) | $O_3$ Range (ppb) Percentiles |
|---|---|---|
| **Longitudinal** | | |
| Islam et al. (2008); New-onset asthma; CHS | 55.2 high vs. 38.4 low communities<br>10:00 a.m. to 6:00 p.m. average | See left |
| Islam et al. (2009); New-onset asthma; CHS | 55.2 high vs. 38.4 low communities<br>10:00 a.m. to 6:00 p.m. | See left |
| Lin et al. (2008b); First asthma hospital admission; New York State - 10 regions | Range of mean $O_3$ concentrations over the 10 New York Regions 37.51 to 47.78<br>8-h max 10:00 a.m. to 6:00 p.m. | See left |
| Salam et al. (2009); Childhood onset asthma; CHS | $O_3$ greater than or less than 50 ppb | See left |
| **Cross-sectional** | | |
| Akinbami et al. (2010); Current asthma U.S. | 12 month median 39.8<br>8hr max | IQR<br>35.9 to 43.7 |
| Hwang et al. (2005); Prevalence of asthma; Taiwan | Mean 23.14 | Range<br>18.65 to 31.17 |
| Jacquemin et al. (2012); Asthma control in adults; Five French cities | Median 46.9 ppb;<br>8-h average | 25th-75th<br>41-52 |
| Lee et al. (2009b); Bronchitic symptoms in asthmatic children; CHS | Above and below 50 ppb | See left |
| Meng et al. (2010); Asthma ED visits or hospitalizations; San Joaquin Valley, CA | Median 30.3 ppb<br>Yearly based on hourly | 25-75% range<br>27.1 to 34.0 |
| Moore et al. (2008); Asthma hospital admissions; South Coast Basin | Median 87.8 ppb<br>Quarterly 1hr daily max | Range<br>28.6 to 199.9 |
| Rage et al. (2009a); Asthma severity; Five French cities | Mean 30 ppb<br>8-h average | 25th-75th<br>21-36 |
| Wenten et al. (2009); Respiratory school absence, U.S. | Median 46.9 ppb;<br>10a.m. – 6 p.m. average | Min-Max<br>27.6-65.3 |

Long-term $O_3$ exposure was related to first childhood asthma hospital admissions in a positive concentration-response relationship in a New York State birth cohort (Lin et al., 2008b). A separate hospitalization cross-sectional study in San Joaquin Valley, California reports similar findings (Meng et al., 2010). Another study relates asthma hospital admissions to quarterly average $O_3$ in the South Coast Air Basin of California (Moore et al., 2008).

Information from toxicological studies indicates that long term exposure to $O_3$ during gestation or development can result in irreversible morphological changes in the lung, which in turn can influence the function of the respiratory tract. Studies by Plopper and colleagues using an allergic asthma model have demonstrated changes in pulmonary function and airway morphology in adult and infant nonhuman primates repeatedly exposed to environmentally relevant concentrations of $O_3$ (Fanucchi et al., 2006; Joad et al., 2006; Schelegle et al., 2003; Harkema et al., 1987b). This nonhuman primate evidence of an $O_3$-induced change in airway responsiveness supports the biologic plausibility of long term exposure to $O_3$ contributing to effects of asthma in children. Results from epidemiologic studies examining long-term $O_3$ exposure and pulmonary function effects are inconclusive with some new studies relating effects at higher exposure levels. The definitive 8-year follow-up analysis of the first cohort of the CHS, which is discussed in Section 7.2 (Gauderman et al., 2004), provided little evidence that long-term exposure to ambient $O_3$ was associated with significant deficits in the growth rate of lung function in children. Other cross-sectional studies provide mixed results.

Several studies (see Table 7-3) provide results adjusted for potential confounders, presenting results for both $O_3$ and PM (single and multipollutant models) as well as other pollutants where PM effects were not provided. As shown in the table, $O_3$ associations are generally robust to adjustment for potential confounding by PM.

## Table 7-3 Studies providing evidence concerning potential confounding by PM for available endpoints.

| Study and Endpoint | Exposure | Single Pollutant $O_3$ | Single Pollutant PM | $O_3$ with PM | PM with $O_3$ |
|---|---|---|---|---|---|
| **Asthma Related Health Effect Endpoint** | | | | | |
| Akinbami et al. (2010)<br>Asthma prevalence in children | IQR<br>35.9-43.7 ppb | 1.56<br>(1.15, 2.10) | $PM_{2.5}$<br>1.43<br>(0.98, 2.10) | Adjusted for $SO_2$,$PM_{2.5}$,$PM_{10}$<br>1.86 (1.02-3.40)<br>Adjusted for $PM_{2.5}$, $PM_{10}$<br>1.36 (0.91-2.02) | $PM_{2.5}$<br>1.24<br>(0.70-2.21)<br>$PM_{2.5}$<br>1.26<br>0.80-1.98) |
| Hwang et al. (2005)<br>Asthma risk in children | 10 ppb $O_3$ | 1.138<br>(1.001, 1.293 | 0.934<br>(0.909, 0.960) | $PM_{10}$<br>1.253<br>(1.089, 1.442) | 0.925<br>(0.899, 0.952) |
| Jacquemin et al. (2012)<br>Asthma control in adults | IQR 25-38 ppb<br>$O_3$ summer | 1.69<br>(1.22, 2.34) | 1.33<br>(1.06, 1.67) | $PM_{10}$<br>1.50<br>(1.07, 2.11) | 1.28<br>(1.06, 1.55) |
| Lee et al. (2009b)<br>Bronchitic symptoms asthmatics | High $O_3$ >50 ppb | 1.42<br>(0.75, 2.70) | NA | No substantial differences<br>$PM_{10}$, $PM_{2.5}$ | NA |
| Lin et al. (2008b)<br>Asthma admissions in children | IQR 2.5% | 1.16<br>(1.15, 1.17) | NA | Air Quality Index<br>1.24<br>(1.23, 1.25) | NA |
| Meng et al. (2007)<br>Asthma control | 1 ppm | 1.70<br>(0.91, 3.18) | $PM_{10}$<br>2.06<br>(1.17, 3.61)<br>women | Did not differ | NA |
| Meng et al. (2010)<br>Asthma ED visits, Hospitalization | 10 ppb | 1.49<br>(1.05, 2.11) | $PM_{10}$<br>1.29<br>(0.99, 1.69) | Did not differ | NA |
| Rage et al. (2009b)<br>Asthma severity in adults | IQR<br>28.5-33.9 ppb | 2.53<br>(1.69, 3.79) | NA | No PM data<br>Three pollutant ($O_3$, $NO_2$, $SO_2$)<br>2.74<br>(1.68, 4.48) | NA |
| **Other Respiratory Health Effect Endpoints** | | | | | |
| Karr et al. (2007)<br>Bronchiolitis<br>Hospitalization | 10 ppb | 0.92<br>(0.88, 0.96) | 1.09<br>(1.04, 1.14) | $PM_{2.5}$<br>1.02<br>(0.94, 1.10) | 1.09<br>(1.03, 1.15) |
| Parker et al. (2009)<br>Respiratory allergy | 10 ppb | 1.24<br>(1.15, 1.34) | 1.23<br>(1.04, 1.46) | Multipollutant<br>1.18<br>(1.09, 1.27) | 1.29 (<br>1.07, 1.56) |
| Rojas-Martinez et al. (2007)<br>$FEV_1$ (mL) Deficit<br>Girls | 11.3 ppb IQR | -24<br>(-30, -19) | $PM_{10}$ IQR<br>36.4 µg/$m^3$<br>-29(-36, -21) | -17<br>(-23, -12) | -24<br>(-31,-16) |

The highest quartile is shown for all results

NA = not available

There is limited evidence for an association between long-term exposure to ambient $O_3$ concentrations and respiratory mortality (Jerrett et al., 2009) and this effect was robust to the inclusion of $PM_{2.5}$. The association between increased $O_3$ concentrations and increased risk of death from respiratory causes was insensitive to a number of different model specifications. Additionally, there is evidence that long-term exposure to $O_3$ is associated with mortality among individuals that had previously experienced an emergency hospital admission due to COPD (Zanobetti and Schwartz, 2011).

Taken together, the recent epidemiologic studies of respiratory health effects (including respiratory symptoms, new-onset asthma and respiratory mortality) combined with toxicological studies in rodents and nonhuman primates, provide biologically plausible evidence that there **is likely to be a causal relationship between long-term exposure to $O_3$ and respiratory effects.** The epidemiologic evidence includes studies that evaluate the relationship between long-term $O_3$ exposure and respiratory effects such as studies that demonstrate interactions between exercise or different genetic variants and long-term measures of $O_3$ exposure on new-onset asthma in children; and increased respiratory symptom effects in asthmatics. Additional studies of respiratory health effects and a study of respiratory mortality provide a collective body of evidence supporting these relationships. Studies considering other pollutants provide data suggesting that the effects related to $O_3$ are independent from potential effects of the other pollutants. Some studies provide evidence for a positive concentration-response relationship. Short-term studies provide supportive evidence with increases in respiratory symptoms and asthma medication use, hospital admissions and ED visits for all respiratory outcomes and asthma, and decrements in lung function in children. The recent epidemiologic and toxicological data base provides a compelling case to support the hypothesis that a relationship exists between long-term exposure to ambient $O_3$ and measures of respiratory health effects.

## 7.3 Cardiovascular Effects

### 7.3.1 Cardiovascular Disease

#### 7.3.1.1 Cardiovascular Epidemiology

Long-term exposure to $O_3$ and its effects on cardiovascular morbidity were not considered in the 2006 $O_3$ AQCD (U.S. EPA, 2006b). However, recent studies have assessed the chronic effects of $O_3$ concentration on cardiovascular morbidity (Chuang et al., 2011; Forbes et al., 2009a; Chen et al., 2007a). The association between $O_3$ concentration and markers of lipid peroxidation and antioxidant capacity was examined among 120 nonsmoking healthy college students, aged 18-22 years, from the University of California, Berkeley (February—June 2002) (Chen et al.,

2007a). By design, students were chosen from geographic areas so they had experienced different concentrations of $O_3$ over their lifetimes and during recent summer vacation in either greater Los Angeles (LA) or the San Francisco Bay Area (SF). A marker of lipid peroxidation, 8-isoprostane (8-iso-PGF) in plasma, was assessed. This marker is formed continuously under normal physiological conditions but has been found at elevated concentrations in response to environmental exposures. A marker of overall antioxidant capacity, ferric reducing ability of plasma (FRAP), was also measured. The lifetime average $O_3$ concentration estimates (from estimated monthly averages) did not show much overlap between the two geographic areas [median (range): LA, 42.9 ppb (28.5-65.3); SF, 26.9 ppb (17.6-33.5)]. Estimated lifetime average $O_3$ concentration was related to 8-iso-PGF [$\beta$ = 0.025 (pg/mL)/8-h ppb $O_3$, p = 0.0007]. For the 17-ppb lifetime $O_3$ concentration difference between LA and SF participants, there was a 17.41-pg/mL (95% CI: 15.43, 19.39) increase in 8-iso-PGF. No evidence of association was observed between lifetime $O_3$ concentration and FRAP [$\beta$ = -2.21 (pg/mL)/8-h ppb $O_3$, p = 0.45]. The authors note that $O_3$ was highly correlated with $PM_{10-2.5}$ and $NO_2$ in this study population; however, their inclusion in the $O_3$ models did not substantially modify the magnitude of the associations with $O_3$. Because the average lifetime concentration results were supported by shorter-term exposure period results from analyses considering $O_3$ concentrations up to 30 days prior to sampling, the authors conclude that persistent exposure to $O_3$ can lead to sustained oxidative stress and increased lipid peroxidation. However, because there was not much overlap in average lifetime $O_3$ concentration estimates between LA and SF, it is possible that the risk estimates involving the lifetime $O_3$ exposures could be confounded by unmeasured factors related to other differences between the two cities.

Forbes et al. (2009a) used the annual average exposures to assess the relationship between chronic ambient air pollution and levels of fibrinogen and C-reactive protein (CRP) in a cross-sectional study conducted in England. Data were collected from the Health Survey of England for 1994, 1998, and 2003. The sampling strategy was designed to obtain a representative sample of the English population; however, due to small group sizes, only data from white ethnic groups were analyzed. For analyses, the annual concentrations of $O_3$ were averaged for the year of data collection and the previous year with the exception of 1994 (because pollutant data were not available for 1993). Median $O_3$ concentrations were 26.7 ppb, 25.4 ppb, and 28 ppb for 1994, 1998, and 2003, respectively. Year specific adjusted effect estimates were created and combined in a meta-analysis. No evidence of association was observed for $O_3$ and levels of fibrinogen or CRP (e.g., the combined estimates for the percent change in fibrinogen and CRP for a 10 ppb increase in $O_3$ were -0.28 [95% CI: -2.43, 1.92] and -3.05 [95% CI: -16.10, 12.02], respectively).

A study was performed in Taiwan to examine the association between long-term $O_3$ concentrations and blood pressure and blood markers using the Social Environment and Biomarkers of Aging Study (SEBAS) (Chuang et al., 2011). Individuals included in the study were 54 years of age and older. The mean annual $O_3$ concentration during the study period was 22.95 ppb (SD 6.76 ppb). Positive associations were observed between $O_3$ concentrations and both systolic and diastolic blood pressure

[changes in systolic and diastolic blood pressure were 21.51mmHg (95% CI: 16.90, 26.13) and 20.56 mmHg (95% CI: 18.14, 22.97) per 8.95 ppb increase in $O_3$, respectively]. Increased $O_3$ concentrations were also associated with increased levels of total cholesterol, fasting glucose, hemoglobin A1c, and neutrophils.
No associations were observed between $O_3$ concentrations and triglyceride and IL-6 levels. The observed associations were reduced when other pollutants were added to the models. Further research will be important for understanding the effects, if any, of chronic $O_3$ exposure on cardiovascular morbidity risk.

#### 7.3.1.2 Cardiovascular Toxicology

Three new studies have investigated the cardiovascular effects of long-term exposure to $O_3$ in animal models (see Table 7-4 for study details). In addition to the short-term exposure effects described in Section 6.3.3, a recent study found that $O_3$ exposure in genetically hyperlipidemic mice enhanced aortic atherosclerotic lesion area compared to air exposed controls (Chuang et al., 2009). Chuang et al. (2009) not only provided evidence for increased atherogenesis in susceptible mice, but also reported an elevated vascular inflammatory and redox state in wild-type mice and infant primates (Section 6.3.3). This study is compelling in that it identifies biochemical and cellular events responsible for transducing the airway epithelial reactions of $O_3$ into proinflammatory responses that are apparent in the extrapulmonary vasculature (Cole and Freeman, 2009).

Another recent study provides further evidence for increased vascular inflammation and oxidation and long term effects in the extrapulmonary space. Rats episodically exposed to $O_3$ for 16 weeks presented marked increases in gene expression of biomarkers of oxidative stress, thrombosis, vasoconstriction, and proteolysis (Kodavanti et al., 2011). Ozone exposure upregulated aortic mRNA expression of heme oxygenase-1 (HO-1), tissue plasminogen activator (tPA), plasminogen activator inhibitor-1 (PAI-1), von Willebrand factor (vWf), thrombomodulin, endothelial nitric oxide synthase (eNOS), endothelin-1 (ET-1), matrix metalloprotease-2 (MMP-2), matrix metalloprotease-3 (MMP-3), and tissue inhibitor of matrix metalloprotease-2 (TIMP-2). In addition, $O_3$ exposure depleted some cardiac mitochondrial phospholipid fatty acids (C16:0 and C18:1), which may be the result of oxidative modifications. The authors speculate that oxidatively modified lipids and proteins produced in the lung and heart promote vascular pathology through activation of lectin-like oxidized-low density lipoprotein receptor-1 (LOX-1). Activated LOX-1 induces expression of a number of the biomarkers induced by $O_3$ exposure and is considered pro-atherogenic. Both LOX-1 mRNA and protein were increased in mouse aorta after $O_3$ exposure. This study provides a possible pathway and further support to the observed $O_3$ induced atherosclerosis.

Vascular occlusion resulting from atherosclerosis can block blood flow through vessels causing ischemia. The restoration of blood flow or reperfusion can cause injury to the tissue from subsequent inflammation and oxidative damage. Ozone

exposure enhanced the sensitivity to myocardial ischemia-reperfusion (I/R) injury in rats while increasing oxidative stress levels and pro-inflammatory mediators and decreasing production of anti-inflammatory proteins (Perepu et al., 2010). Both long- and short-term $O_3$ exposure decreased the left ventricular developed pressure, rate of change of pressure development, and rate of change of pressure decay and increased left ventricular end diastolic pressure in isolated perfused hearts (Section 6.3.3 for short-term exposure discussion). In this ex vivo heart model, $O_3$ induced oxidative stress by decreasing SOD enzyme activity and increasing malondialdehyde levels. Ozone also elicited a proinflammatory state evident by an increase in TNF-α and a decrease in the anti-inflammatory cytokine IL-10. The authors conclude that $O_3$ exposure will result in a greater I/R injury.

Overall, the few animal studies that have been conducted suggest that long-term $O_3$ exposure may result in cardiovascular effects. These studies demonstrate $O_3$-induced atherosclerosis and injury. In addition, evidence is presented for a potential mechanism for the development of vascular pathology that involves increased oxidative stress and proinflammatory mediators, activation of LOX-1 by $O_3$ oxidized lipids and proteins, and upregulation of genes responsible for proteolysis, thrombosis, and vasoconstriction. Further discussion of the mechanisms that may lead to cardiovascular effects from $O_3$ exposure can be found in Section 5.3.8.

**Table 7-4 Characterization of study details for Section 7.3.1.2.**

| Study | Model | $O_3$ (ppm) | Exposure Duration | Effects |
|---|---|---|---|---|
| Chuang et al. (2009) | Mice; ApoE$^{-/-}$; M; 6 weeks | 0.5 | 8 wks, 5 days/week, 8 h/day | Enhanced aortic atherosclerotic lesion area compared to air controls. |
| Kodavanti et al. (2011) | Rat; Wistar; M; 10-12 weeks | 0.4 | 16 wks, 1 day/week, 5 h/day | Increased vascular inflammation and oxidative stress, possibly through activation of LOX-1 signaling. |
| Perepu et al. (2010) | Rat; Sprague-Dawley; Weight: 50-75 g | 0.8 | 56 days, 8 h/day | Enhanced the sensitivity to myocardial I/R injury while increasing oxidative stress and pro-inflammatory mediators and decreasing production of anti-inflammatory proteins. |

No previous studies investigated cardiovascular effects from long-term exposure to $O_3$.
For details, see Section 7.3.1.2.

### 7.3.2 Cardiovascular Mortality

A limited number of epidemiologic studies have assessed the relationship between long-term exposure to $O_3$ and mortality. The 2006 $O_3$ AQCD concluded that an insufficient amount of evidence existed "to suggest a causal relationship between chronic $O_3$ exposure and increased risk for mortality in humans" (U.S. EPA, 2006b). Though total and cardio-pulmonary mortality were considered in these studies, cardiovascular mortality was not specifically considered. In the most recent follow-up analysis of the ACS cohort (Jerrett et al., 2009), cardiopulmonary deaths were

subdivided into respiratory and cardiovascular, separately, as opposed to combined in the Pope et al. (2002) work. A 10-ppb increment in exposure to $O_3$ elevated the risk of death from the cardiopulmonary, cardiovascular, and ischemic heart disease. Inclusion of $PM_{2.5}$ as a copollutant attenuated the association with exposure to $O_3$ for all of the cardiovascular endpoints to become null. Additionally, a recent study (Zanobetti and Schwartz, 2011) observed an association between long-term exposure to $O_3$ and elevated risk of mortality among Medicare enrollees that had previously experienced an emergency hospital admission due to congestive heart failure (CHF) or myocardial infarction (MI).

### 7.3.3 Summary and Causal Determination

Previous AQCDs did not address the cardiovascular effects of long-term $O_3$ exposure due to limited data availability. The evidence remains limited; however the emerging data are supportive of a role for $O_3$ in chronic cardiovascular diseases. Few epidemiologic studies have investigated cardiovascular morbidity after long-term $O_3$ exposure, and the majority only assessed cardiovascular disease related biomarkers. The studies used annual or multi-year averages of air monitoring data for exposure assessment. As described in Section 4.6, this exposure assignment method is typical of long-term epidemiologic studies, and analyses suggest that annual average concentrations are representative of exposure metrics accounting for residential mobility. A study on $O_3$ and cardiovascular mortality reported no association after adjustment for $PM_{2.5}$ levels. Further epidemiologic studies on cardiovascular morbidity and mortality after long-term exposure have not been published.

Toxicological evidence on long-term $O_3$ exposure is also limited but three strong toxicological studies have been published since the previous AQCD. These studies provide evidence for $O_3$ enhanced atherosclerosis and I/R injury, corresponding with development of a systemic oxidative, proinflammatory environment. Further discussion of the mechanisms that may lead to cardiovascular effects can be found in Section 5.3.8. Although questions exist for how $O_3$ inhalation causes systemic effects, a recent study proposes a mechanism for development of vascular pathology that involves activation of LOX-1 by $O_3$ oxidized lipids and proteins. This activation may also be responsible for $O_3$ induced changes in genes involved in proteolysis, thrombosis, and vasoconstriction. Taking into consideration the findings of toxicological studies, and the emerging evidence from epidemiologic studies, the generally limited body of evidence **is suggestive of a causal relationship between long-term exposures to $O_3$ and cardiovascular effects.**

## 7.4 Reproductive and Developmental Effects

Although the body of literature characterizing the health effects associated with exposure to $O_3$ is large and continues to grow, the research focusing on adverse birth outcomes is relatively small. Among these studies, various measures of birth weight

and fetal growth, such as low birth weight (LBW), small for gestational age (SGA), and intrauterine growth restriction (IUGR), and preterm birth (<37-week gestation; [PTB]) have received more attention in air pollution research, while congenital malformations are less studied. There are also recent studies on reproductive and developmental effects and infant mortality.

A major issue in studying environmental exposures and reproductive and developmental effects (including infant mortality) is selecting the relevant exposure period, since the biological mechanisms leading to these outcomes and the critical periods of exposure are poorly understood. To account for this, many epidemiologic studies evaluate multiple exposure periods, including long-term (months to years) exposure periods, such as entire pregnancy, individual trimesters or months of pregnancy, and short-term (days to weeks) exposure periods such as the days and weeks immediately preceding birth. Due to the length of gestation in rodents (18-24 days, on average), animal toxicological studies investigating the effects of $O_3$ generally utilize short-term exposure periods. Thus, an epidemiologic study that uses the entire pregnancy as the exposure period is considered to have a long-term exposure period (about 40 weeks, on average), while a toxicological study conducted with rats that also uses the entire pregnancy as the exposure period is considered to have a short-term exposure period (about 18-24 days, on average). In order to characterize the weight of evidence for the effects of $O_3$ on reproductive and developmental effects in a consistent, cohesive and integrated manner, results from both short-term and long-term exposure periods are included in this section and are identified accordingly in the text and tables throughout this section.

Due to the poorly understood biological mechanisms and uncertainty regarding relevant exposure studies, all of the studies of reproductive and developmental outcomes, including infant mortality, are evaluated in this section. Infant development processes, much like fetal development processes, may be particularly sensitive to $O_3$-induced health effects. Exposures proximate to the effect may be most relevant if exposure causes an acute effect. However, exposure occurring in early life might affect critical growth and development, with results observable later in the first year of life, or cumulative exposure during the first year of life may be the most important determinant. In dealing with the uncertainties surrounding these issues, studies have considered several exposure metrics based on different periods of exposure, including both short- and long-term exposure periods. In the toxicological literature,a challenge in interpreting data from studies that use very young murine pups, is that pups can have differential exposure to $O_3$ doses, versus their respective dams, because of the physiology and behavior associated with the early postnatal period. Namely, young pups tend to nuzzle close to their mothers and are often housed in cages with litter used in nest formation. Both the dam's fur and the bedding can absorb and react with $O_3$, decreasing the dose that a young animal might receive. The reproductive and developmental studies are characterized in this chapter, as they contribute to the weight of evidence for an effect of $O_3$ on reproductive and developmental effects.

Infants and fetal development processes may be particularly at-risk for $O_3$-induced health effects, and although the physical mechanisms are not fully understood, several hypotheses have been proposed; these include: oxidative stress, systemic inflammation, vascular dysfunction and impaired immune function (Section 5.3). Study of these outcomes can be difficult given the need for detailed exposure data and potential residential movement of mothers during pregnancy. Air pollution epidemiologic studies reviewed in the 2006 $O_3$ AQCD (U.S. EPA, 2006b) examined impacts on birth-related endpoints, including intrauterine, perinatal, postneonatal, and infant deaths; premature births; intrauterine growth retardation; very low birth weight (weight <1,500 grams) and low birth weight (weight <2,500 grams); and birth defects. However, in the limited number of studies that investigated $O_3$, no associations were found between $O_3$ and birth outcomes, with the possible exception of birth defects.

Several recent articles have reviewed methodological issues relating to the study of outdoor air pollution and adverse birth outcomes (Chen et al., 2010a; Woodruff et al., 2009; Ritz and Wilhelm, 2008; Slama et al., 2008). Some of the key challenges to interpretation of these study results include the difficulty in assessing exposure as most studies use existing monitoring networks to estimate individual exposure to ambient air pollution; the inability to control for potential confounders such as other risk factors that affect birth outcomes (e.g., smoking); evaluating the exposure window (e.g., trimester) of importance; and limited evidence on the physiological mechanism of these effects (Ritz and Wilhelm, 2008; Slama et al., 2008).

Overall, the evidence for an association between exposure to ambient $O_3$ and reproductive and developmental outcomes is growing, yet remains relatively small. Recently, an international collaboration was formed to better understand the relationships between air pollution and adverse birth outcomes and to examine some of these methodological issues through standardized parallel analyses in datasets from different countries (Woodruff et al., 2010). Initial results from this collaboration have examined PM and birth weight (Parker et al., 2011); work on $O_3$ has not yet been performed. Although early animal studies (Kavlock et al., 1980) found that exposure to $O_3$ in the late gestation of pregnancy in rats led to some abnormal neurological and behavioral performances for neonates, to date human studies have reported inconsistent results for the association of ambient $O_3$ concentrations and birth outcomes.

### 7.4.1 Effects on Sperm

A limited amount of research has been conducted to examine the association between air pollution and male reproductive outcomes, specifically semen quality. To date, the epidemiologic studies have considered various exposure durations before semen collection that encompass either the entire period of spermatogenesis (i.e., 90 days) or key periods of sperm development that correspond to epididymal storage, development of sperm motility, and spermatogenesis. In an analysis conducted as

part of the Teplice Program, 18-year-old men residing in the heavily polluted district of Teplice in the Czech Republic were found to be at greater risk of having abnormalities in sperm morphology and chromatin integrity than men of similar age residing in Prachatice, a less polluted district (Selevan et al., 2000; Sram et al., 1999). A follow-up longitudinal study conducted on a subset of the same men from Teplice revealed associations between total episodic air pollution and abnormalities in sperm chromatin (Rubes et al., 2005). A limitation of these studies is that they did not identify specific pollutants or their concentrations.

More recent epidemiologic studies conducted in the U.S. have also reported associations between ambient air pollution and sperm quality for individual air pollutants, including $O_3$ and $PM_{2.5}$. In a repeated measures study in Los Angeles, CA, Sokol et al. (2006) reported a reduction in average sperm concentration during three exposure windows (short-term exposures of 0-9, 10-14, and 70-90 days before semen collection, as well as long-term exposures of 0-90 days before semen collection) associated with high ambient levels of $O_3$ in healthy sperm donors. This effect persisted under a joint additive model for $O_3$, CO, $NO_2$ and $PM_{10}$. The authors did not detect a reduction in sperm count. Hansen et al. (2010) investigated the effect of exposure to $O_3$ and $PM_{2.5}$ (using the same exposure windows used by Sokol et al. (2006) on sperm quality in three southeastern counties (Wake County, NC; Shelby County, TN; Galveston County, TX). Outcomes included sperm concentration and count, morphology, DNA integrity and chromatin maturity. Overall, the authors found both protective and adverse effects, although some results suggested adverse effects on sperm concentration, count and morphology.

The biological mechanisms linking ambient air pollution to decreased sperm quality have yet to be determined, though $O_3$-induced oxidative stress, inflammatory reactions, and the induction of the formation of circulating toxic species have been suggested as possible mechanisms (see Section 5.3.8). Decremental effects on testicular morphology have been demonstrated in a toxicological study with histological evidence of $O_3$-induced depletion of germ cells in testicular tissue and decreased seminiferous tubule epithelial layer. Jedlinska-Krakowska et al. (2006) demonstrated histopathological evidence of impaired spermatogenesis (round spermatids/ spermatocytes, giant spermatid cells, and focal epithelial desquamation with denudation to the basement membrane). The exposure protocol used five-month-old adult rats exposed to $O_3$ as adults (long-term exposure, 0.5 ppm, 5 h/day for 50 days). This degeneration could be rescued by vitamin E administration, indicating an antioxidant effect. Vitamin C administration had no effect at low doses of ascorbic acid and exacerbated the $O_3$-dependent damage at high doses, as would be expected as vitamin C can be a radical generator instead of an antioxidant at higher doses. In summary, this study provided toxicological evidence of impaired spermatogenesis with $O_3$ exposure that was rescued with certain antioxidant supplementation.

Overall, there is limited epidemiologic evidence for an association with $O_3$ concentration and decreased sperm concentration. A recent toxicological study provides limited evidence for a possible biological mechanism (histopathology showing impaired spermatogenesis) for such an association.

### 7.4.2 Effects on Reproduction

Evidence suggests that exposure to air pollutants during pregnancy may be associated with adverse birth outcomes, which has been attributed to the increased sensitivity of the fetus due to physiologic immaturity. Gametes (i.e., ova and sperm) may be even more at-risk, especially outside of the human body, as occurs with assisted reproduction. Smokers require twice the number of in vitro fertilization (IVF) attempts to conceive as non-smokers (Feichtinger et al., 1997), suggesting that a preconception exposure can be harmful to pregnancy. A recent study used an established national-scale, log-normal kriging method to spatially estimate daily mean concentrations of criteria pollutants at addresses of women undergoing their first IVF cycle and at their IVF labs from 2000 to 2007 in the northeastern U.S. (Legro et al., 2010). Increasing $O_3$ concentration at the patient's address during ovulation induction (short-term exposure, ~12 days) was significantly associated with an increased chance of live birth (OR = 1.13, [95% CI: 1.05, 1.22] per 10 ppb increase), but with decreased odds of live birth when exposed from embryo transfer to live birth (long-term exposure, ~200 days) (OR = 0.79, [95% CI: 0.69, 0.90] per 10 ppb increase). After controlling for $NO_2$ in a copollutant model, however, $O_3$ was no longer significantly associated with IVF failure. The results of this study suggest that short-term exposure to $O_3$ during ovulation was beneficial (perhaps due to early conditioning to $O_3$), whereas long-term exposure to $O_3$ (e.g., during gestation) was detrimental, and reduced the likelihood of a live birth.

In most toxicological studies, reproductive success appears to be unaffected by $O_3$ exposure. Nonetheless, one study has reported that 25% of the BALB/c mouse dams in the highest $O_3$ exposure group (1.2 ppm, short-term exposure GD9-18) did not complete a successful pregnancy, a significant reduction (Sharkhuu et al., 2011). Ozone administration (continuous 0.4, 0.8 or 1.2 ppm $O_3$) to CD-1 mouse dams during the majority of pregnancy (short-term exposure, PD7-17, which excludes the pre-implantation period), led to no adverse effects on reproductive success (proportion of successful pregnancies, litter size, sex ratio, frequency of still birth, or neonatal mortality) (Bignami et al., 1994). There was a nearly statistically significant increase in pregnancy duration (0.8 and 1.2 ppm $O_3$). Initially, dam body weight (0.8 and 1.2 ppm $O_3$), water consumption (0.4, 0.8 and 1.2 ppm $O_3$) and food consumption (0.4, 0.8 and 1.2 ppm $O_3$) during pregnancy were decreased with $O_3$ exposure but these deficits dissipated a week or two after the initial exposure (Bignami et al., 1994). The anorexigenic effect of $O_3$ exposure on the pregnant dam appears to dissipate with time; the dams seem to adapt to the $O_3$ exposure. In males, data exist showing morphological evidence of altered spermatogenesis in $O_3$ exposed animals (Jedlinska-Krakowska et al., 2006). Some evidence suggests that $O_3$ may

affect reproductive success when combined with other chemicals. Kavlock et al. (1979) showed that $O_3$ acted synergistically with sodium salicylate to increase the rate of pup resorptions after midgestational exposure (1.0 ppm $O_3$, short-term exposure, GD9-GD12). At low concentrations of $O_3$ exposure, toxicological studies show reproductive effects to include a transient anorexigenic effect of $O_3$ on gestational weight gain, and a synergistic effect of $O_3$ on salicylate-induced pup resorptions; other fecundity, pregnancy- and gestation-related outcomes appear unaffected by $O_3$ exposure.

Collectively, there is very little epidemiologic evidence for the effect of short- or long-term exposure to $O_3$ on reproductive success, and the reproductive success in rats appears to be unaffected in toxicological studies of short-term exposure to $O_3$.

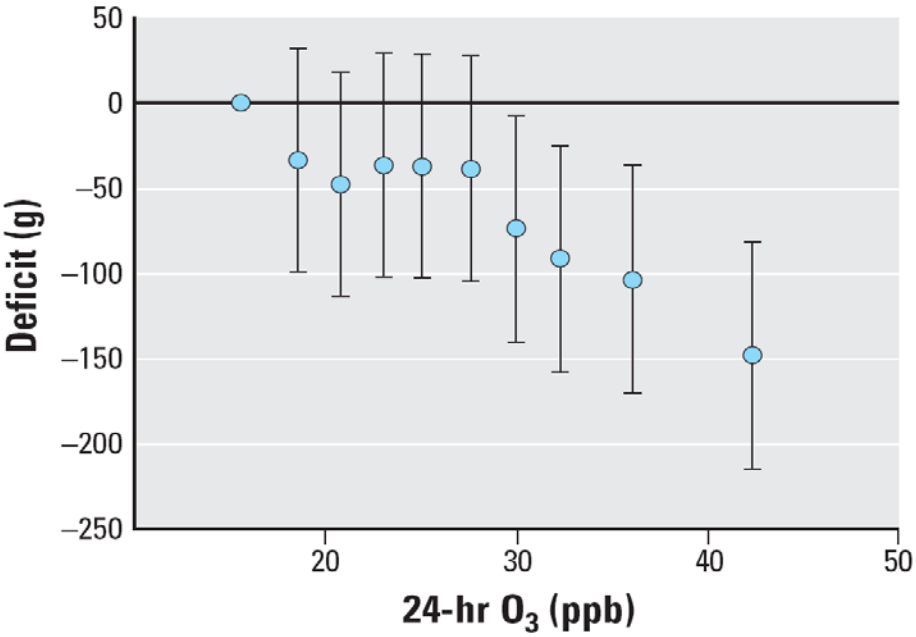
### 7.4.3 Birth Weight

With birth weight routinely collected in vital statistics and being a powerful predictor of infant mortality, it is the most studied outcome within air pollution-birth outcome research. Air pollution researchers have analyzed birth weight as a continuous variable and/or as a dichotomized variable in the form of LBW (<2,500 g [5 lbs, 8 oz]).

Birth weight is primarily determined by gestational age and intrauterine growth, but also depends on maternal, placental and fetal factors as well as on environmental influences. In both developed and developing countries, LBW is the most important predictor for neonatal mortality and is a significant determinant of postneonatal mortality and morbidity. Studies report that infants who are smallest at birth have a higher incidence of diseases and disabilities, which continue into adulthood (Hack and Fanaroff, 1999).

The strongest evidence for an effect of $O_3$ on birth weight comes from the Children's Health Study conducted in southern California. In this study, Salam et al. (2005) report that maternal exposure to 24-h avg $O_3$ concentrations averaged over the entire pregnancy was associated with reduced birth weight (39.3 g decrease [95% CI: -55.8, -22.8] in birth weight per 10 ppb and 8-h avg (19.2-g decrease [95% CI: -27.7, -10.7] in birth weight per 10 ppb). This effect was stronger for concentrations averaged over the second and third trimesters. $PM_{10}$, $NO_2$ and CO concentrations averaged over the entire pregnancy were not statistically significantly associated with birth weight, although CO concentrations in the first trimester and $PM_{10}$ concentrations in the third trimester were associated with a decrease in birth weight. Additionally, the authors observed a concentration-response relationship of birth weight with 24-h avg $O_3$ concentrations averaged over the entire pregnancy that was clearest above the 30-ppb level (see Figure 7-4). Relative to the lowest decile of 24-h avg $O_3$, estimates for the next 5 lowest deciles were approximately -40 g to -50 g, with no clear trend and with 95% confidence bounds that included zero. The highest four deciles of $O_3$ exposure showed an approximately linear decrease in birth weight, and all four 95% CIs

excluded zero, and ranged from mean decreases of 74 grams to decreases of 148 grams.




Note: Deficits are plotted against the decile-group-specific median $O_3$ exposure. Error bars represent 95% CIs. Indicator variables for each decile of $O_3$ exposure (except the least-exposed group) were included in a mixed model.

Source: Salam et al. (2005).'

**Figure 7-4 Birthweight deficit by decile of 24-h avg $O_3$ concentration averaged over the entire pregnancy compared with the decile group with the lowest $O_3$ exposure.**

Several additional studies conducted in the U.S. and Canada also investigated the association between ambient $O_3$ concentrations and birth weight and report some weak evidence for an association. Morello-Frosch et al. (2010) estimated ambient $O_3$ concentrations throughout pregnancy and for each trimester in the neighborhoods of women who delivered term singleton births between 1996 and 2006 in California. A 10-ppb increase in the $O_3$ concentration averaged across the entire pregnancy was associated with a 5.7-g decrease (95% CI: -6.6, -4.9) in birth weight when exposures were calculated using monitors within 10 km of the maternal address at date of birth. When the distance from the monitor was restricted to 3 km, the decrease in birth weight associated with a 10-ppb increase in $O_3$ concentration was 8.9 g (95% CI: -10.6, -7.1). These results persisted in copollutant models and in models that stratified by trimester of exposure, SES, and race. Darrow et al. (2011b) did not observe an association with birth weight and $O_3$ concentrations during two exposure periods of interest (i.e., the first month and last trimester), but did find an association

with reduced birth weight when examining the cumulative air pollution concentration during the entire pregnancy period. Additionally, they observed effect modification by race and ethnicity, such that associations between birth weight and third-trimester $O_3$ concentrations were significantly stronger in Hispanics and non-Hispanic African Americans than in non-Hispanic whites. Chen et al. (2002) used 8-h avg $O_3$ concentrations to create exposure variables based on average maternal exposure for each trimester. Ozone was not found to be related to birth weight in single-pollutant models, though the $O_3$ effect during the third trimester was borderline statistically significant in a copollutant model with $PM_{10}$.

Several studies found no association between ambient $O_3$ concentrations and birth weight. Wilhelm and Ritz (2005) extended previous analyses of term LBW (Ritz et al., 2000; Ritz and Yu, 1999) to include the period 1994-2000. The authors examined varying residential distances from monitoring stations to see if the distance affected risk estimation, exploring the possibility that effect attenuation may result from local pollutant heterogeneity inadequately captured by ambient monitors. As in their previous studies, the authors observed associations between elevated concentrations of CO and $PM_{10}$ both early and late in pregnancy and risk of term LBW. After adjusting for CO and/or $PM_{10}$ the authors did not observe associations between $O_3$ and term LBW in any of their models. Brauer et al. (2008) evaluated the impacts of air pollution (CO, $NO_2$, NO, $O_3$, $SO_2$, $PM_{2.5}$, $PM_{10}$) on birth weight for the period 1999-2002 using spatiotemporal residential exposure metrics by month of pregnancy in Vancouver, BC. Quantitative results were not presented for the association between $O_3$ and LBW, though the authors observed associations that were largely protective. Dugandzic et al. (2006) examined the association between LBW and ambient levels of air pollutants by trimester of exposure among a cohort of term singleton births from 1988-2000. Though there was some indication of an association with $SO_2$ and $PM_{10}$, there were no effects for $O_3$.

Similarly, studies conducted in Australia, Latin America, and Asia report limited evidence for an association between ambient $O_3$ and measures of birth weight. In Sydney, Australia, Mannes et al. (2005) found that $O_3$ concentrations in the second trimester of pregnancy had small adverse effects on birth weight (7.5-g decrease; [95% CI: -13.8, 1.2] per 10 ppb), although this effect disappeared when the analysis was limited to births with a maternal address within 5 km of a monitoring station (87.7-g increase; [95% CI: 10.5, 164.9] per 10 ppb). Hansen et al. (2007) reported that trimester and monthly specific exposures to all pollutants were not statistically significantly associated with a reduction in birth weight in Brisbane, Australia. In Sao Paulo, Brazil, Gouveia et al. (2004) found that $O_3$ exhibited a small inverse relation with birth weight over the third trimester (6.0-g decrease; [95% CI: -30.8, 18.8] per 10 ppb). Lin et al. (2004b) reported a positive, though not statistically significant, exposure-response relationship for $O_3$ during the entire pregnancy in a Taiwanese study. In a study performed in Korea, Ha et al. (2001) reported no $O_3$ effect during the first trimester of pregnancy, but they found that during the third trimester of pregnancy $O_3$ was associated with LBW (RR = 1.05 [95% CI: 1.02, 1.08] per 10 ppb).

## Table 7-5 Brief summary of epidemiologic studies of birth weight.

| Study | Location Sample Size | Mean $O_3$ (ppb) | Exposure assessment | Effect Estimate[a] (95% CI) |
|---|---|---|---|---|
| Salam et al. (2005) | California, U.S.<br>(n = 3,901) | 24-h avg:<br>27.3<br>8 h:<br>50.6 | ZIP code level | Entire pregnancy:<br>-39.3 g (-55.8, -22.8)<br>T1: -6.1 g (-16.8, 4.8)<br>T2: -20.0 g (-31.7, -8.4)<br>T3: -20.7 g (-32.1, -9.3) |
| Morello-Frosch et al. (2010) | California, U.S.<br>(n = 3,545,177) | 24-h avg:<br>23.5 | Nearest Monitor<br>(within 10, 5, 3 km) | Entire pregnancy:<br>-5.7 g (-6.6, -4.9)<br>T1: -2.1 g (-2.9, -1.4)<br>T2: -2.3 g (-3.1, -1.5)<br>T3: -1.3 g (-2.1, -0.6) |
| Darrow et al. (2011b) | Atlanta, GA<br>(N=406,627) | 8-h max:<br>44.8 | Population-weighted spatial average | Entire pregnancy:<br>-12.3 g (-17.8, -6.8)<br>First 28 days<br>-0.5 g (-3.0, 2.1)<br>T3: -0.9g (-4.5, 2.8) |
| Chen et al. (2002) | Northern Nevada, U.S.<br>(n = 36,305) | 8-h:<br>27.2 | County level | Entire pregnancy:<br>20.9 g (6.3, 35.5)<br>T1: 23.4 g (-35.6, 82.4)<br>T2: -19.4 g (-77.0, 38.2)<br>T3: 7.7 g (-50.9, 66.3) |
| Wilhelm and Ritz (2005) | Los Angeles County, CA<br>(n = 136,134) | 1-h:<br>21.1-22.2 | Varying distances from monitor | T1: NR<br>T3: NR<br>6 weeks before birth: NR |
| Brauer et al. (2008) | Vancouver, BC, Canada<br>(n = 70,249) | 24-h avg:<br>14 | Nearest Monitor<br>(within 10 km)<br>Inverse Distance Weighting (IDW) | Entire pregnancy: NR<br>First 30 days of pregnancy: NR<br>Last 30 days of pregnancy: NR<br>T1: NR<br>T3: NR |
| Dugandzic et al. (2006) | Nova Scotia, Canada<br>(n = 74,284) | 24-h avg:<br>21 | Nearest Monitor<br>(within 25 km) | T1: 0.97 (0.81, 1.18)[d]<br>T2: 1.06 (0.87, 1.27)[d]<br>T3: 1.01 (0.83-1.24)[d] |
| Mannes et al. (2005) | Sydney, Australia<br>(n = 138,056) | 1-h max:<br>31.6 | Citywide avg and<br><5 km from monitor | T1: -0.9 g (-6.6, 4.8)<br>T2: -7.5 g (-13.8, 1.2)<br>T3: -4.5 g (-10.8, 1.8)<br>Last 30 days:<br>-1.1 g (-5.6, 3.4) |
| Hansen et al. (2007) | Brisbane, Australia<br>(n = 26,617) | 8 h max:<br>26.7 | Citywide avg | T1: 2.8 g (-10.5, 16.0)<br>T2: 4.4 g (-11.4, 20.1)<br>T3: 11.3 g (-4.4, 27.1) |
| Gouveia et al. (2004) | Sao Paulo, Brazil<br>(n = 179,460) | 1-h max:<br>31.5 | Citywide avg | T1: -3.2 g (-25.6, 19)<br>T2: -0.2 g (-23.8, 23.4)<br>T3: -6.0 g (-30.8, -18.8) |

| Study | Location<br>Sample Size | Mean $O_3$ (ppb) | Exposure assessment | Effect Estimate[a]<br>(95% CI) |
|---|---|---|---|---|
| Lin et al. (2004b) | Kaohsiung and Taipei, Taiwan<br>(n = 92,288) | 24-h avg: 15.86-47.78 | Nearest monitor (within 3 km) | Entire pregnancy: 1.13 (0.92, 1.38)[c]<br>T1: 1.02 (0.85, 1.22)[c]<br>T2: 0.93 (0.78, 1.12)[c]<br>T3: 1.05 (0.87, 1.26)[c] |
| Ha et al. (2001) | Seoul, Korea<br>(n = 276,763) | 8-h avg: 22.4-23.3[b] | Citywide avg | T1: 0.87 (0.81, 0.94)[c]<br>T3: 1.05 (1.02, 1.08)[c] |

[a]Change in birthweight per 10 ppb change in $O_3$

[b]Median

[c]Odds ratios of LBW; Highest quartile of exposure compared to lowest quartile of exposure

[d]Relative risk of LBW per 10 ppb change in $O_3$

T1 = First Trimester, T2 = Second Trimester, T3 = Third Trimester

NR: No quantitative results reported

Table 7-5 provides a brief overview of the epidemiologic studies of birth weight. In summary, only the Children's Health Study conducted in southern California (Salam et al., 2005) provides strong evidence for an effect of ambient $O_3$ on birth weight. The study by Morello-Frosch et al. (2010), also conducted in California, provides support for the results of the Children's Health Study. Additional studies, conducted in the U.S., Canada, Australia, Latin America, and Asia, provide limited and inconsistent evidence to support the effect reported in the Children's Health Study. The toxicological literature on the effect of $O_3$ on birth weight is sparse. In some studies, the reporting of birth weight may be avoided because birth weight can be confounded by decreased litter size resulting from an increased rate of pup resorption (aborted pups) in $O_3$ exposed dams. In one toxicological study by Haro and Paz (1993), no differences in litter size were observed and decreased birth weight in pups from dams who were exposed to 1ppm $O_3$ during pregnancy (short-term exposure, ~22 days) was reported. A second animal toxicology study recapitulated these finding with pregnant BALB/c mice exposed to $O_3$ (1.2 ppm, short-term exposure, GD9-18) that produced pups with significantly decreased birth weights (Sharkhuu et al., 2011).

### 7.4.4 Preterm Birth

Preterm birth (PTB) is a syndrome (Romero et al., 2006) that is characterized by multiple etiologies. It is therefore unusual to be able to identify an exact cause for each PTB. In addition, PTB is not an adverse outcome in itself, but an important determinant of health status (i.e., neonatal morbidity and mortality). Although some overlap exists for common risk factors, different etiologic entities related to distinct risk factor profiles and leading to different neonatal and postneonatal complications are attributed to PTB and measures of fetal growth. Although both restricted fetal growth and PTB can result in LBW, prematurity does not have to result in LBW or growth restricted babies.

A major issue in studying environmental exposures and PTB is selecting the relevant exposure period, since the biological mechanisms leading to PTB and the critical periods of vulnerability are poorly understood (Bobak, 2000). Short-term exposures proximate to the birth may be most relevant if exposure causes an acute effect. However, exposure occurring in early gestation might affect placentation, with results observable later in pregnancy, or cumulative exposure during pregnancy may be the most important determinant. The studies reviewed have dealt with this issue in different ways. Many have considered several exposure metrics based on different periods of exposure. Often the time periods used are the first month (or first trimester) of pregnancy and the last month (or 6 weeks) prior to delivery. Using a time interval prior to delivery introduces an additional problem since cases and controls are not in the same stage of development when they are compared. For example, a preterm infant delivered at 36 weeks is a 32-week fetus 4 weeks prior to birth, while an infant born at term (40 weeks) is a 36-week fetus 4 weeks prior to birth.

Recently, investigators have examined the association of PTB with both short-term (i.e., hours, days, or weeks) and long-term (i.e., months or years) exposure periods. Time-series studies have been used to examine the association between air pollution concentrations during the days immediately preceding birth. An advantage of these time-series studies is that this approach can remove the influence of covariates that vary across individuals over a short period of time. Retrospective cohort and case-control studies have been used to examine long-term exposure periods, often averaging air pollution concentrations over months or trimesters of pregnancy.

Studies of PTB fail to show consistency in pollutants and periods during pregnancy when an effect occurs. For example, while some studies find the strongest effects associated with exposures early in pregnancy, others report effects when the exposure is limited to the second or third trimester. However, the effect of air pollutant exposure during pregnancy on PTB has a biological basis. There is an expanding list of possible mechanisms that may explain the association between $O_3$ exposure and PTB (see Section 5.4.2.4).

Many studies of PTB compare exposure in quartiles, using the lowest quartile as the reference (or control) group. No studies use a truly unexposed control group. If exposure in the lowest quartile confers risk, than it may be difficult to demonstrate additional risk associated with a higher quartile. Thus negative studies must be interpreted with caution.

Preterm birth occurs both naturally (idiopathic PTB), and as a result of medical intervention (iatrogenic PTB). Ritz et al. (2007); (2000) excluded all births by Cesarean section to limit their studies to idiopathic PTB. No other studies attempted to distinguish the type of PTB, although air pollution exposure maybe associated with only one type. This is a source of potential effect misclassification.

Generally, studies of air pollution and birth outcomes conducted in North America and the United Kingdom have not identified an association between PTB and maternal exposure to $O_3$. Most recently, Darrow et al. (2009) used vital record data

to construct a retrospective cohort of 476,489 births occurring between 1994 and 2004 in 5 central counties of metropolitan Atlanta, GA. Using a time-series approach, the authors examined aggregated daily counts of PTB in relation to ambient levels of CO, $NO_2$, $SO_2$, $O_3$, $PM_{10}$, $PM_{2.5}$ and speciated PM measurements. This study investigated 3 gestational windows of short- and long-term exposure: the final week of gestation (short-term exposure), and the first month of gestation and the final 6 weeks of gestation (long-term exposure). The authors did not observe associations of PTB with $O_3$ concentrations for any of the exposure periods.

A number of U.S. studies were conducted in southern California, and report somewhat inconsistent results. Ritz et al. (2000) evaluated the effect of air pollution (CO, $NO_2$, $O_3$, $PM_{10}$) exposure during pregnancy on the occurrence of PTB in a cohort of 97,518 neonates born in southern California between 1989 and 1993. The authors use both short- and long-term exposure windows, averaging pollutant measures taken at the closest air-monitoring station over distinct periods, such as 1, 2, 4, 6, 8, 12, and 26 weeks before birth and the whole pregnancy period. Additionally, they calculated average exposures for the first and second months of pregnancy. The authors found no consistent effects associated with $O_3$ concentration over any of the pregnancy periods in single or multipollutant models. Wilhelm and Ritz (2005) extended previous analyses of PTB (Ritz et al., 2000; Ritz and Yu, 1999) in California to include 1994-2000. The authors examined varying residential distances from monitoring stations to see if the distance affected risk estimation, because effect attenuation may result from local pollutant heterogeneity inadequately captured by ambient monitors. The authors analyzed the association between long-term $O_3$ exposure during varying periods of pregnancy and PTB, finding a positive association between $O_3$ levels in both the first trimester of pregnancy (RR = 1.23 [95% CI: 1.06, 1.42] per 10 ppb increase in 24-h avg $O_3$) and the first month of pregnancy (results for first trimester exposure were similar, but slightly smaller, quantitative results not presented) in models containing all pollutants. No association was observed between $O_3$ in the 6 weeks before birth and preterm delivery. Finally, Ritz et al. (2007) conducted a case-control survey nested within a birth cohort and assessed the extent to which residual confounding and exposure misclassification impacted air pollution effect estimates. The authors calculated mean long-term exposure levels for three gestational periods: the entire pregnancy, the first trimester, and the last 6 weeks before delivery. Though positive associations were observed for CO and $PM_{2.5}$, no consistent patterns of increase in the odds of PTB for $O_3$ or $NO_2$ were observed.

A study conducted in Canada evaluated the impacts of air pollution (including CO, $NO_2$, NO, $O_3$, $SO_2$, $PM_{2.5}$, and $PM_{10}$) on PTBs (1999-2002) using spatiotemporal residential exposure metrics by month of pregnancy (long-term exposure) in Vancouver, BC (Brauer et al., 2008). The authors did not observe consistent associations with any of the pregnancy average exposure metrics except for $PM_{2.5}$ for PTB. The $O_3$ associations were largely protective, and no quantitative results were presented for $O_3$. Additionally, Lee et al. (2008c) used time-series techniques to investigate the associations of short-term exposure to $O_3$ and PTB in London, England. In addition to exposure on the day of birth, cumulative exposure up to

1 week before birth was investigated. The risk of PTB did not increase with exposure to the levels of ambient air pollution experienced by this population.

Conversely, studies conducted in Australia and China provide evidence for an association between ambient $O_3$ and PTB. Hansen et al. (2006) reported that long-term exposure to $O_3$ during the first trimester was associated with an increased risk of PTB (OR = 1.38, [95% CI: 1.14, 1.69] per 10 ppb increase). Although the test for trend was significant due to the strong effect in the highest quartile, there was not an obvious exposure-response pattern across the quartiles of $O_3$ during the first trimester. The effect estimate was diminished and lost statistical significance when $PM_{10}$ was included in the model (OR = 1.23, [95% CI: 0.97, 1.59] per 10 ppb increase). Maternal exposure to $O_3$ during the 90 days prior to birth showed a weak, positive association with PTB (OR = 1.09, [95% CI: 0.85, 1.39] per 10 ppb increase). Jalaludin et al. (2007) found that $O_3$ levels in the month and three months preceding birth had a statistically significant association with PTB. Ozone levels in the first trimester of pregnancy were associated with increased risks for PTBs (OR = 1.15 [95% CI: 1.05, 1.24] per 10 ppb increase in 1-h max $O_3$ concentration), and remained a significant predictor of PTB in copollutant models (ORs between 1.07 and 1.10). Jiang et al. (2007) examined the effect of short- and long-term exposure to air pollution on PTB, including risk in relation to levels of pollutants for a single day exposure window with lags from 0 to 6 days before birth. An increase of 10 ppb of the 8-week avg of $O_3$ corresponded to 9.47% (95% CI: 0.70, 18.7%) increase in PTBs. Increases in PTB were also observed for $PM_{10}$, $SO_2$, and $NO_2$. The authors did not observe any significant effect of short-term exposure to outdoor air pollution on PTB among the 1-day time windows examined in the week before birth.

Few data are available from toxicological studies; a study reported a nearly statistically significant increase in pregnancy duration (short-term exposure) in mice when exposed to 0.8 or 1.2 ppm $O_3$. This phenomenon was most likely due to the anorexigenic effect of relatively high $O_3$ concentrations (Bignami et al., 1994).

Table 7-6 provides a brief overview of the epidemiologic studies of PTB.
In summary, the evidence is consistent when examining short-term exposure to $O_3$ during late pregnancy and reports no association with PTB. However when long-term exposure to $O_3$ early in pregnancy is examined the results are inconsistent. Generally, studies conducted in the U.S., Canada, and England find no association with $O_3$ and PTB, while studies conducted in Australia and China report an $O_3$ effect on PTB.

## Table 7-6 Brief summary of epidemiologic studies of preterm birth (PTB).

| Study | Location Sample Size | Mean $O_3$ (ppb) | Exposure assessment | Effect Estimate[a] (95% CI) |
|---|---|---|---|---|
| Darrow et al. (2009) | Atlanta, GA<br>(n = 476,489) | 8-h max: 44.1 | Population-weighted spatial averages<br>Nearest Monitor (within 4 miles) | First month: 0.98 (0.97, 1.00)<br>Last week: 0.99 (0.98, 1.00)<br>Last 6 weeks: 1.00 (0.98, 1.02) |
| Ritz et al. (2000) | California, U.S.<br>(n = 97,158) | 8 h: 36.9 | <2 mi of monitor | First month: NR<br>Last 6 weeks: NR |
| Wilhelm and Ritz (2005) | Los Angeles, CA<br>(n = 106,483) | 1 h: 21.1-22.2 | Varying distances to monitor | First month: 1.23 (1.06, 1.42)<br>T1: NR<br>T2: 1.38 (1.14, 1.66)<br>Last 6 weeks: NR |
| Ritz et al. (2007) | Los Angeles, CA<br>(n = 58,316) | 24-h avg: 22.5 | Nearest monitor to ZIP code | Entire pregnancy: NR<br>T1: 0.93 (0.82, 1.06)<br>Last 6 weeks: NR |
| Brauer et al. (2008) | Vancouver, BC, Canada<br>(n = 70,249) | 24-h avg: 14 | Nearest Monitor (within 10 km)<br>Inverse Distance Weighting (IDW) | Entire pregnancy: NR<br>First 30 days of pregnancy: NR<br>Last 30 days of pregnancy: NR<br>T1: NR<br>T3: NR |
| Lee et al. (2008c) | London, UK | 24-h avg: NR | 1 monitor | Lag 0: 1.00 (1.00, 1.01) |
| Hansen et al. (2006) | Brisbane, Australia<br>(n = 28,200) | 8-h max: 26.7 | Citywide avg | T1: 1.39 (1.15, 1.70)<br>T3: 1.09 (0.88, 1.39) |
| Jalaludin et al. (2007) | Sydney, Australia<br>(n = 123,840) | 1-h max: 30.9 | Citywide avg and <5 km from monitor | First month: 1.04 (0.95, 1.13)<br>T1: 1.15 (1.05, 1.24)<br>T3: 0.98 (0.89, 1.07)<br>Last month: 0.98 (0.88, 1.06) |
| Jiang et al. (2007) | Shanghai, China<br>(n = 3,346 preterm births) | 8-h avg: 32.7 | Citywide avg | 4 wks before birth: 1.06 (1.00, 1.12)<br>6 wks before birth: 1.06 (0.99, 1.13)<br>8 wks before birth: 1.09 (1.01, 1.19)<br>Acute effects, L0 to L6:<br>NR* (relative risk results presented in figure 1)<br>*NR: No quantitative results reported; however, preterm birth was not significantly associated with outdoor $O_3$ air pollution in any lag day (0 – 6) that was considered in the Jiang et al. (2007) study. |

[a]Relative risk of PTB per 10 ppb change in $O_3$.

T1 = First Trimester, T2 = Second Trimester, T3 = Third Trimester.

L0 = Lag 0, L1= Lag 1, L2 = Lag 2, L3 = Lag 3, L4 = Lag 4, L5 = Lag 5, L6 = Lag 6.

NR: No quantitative results reported.

### 7.4.5 Fetal Growth

Low birth weight has often been used as an outcome measure because it is easily available and accurately recorded on birth certificates. However, LBW may result from either short gestation, or inadequate growth in utero. Most of the studies investigating air pollution exposure and LBW limited their analyses to term infants to focus on inadequate growth. A number of studies were identified that specifically addressed growth restriction in utero by identifying infants who failed to meet specific growth standards. Usually these infants had birth weight less than the 10th percentile for gestational age, using an external standard. Many of these studies have been previously discussed, since they also examined other reproductive outcomes (i.e., LBW or PTB).

Fetal growth is influenced by maternal, placental, and fetal factors. The biological mechanisms by which air pollutants may influence the developing fetus remain largely unknown. Several mechanisms have been proposed, and are the same as those hypothesized for birth weight (see Section 5.4.2.4). Additionally, in animal toxicology studies, $O_3$ causes transient anorexia in exposed pregnant dams. This may be one of many possible contributors to $O_3$-dependent decreased fetal growth.

A limitation of environmental studies that use birth weight as a proxy measure of fetal growth is that patterns of fetal growth during pregnancy cannot be assessed. This is particularly important when investigating pollutant exposures during early pregnancy as birth weight is recorded many months after the exposure period. The insult of air pollution may have a transient effect on fetal growth, where growth is hindered at one point in time but catches up at a later point. For example, maternal smoking during pregnancy can alter the growth rate of individual body segments of the fetus at variable developmental stages, as the fetus experiences selective growth restriction and augmentation (Lampl and Jeanty, 2003).

The terms small-for-gestational-age (SGA), which is defined as a birth weight <10th percentile for gestational age (and often sex and/or race), and intrauterine growth retardation (IUGR) are often used interchangeably. However, this definition of SGA does have limitations. For example, using it for IUGR may overestimate the percentage of "growth-restricted" neonates as it is unlikely that 10% of neonates have growth restriction (Wollmann, 1998). On the other hand, when the 10th percentile is based on the distribution of live births at a population level, the percentage of SGA among PTB is most likely underestimated (Hutcheon and Platt, 2008). Nevertheless, SGA represents a statistical description of a small neonate, whereas the term IUGR is reserved for those with clinical evidence of abnormal growth. Thus all IUGR neonates will be SGA, but not all SGA neonates with be IUGR (Wollmann, 1998). In the following section the terms SGA and IUGR are referred to as each cited study used the terms.

Over the past decade a number of studies examined various metrics of fetal growth restriction. Salam et al. (2005) assessed the effect of increasing $O_3$ concentrations on IUGR in a population of infants born in California from 1975-1987 as part of the

Children's Health Study. The authors reported that maternal $O_3$ exposures averaged over the entire pregnancy and during the third trimester were associated with increased risk of IUGR. A 10-ppb difference in 24-h maternal $O_3$ exposure during the third trimester increased the risk of IUGR by 11% (95% CI: 0, 20%). Brauer et al. (2008) evaluated the impacts of air pollution (CO, $NO_2$, NO, $O_3$, $SO_2$, $PM_{2.5}$, $PM_{10}$) on SGA (1999-2002) using spatiotemporal residential exposure metrics by month of pregnancy in Vancouver, BC. The $O_3$ associations were largely protective (OR = 0.87, [95% CI: 0.81, 0.93] for a 10 ppb increase in inverse distance weighted SGA), and no additional quantitative results were presented for $O_3$. Liu et al. (2007b) examined the association between IUGR among singleton term live births and $SO_2$, $NO_2$, CO, $O_3$, and $PM_{2.5}$ in 3 Canadian cities for the period 1985-2000. No increase in the risk of IUGR in relation to exposure to $O_3$ averaged over each month and trimester of pregnancy was noted.

Three studies conducted in Australia provide evidence for an association between ambient $O_3$ and fetal growth restriction. Hansen et al. (2007) examined SGA among singleton, full-term births in Brisbane, Australia in relation to ambient air pollution (bsp, $PM_{10}$, $NO_2$, $O_3$) during pregnancy. They also examined head circumference and crown-heel length in a subsample of term neonates. Trimester specific exposures to all pollutants were not statistically significantly associated with a reduction in head circumference or an increased risk of SGA. When monthly-specific exposures were examined, the authors observed an increased risk of SGA associated with exposure to $O_3$ during month 4 (OR = 1.11 [95% CI: 1.00, 1.24] per 10 ppb increase). In a subsequent study, Hansen et al. (2008) examined the possible associations between fetal ultrasonic measurements and ambient air pollution ($PM_{10}$, $O_3$, $NO_2$, $SO_2$) during early pregnancy. This study had two strengths: (1) fetal growth was assessed during pregnancy as opposed to at birth; and (2) there was little delay between exposures and fetal growth measurements, which reduces potential confounding and uses exposures that are concurrent with the observed growth pattern of the fetus. Fetal ultrasound biometric measurements were recorded for biparietal diameter (BPD), femur length, abdominal circumference, and head circumference. To further improve exposure assessment, the authors restricted the samples to include only scans from women for whom the centroid of their postcode was within 14 km of an air pollution monitoring site. Ozone during days 31-60 was associated with decreases in all of the fetal growth measurements, and a 1.78 mm reduction in abdomen circumference per 10 ppb increase in $O_3$ concentration, though this effect did not persist in copollutant models. The change in ultrasound measurements associated with $O_3$ during days 31-60 of gestation indicated that increasing $O_3$ concentration decreased the magnitude of ultrasound measurements for women living within 2 km of the monitoring site. The relationship decreased toward the null as the distance from the monitoring sites increased. When assessing effect modification due to SES, there was some evidence of effect modification for most of the associations, with the effects of air pollution stronger in the highest SES quartile. In the third study, Mannes et al. (2005) estimated the effects of pollutant ($PM_{10}$, $PM_{2.5}$, $NO_2$, CO and $O_3$) exposure in the first, second and third trimesters of pregnancy and risk of SGA in Sydney, Australia. Citywide average air pollutant concentrations in the last month, third trimester, and first trimester of pregnancy had no effect on SGA.

Concentrations of $O_3$ in the second trimester of pregnancy had small but adverse effects on SGA (OR = 1.10 [95% CI: 1.00, 1.14] per 10 ppb increment). This effect disappeared when the analysis was limited to births with a maternal address within 5 km of a monitoring station (OR = 1.00 [95% CI: 0.60, 1.79] per 10 ppb increment).

Very little information from toxicological studies is available to address effects on fetal growth. However, there is evidence to suggest that prenatal (short-term) exposure to $O_3$ can affect postnatal growth. A few studies reported that mice or rats exposed developmentally (gestationally ± lactationally) to $O_3$ had deficits in body weight gain in the postpartum period (Bignami et al., 1994; Haro and Paz, 1993; Kavlock et al., 1980).

Table 7-7 provides a brief overview of the epidemiologic studies of fetal growth restriction. In summary, the evidence is inconsistent when examining exposure to $O_3$ and fetal growth restriction. Similar to PTB, studies conducted in Australia have reported an effect of $O_3$ on fetal growth, whereas studies conducted in other areas generally have not found such an effect. This may be due to the restriction of births to those within 2-14 km of a monitoring station, as was done in the Australian studies.

**Table 7-7 Brief summary of epidemiologic studies of fetal growth.**

| Study | Location (Sample Size) | Mean $O_3$ (ppb) | Exposure assessment | Effect Estimate[a] (95% CI) |
|---|---|---|---|---|
| Salam et al. (2005) | California, U.S.<br>(n = 3,901) | 24-h avg:<br>27.3<br>8 h:<br>50.6 | ZIP code level | Entire pregnancy: 1.16 (1.00, 1.32)<br>T1:1.00 (0.94, 1.11)<br>T2: 1.06 (1.00, 1.12)<br>T3: 1.11 (1.00, 1.17) |
| Brauer et al. (2008) | Vancouver, BC, Canada<br>(n = 70,249) | 24-h avg:<br>14 | Nearest Monitor (within 10 km)<br>Inverse Distance Weighting (IDW) | Entire pregnancy: NR<br>First 30 days of pregnancy: NR<br>Last 30 days of pregnancy: NR<br>T1: NR<br>T3: NR |
| Liu et al. (2007b) | Calgary, Edmonton, and Montreal, Canada<br>(n = 16,430) | 24-h avg:<br>16.5<br>1-h max:<br>31.2 | Census Subdivision avg | Entire pregnancy: NR (results presented in figure)<br>T1: NR (results presented in figure)<br>T2: NR (results presented in figure)<br>T3: NR (results presented in figure) |
| Hansen et al. (2007) | Brisbane, Australia<br>(n = 26,617) | 8-h max:<br>26.7 | Citywide avg | T1: 1.01 (0.89, 1.15)<br>T2: 1.00 (0.86, 1.17)<br>T3: 0.83 (0.71, 0.97) |
| Hansen et al. (2008) | Brisbane, Australia<br>(n = 15,623) | 8-h avg:<br>24.8 | Within 2 km of monitor | M1: -0.32 (-1.56, 0.91)[b]<br>M2: -0.58 (-1.97, 0.80)[b]<br>M3: 0.26 (-1.07, 1.59)[b]<br>M4: 0.11 (-0.98, 1.21)[b] |
| Mannes et al. (2005) | Sydney, Australia<br>(n = 138,056) | 1-h max:<br>31.6 | Citywide avg and <5 km from monitor | T1: 0.90 (0.48, 1.34)<br>T2: 1.00 (0.60, 1.79)<br>T3: 1.10 (0.66, 1.97)<br>Last 30 days of pregnancy: 1.10 (0.74, 1.79) |

[a]Relative risk of fetal growth restriction per 10 ppb change in $O_3$, unless otherwise noted.

[b]Mean change in fetal ultrasonic measure of head circumference recorded between 13 and 26 weeks gestation for a 10-ppb increase in maternal exposure to $O_3$ during early pregnancy

T1 = First Trimester, T2 = Second Trimester, T3 = Third Trimester

M1 = Month 1, M2 = Month 2, M3 = Month 3, M4 = Month 4

NR: No quantitative results reported

### 7.4.6 Postnatal Growth

Postnatal weight and height are routinely measured in children as indicators of growth and somatic changes. Toxicological studies often follow these endpoints to ascertain if a known exposure has an effect in the postnatal window, an effect which can be permanent. Time-pregnant BALB/c mice were exposed to $O_3$ (0, 0.4, 0.8, or 1.2 ppm) GD9-18 (short-term exposure) with parturition at GD20-21 (Sharkhuu et al., 2011). As the offspring aged, postnatal litter body weight continued to be significantly decreased in the highest concentration (1.2 ppm) $O_3$ group at PND3 and PND7. When the pups were weighed separately by sex at PND42, the males with the

highest concentration of $O_3$ exposure (1.2 ppm, GD9-18) had significant decrements in body weight (Sharkhuu et al., 2011).

Significant decrements in body weight at 4 weeks of age were reported in C57Bl/6 mice that were exposed to postnatal $O_3$ (short-term exposure, PND2-28 exposure, 1 ppm $O_3$, 3 hours/day, 3 days/week) (Auten et al., 2012). Animals with co-exposure to in utero DE (short-term exposure, dam GD9-GD17; inhalation 0.5 or 2.0 mg/$m^3$ $O_3$; 4 h/day via inhalation; or oropharyngeal aspiration DEPs, 2×/week) + postnatal $O_3$ (aforementioned short-term exposure) also had significantly reduced body weight.

### 7.4.7 Birth Defects

Despite the growing body of literature evaluating the association between ambient air pollution and various adverse birth outcomes, relatively few studies have investigated the effect of temporal variations in ambient air pollution on birth defects. Heart defects and oral clefts have been the focus of the majority of these recent studies, given the higher prevalence than other birth defects and associated mortality. Mechanistically, air pollutants could be involved in the etiology of birth defects via a number of key events (see Section 5.4.2.4).

Several studies have been conducted examining the relationship between $O_3$ exposure during pregnancy and birth defects and reported a positive association with cardiac defects. The earliest of these studies was conducted in southern California (Ritz et al., 2002). This study evaluated the effect of air pollution on the occurrence of cardiac birth defects in neonates and fetuses delivered in southern California in 1987-1993. Maternal exposure estimates were based on data from the fixed site closest to the mother's ZIP code area. When using a case-control design where cases were matched to 10 randomly selected controls, results showed increased risks for aortic artery and valve defects (OR = 1.56 [95% CI: 1.16, 2.09] per 10 ppb $O_3$), pulmonary artery and valve anomalies (OR = 1.34 [95% CI: 0.96, 1.87] per 10 ppb $O_3$), and conotruncal defects (OR = 1.36 [95% CI: 0.91, 2.03] per 10 ppb $O_3$) in a dose-response manner with second-month $O_3$ exposure. A study conducted in Texas (Gilboa et al., 2005) looked at a similar period of exposure but reported no association with most of the birth defects studied ($O_3$ concentration was studied using quartiles with the lowest representing <18 ppb and the highest representing ≥ 31 ppb). The authors found slightly elevated odds ratios for pulmonary artery and valve defects. They also detected an inverse association between $O_3$ exposure and isolated ventricular septal defects. Overall, this study provided some weak evidence that air pollution increases the risk of cardiac defects. Hansen et al. (2009) investigated the possible association between ambient air pollution concentrations averaged over weeks 3-8 of pregnancy and the risk of cardiac defects. When analyzing all births with exposure estimates for $O_3$ from the nearest monitor there was no indication for an association with cardiac defects. There was also no adverse association when restricting the analyses to only include births where the mother

resided within 12 km of a monitoring station. However, among births within 6 km of a monitor, a 10 ppb increase in $O_3$ was associated with an increased risk of pulmonary artery and valve defects (OR = 8.76 [95% CI: 1.80, 56.55]). As indicated by the very wide credible intervals, there were very few cases in the sensitivity analyses for births within 6 km of a monitor, and this effect could be a result of type I errors. Dadvand et al. (2011) investigated the association between maternal exposure to ambient air pollution concentrations averaged over weeks 3-8 of pregnancy and the occurrence of cardiac birth defects in England. Similar to Hansen et al. (2009), they found no associations with maternal exposure to $O_3$ except for when the analysis was limited to those subjects residing within a 16 km distance of a monitoring station (OR for malformations of pulmonary and tricuspid valves=1.64 [95% CI: 1.04, 2.60] per 10 ppb increase in $O_3$).

Despite the association between $O_3$ and cardiac defects observed in the above studies, a recent study did not observe an increased risk of cardiac birth defects associated with ambient $O_3$ concentrations. The study, conducted in Atlanta, GA, examined $O_3$ exposure during weeks 3-7 of of pregnancy and reported no association with risk of cardiovascular malformations (Strickland et al., 2009).

Several of these studies have also examined the relationship between $O_3$ exposure during pregnancy and oral cleft defects. The study by Ritz et al. (2002) evaluated the effect of air pollution on the occurrence of orofacial birth defects and did not observe strong associations between ambient $O_3$ concentration and orofacial defects. They did report an OR of 1.13 (95% CI: 0.90, 1.40) per 10 ppb during the second trimester for cleft lip with or without cleft palate. Similarly, Gilboa et al. (2005) reported an OR of 1.09 (95% CI: 0.70, 1.69) for oral cleft defects when the fourth quartile was contrasted with the first quartile of exposure during 3-8 weeks of pregnancy. Hansen et al. (2009) reported no indication for an association with cleft defects and air pollution concentrations averaged over weeks 3-8 of pregnancy. Hwang and Jaakkola (2008) conducted a population-based case-control study to investigate exposure to ambient air pollution and the risk of cleft lip with or without cleft palate in Taiwan. The risk of cleft lip with or without cleft palate was increased in relation to $O_3$ levels in the first gestational month (OR = 1.17 [95% CI: 1.01, 1.36] per 10 ppb) and second gestational month (OR = 1.22 [95% CI: 1.03, 1.46] per 10 ppb), but was not related to any of the other pollutants. In three-pollutant models, the effect estimates for $O_3$ exposure were stable for the four different combinations of pollutants and were all statistically significant. Marshall et al. (2010) compared estimated exposure to ambient pollutants during early pregnancy (6 week period from 5 to 10 weeks into the gestational period) among mothers of children with oral cleft defects to that among mothers of controls. The authors observed no consistent elevated associations between any of the air pollutants examined and cleft malformations, though there was a weak association between cases of cleft palate only and increasing $O_3$ concentrations. This association increased when cases and controls were limited to those with residences within 10 km of the closest $O_3$ monitor (OR = 2.2 [95% CI: 1.0, 4.9], comparing highest quartile [>33 ppb] to lowest quartile [<15 ppb]).

A limited number of toxicological studies have examined birth defects in animals exposed gestationally to $O_3$. Kavlock et al. (1979) exposed pregnant rats to $O_3$ for precise periods during organogenesis. No significant teratogenic effects were found in rats exposed 8 h/day to concentrations of $O_3$ varying from 0.44 to 1.97 ppm during early (days 6-9), mid (days 9-12), or late (days 17 to 20) gestation, or the entire period of organogenesis (days 6-15) (short-term exposures). Earlier research found eyelid malformation following gestational and postnatal exposure to 0.2 ppm $O_3$ (Veninga, 1967).

Table 7-8 provides a brief overview of the epidemiologic studies of birth defects. These studies have focused on cardiac and oral cleft defects, and the results from these studies are not entirely consistent. This inconsistency could be due to the absence of true associations between $O_3$ and risks of cardiovascular malformations and oral cleft defects; it could also be due to differences in populations, pollution levels, outcome definitions, or analytical approaches. The lack of consistency of associations between $O_3$ and cardiovascular malformations or oral cleft defects might be due to issues relating to statistical power or measurement error. A recent meta-analysis of air pollution and congenital anomalies concluded that there was no statistically significant increase in risk of congenital anomalies with $O_3$ exposure (Vrijheid et al., 2011). These authors note that heterogeneity in the results of these studies may be due to inherent differences in study location, study design, and/or analytic methods, and comment that these studies have not employed some recent advances in exposure assessment used in other areas of air pollution research that may help refine or reduce this heterogeneity.

**Table 7-8 Brief summary of epidemiologic studies of birth defects.**

| Study | Outcomes Examined | Location (Sample Size) | Mean $O_3$ (ppb) | Exposure Assessment | Exposure Window |
|---|---|---|---|---|---|
| Ritz et al. (2002) | Cardiac and Cleft Defects | Southern California (n = 3,549 cases; 10,649 controls) | 24-h avg: NR | Nearest Monitor (within 10 mi) | Month 1,2,3<br>Trimester 2,3<br>3-mo period prior to conception |
| Gilboa et al. (2005) | Cardiac and Cleft Defects | 7 Counties in TX (n = 5,338 cases; 4,580 controls) | 24-h avg: NR | Nearest Monitor | Weeks 3-8 of gestation |
| Hwang and Jaakkola (2008) | Oral Cleft Defects | Taiwan (n = 653 cases; 6,530 controls) | 24-h avg: 27.31 | Inverse Distance Weighting (IDW) | Months 1,2,3 |
| Strickland et al. (2009) | Cardiac Defects | Atlanta, GA (n = 3,338 cases) | 8-h max: 39.8-43.3 | Weighted citywide avg | Weeks 3-7 of gestation |
| Hansen et al. (2009) | Cardiac and Cleft Defects | Brisbane, Australia (n = 150,308 births) | 8-h max: 25.8 | Nearest Monitor | Weeks 3-8 of gestation |
| Marshall et al. (2010) | Oral Cleft Defects | New Jersey (n = 717 cases; 12,925 controls) | 24-h avg: 25 | Nearest Monitor (within 40 km) | Weeks 5-10 of gestation |
| Dadvand et al. (2011) | Cardiac Defects | Northeast England (n = 2,140 cases; 14,256 controls) | 24-h avg: 18.8 | Nearest Monitor | Weeks 3-8 of gestation`1 |

### 7.4.8 Developmental Respiratory Effects

The issue of prenatal exposure has assumed increasing importance since ambient air pollution exposures of pregnant women have been shown to lead to adverse pregnancy outcomes, as well as to respiratory morbidity and mortality in the first year of life. Growth and development of the respiratory system take place mainly during the prenatal and early postnatal periods. This early developmental phase is thought to be very important in determining long-term lung growth. Studies have recently examined this emerging issue. Several studies were included in Section 7.2.1 and Section 7.2.3, and are included here because they reported both prenatal and post-natal exposure periods.

Mortimer et al. (2008a, b) examined the association of prenatal and lifetime exposures to air pollutants with pulmonary function and allergen sensitization in a subset of asthmatic children (ages 6-11) included in the Fresno Asthmatic Children's Environment Study (FACES). Monthly means of pollutant levels for the years 1989-2000 were created and averaged separately across several important developmental time-periods, including the entire pregnancy, each trimester, the first 3 years of life, the first 6 years of life, and the entire lifetime. The 8-h avg $O_3$ concentrations were approximately 50 ppb for each of the exposure metrics

(estimated from figure). In the first analysis (Mortimer et al., 2008a), negative effects on pulmonary function were found for exposure to $PM_{10}$, $NO_2$, and CO during key neonatal and early life developmental periods. The authors did not find a negative effect of exposure to $O_3$ among this cohort. In the second analysis (Mortimer et al., 2008b), sensitization to at least one allergen was associated, in general, with higher levels of CO and $PM_{10}$ during the entire pregnancy and second trimester and higher $PM_{10}$ during the first 2 years of life. Lower exposure to $O_3$ during the entire pregnancy or second trimester was associated with an increased risk of allergen sensitization. Although the pollutant metrics across time periods are correlated, the strongest associations with the outcomes were observed for prenatal exposures. Though it may be difficult to disentangle the effect of prenatal and postnatal exposures, the models from this group of studies suggest that each time period of exposure may contribute independently to different dimensions of school-aged children's pulmonary function. For 4 of the 8 pulmonary-function measures (FVC, $FEV_1$, PEF, $FEF_{25-75}$), prenatal exposures were more influential on pulmonary function than early-lifetime metrics, while, in contrast, the ratio of measures ($FEV_1$/FVC and $FEF_{25-75}$/FVC) were most influenced by postnatal exposures. When lifetime metrics were considered alone, or in combination with the prenatal metrics, the lifetime measures were not associated with any of the outcomes, suggesting the timing of the exposure may be more important than the overall dose and prenatal exposures are not just markers for lifetime or current exposures.

Clark et al. (2010) investigated the effect of exposure to ambient air pollution in utero and during the first year of life on risk of subsequent asthma diagnosis (incident asthma diagnosis up to age 3-4) in a population-based nested case-control study. Air pollution exposure for each subject based on their residential address history was estimated using regulatory monitoring data, land use regression modeling, and proximity to stationary pollution sources. An average exposure was calculated for the duration of pregnancy (~15 ppb) and the first year of life (~14 ppb). In contrast to the Mortimer et al. (2008a, b) studies, the effect estimates for first-year exposure were generally larger than for in utero exposures. However, similar to the Mortimer et al. (2008a, b), the observed associations with $O_3$ were largely protective. Because of the relatively high correlation between in utero and first-year exposures for many pollutants, it was difficult to discern the relative importance of the individual exposure periods.

Latzin et al. (2009) examined whether prenatal exposure to air pollution was associated with lung function changes in the newborn. Tidal breathing, lung volume, ventilation inhomogeneity and eNO were measured in 241 unsedated, sleeping neonates (age = 5 weeks). The median of the 24-h avg $O_3$ concentrations averaged across the post-natal period was ~44 ppb. Consistent with the previous studies, no association was found for prenatal exposure to $O_3$ and lung function.

The new toxicological literature since the 2006 $O_3$ AQCD, covering respiratory changes related to developmental $O_3$ exposure, reports ultrastructural changes in bronchiole development, alterations in placental and pup cytokines, and increased pup airway hyper-reactivity. These studies are detailed below.

Fetal rat lung bronchiole development is triphasic, comprised of the glandular phase (measured at GD18), the canalicular phase (GD20), and the saccular phase (GD21). The ultrastructural lung development in fetuses of pregnant rats exposed to 1-ppm $O_3$ (12 h/day, out to either GD18, GD20 or GD21) was examined by electron microscopy during these three phases. In the glandular phase, bronchiolar columnar epithelial cells in fetuses of dams exposed to $O_3$ had cytoplasmic damage and swollen mitochondria. Bronchial epithelium at the canalicular phase in $O_3$ exposed pups had delayed maturation in differentiation, i.e., glycogen abundance in secretory cells had not diminished as it should with this phase of development. Congruent with this finding, delayed maturation of tracheal epithelium following early neonatal $O_3$ exposure (1 ppm, 4-5 h/day for first week of life) in lambs has been previously reported (Mariassy et al., 1990; Mariassy et al., 1989). Also at the canalicular phase, atypical cells were seen in the bronchiolar lumen of $O_3$-exposed rat fetuses. Finally, in the saccular phase, mitochondrial degradation was present in the non-ciliated bronchiolar cells of rats exposed in utero to $O_3$. In conclusion, $O_3$ exposure of pregnant rats produced ultra-structural damage to near-term fetal bronchiolar epithelium (López et al., 2008).

Exposure of laboratory animals to multiple airborne pollutants can differentially affect pup physiology. One study showed that exposure of C57BL/6 mouse dams to 0.48 mg PM intratracheally twice weekly for 3 weeks during pregnancy augmented $O_3$-induced airway hyper-reactivity in juvenile offspring. Maternal PM exposure also significantly increased placental cytokines above vehicle-instilled controls. Pup postnatal $O_3$ exposure (1 ppm 3 h/day, every other day, thrice weekly for 4 weeks) induced significantly increased cytokine levels (IL-1β, TNF-α, KC, and IL-6) in whole lung versus postnatal air exposed groups; this was further exacerbated with gestational PM exposure (Auten et al., 2009). In further studies by the same laboratory, $O_3$-induced AHR was studied in rodent offspring after dam gestational exposure to inhaled diesel exhaust (Auten et al., 2012). Pregnant C57Bl/6 mice were exposed to diesel exhaust GD9-17 (0.5 or 2.0 mg/m$^3$ $O_3$, 4h/day) via inhalation or in a separate set of animals via oropharyngeal aspiration of freshly generated DEPs (2×/week). Postnatally, the offspring were exposed to $O_3$ starting at PND2 (1 ppm $O_3$, 3 hours/day, 3 days/week for 4 weeks). Juvenile mice were then subjected to measurements of pulmonary mechanisms (at 4 weeks of age and then at 8 weeks of age). Increased inflammation of the placenta and lungs of DE exposed fetuses was reported at GD18. In animals with postnatal $O_3$ exposure alone, elevated inflammation was seen with significant increased levels of BAL cytokines; these $O_3$-related elevated levels were significantly exacerbated with prenatal DE exposure (DE+$O_3$). At PND28, DE+$O_3$ exposed offspring had significant impairment of alveolar development as measured with secondary alveolar crest development, a finding that was absent in all other exposure groups ($O_3$ alone, DE alone). Postnatal $O_3$ exposure induced AHR in methacholine challenged animals at 4 weeks of age and was exacerbated with the higher dose of DE exposure (DE+$O_3$). At 8 weeks of age, $O_3$ exposed pups had persistent AHR (+/-DE) that was significantly augmented in DE+$O_3$ pups. In summary, gestational DE exposure induced an inflammatory response which, when combined with postnatal $O_3$ exposure impaired alveolar

development, and caused an exacerbated and longer-lasting $O_3$-induced AHR in offspring.

A series of experiments using infant rhesus monkeys repeatedly exposed to 0.5 ppm $O_3$ starting at one-month of age have examined the effect of $O_3$ alone or in combination with an inhaled allergen on morphology and lung function (Plopper et al., 2007). Exposure to $O_3$ alone or allergen alone produced small but not statistically significant changes in baseline airway resistance and airway responsiveness, but the combined exposure to both $O_3$ + antigen produced statistically significant and greater than additive changes in both functional measurements. Additionally, cellular changes and significant structural changes in the respiratory tract have been observed in infant rhesus monkeys exposed to $O_3$ (Fanucchi et al., 2006). A more detailed description of these studies can be found in Section 7.2.3 (Pulmonary Structure and Function), with mechanistic information found in Section 5.4.2.4.

Lung immunological response in $O_3$ exposed pups was followed by analyzing BAL and lung tissue. Sprague Dawley (SD) pups were exposed to a single 3h exposure of air or $O_3$ (0.6 ppm) on PND 13 (Han et al., 2011). Bronchoalveolar lavage (BAL) was performed 10 hours after the end of $O_3$ exposure. BALF polymorphonuclear leukocytes (PMNs) and total BALF protein were significantly elevated in $O_3$ exposed pups. Lung tissue from $O_3$ exposed pups had significant elevations of manganese superoxide dismutase (SOD) protein and significant decrements of extra-cellular SOD protein.

Various immunological outcomes were followed in offspring after their pregnant dams (BALB/c mice) were exposed gestationally to $O_3$ (0, 0.4, 0.8, or 1.2 ppm, GD9-18) (Sharkhuu et al., 2011). Delayed type hypersensitivity (DTH) was initiated with initial BSA injection at 6 weeks of age and then challenge 7 days later. The normal edematous response of the exposed footpad (thickness after BSA injection) was recorded as an indicator of DTH. In female offspring, normal footpad swelling with BSA injection that was seen in air exposed animals was significantly attenuated with $O_3$ exposure (0.8 and 1.2 ppm $O_3$), implying immune suppression of $O_3$ exposure specifically in DTH. Humoral immunity was measured with the sheep red blood cell (SRBC) response. Animals received primary immunization with SRBC and then blood was drawn for SRBC IgM measurement. A SRBC booster was given 2 weeks later with blood collected 5 days after booster for IgG measurement. Maternal $O_3$ exposure had no effect on humoral immunity in the offspring as measured by IgG and IgM titers after SRBC primary and booster immunizations (Sharkhuu et al., 2011).

Toxicity assessment and allergen sensitization was also assessed in these $O_3$ exposed offspring. At PND42, animals were euthanized for analysis of immune and inflammatory markers (immune proteins, inflammatory cells, T-cell populations in the spleen). A subset of the animals was intra-nasally instilled or sensitized with ovalbumin on either PND2 and 3 or PND42 and 43. All animals were challenged with OVA on PND54, 55, and 56. One day after final OVA challenge, lung function, lung inflammation and immune response were determined. Offspring of $O_3$ exposed dams that were initially sensitized at PDN3 (early) or PND42 (late) were tested to

determine the level of allergic sensitization or asthma-like inflammation after OVA challenge. Female offspring sensitized early in life developed significant eosinophilia (1.2 ppm $O_3$) and elevated serum OVA-specific IgE (1.2 ppm $O_3$), which is a marker of airway allergic inflammation. The females that were sensitized early also had significant decrements in BALF total cells, macrophages, and lymphocytes (1.2 ppm $O_3$). Offspring that were sensitized later (PND42) in life did not develop the aforementioned changes in BALF, but these animals did develop modest, albeit significant neutropenia (0.8 and 1.2 ppm $O_3$) (Sharkhuu et al., 2011).

BALF cytology in non-sensitized animals was followed. BALF of offspring born to dams exposed to $O_3$ was relatively unaffected (cytokines, inflammatory cell numbers/types) as were splenic T-cell subpopulations. LDH was significantly elevated in BALF of females whose mothers were exposed to 1.2 ppm during pregnancy (Sharkhuu et al., 2011). In summary, the females born to mothers exposed to $O_3$ developed modest immunocompromise. Males were unaffected (Sharkhuu et al., 2011).

Overall, animal toxicological studies have reported ultrastructural changes in bronchiole development, alterations in placental and pup cytokines, and increased pup airway hyper-reactivity related to exposure to $O_3$ during the developmental period. Epidemiologic studies have found no association between prenatal exposure to $O_3$ and growth and development of the respiratory system. Fetal origins of disease have received a lot of attention recently, thus additional research to further explore the inconsistencies between these two lines of evidence is warranted.

## 7.4.9 Developmental Central Nervous System Effects

The following sections describe the results of toxicological studies of $O_3$ and developmental central nervous system effects. No epidemiologic studies of this association have been published.

### 7.4.9.1 Laterality

Two reports of laterality changes in mice developmentally exposed to $O_3$ have been reported in the literature. Mice developmentally exposed to 0.6 ppm $O_3$ (6 days before breeding to weaning at PND21) showed a turning preference (left turns) distinct from air exposed controls (clockwise turns) (Dell'Omo et al., 1995); in previous studies this behavior in mice has been found to correlate with specific structural asymmetries of the hippocampal mossy fiber projections (Schöpke et al., 1991). The 2006 $O_3$ AQCD evidence for the effect of $O_3$ on laterality or handedness demonstrated that rats exposed to $O_3$ during fetal and neonatal life showed limited, sex-specific changes in handedness after exposure to the intermediate concentration of $O_3$ (only seen in female mice exposed to 0.6 ppm $O_3$, and not in males at 0.6 ppm

or in either sex of 0.3 or 0.9 ppm $O_3$ with exposure from 6 days before breeding to PND26) (Petruzzi et al., 1999).

### 7.4.9.2 Brain Morphology and Neurochemical Changes

The nucleus tractus solitarius (NTS), a medullary area of respiratory control, of adult animals exposed prenatally to 0.5 ppm $O_3$ (12h/day, ED5-eD20) had significantly less tyrosine hydroxylase staining versus control (Boussouar et al., 2009). Tyrosine hydroxylase is the rate-limiting enzyme for dopamine synthesis and serves as a precursor for catecholamine synthesis; thus, decreased staining is used as a marker of dopaminergic or catecholaminergic cell or activity loss in these regions and thus functions in neuronal plasticity. After physical restraint stress, control animals respond at the histological level with Fos activation, a marker of neuronal activity, and tyrosine hydroxylase activation in the NTS, a response which is absent or attenuated in adult animals exposed prenatally to 0.5 ppm $O_3$ (Boussouar et al., 2009) when compared to control air exposed animals who also were restrained. The $O_3$-exposed offspring in this study were cross-fostered to control air exposed dams to avoid $O_3$-dependent dam related neonatal effects on offspring outcomes (i.e., dam behavioral or lactational contributions to pup outcomes) (Boussouar et al., 2009).

Developmental exposure to 0.3 or 0.6 ppm $O_3$ prior to mating pair formation through GD17 induced significant increased levels of BDNF in the striatum of adult (PND140) $O_3$-exposed offspring as compared to control air exposed animals; these $O_3$-exposed animals also had significantly decreased level of NGF in the hippocampus versus control (Santucci et al., 2006).

Changes in the pup cerebellum with prenatal 1 ppm $O_3$ exposure include altered morphology (Romero-Velazquez et al., 2002; Rivas-Manzano and Paz, 1999), decreased total area (Romero-Velazquez et al., 2002), decreased number of Purkinje cells (Romero-Velazquez et al., 2002), and altered monoamine neurotransmitter content with the catecholamine system affected and the indoleamine system unaffected by $O_3$ (Gonzalez-Pina et al., 2008).

### 7.4.9.3 Neurobehavioral Outcomes

Ozone administration to dams during pregnancy with or without early neonatal exposure has been shown to contribute to multiple neurobehavioral outcomes in offspring that are described in further detail below.

Ozone administration (0.4, 0.8 or 1.2 ppm $O_3$) during the majority of pregnancy (PD7-17) of CD-1 mice did not affect pup behavioral outcomes including early behavioral ultrasonic vocalizations and more permanent later measurements (PND60 or 61) including pup activity, habituation and exploration and d-

amphetamine-induced hyperactivity (Bignami et al., 1994); these pups were all cross-fostered or reared on non- $O_3$ exposed dams.

Testing for aggressive behavior in mice continuously exposed to $O_3$ (0.3 or 0.6 ppm from 30 days prior to mating to GD17) revealed that mice had significantly increased defensive/ submissive behavior (increased freezing posturing on the first day only of a multiple-day exam) versus air exposed controls (Santucci et al., 2006). Similarly, continuous exposure of adult animals to $O_3$ induced significant increases in fear behavior and decreased aggression as measured by significantly decreased freezing behavior (Petruzzi et al., 1995).

Developmentally exposed animals also had significantly decreased amount of time spent nose sniffing other mice (Santucci et al., 2006); this social behavior deficit, decreased sniffing time, was not found in an earlier study with similar exposures (Petruzzi et al., 1995), but sniffing of specific body areas was measured in Santucci et al. (2006) and total number of sniffs of the entire body was measured in Petruzzi et al. (1995). The two toxicology studies exploring social behavior (sniffing) employ different study designs and find opposite effects in animals exposed to $O_3$.

#### 7.4.9.4 Sleep Aberrations after Developmental Ozone Exposure

The effect of gestational $O_3$ exposure (1 ppm $O_3$ daily for 12h/day, during dark period for the entire pregnancy) on sleep patterns in rat offspring was followed using 24 h polysomnographic recordings at 30, 60 and 90 days of age (Haro and Paz, 1993). Ozone-exposed pups manifested with inverted sleep-wake patterns or circadian rhythm phase-shift. Rat vigilance was characterized in wakefulness, slow wave sleep (SWS), and paradoxical sleep (PS) using previously characterized criteria. The $O_3$ exposed offspring spent longer time in the wakefulness state during the light period, more time in SWS during the period of darkness, and showed significant decrements in PS. Chronic $O_3$ inhalation significantly decreased the duration of PS during both the light and dark periods (Haro and Paz, 1993). These effects were consistent at all time periods measured (30, 60 and 90 days of age). These sleep effects reported after developmental exposures expand upon the existing literature on sleep aberrations in adult animals exposed to $O_3$ [rodents: (Paz and Huitron-Resendiz, 1996; Arito et al., 1992); and cats: (Paz and Bazan-Perkins, 1992)]. A role for inhibition of cyclooxygenase-2 and the interleukins and prostaglandins in the $O_3$-dependent sleep changes potentially exists with evidence from a publication on indomethacin pretreatment attenuating $O_3$-induced sleep aberrations in adult male animals (Rubio and Paz, 2003).

### 7.4.10 Early Life Mortality

Infants may be particularly at risk for the effects of air pollution. Within the first year of life, infants develop rapidly; therefore their sensitivity may change within weeks

or months. During the neonatal and post-neonatal periods, the developing lung is highly sensitive to environmental toxicants. The lung is not well developed at birth, with 80% of alveoli being formed postnatally. An important question regarding the association between $O_3$ and infant mortality is the critical window of exposure during development for which infants are at risk. Several age intervals have been explored: neonatal (<1 month); postneonatal (1 month to 1 year); and an overall interval for infants that includes both the neonatal and postneonatal periods (<1 year). Within these various age categories, multiple causes of deaths have been investigated, particularly total deaths and respiratory-related deaths. The studies reflect a variety of study designs, exposure periods, regions, and adjustment for confounders. As discussed below, a handful of studies have examined the effect of ambient air pollution on neonatal and postneonatal mortality, with the former the least studied. These studies varied somewhat with regard to the outcomes and exposure periods examined and study designs employed.

#### 7.4.10.1 Stillbirth

Pereira et al. (1998) investigated the association among daily counts of intrauterine mortality (over 28 weeks of gestation) and air pollutant concentrations in Sao Paulo, Brazil from 1991 through 1992. The association was strong for $NO_2$, but lesser for $SO_2$ and CO. These associations exhibited a short lag time, less than 5 days. No significant association was detected between short-term $O_3$ exposure and intrauterine mortality.

#### 7.4.10.2 Infant Mortality, Less than 1 Year

Ritz et al. (2006) linked birth and death certificates for infants who died between 1989 and 2000 to evaluate the influence of outdoor air pollution on infant death in the South Coast Air Basin of California. The authors examined short- and long-term exposure periods 2 weeks, 1 month, 2 months, and 6 months before each case subject's death and reported no association between ambient levels of $O_3$ and infant mortality. Similarly, Diaz et al. (2004) analyzed the effects of extreme temperatures and short-term exposure to air pollutants on daily mortality in children less than 1 year of age in Madrid, Spain, from 1986 to 1997 and observed no statistically significant association between mortality and $O_3$ concentrations. Hajat et al. (2007) analyzed time-series data of daily infant mortality counts in 10 major cities in the UK to quantify any associations with short-term changes in air pollution. When the results from the 10 cities were combined there was no relationship between $O_3$ and infant mortality, even after restricting the analysis to just the summer months.

Conversely, a time-series study of infant mortality conducted in the southwestern part of Mexico City in the years 1993-1995 found that infant mortality was associated with short-term exposure to $NO_2$ and $O_3$ 3-5 days before death, but not as consistently as with PM. A 10-ppb increase in 24-h avg $O_3$ was associated with a

2.78% increase (95% CI: 0.29, 5.26%) in infant mortality (lag 3) (Loomis et al., 1999). This increase was attenuated, although still positive when evaluated in a two-pollutant model with $PM_{2.5}$. One-hour max concentrations of $O_3$ exceeded prevailing Mexican and international standards nearly every day.

#### 7.4.10.3 Neonatal Mortality, Less than 1 Month

Several studies have evaluated ambient $O_3$ concentrations and neonatal mortality and observed no association. Ritz et al. (2006) linked birth and death certificates for infants who died between 1989 and 2000 to evaluate the influence of outdoor air pollution on infant death in the South Coast Air Basin of California. The authors examined short- and long-term exposure periods 2 weeks, 1 month, 2 months, and 6 months before each case subject's death and reported no association between ambient levels of $O_3$ and neonatal mortality. Hajat et al. (2007) analyzed time-series data of daily infant mortality counts in 10 major cities in the UK to quantify any associations with short-term changes in air pollution. When the results from the 10 cities were combined there was no relationship between $O_3$ and neonatal mortality, even after restricting the analysis to just the summer months. Lin et al. (2004a) assessed the impact of short-term changes in air pollutants on the number of daily neonatal deaths in Sao Paulo, Brazil. The authors observed no association between ambient levels of $O_3$ and neonatal mortality.

#### 7.4.10.4 Postneonatal Mortality, 1 Month to 1 Year

A number of studies focused on the postneonatal period when examining the effects of $O_3$ on infant mortality. Ritz et al. (2006) linked birth and death certificates for infants who died between 1989 and 2000 to evaluate the influence of outdoor air pollution on infant death in the South Coast Air Basin of California. The authors examined short- and long-term exposure periods 2 weeks, 1 month, 2 months, and 6 months before each case subject's death and reported no association between ambient levels of $O_3$ and postneonatal mortality. Woodruff et al. (2008) evaluated the county-level relationship between cause-specific postneonatal infant mortality and long-term early-life exposure (first 2 months of life) to air pollutants across the United States. Similarly, they found no association between $O_3$ exposure and deaths from respiratory causes. In the U.K., Hajat et al. (2007) analyzed time-series data of daily infant mortality counts in 10 major cities to quantify any associations with short-term changes in air pollution. When the results from the 10 cities were combined there was no relationship between $O_3$ and postneonatal mortality, even after restricting the analysis to just the summer months. In Ciudad Juarez, Mexico, Romieu et al. (2004a) examined the daily number of deaths between 1997 and 2001, estimating the modifying effect of SES on the risk of postneonatal mortality. Ambient $O_3$ concentrations were not related to infant mortality overall, or in any of the SES groups. In a follow-up study, Carbajal-Arroyo et al. (2011) evaluated the

relationship of 1-h daily max $O_3$ levels with postneonatal infant mortality in the Mexico City Metropolitan Area between 1997 and 2005. Generally, short-term exposure to $O_3$ was not significantly related to infant mortality. However, upon estimating the modifying effect of SES on the risk of postneonatal mortality, the authors found that $O_3$ was statistically significantly related to respiratory mortality among those with low SES. In a separate analysis, the effect of $PM_{10}$ was evaluated with $O_3$ level quartiles. $PM_{10}$ alone was related to a significant increase in all-cause mortality. The magnitude of this effect remained the same when only the days when $O_3$ was in the lowest quartile were included in the analyses. However, when only the days when $O_3$ was in the highest quartile were included in the analyses, the magnitude of the $PM_{10}$ effect increased dramatically (OR = 1.06 [95% CI: 0.909, 1.241] for $PM_{10}$ on days with $O_3$ in lowest quartile; OR = 1.26 [95% CI: 1.08, 1.47] for $PM_{10}$ on days with $O_3$ in the highest quartile. These results suggest that while $O_3$ alone may not have an effect on infant mortality, it may serve to potentiate the observed effect of $PM_{10}$ on infant mortality.

Tsai et al. (2006) used a case-crossover analysis to examine the relationship between short-term exposure to air pollution and postneonatal mortality in Kaohsiung, Taiwan during the period 1994-2000. The risk of postneonatal deaths was 1.023 (95% CI: 0.564, 1.858) per 10-ppb increase in 24-h avg $O_3$. The confidence interval for this effect estimate is very wide, likely due to the small number of infants that died each day, making it difficult to interpret this result. Several other studies conducted in Asia did not find any association between $O_3$ concentrations and infant mortality in the postneonatal period. Ha et al. (2003) conducted a daily time-series study in Seoul, Korea to evaluate the effect of short-term changes in ambient 8-h $O_3$ concentrations on postneonatal mortality. Son et al. (2008) examined the relationship between air pollution and postneonatal mortality from all causes among firstborn infants in Seoul, Korea during 1999-2003. Yang et al. (2006) used a case-crossover analysis to examine the relationship between air pollution exposure and postneonatal mortality in Taipei, Taiwan for the period 1994-2000. The authors observed no associations between ambient levels of $O_3$ and postneonatal mortality.

#### 7.4.10.5 Sudden Infant Death Syndrome

The strongest evidence for an association between ambient $O_3$ concentrations and SIDS comes from a study that evaluated the county-level relationship between SIDS and long-term early-life exposure (first 2 months of life) to air pollutants across the U.S. (Woodruff et al., 2008). The authors observed a 1.20 (95% CI: 1.09, 1.32) odds ratio for a 10-ppb increase in $O_3$ and deaths from SIDS. There was a monotonic increase in odds of SIDS for each quartile of $O_3$ exposure compared with the lowest quartile (highest quartile OR = 1.51; [95% CI: 1.17, 1.96]). In a multipollutant model including $PM_{10}$ or $PM_{2.5}$, CO and $SO_2$, the OR for SIDS and $O_3$ was not substantially lower than that found in the single-pollutant model. When examined by season, the relationship between SIDS deaths and $O_3$ was generally consistent across seasons with a slight increase for those babies born in the summer. When stratified

by birth weight, the OR for LBW babies was 1.27 (95% CI: 0.95, 1.69) per 10-ppb increase in $O_3$ and the OR for normal weight babies was 1.16 (95% CI: 1.01, 1.32) per 10-ppb increase in $O_3$.

Conversely, two additional studies reported no association between ambient levels of $O_3$ and SIDS. Ritz et al. (2006) linked birth and death certificates for infants who died between 1989 and 2000 to evaluate the influence of outdoor air pollution on infant death in the South Coast Air Basin of California. The authors examined short- and long-term exposure periods 2 weeks, 1 month, 2 months, and 6 months before each case subject's death and reported no association between ambient levels of $O_3$ and SIDS. Dales et al. (2004) used time-series analyses to compare the daily mortality rates for SIDS and short-term air pollution concentrations in 12 Canadian cities during the period of 1984-1999. Increased daily rates of SIDS were associated with previous day increases in the levels of $SO_2$, $NO_2$, and CO, but not $O_3$ or $PM_{2.5}$.

Table 7-9 provides a brief overview of the epidemiologic studies of infant mortality. These studies have focused on short-term exposure windows (e.g., 1-3 days) and long-term exposure windows (e.g., up to 6 months). Collectively, they provide no evidence for an association between ambient $O_3$ concentrations and infant mortality.

**Table 7-9 Brief summary of infant mortality studies.**

| Study | Location | Mean $O_3$ (ppb) | Exposure Assessment | Effect Estimate[a] (95% CI): |
|---|---|---|---|---|
| Pereira et al. (1998) | Sao Paulo, Brazil | 1-h max: 33.8 | Citywide avg | L0-2: 1.00 (0.99, 1.01) |
| Diaz et al. (2004) | Madrid, Spain | 24-h avg: 11.4 | Citywide avg | NR |
| Loomis et al. (1999) | Mexico City, Mexico | 24-h avg: 44.1<br>1-h max: 163.5 | 1 monitor | L0: 0.99 (0.97, 1.02)<br>L1: 0.99 (0.96, 1.01)<br>L2: 1.00 (0.98, 1.03)<br>L3: 1.03 (1.00, 1.05)<br>L4: 1.01 (0.98, 1.03)<br>L5: 1.02 (0.99, 1.04)<br>L0-2: 1.02 (0.99, 1.05) |
| Ritz et al. (2006) | Southern California | 24-h avg: 21.9-22.1 | Nearest Monitor | 2 weeks before death: 1.03 (0.93, 1.14)<br>1 mo before death: NR<br>2 mo before death: 0.93 (0.89, 0.97)<br>6 mo before death: NR |
| Hajat et al. (2007) | 10 Cities in the UK | 24-h avg: 20.5-42.6 | Citywide avg | L0-2: 1.00 (0.96, 1.06) |
| Lin et al. (2004a) | Sao Paulo, Brazil | 24-h avg: 38.06 | Citywide avg | L0: 1.00 (0.99, 1.01) |

| Study | Location | Mean $O_3$ (ppb) | Exposure Assessment | Effect Estimate[a] (95% CI): |
|---|---|---|---|---|
| Ha et al. (2003) | Seoul, South Korea | 8-h avg: 21.2 | Citywide avg | L0: 0.93 (0.90, 0.96) |
| Romieu et al. (2004a) | Ciudad Juarez, Mexico | 8-h avg: 43.43-55.12 | Citywide avg | L1: 0.96 (0.90, 1.03)<br>L2: 0.97 (0.91, 1.04)<br>L0-1 cum: 0.96 (0.89, 1.04)<br>L0-2 cum: 0.94 (0.87, 1.02) |
| Carbajal-Arroyo et al. (2011) | Mexico City, Mexico | 1-h max: 103.0 | Citywide avg | L0: 1.00 (0.99, 1.00)<br>L1: 0.99 (0.99, 0.99)<br>L2: 0.99 (0.99, 1.00)<br>L0-2: 0.99 (0.99, 1.00) |
| Son et al. (2008) | Seoul, South Korea | 8-h avg: 25.61 | Citywide avg | L(NR): 0.984 (0.976, 0.992)[b] |
| Tsai et al. (2006) | Kaohsiung, Taiwan | 24-h avg: 23.60 | Citywide avg | L0-2 cum: 1.02 (0.56, 1.86) |
| Woodruff et al. (2008) | Nationwide, U.S. | 24-h avg: 26.6 | County wide avg | First 2 mo of life: 1.04 (0.98, 1.10) |
| Yang et al. (2006) | Taipei, Taiwan | 24-h avg: 18.14 | Citywide avg | L0-2 cum:1.00 (0.62, 1.61) |
| Dales et al. (2004) | 12 Canadian cities | 24-h: 31.77 | Citywide avg | L0: NR<br>L1: NR<br>L2: NR<br>L3: NR<br>L4: NR<br>L5: NR<br>Multiday lags of 2-6 days: NR |

[a]Relative risk of infant mortality per 10 ppb change in $O_3$

[b]No increment provided

L0 = Lag 0, L1= Lag 1, L2 = Lag 2, L3 = Lag 3, L4 = Lag 4, L5 = Lag 5, L6 = Lag 6

NR: No quantitative results reported

## Table 7-10 Summary of key reproductive and developmental toxicological studies.

| Study | Model | $O_3$ (ppm) | Exposure Duration | Effects |
|---|---|---|---|---|
| Sharkhuu et al. (2011) | Pregnant mice; BALB/c; F; GD9-18; effects in offspring | 0.4, 0.8, or 1.2 | Continuously for 10 consecutive days | Dams: Decreased number of dams reaching parturition. Offspring: (1)-Decreased birth weights. (2)-Decreased rate of postnatal growth (body weight). (3)-impaired delayed type hypersensitivity.(4)-No effect on humoral immunity. (5)-Significantly affected allergic airway inflammation markers (eosinophilia, IgE) in female offspring sensitized early in life. 6-BALF LDH significantly elevated in female offspring. |
| Bignami et al. (1994) | Pregnant CD-1 dams (PD7-17) | 0.4, 0.8 or 1.2 | Continuous | Reproductive success was not affected by $O_3$ exposure (PD7-17, proportion of successful pregnancies, litter size, ex ratio, frequency of still birth, or neonatal mortality). Ozone acted as a transient anorexigen in pregnant dams. |
| Haro and Paz (1993) | Rat dams, Exposure over the entirety of pregnancy; | 1.0 | 12h/day during dark cycle | Decreased birth weight and postnatal body weight of offspring out to PND 90. Ozone-exposed pups manifested with inverted sleep-wake patterns or circadian rhythm phase-shift. |
| López et al. (2008) | Rats; Pregnant dams; GD1-GD18, GD20, or GD21. | 1.0 | (12 h/day, out to either GD18, GD20 or GD21) | $O_3$ induced delayed maturation of near term rodent bronchioles, with ultra-structural damage to bronchiolar epithelium. |
| Auten et al. (2009) | C57BL/6 mouse pups | 1.0 | 3 h/day, every other day, thrice weekly for 4 weeks | Postnatal $O_3$ exposure significantly increased lung inflammatory cytokine levels; this was further exacerbated with gestational PM exposure. |
| Plopper et al. (2007) | Infant rhesus monkeys | 0.5 | Postnatal, PND30-6month of age, 5 months of cyclic exposure, 5 days $O_3$ followed by 9 days of filtered air, 8h/day. | Non-significant increases airway resistance and airway responsiveness with $O_3$ or inhaled allergen alone. Allergen + $O_3$ produced additive changes in both measures. |
| Fanucchi et al. (2006) | Infant male Rhesus monkeys, post-natal exposure | 0.5 | 5 months of episodic exposure, age 1 month-age 6 months, 5 days $O_3$ followed by 9 days of filtered air, 8h/day. | Cellular changes and significant structural changes in the distal respiratory tract in infant rhesus monkeys exposed to $O_3$ postnatally. |
| Dell'Omo et al. (1995) | CD-1 Mouse dams and pups | 0.6 | 6 days before breeding to weaning at PND21 | Laterality changes in offspring: Ozone exposed pups showed a turning preference (left turns) distinct from air exposed controls (clockwise turns) as adults. |
| Santucci et al. (2006) | CD-1 Mouse dams | 0.3 or 0.6 | Dam exposure prior to mating through GD17. | Developmental $O_3$ caused increased defensive/submissive behavior in offspring. Ozone exposed offspring also had significant elevations of striatal BDNF and hippocampal NGF v. air exposed controls. |

| Study | Model | $O_3$ (ppm) | Exposure Duration | Effects |
|---|---|---|---|---|
| Han et al. (2011) | Rat; Sprague Dawley, M & F; PND13 | 0.6 | 3 h, BALF examined 10h after $O_3$ exposure | BALF polymorphonuclear leukocytes and total BALF protein were significantly elevated in $O_3$ exposed pups. Lung tissue from $O_3$ exposed pups had significant elevations of manganese superoxide dismutase (SOD) protein and significant decrements of extra-cellular SOD protein. |
| Campos-Bedolla et al. (2002) | Pregnant Rats; Sprague Dawley (GD5, GD10, or GD18) | 3.0 | 1 h on one day of gestation, uteri collected 16-18 h later | Ozone inhalation modifies the contractile response of the pregnant uterus. The $O_3$ exposed pregnant uteri had significant increases in the maximum response to acetyl choline stimulation at GD5 and 10; they also had a significant increase in maximal response to oxytocin at GD 5. |
| Kavlock et al. (1980) | CD-1 mice; (pregnancy day 7-17) | 0.4, 0.8 and 1.2 | Continuous, pregnancy day 7-17 | $O_3$ induced decrements in postnatal body weight gain. When $O_3$ was co-administered with sodium salicylate, $O_3$ synergistically increased the rate of pup resorption (1.0 ppm GD9-12). |
| Jedlinska-Krakowska et al. (2006) | 5 month old male Wistar Hannover rats | 3.0 | 0.5 ppm, 5h/day for 50 days | Histopathological evidence of impaired spermatogenesis (round spermatids/ 21 spermatocytes, giant spermatid cells, and focal epithelial desquamation with denudation to the 22 basement membrane). Vitamin E exposure concomitant with $O_3$ protected against pathological changes but Vitamin C did not. |

### 7.4.11 Summary and Causal Determination

The 2006 $O_3$ AQCD concluded that the limited number of studies that investigated $O_3$ demonstrated no associations between $O_3$ and birth outcomes, with the possible exception of birth defects. The current review included an expanded body of evidence on the associations between $O_3$ and reproductive and developmental effects. Recent epidemiologic and toxicological studies provide evidence for an effect of prenatal exposure to $O_3$ on pulmonary structure and function, including lung function changes in the newborn, incident asthma, ultrastructural changes in bronchiole development, alterations in placental and pup cytokines, and increased pup airway hyper-reactivity. Also, there is limited toxicological evidence for an effect of prenatal and early life exposure on central nervous system effects, including laterality, brain morphology, neurobehavioral abnormalities, and sleep aberration. Recent epidemiologic studies have begun to explore the effects of $O_3$ on sperm quality, and provide limited evidence for decrements in sperm concentration, while there is limited toxicological evidence for testicular degeneration associated with $O_3$.

While the collective evidence for many of the birth outcomes examined is generally inconsistent (including birth defects), there are several well-designed, well-conducted studies that indicate an association between $O_3$ and adverse outcomes. For example, as part of the southern California Children's Health Study, Salam et al. (2005)

observed a concentration-response relationship of decreasing birth weight with increasing $O_3$ concentrations averaged over the entire pregnancy that was clearest above the 30-ppb level (see Figure 7-4). Similarly, Hansen et al. (2008) utilized fetal ultrasonic measurements and found a change in ultrasound measurements associated with $O_3$ during days 31-60 of gestation indicated that increasing $O_3$ concentration decreased an ultrasound measurement for women living within 2 km of the monitoring site.

The weight of evidence does not indicate that prenatal or early life $O_3$ concentrations are associated with infant mortality. Collectively, there is limited though positive toxicological evidence for $O_3$-induced developmental effects, including effects on pulmonary structure and function and central nervous system effects. Limited epidemiologic evidence for an effect on prenatal $O_3$ exposure on respiratory development provides coherence with the effects observed in toxicological studies. There is also limited epidemiologic evidence for an association with $O_3$ concentration and decreased sperm concentration. A recent toxicological study provides limited evidence for a possible biological mechanism (histopathology showing impaired spermatogenesis) for such an association. Additionally, though the evidence for an association between $O_3$ concentrations and adverse birth outcomes is generally inconsistent, there are several influential studies that indicate an association with reduced birth weight and restricted fetal growth.

Some of the key challenges to interpretation of these study results include the difficulty in assessing exposure as most studies use existing monitoring networks to estimate individual exposure to ambient air pollution (see Section 4.6); the inability to control for potential confounders such as other risk factors that affect birth outcomes (e.g., smoking); evaluating the exposure window (e.g., trimester) of importance; integrating the results from both short- and long-term exposure periods; integrating the results across a variety of reproductive and developmental outcomes; and limited evidence on the physiological mechanism of these effects.

Taking into consideration the positive evidence for developmental and reproductive outcomes from toxicological and epidemiological studies, and the few influential birth outcome studies, the evidence **is suggestive of a causal relationship between exposures to $O_3$ and reproductive and developmental effects.**

## 7.5 Central Nervous System Effects

### 7.5.1 Effects on the Brain and Behavior

The 2006 $O_3$ AQCD (U.S. EPA, 2006b) included toxicological evidence that acute exposures to $O_3$ are associated with alterations in neurotransmitters, motor activity, short and long term memory, and sleep patterns. Additionally, histological signs of neurodegeneration have been observed. Reports of headache, dizziness, and irritation

of the nose with $O_3$ exposure are common complaints in humans, and some behavioral changes in animals may be related to these symptoms rather than indicative of neurotoxicity. Research in the area of $O_3$-induced neurotoxicity has notably increased over the past few years, and recent studies examining the effects of long-term exposure have demonstrated progressive damage in various regions of the brains of rodents in conjunction with altered behavior. Evidence from epidemiologic studies has been more limited. A recently published epidemiologic study examined the association between $O_3$ concentration and neurobehavioral effects. Chen and Schwartz (2009) utilized data from the NHANES III cohort to study the relationship between $O_3$ concentrations (mean annual $O_3$ concentration 26.5 ppb) and neurobehavioral effects among adults aged 20-59 years. Annual $O_3$ concentration was determined using inverse distance weighting for county of residence and adjacent counties (for more information on inverse distance weighting and other methods for exposure assessment, see Sections 4.5.1 and 4.6). The authors observed an association between annual $O_3$ concentration and tests measuring coding ability (symbol-digit substitution test) and attention/short-term memory (serial-digit learning test). Each 10-ppb increase in annual $O_3$ concentration corresponded to an aging-related cognitive performance decline of 3.5 yr for coding ability and 5.3 years for attention/short-term memory. These associations persisted in both crude and adjusted models. There was no association between $O_3$ concentration and reaction time tests. The authors concluded that overall, there is an association between long-term $O_3$ concentration and reduced performance on neurobehavioral tests.

A number of recent toxicological studies demonstrate various perturbations in neurologic function or histology with long-term exposure to $O_3$, including changes similar to those observed in neurodegenerative disorders such as Parkinson's and Alzheimer's disease pathologies in relevant regions of the brain (Table 7-11).
The central nervous system is very sensitive to oxidative stress, due in part to its high content of polyunsaturated fatty acids, high rate of oxygen consumption, and low antioxidant enzyme capacity. Oxidative stress has been identified as one of the pathophysiological mechanisms underlying neurodegenerative disease (Simonian and Coyle, 1996), and it is believed to play a role in altering hippocampal function, which causes cognitive deficits with aging (Vanguilder and Freeman, 2011).
A particularly common finding in studies of $O_3$-exposed rats is lipid peroxidation in the brain, especially in the hippocampus, which is important for higher cognitive function including contextual memory acquisition. Performance in passive avoidance learning tests is impaired when the hippocampus is injured. For example, in a subchronic study, exposure of rats to 0.25 ppm $O_3$ (4 h/day) for 15-90 days caused a complex array of responses, including a time-dependent increase in lipid peroxidation products and immunohistochemical changes in the hippocampus that were correlated with decrements in passive avoidance behavioral tests (Rivas-Arancibia et al., 2010). Changes included increased numbers of activated microglia, a sign of inflammation, and progressive neurodegeneration. Notably, continued exposure tends to bring about progressive, cumulative damage, as shown by this study (Rivas-Arancibia et al., 2010) and others (Santiago-López et al., 2010; Guevara-Guzmán et al., 2009; Angoa-Pérez et al., 2006). The effects of $O_3$ on passive avoidance test performance were particularly evident at 90 days for both

short- and long-term memory. The greatest extent of cell loss was also observed at this time point, whereas lipid peroxidation did not increase much beyond 60 days of exposure.

The substantia nigra is another region of the brain affected by $O_3$, and seems particularly sensitive to oxidative stress because the metabolism of dopamine, central to its function, is an oxidative process perturbed by redox imbalance. Oxidative stress has been implicated in the premature death of substantia nigra dopamine neurons in Parkinson's disease. Progressive damage has been found in the substantia nigra of male rats after 15, 30, and 60 days of exposure to 0.25 ppm $O_3$ for 4 h/day. Santiago-López et al. (2010) observed a reduction dopaminergic neurons within the substantia nigra over time, with a complete loss of normal morphology in the remaining cells and virtually no dopamine immunoreactivity at 60 days. This was accompanied by an increase in p53 levels and nuclear translocation, a process associated with programmed cell death. Similarly, Angoa-Pérez et al. (2006) have shown progressive lipoperoxidation in the substantia nigra and a decrease in nigral neurons in ovariectomized female rats exposed to 0.25 ppm $O_3$, 4h/day, for 7 - 60 days. Lipid peroxidation effectively doubled between the 30 and 60 day time points. Total nigral cell number was also diminished to the greatest extent at 60 days, and cell loss was particularly evident in the tyrosine hydroxylase positive cell population (90%), indicating a selective loss of dopamine neurons or a loss of dopamine pathway functionality.

The olfactory bulb also undergoes oxidative damage in $O_3$-exposed animals, in some cases altering olfactory-dependent behavior. Lipid peroxidation was observed in the olfactory bulbs of ovariectomized female rats exposed to 0.25 ppm $O_3$ (4 h/day) for 30 or 60 days (Guevara-Guzmán et al., 2009). Ozone also induced decrements in a selective olfactory recognition memory test, which were significantly greater at 60 days compared to 30 days, and the authors note that early deficits in odor perception and memory are components of human neurodegenerative diseases. The decrements in olfactory memory did not appear to be due to damaged olfactory perception based on other tests early on, but by 60 days deficits in olfactory perception had emerged.

Memory deficits and associated morphological changes can be attenuated by administration of α-tocopherol (Guerrero et al., 1999), taurine (Rivas-Arancibia et al., 2000), and estradiol (Guevara-Guzmán et al., 2009; Angoa-Pérez et al., 2006), all of which have antioxidant properties. In the study by Angoa-Pérez et al. (2006) described above, estradiol seemed particularly effective at protecting against lipid peroxidation and nigral cell loss at 60 days compared to shorter exposure durations. The same was true for amelioration of decrements in olfactory recognition memory (Guevara-Guzmán et al., 2009), although protection against lipid peroxidation was similar for the 30 and 60 day exposures.

CNS effects have also been demonstrated in adult mice whose only exposure to $O_3$ occurred while in utero, a period particularly critical for brain development. Santucci et al. (2006) investigated behavioral effects and gene expression after in utero exposure of mice to 0.3 or 0.6 ppm $O_3$. Exposure began 30 days prior to mating and

continued throughout gestation. Testing of adult animals demonstrated increased defensive/submissive behavior and reduced social investigation in both the 0.3 and 0.6 ppm $O_3$ groups. Changes in gene expression of brain-derived neurotrophic factor (BDNF, increased in striatum) and nerve growth factor (NGF, decreased in hippocampus) accompanied these behavioral changes. BDNF and NGF are involved in neuronal organization and the growth, maintenance, and survival of neurons during early development and in adulthood. This study and two others using short-term exposures demonstrate that CNS effects can occur as a result of in utero exposure to $O_3$, and although the mode of action of these effects is not known, it has been suggested that circulating lipid peroxidation products may play a role (Boussouar et al., 2009). Importantly, these CNS effects occurred in rodent models after in utero only exposure to (semi-) relevant concentrations of $O_3$.

**Table 7-11 Central nervous system effects of long-term $O_3$ exposure in rats.**

| Study | Model | $O_3$ (ppm) | Exposure Duration | Effects |
|---|---|---|---|---|
| Angoa-Pérez et al. (2006) | Rat; Wistar; F; Weight: 300 g; Ovariectomized | 0.25 | 7 to 60 days, 4 h/day, 5 days/week | Long-term estradiol treatment protected against $O_3$-induced oxidative damage to nigral dopamine neurons, lipid peroxidation, and loss of tyrosine hydrolase-immunopositive cells. |
| Guevara-Guzmán et al. (2009) | Rat; Wistar; F; Weight: 264 g; Ovariectomized | 0.25 | 30 and 60 days, 4h/day | Long-term estradiol treatment protected against $O_3$-induced oxidative stress and decreases in α and β estrogen receptors and dopamine β-hydroxlyase in olfactory bulb, and deficits in olfactory social recognition memory and chocolate recognition. |
| Rivas-Arancibia et al. (2010) | Rat; Wistar; M; Weight: 250-300 g | 0.25 | 15 to 90 days, 4h/day | Ozone produced significant increases in lipid peroxidation in the hippocampus, and altered the number of p53 positive immunoreactive cells, activated and phagocytic microglia, GFAP immunoreactive cells, double cortine cells, and short- and long-term memory-retention latency |
| Santiago-López et al. (2010) | Rat; Wistar; M; Weight: 250-300 g | 0.25 | 15, 30, and 60 days, 4 h/day | Progressive loss of dopamine reactivity in the substantia nigra, along with morphological changes. Increased p53 levels and nuclear translocation. |
| Santucci et al. (2006) | Mice; CD-1; M; 18 weeks old | 0.3; 0.6 | Females continuously exposed from 30 days prior to breeding until GD17 | Upon behavioral challenge with another male, there was a significant increase in defensive and freezing postures and decrease in the frequency of nose-sniffing. These behavioral changes were accompanied by a significant increase in BDNF in the striatum and a decrease of NGF in the hippocampus. |

### 7.5.2 Summary and Causal Determination

The 2006 $O_3$ AQCD included toxicological evidence that acute exposures to $O_3$ are associated with alterations in neurotransmitters, motor activity, short and long term memory, and sleep patterns. Additionally, histological signs of neurodegeneration have been observed. However, evidence regarding chronic exposure and neurobehavioral effects was not available. Recent research in the area of $O_3$-induced neurotoxicity has included several long-term exposure studies. Notably, the first

epidemiologic study to examine the relationship between $O_3$ exposure and neurobehavioral effects observed an association between annual $O_3$ levels and an aging-related cognitive performance decline in tests measuring coding ability and attention/short-term memory. This observation is supported by studies in rodents which demonstrate progressive oxidative stress and damage in the brain and associated decrements in behavioral tests, including those measuring memory, after subchronic exposure to 0.25 ppm $O_3$. Additionally, neurobehavioral changes are evident in animals whose only exposure to $O_3$ occurred in utero. Collectively, the limited epidemiologic and toxicological evidence is coherent and **suggestive of a causal relationship between $O_3$ exposure and CNS effects.**

## 7.6 Carcinogenic and Genotoxic Potential of Ozone

### 7.6.1 Introduction

The radiomimetic and clastogenic qualities of $O_3$, combined with its ability to stimulate proliferation of cells in the respiratory tract, have suggested that $O_3$ could act as a carcinogen. However, toxicological studies of tumorigenesis in the rodent lung have yielded mixed and often confusing results, and the epidemiologic evidence is equally conflicted. The 2006 $O_3$ AQCD concluded that, "the weight of evidence from recent animal toxicological studies and a very limited number of epidemiologic studies do not support ambient $O_3$ as a pulmonary carcinogen"[1] (U.S. EPA, 2006b).

Multiple epidemiologic studies reported in the 2006 $O_3$ AQCD examined the association between $O_3$ concentration and cancer. The largest of these studies, by Pope et al. (2002), included 500,000 adults from the American Cancer Society's (ACS) Cancer Prevention II study. In this study, no association was observed between $O_3$ concentration and lung cancer mortality. The Adventist Health Study of Smog (AHSMOG) also examined the association between $O_3$ concentration and lung cancer mortality (Abbey et al., 1999). There was a positive association between $O_3$ concentrations and lung cancer mortality among men. No association was reported for women. Another study using the AHSMOG cohort assessed the risk of incident lung cancer (Beeson et al., 1998). Among males, an association with incidence of lung cancer was observed with increasing $O_3$ concentrations. When stratified by smoking status, the association persisted among never smokers but was null for former smokers. No association was detected for females. The Six Cities Study examined various air pollutants and mortality but did not specifically explore the association between $O_3$ concentrations and lung cancer mortality due to low variability in $O_3$ concentrations across the cities (Dockery et al., 1993). An ecologic study performed in Sao Paulo City, Brazil examined the correlations between $O_3$ concentrations in four of the city districts and incident cancer of the larynx and lung

[1] The toxicological evidence is presented in detail in Table 6-18 on page 6-116 of the 1996 $O_3$ AQCD (U.S. EPA, 1996a) and Table AX5-13 on page AX5-43 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b).

reported in 1997 (Pereira et al., 2005). A correlation between the average number of days $O_3$ concentrations exceeded air quality standards from 1981 to 1990 and cancer incidence was present for larynx cancer but not for lung cancer.

Early toxicological research demonstrated lung adenoma[1] acceleration in mice with daily exposure to 1 ppm over 15 months (Stokinger, 1962). Later work demonstrated a significant increase in lung tumor numbers in one strain of mouse (A/J) but not another after exposure to 0.3-0.8 ppm $O_3$ (Last et al., 1987; Hassett et al., 1985). The A/J mouse strain is known to have a high incidence of spontaneous adenomas, and further studies using this strain found a statistically significant increase in lung tumor incidence after a 9-month exposure to 0.5 ppm and incidence and multiplicity after a 5 month exposure to 0.12 ppm with a 4-month recovery period (Witschi et al., 1999). However, these findings were discounted by the study authors due to the lack of a clear concentration-response, and results from the Hassett et al. (1985) and Last et al. (1987) studies were retrospectively deemed spurious based on what appeared to be unusually low spontaneous tumor incidences in the control groups (Witschi, 1991). A study of carcinogenicity of $O_3$ by the National Toxicology Program (NTP, 1994) reported increased incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in female B6C3F$_1$ mice exposed over 2 years to 1.0 ppm $O_3$, but not 0.12 or .5 ppm. No effect was detected in male mice. For a lifetime exposure to 0.5 or 1.0 ppm $O_3$, an increase in the number of female mice with adenomas (but not carcinomas or total neoplasms) was found. The number of total neoplasms was also unaffected in male mice, but there was a marginally increased incidence of carcinoma in males exposed to 0.5 and 1.0 ppm. Thus there was equivocal evidence of carcinogenic activity in male mice and some evidence of carcinogenic activity of $O_3$ in females. Experimental details of the NTP mouse study are available in Table 6-19 on page 6-121 (U.S. EPA, 1996o) of the 1996 $O_3$ AQCD (U.S. EPA, 1996a).

In Fischer-344/N rats (50 of each sex per group), neither a 2-year nor lifetime exposure to $O_3$ ranging from 0.12 to 1.0 ppm was found to be carcinogenic (Boorman et al., 1994; NTP, 1994). However, a marginally significant carcinogenic effect of 0.2 ppm $O_3$ was reported in a study of male Sprague-Dawley rats exposed for 6 months (n = 50) (Monchaux et al., 1996). These two studies also examined co-carcinogenicity of $O_3$ with NNK[2] (Boorman et al., 1994) or a relatively high dose of radon (Monchaux et al., 1996), finding no enhancement of NNK related tumors and a slight non-significant increase in tumor incidence after combined exposure with radon, respectively. Another study exploring co-carcinogenicity was conducted in hamsters. Not only was there no enhancement of chemically induced tumors in the peripheral lung or nasal cavity, but results suggested that $O_3$ could potentially delay or inhibit tumor development (Witschi et al., 1993). Thus there is no concrete evidence that $O_3$ can act as a co-carcinogen.

[1] NOTE: Although adenomas are benign, over time they may progress to become malignant, at which point they are called adenocarcinomas. Adenocarcinoma is the predominant lung cancer subtype in most countries, and is the only lung cancer found in nonsmokers. From page 8-33 of the 1970 $O_3$ AQCD: "No true lung cancers have been reported, however, from experimental exposures to either $O_3$ alone or any other combination or ingredient of photochemical oxidants."

[2] 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone

Immune surveillance is an important defense against cancer, and it should be noted that natural killer (NK) cells, which destroy tumor cells in the lung, appear to be inhibited by higher concentrations of $O_3$ and either unaffected or stimulated at lower concentrations (Section 6.2.5.4, Infection and Adaptive Immunity). This aspect of tumorigenesis adds yet another layer of complexity which may be reflected by conflicting results across studies.

The following sections will examine epidemiologic studies of cancer incidence and mortality and toxicological studies that have been published since the 2006 $O_3$ AQCD. An epidemiologic study has been published with cancer as the outcome; most epidemiologic studies examine markers of exposure.

## 7.6.2 Lung Cancer Incidence and Mortality

A recent re-analysis of the full ACS CPSII cohort by the Health Effects Institute is the only epidemiologic study that has explored the association between $O_3$ concentration and cancer mortality since the last $O_3$ AQCD. Krewski et al. (2009) conducted an extended follow-up of the cohort (1982-2000). Mean $O_3$ concentration [obtained from the Aerometric Information Retrieval System (AIRS) for 1980] were 22.91 ppb for the full year and 30.15 ppb for the summer months (April-September). No association was reported between lung cancer mortality and $O_3$ concentration (HR = 1.00 [95% CI: 0.96-1.04] per 10 ppb $O_3$). Additionally, no association was observed when the analysis was restricted to the summer months. There was also no association present in a sub-analysis of the cohort examining the relationship between $O_3$ concentration and lung cancer mortality in the Los Angeles area.

Since the 2006 $O_3$ AQCD, two toxicological studies have examined potential carcinogenicity of $O_3$ (Kim and Cho, 2009a, b). Looking across both studies, which used the same mouse strain as the National Toxicology Program study described above (NTP, 1994), 0.5 ppm $O_3$ alone or in conjunction with chemical tumor inducers did not enhance lung tumor incidence in males or females. However, a 10% incidence of oviductal carcinoma was observed in mice exposed to 0.5 ppm $O_3$ for 16 weeks. The implications of this observation are unclear, particularly in light of the lack of statistical information reported. Additionally, there is no mention of oviductal carcinoma after 32 weeks of exposure, and no oviductal carcinoma was observed after one year of exposure. The NTP study did not report any increase in tumors at extrapulmonary sites.

## 7.6.3 DNA Damage

The potential for genotoxic effects relating to $O_3$ exposure was predicted from the radiomimetic properties of $O_3$. The decomposition of $O_3$ in water produces OH and $HO_2$ radicals, the same species that are generally considered to be the biologically active products of ionizing radiation. Ozone has been observed to cause degradation

of DNA in a number of different models and bacterial strains. The toxic effects of $O_3$ have been generally assumed to be confined to the tissues directly in contact with the gas, such as the respiratory epithelium. Due to the highly reactive nature of $O_3$, little systemic absorption is predicted. Zelac et al. (1971a, b); however, reported a significant increase in chromosome aberrations in peripheral blood lymphocytes from Chinese hamsters exposed to 0.2 ppm for 5 hours. Other in vivo exposure studies found increased DNA strand breaks in respiratory cells from guinea pigs (Ferng et al., 1997) and mice (Bornholdt et al., 2002) but only with exposure to higher concentrations of $O_3$ (1 ppm for 72 hours and 1 or 2 ppm for 90 minutes, respectively). In other studies there were no observations of chromosomal aberrations in germ cells, but mutagenic effects have been seen in offspring of mice exposed to 0.2 ppm during gestation (blepharophimosis or dysplasia of the eyelids). The overall evidence for mutagenic activity from in vitro studies is positive, and in the National Toxicology Program report described above, $O_3$ was found to be mutagenic in Salmonella, with and without S9 metabolic activation. No recent toxicological studies of DNA damage have become available since the 2006 $O_3$ AQCD.

A number of epidemiologic studies looked at the association between $O_3$ and DNA and cellular level damages. These changes may be relevant to mechanisms leading to cancers development and serve as early indicators of elevated risk of mutagenicity.

Two studies performed in California examined cytogenetic damage in relation to $O_3$ exposures. Huen et al. (2006) examined cytogenetic damage among African American children and their mothers in Oakland, CA. Increased $O_3$ (mean monthly 8-h $O_3$ concentrations ranged from about 30 ppb in April to 14 ppb in November) was associated with increased cytogenetic damage (micronuclei frequency among lymphocytes and buccal cells) even after adjustment for household/personal smoking status and distance-weighted traffic density. Chen et al. (2006a) recruited college students at the University or California, Berkeley who reported never smoking and compared their levels of cytogenetic damage (micronuclei frequency from buccal cells) in the spring and fall. Cytogenetic damage was greater in the fall, which the authors attributed to the increase in $O_3$ over the summer. However, $O_3$ levels over 2, 7, 10, 14, or 30 days (concentrations not given) before collection of buccal cells did not correlate with cytogenetic damage. Estimated lifetime $O_3$ exposure was also not correlated with cytogenetic damage. Additionally, the authors exposed a subset of the students (n = 15) to 200 ppb $O_3$ for 4 hours while the students exercised intermittently. Ozone was found to be associated with an increase in cytogenetic damage in degenerated cells but not in normal cells 9-10 days after exposure. Increased cytogenetic damage was also noted in peripheral blood lymphocytes collected 18 hours after exposure.

A study performed in Mexico recruited 55 male workers working indoors (n = 27) or outdoors (n = 28) in Mexico City or Puebla, Mexico in order to study the relationship between $O_3$ and DNA damage (detected from peripheral blood samples using the Comet assay) (Tovalin et al., 2006). The median estimated daily $O_3$ concentrations were estimated to be 28.5 ppb for outdoor workers and 5.1 ppb for indoor workers in

Mexico City and 36.1 ppb for outdoor workers and 19.5 ppb for indoor workers in Puebla. Overall, a positive correlation between $O_3$ levels and DNA damage was observed. However, when examining the relationship by city and workplace, only DNA damage in outdoor workers in Mexico City remained correlated with $O_3$ levels.

Three studies examining the relationship between $O_3$ concentration and DNA-level damage have been performed in Europe. The largest of these studies was the GenAir case-control study, which was nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) study, and included individuals recruited between 1993 and 1998 from ten European countries. Only non-smokers (must not have smoked for at least 10 years prior to enrollment) were enrolled in the study. The researchers examined DNA adduct levels (DNA bonded to cancer-causing chemicals) and their relationship with $O_3$ concentrations (concentrations not given) (Peluso et al., 2005). A positive association was seen between DNA adduct levels and $O_3$ concentrations from 1990-1994 but not $O_3$ concentrations from 1995-1999. In adjusted analyses with DNA adduct levels dichotomized as high and low (detectable versus non-detectable), the OR was 1.97 (95% CI: 1.08, 3.58) when comparing the upper tertile of $O_3$ concentration to the lower two tertiles. Two other European studies were conducted in Florence, Italy. The most recent of these enrolled individuals from the EPIC study into a separate study between March and September of 1999 (Palli et al., 2009). The purpose of the study was to examine oxidative DNA damage (determined by Comet assay using blood lymphocytes) in association with varying periods of $O_3$ exposure. The researchers observed that longer periods of high $O_3$ concentrations (values not given) were more strongly correlated with oxidative DNA damage than shorter periods of time (i.e., the rho [p-value] was 0.26 [0.03] for 0-10 days and 0.35 [0.002] for 0-90 days). This correlation was stronger among men compared to women. The correlations for all time periods had p-values $<0.05$ for ex- and never-smokers. For current smokers, the correlation was only observed among time periods $\leq 25$ days. When adjusted for age, sex, smoking history, traffic pollution exposure, period of blood draw, and area of residence, the association between $O_3$ concentrations and oxidative DNA damage was positive for $O_3$ concentrations 0-60 days, 0-75 days, and 0-90 days prior to blood draw. Positive, statistically significant associations were not observed among shorter time periods. The other study performed in Florence recruited healthy volunteers who reported being non-smokers or light smokers (Giovannelli et al., 2006). The estimated $O_3$ concentrations during the study ranged from approximately 4-40 ppb for 3-day averages, 5-35 ppb for 7-day averages, and 7.5-32.5 ppb for 30-day averages. Ozone concentrations were correlated with DNA strand breaks (measured from blood lymphocytes) over longer exposure periods (p-value: 0.002 at 30 days, p-value: 0.04 at 7 days; p-value: 0.17 at 3 days). This association was robust to control for temperature, solar radiation, sex, and age. No association was seen between $O_3$ concentrations and measures of oxidative DNA damage at 3, 7, or 30 days.

### 7.6.4 Summary and Causal Determination

The 2006 $O_3$ AQCD reported that evidence did not support ambient $O_3$ as a pulmonary carcinogen. Since the 2006 $O_3$ AQCD, very few epidemiologic and toxicological studies have been published that examine $O_3$ as a carcinogen, but collectively, study results indicate that $O_3$ may contribute to DNA damage. Ozone concentrations in most epidemiologic studies were measured using air monitoring data. For more information on long-term exposure assessment, see Section 4.6.3.2. Overall, the evidence **is inadequate to determine if a causal relationship exists between ambient $O_3$ exposures and cancer.**

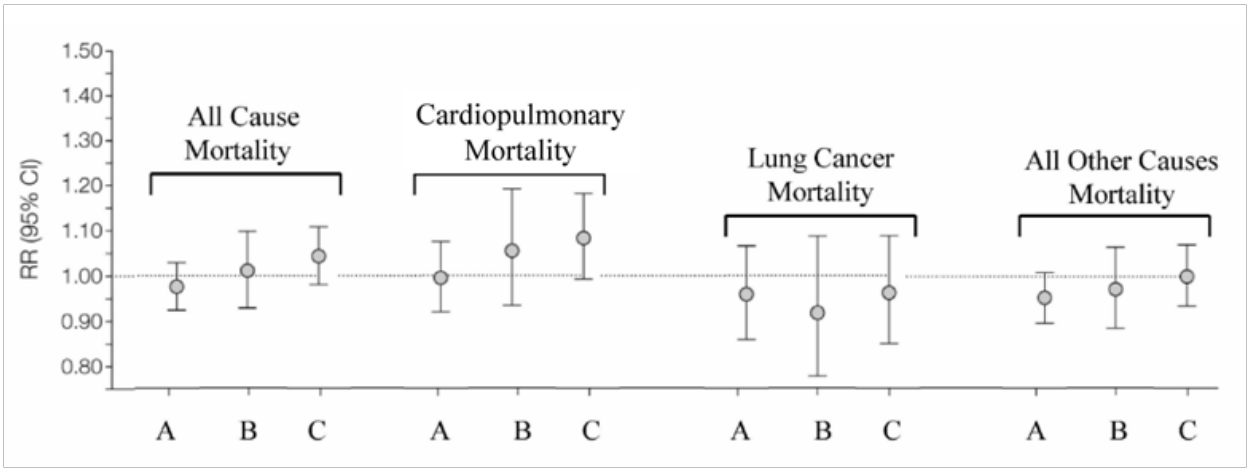
## 7.7 Mortality

A limited number of epidemiologic studies have assessed the relationship between long-term exposure to $O_3$ and mortality in adults. The 2006 $O_3$ AQCD concluded that an insufficient amount of evidence existed "to suggest a causal relationship between chronic $O_3$ exposure and increased risk for mortality in humans" (U.S. EPA, 2006b). In addition to the infant mortality studies discussed in Section 7.4.10, additional studies have been conducted among adults since the last review; an ecologic study that finds no association between mortality and $O_3$, several re-analyses of the ACS cohort, one of which specifically points to a relationship between long-term $O_3$ exposure and an increased risk of respiratory mortality, and a study of four cohorts of persons with potentially predisposing conditions. These studies supplement the evidence from long-term cohort studies characterized in previous reviews of $O_3$, and are summarized here briefly.

In the Harvard Six Cities Study (Dockery et al., 1993), adjusted mortality rate ratios were examined in relation to long-term mean $O_3$ concentrations in six cities: Topeka, KS; St. Louis, MO; Portage, WI; Harriman, TN; Steubenville, OH; and Watertown, MA. Mean $O_3$ concentrations from 1977 to 1985 ranged from 19.7 ppb in Watertown to 28.0 ppb in Portage. Long-term mean $O_3$ concentrations were not found to be associated with mortality in the six cities. However, the authors noted that "The small differences in $O_3$ levels among the (six) cities limited the power of the study to detect associations between mortality and $O_3$ levels." In addition, while total and cardio-pulmonary mortality were considered in this study, respiratory mortality was not specifically considered.

In a subsequent large prospective cohort study of approximately 500,000 U.S. adults, Pope et al. (2002) examined the effects of long-term exposure to air pollutants on mortality (American Cancer Society, Cancer Prevention Study II). All-cause, cardiopulmonary, lung cancer and other mortality risk estimates for long-term $O_3$ exposure are shown in Figure 7-5. While consistently positive associations were not observed between $O_3$ and mortality (effect estimates labeled "A" in Figure 7-5), the mortality risk estimates were larger in magnitude when analyses considered more accurate exposure metrics, increasing when the entire period was considered (effect

estimates labeled "B" in Figure 7-5) and becoming marginally significant when the exposure estimate was restricted to the summer months (July to September; effect estimates labeled "C" in Figure 7-5), especially when considering cardiopulmonary deaths. In contrast, consistent positive and significant effects of $PM_{2.5}$ were observed for both lung cancer and cardio-pulmonary mortality.




| | Years of Data Collection | Number of Metropolitan Areas | Number of Participants (in thousands) | 1-h max $O_3$ Mean (SD) |
|---|---|---|---|---|
| **A** | 1980-1981 | 134 | 559 | 47.9 (11.0) |
| **B** | 1982-1998 | 119 | 525 | 45.5 (7.3) |
| **C** | 1982-1998 (July – Sept) | 134 | 557 | 59.7 (12.8) |

Source: Reprinted with permission of American Medical Association Pope et al. (2002).

**Figure 7-5 Adjusted $O_3$-mortality relative risk estimates (95% CI) by time period of analysis per subject-weighted mean $O_3$ concentration in the Cancer Prevention Study II by the American Cancer Society.**

A study by Abbey et al. (1999) examined the effects of long-term air pollution exposure, including $O_3$, on all-cause (n = 1,575), cardiopulmonary (n = 1,029), nonmalignant respiratory (n = 410), and lung cancer (n = 30) mortality in the long-term prospective Adventist Health Study of Smog (AHSMOG) of 6,338 nonsmoking, non-Hispanic white individuals living in California. A particular strength of this study was the extensive effort devoted to assessing long-term air pollution exposures, including interpolation to residential and work locations from monitoring sites over time and space. No associations with long-term $O_3$ exposure were observed for all cause, cardiopulmonary, and nonmalignant respiratory mortality. In a follow-up, Chen et al. (2005) utilized data from the AHSMOG study and reported no evidence of associations between long-term $O_3$ exposure (mean $O_3$ concentration 26.2 ppb)

and fatal coronary heart disease. Thus, no association of chronic $O_3$ exposure with mortality outcomes has been detected in this study.

Lipfert et al. (2003, 2000) reported positive effects on all-cause mortality for peak $O_3$ exposures (95th percentile levels) in the U.S. Veterans Cohort study of approximately 50,000 middle-aged men recruited with a diagnosis of hypertension. The actual analysis involved smaller subcohorts based on exposure and mortality follow-up periods. Four separate exposure periods were associated with three mortality follow-up periods. For concurrent exposure periods, peak $O_3$ was positively associated with all-cause mortality, with a 9.4% (95% CI: 0.4, 18.4) excess risk per mean 95th percentile $O_3$ less estimated background level (not stated). "Peak" refers, in this case, to the 95th percentile of the hourly measurements, averaged by year and county. In a further analysis, Lipfert et al. (2003) reported the strongest positive association for concurrent exposure to peak $O_3$ for the subset of subjects with low diastolic blood pressure during the 1982 to 1988 period. Two more recent studies of this cohort focused specifically on traffic density (Lipfert et al., 2006a; 2006b). Lipfert et al. (2006b) concluded that: "Traffic density is seen to be a significant and robust predictor of survival in this cohort, more so than ambient air quality, with the possible exception of $O_3$," reporting a significant $O_3$ effect even with traffic density included in the model: RR = 1.080 per 40 ppb peak $O_3$ (95% CI: 1.019, 1.146). However, in Lipfert et al. (2006a), which considers only the EPA Speciation Trends Network (STN) sites, $O_3$ drops to non-significant predictor of total mortality for this cohort. The authors acknowledge that: "Peak $O_3$ has been important in analyses of this cohort for previous periods, but in the STN data set, this variable has limited range and somewhat lower values and its small coefficient of variation results in a relatively large standard error." The restriction to subjects near STN sites likely reduced the power of this analysis, though the size of the remaining subjects considered was not reported in this paper. In addition, these various Veterans Cohort studies considered only total mortality, and did not consider mortality on a by-cause basis.

An ecological study in Brisbane, Australia used a geospatial approach to analyze the association of long-term exposure to gaseous air pollution with cardio-respiratory mortality, in the period 1996-2004 (Wang et al., 2009c). A generalized estimating equations model was employed to investigate the impact of $NO_2$, $O_3$ and $SO_2$, but PM was not addressed. The results indicated that long-term exposure to $O_3$ was not associated with cardio-respiratory mortality, but the fact that this study considered only one city, and that the range of $O_3$ exposure across that city (23.7-35.6 ppb) was low and slight in variation in comparison to the range of other pollutants across the city, limited study power. In addition, confounding factors (e.g., smoking) could not be addressed at the individual level in this ecological study. Respiratory mortality was not evaluated separately.

A recent study by Zanobetti and Schwartz examined whether year-to-year variations in 8-h mean daily $O_3$ concentrations for the summer (May-September) around their city-specific long-term trend were associated with year-to-year variations in mortality around its long-term trend. This association was examined among Medicare

participants with potentially predisposing conditions, including COPD, diabetes, CHF, and MI, defined as patients discharged alive after an emergency admission for one of these four conditions. The analyses was repeated in 105 cities using available data from 1985 through 2006, and the results were combined using methods previously employed by these authors (Zanobetti et al., 2008; Zanobetti and Schwartz, 2007). This study design eliminated potential confounding by factors that vary across city, which is a common concern in most air pollution cohort studies, and also avoided both confounding by cross-sectional factors that vary by city and the short-term factors that confound daily time-series studies, but are not present in annual analyses. The average 8-h mean daily summer $O_3$ concentrations ranged from 15.6 ppb (Honolulu, HI) to 71.4 ppb (Bakersfield, CA) for the 105 cities. The authors observed associations between yearly fluctuations in summer $O_3$ concentrations and mortality in each of the four cohorts; the hazard ratios (per 10 ppb increment) were 1.12 (95% CI: 1.06, 1.17) for the CHF cohort, 1.19 (95% CI 1.12, 1.25) for the MI cohort, 1.14 (95% CI: 1.10, 1.21) for the diabetes cohort, and 1.14 (95% CI: 1.08, 1.19) for the COPD cohort. A key advantage to this study is that fluctuations from summer to summer in $O_3$ concentrations around long-term level and trend in a specific city are unlikely to be correlated with most other predicators of mortality risk; except for temperature, which was controlled for in the regression. Key limitations of the study were the inability to control for $PM_{2.5}$, since it was not reliably measured in these cities until 1999, and the inability to separate specific causes of death (e.g., respiratory, cardiovascular), since Medicare does not provide the underlying cause of death.

In the most recent follow-up analyses of the ACS cohort (Jerrett et al., 2009; Smith et al., 2009a), the effects of long-term exposure to $O_3$ were evaluated alone, as well as in copollutant models with $PM_{2.5}$ and components of $PM_{2.5}$. Jerrett et al. (2009) utilized the ACS cohort with data from 1977 through 2000 (mean $O_3$ concentration ranged from 33.3 to 104.0 ppb) and subdivided cardiopulmonary deaths into respiratory and cardiovascular, separately, as opposed to combined into one category, as was done by Pope et al. (2002). Increases in exposure to $O_3$ were associated with an elevated risk of death from cardiopulmonary, cardiovascular, ischemic heart disease, and respiratory causes. Consistent with study hypotheses, inclusion of $PM_{2.5}$ concentrations measured in 1999-2000 (the earliest years for which it was available) as a copollutant attenuated the association with $O_3$ for all end points except death from respiratory causes, for which a significant association persisted (Table 7-12). The association between increased $O_3$ concentrations and increased risk of death from respiratory causes was insensitive to the use of a random-effects survival model allowing for spatial clustering within the metropolitan area and state of residence, and adjustment for several ecologic variables considered individually. Subgroup analyses showed that temperature and region of country, but not sex, age at enrollment, body-mass index, education, or $PM_{2.5}$ concentration, modified the effects of $O_3$ on the risk of death from respiratory causes (i.e., risks were higher at higher temperature, and in the Southeast, Southwest, and Upper Midwest). Ozone threshold analyses indicated that the threshold model was not a better fit to the data ($p > 0.05$) than a linear representation of the overall $O_3$-mortality association. Overall, this new analysis indicates that long-term exposure to $PM_{2.5}$ increases risk of cardiac death,

while long-term exposure to $O_3$ is specifically associated with an increased risk of respiratory death, and suggests that combining cardiovascular and respiratory causes of mortality into one category for analysis may obscure any effect that $O_3$ may have on respiratory-related causes of mortality.

**Table 7-12 Relative risk (and 95% CI) of death attributable to a 10-ppb change in the ambient $O_3$ concentration.**

| Cause of Death | $O_3$ (96 MSAs)[a] | $O_3$ (86 MSAs)[a] | $O_3$ +$PM_{2.5}$ (86 MSAs)[a] |
|---|---|---|---|
| Any Cause | 1.001 (0.996, 1.007) | 1.001 (0.996, 1.007) | 0.989 (0.981, 0.996) |
| Cardiopulmonary | 1.014 (1.007, 1.022) | 1.016 (1.008, 1.024) | 0.992 (0.982, 1.003) |
| Respiratory | 1.029 (1.010, 1.048) | 1.027 (1.007, 1.046) | 1.040 (1.013, 1.067) |
| Cardiovascular | 1.011 (1.003, 1.023) | 1.014 (1.005, 1.023) | 0.983 (0.971, 0.994) |
| Ischemic Heart Disease | 1.015 (1.003, 1.026) | 1.017 (1.006, 1.029) | 0.973 (0.958, 0.988) |

[a]Ozone concentrations were measured from April to September during the years from 1977 to 2000, with follow-up from 1982 to 2000; changes in the concentration of $PM_{2.5}$ of 10 µg/$m^3$ were recorded for members of the cohort in 1999 and 2000.

Source: Reprinted with permission of Massachusetts Medical Society (Jerrett et al., 2009).

In a similar analysis, Smith et al. (2009a) used data from 66 Metropolitan Statistical Areas (MSAs) in the ACS cohort to examine the association of $O_3$ concentrations during the warm season and all-cause and cardiopulmonary mortality. Mortality effects were estimated in single pollutant and copollutant models, adjusting for two $PM_{2.5}$ constituents, sulfate, and EC. When all-cause mortality was investigated, there was a 0.8% (95% CI: -0.31, 1.9) increase associated with a 10 ppb increase in $O_3$ concentration. This association was diminished when sulfate or EC were included in the model. There was a 2.48% (95% CI: 0.74, 4.3) increase in cardiopulmonary mortality associated with a 10 ppb increase in $O_3$ concentration.
The cardiopulmonary association was robust to adjustment for sulfate, and diminished, though still positive, after adjustment for EC (1.63% increase; 95% CI: -0.41, 3.7). Smith et al. (2009a) did not specifically separate out cardiovascular and respiratory causes of death from the cardiopulmonary category, as was done by Jerrett et al. (2009).

### 7.7.1 Summary and Causal Determination

The The 2006 $O_3$ AQCD concluded that an insufficient amount of evidence existed "to suggest a causal relationship between chronic $O_3$ exposure and increased risk for mortality in humans" (U.S. EPA, 2006b). Several additional studies have been conducted since the last review that evaluate cause-specific and total mortality.
An ecologic study conducted in Australia observed no association between cardiopulmonary mortality and $O_3$ (Wang et al., 2009c). Two reanalyses of the ACS cohort were conducted; one provides weak evidence for an association with cardiopulmonary mortality (Smith et al., 2009a) while the other specifically points to

a relationship between long-term $O_3$ exposure and an increased risk of respiratory mortality (Jerrett et al., 2009). Most recently, a study of four cohorts of Medicare enrollees with potentially predisposing conditions observed associations between $O_3$ and total mortality among each of the cohorts (Zanobetti and Schwartz, 2011).

When considering the entire body of evidence, there is limited support for an association with long-term exposure to ambient $O_3$ and total mortality. There is inconsistent evidence for an association between long-term exposure to ambient $O_3$ and cardiopulmonary mortality, with several analyses from the ACS cohort reporting some positive associations (Smith et al., 2009a; Pope et al., 2002) while other studies reported no association (Wang et al., 2009c; Abbey et al., 1999; Dockery et al., 1993). The strongest evidence for an association between long-term exposure to ambient $O_3$ concentrations and mortality is derived from associations reported in the Jerrett et al. (2009) study for respiratory mortality that remained robust after adjusting for $PM_{2.5}$ concentrations. Finally, a recent analysis reported associations of ambient $O_3$ concentrations and total mortality in potentially at-risk populations in the Medicare Cohort (Zanobetti and Schwartz, 2011), while earlier studies generally report no associations with total mortality (Lipfert et al., 2006a; Lipfert et al., 2003; Pope et al., 2002; Abbey et al., 1999; Dockery et al., 1993). Studies of cardiopulmonary and total mortality provide limited evidence for an association with long-term exposure to ambient $O_3$ concentrations. The study by Jerrett et al. (2009) observes an association between long-term exposure to ambient $O_3$ concentrations and respiratory mortality that remained robust after adjusting for $PM_{2.5}$ concentrations. Coherence and biological plausibility for this observation is provided by evidence from epidemiologic, controlled human exposure, and animal toxicological studies for the effects of short- and long-term exposure to $O_3$ on respiratory effects (see Sections 6.2 and 7.2). Respiratory mortality is a relatively small portion of total mortality [about 7.6% of all deaths in 2010 were due to respiratory causes (Murphy et al., 2012)], thus it is not surprising that the respiratory mortality signal may be difficult to detect in studies of cardiopulmonary or total mortality. Based on the recent evidence for respiratory mortality along with limited evidence for total and cardiopulmonary mortality, the evidence **is suggestive of a causal relationship between long-term $O_3$ exposures and total mortality.**

## 7.8 Overall Summary

The evidence reviewed in this chapter describes the recent findings regarding the health effects of long-term exposure to ambient $O_3$ concentrations. Table 7-13 provides an overview of the causal determinations for each of the health categories evaluated.

**Table 7-13 Summary of causal determinations for long-term exposures to $O_3$.**

| Health Category | Causal Determination |
|---|---|
| Respiratory Effects | Likely to be a causal relationship |
| Cardiovascular Effects | Suggestive of a causal relationship |
| Reproductive and Developmental Effects | Suggestive of a causal relationship |
| Central Nervous System Effects | Suggestive of a causal relationship |
| Carcinogenicity and Genotoxicity | Inadequate to infer a causal relationship |
| Total Mortality | Suggestive of a causal relationship |

## References


Abbey, DE; Nishino, N; McDonnell, WF; Burchette, RJ; Knutsen, SF; Beeson, WL; Yang, JX. (1999). Long-term inhalable particles and other air pollutants related to mortality in nonsmokers. Am J Respir Crit Care Med 159: 373-382.

Akinbami, LJ; Lynch, CD; Parker, JD; Woodruff, TJ. (2010). The association between childhood asthma prevalence and monitored air pollutants in metropolitan areas, United States, 2001-2004. Environ Res 110: 294-301. http://dx.doi.org/10.1016/j.envres.2010.01.001

Andersen, ZJ; Raaschou-Nielsen, O; Ketzel, M; Jensen, SS; Hvidberg, M; Loft, S; Tjønneland, A; Overvad, K; Sørensen, M. (2012). Diabetes Incidence and Long-Term Exposure to Air Pollution: A cohort study. Diabetes Care 35: 92-98. http://dx.doi.org/10.2337/dc11-1155

Angoa-Pérez, M; Jiang, H; Rodríguez, AI; Lemini, C; Levine, RA; Rivas-Arancibia, S. (2006). Estrogen counteracts ozone-induced oxidative stress and nigral neuronal death. Neuroreport 17: 629-633.

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

Auten, RL; Gilmour, MI; Krantz, QT; Potts, EN; Mason, SN; Foster, WM. (2012). Maternal diesel inhalation increases airway hyperreactivity in ozone-exposed offspring. Am J Respir Cell Mol Biol 46: 454-460. http://dx.doi.org/10.1165/rcmb.2011-0256OC

Auten, RL; Potts, EN; Mason, SN; Fischer, B; Huang, Y; Foster, WM. (2009). Maternal exposure to particulate matter increases postnatal ozone-induced airway hyperreactivity in juvenile mice. Am J Respir Crit Care Med 180: 1218-1226. http://dx.doi.org/10.1164/rccm.200901-0116OC

Barry, BE; Miller, FJ; Crapo, JD. (1985). Effects of inhalation of 0.12 and 0.25 parts per million ozone on the proximal alveolar region of juvenile and adult rats. Lab Invest 53: 692-704.

Barry, BE; Miller, FJ; Crapo, JD. (1983). Alveolar epithelial injury caused by inhalation of 0.25 ppm of ozone. In SD Lee; MG Mustafa; MA Mehlman (Eds.), The biomedical effects of ozone and related photochemical oxidants (pp. 299-308). Princeton, NJ: Princeton Scientific Publishers.

Beeson, WL; Abbey, DE; Knutsen, SF. (1998). Long-term concentrations of ambient air pollutants and incident lung cancer in California adults: Results from the AHSMOG study. Environ Health Perspect 106: 813-823.

Berhane, K; Gauderman, WJ; Stram, DO; Thomas, DC. (2004). Statistical issues in studies of the long-term effects of air pollution: The Southern California Childrens Health Study. Stat Sci 19: 414-449. http://dx.doi.org/10.1214/088342304000000413

Bignami, G; Musi, B; Dell'Omo, G; Laviola, G; Alleva, E. (1994). Limited effects of ozone exposure during pregnancy on physical and neurobehavioral development of CD-1 mice. Toxicol Appl Pharmacol 129: 264-271. http://dx.doi.org/10.1006/taap.1994.1251

Bobak, M. (2000). Outdoor air pollution, low birth weight, and prematurity. Environ Health Perspect 108: 173-176.

Boorman, GA; Hailey, R; Grumbein, S; Chou, BJ; Herbert, RA; Goehl, T; Mellick, PW; Roycroft, JH; Haseman, JK; Sills, R. (1994). Toxicology and carcinogenesis studies of ozone and ozone 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone in Fischer-344/N rats. Toxicol Pathol 22: 545-554.

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

Boussouar, A; Araneda, S; Hamelin, C; Soulage, C; Kitahama, K; Dalmaz, Y. (2009). Prenatal ozone exposure abolishes stress activation of Fos and tyrosine hydroxylase in the nucleus tractus solitarius of adult rat. Neurosci Lett 452: 75-78.

Brauer, M; Lencar, C; Tamburic, L; Koehoorn, M; Demers, P; Karr, C. (2008). A cohort study of traffic-related air pollution impacts on birth outcomes. Environ Health Perspect 116: 680-686. http://dx.doi.org/10.1289/ehp.10952

Breton, CV; Salam, MT; Vora, H; Gauderman, WJ; Gilliland, FD. (2011). Genetic variation in the glutathione synthesis pathway, air pollution, and children's lung function growth. Am J Respir Crit Care Med 183: 243-248. http://dx.doi.org/10.1164/rccm.201006-0849OC

Calderón-Garcidueñas, L; Mora-Tiscareno, A; Fordham, LA; Chung, CJ; Valencia-Salazar, G; Flores-Gomez, S; Solt, AC; Gomez-del Campo, A; Jardon-Torres, R; Henriquez-Roldan, C; Hazucha, MJ; Reed, W. (2006). Lung radiology and pulmonary function of children chronically exposed to air pollution. Environ Health Perspect 114: 1432-1437.

Campos-Bedolla, P; Vargas, MH; Montano, LM. (2002). Effect of acute ozone exposure on pregnant rat uterus contractile responses. Reprod Toxicol 16: 269-273.

Carbajal-Arroyo, L; Miranda-Soberanis, V; Medina-Ramón, M; Rojas-Bracho, L; Tzintzun, G; Solís-Gutiérrez, P; Méndez-Ramírez, I; Hurtado-Díaz, M; Schwartz, J; Romieu, I. (2011). Effect of PM10 and O3 on infant mortality among residents in the Mexico City Metropolitan Area: A case-crossover analysis, 19972005. J Epidemiol Community Health 65: 715-721. http://dx.doi.org/10.1136/jech.2009.101212

Carey, SA; Ballinger, CA; Plopper, CG; McDonald, RJ; Bartolucci, AA; Postlethwait, EM; Harkema, JR. (2011). Persistent rhinitis and epithelial remodeling induced by cyclic ozone exposure in the nasal airways of infant monkeys. Am J Physiol Lung Cell Mol Physiol 300: L242-L254. http://dx.doi.org/10.1152/ajplung.00177.2010

Carey, SA; Minard, KR; Trease, LL; Wagner, JG; Garcia, GJ; Ballinger, CA; Kimbell, JS; Plopper, CG; Corley, RA; Postlethwait, EM; Harkema, JR. (2007). Three-dimensional mapping of ozone-induced injury in the nasal airways of monkeys using magnetic resonance imaging and morphometric techniques. Toxicol Pathol 35: 27-40. http://dx.doi.org/10.1080/01926230601072343

Carter, EP; Garat, C; Imamura, M. (2004). Continual emerging roles of HO-1: protection against airway inflammation [Review]. Am J Physiol Lung Cell Mol Physiol 287: L24-L25. http://dx.doi.org/10.1152/ajplung.00097.2004

Catalano, PJ; Chang, LY; Harkema, JR; Kaden, DA; Last, JA; Mellick, PW; Parks, WC; Pinkerton, KE; Radhakrishnamurthy, B; Ryan, LM; Szarek, JL. (1995a). Consequences of prolonged inhalation of ozone on F344/N rats: Collaborative studies Part XI: Integrative summary. Cambridge, MA: Health Effects Institute.

Catalano, PJ; Rogus, J; Ryan, LM. (1995b). Consequences of prolonged inhalation of ozone on F344/N rats: collaborative studies Part X: robust composite scores based on median polish analysis. Health Effects Institute.

Chang, L; Miller, FJ; Ultman, J; Huang, Y; Stockstill, BL; Grose, E; Graham, JA; Ospital, JJ; Crapo, JD. (1991). Alveolar epithelial cell injuries by subchronic exposure to low concentrations of ozone correlate with cumulative exposure. Toxicol Appl Pharmacol 109: 219-234. http://dx.doi.org/10.1016/0041-008X(91)90170-J

Chang, LY; Huang, Y; Stockstill, BL; Graham, JA; Grose, EC; Menache, MG; Miller, FJ; Costa, DL; Crapo, JD. (1992). Epithelial injury and interstitial fibrosis in the proximal alveolar regions of rats chronically exposed to a simulated pattern of urban ambient ozone. Toxicol Appl Pharmacol 115: 241-252. http://dx.doi.org/10.1016/0041-008X(92)90329-Q

Chang, LY; Stockstill, BL; Ménache, MG; Mercer, RR; Crapo, JD. (1995). Consequences of prolonged inhalation of ozone on F344/N rats: Collaborative studies. Part VIII: Morphometric analysis of structural alterations in alveolar regions [HEI] (pp. 3-39). (Research Report 65-VIII). Health Effects Institute. http://pubs.healtheffects.org/view.php?id=82

Charpin, D; Pascal, L; Birnbaum, J; Armengaud, A; Sambuc, R; Lanteaume, A; Vervloet, D. (1999). Gaseous air pollution and atopy. Clin Exp Allergy 29: 1474-1480.

Chen, C; Arjomandi, M; Balmes, J; Tager, I; N, H. (2007a). Effects of chronic and acute ozone exposure on lipid peroxidation and antioxidant capacity in healthy young adults. Environ Health Perspect 115: 1732-1737. http://dx.doi.org/10.1289/ehp.10294

Chen, C; Arjomandi, M; Qin, H; Balmes, J; Tager, I; Holland, N. (2006a). Cytogenetic damage in buccal epithelia and peripheral lymphocytes of young healthy individuals exposed to ozone. Mutagenesis 21: 131-137. http://dx.doi.org/10.1093/mutage/gel007

Chen, JC; Schwartz, J. (2009). Neurobehavioral effects of ambient air pollution on cognitive performance in US adults. Neurotoxicology 30: 231-239. http://dx.doi.org/10.1016/j.neuro.2008.12.011

Chen, L; Bell, EM; Caton, AR; Druschel, CM; Lin, S. (2010a). Residential mobility during pregnancy and the potential for ambient air pollution exposure misclassification. Environ Res 110: 162-168. http://dx.doi.org/10.1016/j.envres.2009.11.001

Chen, L; Yang, W; Jennison, BL; Goodrich, A; Omaye, ST. (2002). Air pollution and birth weight in northern Nevada, 1991-1999. Inhal Toxicol 14: 141-157.

Chen, LH; Knutsen, SF; Shavlik, D; Beeson, WL; Petersen, F; Ghamsary, M; Abbey, D. (2005). The association between fatal coronary heart disease and ambient particulate air pollution: Are females at greater risk? Environ Health Perspect 113: 1723-1729.

Chuang, GC; Yang, Z; Westbrook, DG; Pompilius, M; Ballinger, CA; White, RC; Krzywanski, DM; Postlethwait, EM; Ballinger, SW. (2009). Pulmonary ozone exposure induces vascular dysfunction, mitochondrial damage, and atherogenesis. Am J Physiol Lung Cell Mol Physiol 297: L209-L216. http://dx.doi.org/10.1152/ajplung.00102.2009

Chuang, KJ; Yan, YH; Chiu, SY; Cheng, TJ. (2011). Long-term air pollution exposure and risk factors for cardiovascular diseases among the elderly in Taiwan. Occup Environ Med 68: 64-68. http://dx.doi.org/10.1136/oem.2009.052704

Clark, NA; Demers, PA; Karr, CJ; Koehoorn, M; Lencar, C; Tamburic, L; Brauer, M. (2010). Effect of early life exposure to air pollution on development of childhood asthma. Environ Health Perspect 118: 284-290. http://dx.doi.org/10.1289/ehp.0900916

Cole, MP; Freeman, BA. (2009). Promotion of cardiovascular disease by exposure to the air pollutant ozone [Review]. Am J Physiol Lung Cell Mol Physiol 297: L209-L216. http://dx.doi.org/10.1152/ajplung.00187.2009

Conti, DV; Cortessis, V; Molitor, J; Thomas, DC. (2003). Bayesian modeling of complex metabolic pathways. Hum Hered 56: 83-93. http://dx.doi.org/10.1159/000073736

Dadvand, P; Rankin, J; Rushton, S; Pless-Mulloli, T. (2011). Ambient air pollution and congenital heart disease: A register-based study. Environ Res 111: 435-441. http://dx.doi.org/10.1016/j.envres.2011.01.022

Dales, R; Burnett, RT; Smith-Doiron, M; Stieb, DM; Brook, JR. (2004). Air pollution and sudden infant death syndrome. Pediatrics 113: 628-631.

Darrow, LA; Klein, M; Flanders, WD; Waller, LA; Correa, A; Marcus, M; Mulholland, JA; Russell, AG; Tolbert, PE. (2009). Ambient air pollution and preterm birth: A time-series analysis. Epidemiology 20: 689-698. http://dx.doi.org/10.1097/EDE.0b013e3181a7128f

Darrow, LA; Klein, M; Strickland, MJ; Mulholland, JA; Tolbert, PE. (2011b). Ambient air pollution and birth weight in full-term infants in Atlanta, 1994-2004. Environ Health Perspect 119: 731-737. http://dx.doi.org/10.1289/ehp.1002785

Dell'Omo, G; Wolfer, D; Alleva, E; Lipp, HP. (1995). Developmental exposure to ozone induces subtle changes in swimming navigation of adult mice. Toxicol Lett 81: 91-99.

Diaz, J; Linares, C; Garcia-Herrera, R; Lopez, C; Trigo, R. (2004). Impact of temperature and air pollution on the mortality of children in Madrid. J Occup Environ Med 46: 768-774.

Dockery, DW; Pope, CA, III; Xu, X; Spengler, JD; Ware, JH; Fay, ME; Ferris, BG, Jr; Speizer, FE. (1993). An association between air pollution and mortality in six US cities. N Engl J Med 329: 1753-1759. http://dx.doi.org/10.1056/NEJM199312093292401

Dugandzic, R; Dodds, L; Stieb, D; Smith-Doiron, M. (2006). The association between low level exposures to ambient air pollution and term low birth weight: A retrospective cohort study. Environ Health 5: 3. http://dx.doi.org/10.1186/1476-069X-5-3

Ercan, H; Birben, E; Dizdar, EA; Keskin, O; Karaaslan, C; Soyer, OU; Dut, R; Sackesen, C; Besler, T; Kalayci, O. (2006). Oxidative stress and genetic and epidemiologic determinants of oxidant injury in childhood asthma. J Allergy Clin Immunol 118: 1097-1104. http://dx.doi.org/10.1016/j.jaci.2006.08.012

Eustis, SL; Schwartz, LW; Kosch, PC; Dungworth, DL. (1981). Chronic bronchiolitis in nonhuman primates after prolonged ozone exposure. Am J Pathol 105: 121-137.

Evans, MJ; Fanucchi, MV; Baker, GL; Van Winkle, LS; Pantle, LM; Nishio, SJ; Schelegle, ES; Gershwhin, LJ; Miller, LA; Hyde, DM; Sannes, PL; Plopper, CG. (2003). Atypical development of the tracheal basement membrane zone of infant rhesus monkeys exposed to ozone and allergen. Am J Physiol 285: L931-L939. http://dx.doi.org/10.1152/ajplung.00175.2003

Evans, MJ; Fanucchi, MV; Baker, GL; Van Winkle, LS; Pantle, LM; Nishio, SJ; Schelegle, ES; Gershwin, LJ; Miller, LA; Hyde, DM; Plopper, CG. (2004). The remodelled tracheal basement membrane zone of infant rhesus monkeys after 6 months of recovery. Clin Exp Allergy 34: 1131-1136. http://dx.doi.org/10.1111/j.1365-2222.2004.02004.x CEA2004

Exner, M; Minar, E; Wagner, O; Schillinger, M. (2004). The role of heme oxygenase-1 promoter polymorphisms in human disease [Review]. Free Radic Biol Med 37: 1097-1104. http://dx.doi.org/10.1016/j.freeradbiomed.2004.07.008

Fanucchi, MV; Plopper, CG; Evans, MJ; Hyde, DM; Van Winkle, LS; Gershwin, LJ; Schelegle, ES. (2006). Cyclic exposure to ozone alters distal airway development in infant rhesus monkeys. Am J Physiol Lung Cell Mol Physiol 291: L644-L650. http://dx.doi.org/10.1152/ajplung.00027.2006

Fanucchi, MV; Wong, VJ; Hinds, D; Tarkington, BK; Van Winkle, LS; Evans, MJ; Plopper, CG. (2000). Repeated episodes of exposure to ozone alters postnatal development of distal conducting airways in infant rhesus monkeys [Abstract]. Am J Respir Crit Care Med 161: A615.

Feichtinger, W; Papalambrou, K; Poehl, M; Krischker, U; Neumann, K. (1997). Smoking and in vitro fertilization: A meta-analysis. J Assist Reprod Genet 14: 596-599. http://dx.doi.org/10.1023/A:1022584802711

Ferng, SF; Castro, CE; Afifi, AA; Bermudez, E; Mustafa, MG. (1997). Ozone-induced DNA strand breaks in guinea pig tracheobronchial epithelial cells. J Toxicol Environ Health 51: 353-367.

Forbes, LJ; Patel, MD; Rudnicka, AR; Cook, DG; Bush, T; Stedman, JR; Whincup, PH; Strachan, DP; Anderson, RH. (2009a). Chronic exposure to outdoor air pollution and markers of systemic inflammation. Epidemiology 20: 245-253. http://dx.doi.org/10.1097/EDE.0b013e318190ea3f

Forbes, LJL; Kapetanakis, V; Rudnicka, AR; Cook, DG; Bush, T; Stedman, JR; Whincup, PH; Strachan, DP; Anderson, HR. (2009b). Chronic exposure to outdoor air pollution and lung function in adults. Thorax 64: 657-663. http://dx.doi.org/10.1136/thx.2008.109389

Frischer, T; Studnicka, M; Gartner, C; Tauber, E; Horak, F; Veiter, A; Spengler, J; Kuhr, J; Urbanek, R. (1999). Lung function growth and ambient ozone: A three-year population study in school children. Am J Respir Crit Care Med 160: 390-396.

Frischer, T; Studnicka, M; Halmerbauer, G; Horak, F; Gartner, C; Tauber, E; Koller, DY. (2001). Ambient ozone exposure is associated with eosinophil activation in healthy children. Clin Exp Allergy 31: 1213-1219.

Fujinaka, LE; Hyde, DM; Plopper, CG; Tyler, WS; Dungworth, DL; Lollini, LO. (1985). Respiratory bronchiolitis following long-term ozone exposure in bonnet monkeys: A morphometric study. Exp Lung Res 8: 167-190.

Gauderman, WJ; Avol, E; Gilliland, F; Vora, H; Thomas, D; Berhane, K; McConnell, R; Kuenzli, N; Lurmann, F; Rappaport, E; Margolis, H; Bates, D; Peters, J. (2004). The effect of air pollution on lung development from 10 to 18 years of age. N Engl J Med 351: 1057-1067.

Gauderman, WJ; McConnell, R; Gilliland, F; London, S; Thomas, D; Avol, E; Vora, H; Berhane, K; Rappaport, EB; Lurmann, F; Margolis, HG; Peters, J. (2000). Association between air pollution and lung function growth in southern California children. Am J Respir Crit Care Med 162: 1383-1390.

Gilboa, SM; Mendola, P; Olshan, AF; Langlois, PH; Savitz, DA; Loomis, D; Herring, AH; Fixler, DE. (2005). Relation between ambient air quality and selected birth defects, seven county study, Texas, 1997-2000. Am J Epidemiol 162: 238-252. http://dx.doi.org/10.1093/aje/kwi189

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

Gilliland, FD; McConnell, R; Peters, J; Gong, J, r, H. (1999). A theoretical basis for investigating ambient air pollution and children's respiratory health [Review]. Environ Health Perspect 107: 403-407.

Gilliland, FD; Rappaport, EB; Berhane, K; Islam, T; Dubeau, L; Gauderman, WJ; McConnell, R. (2002). Effects of glutathione S-Transferase P1, M1, and T1 on acute respiratory illness in school children. Am J Respir Crit Care Med 166: 346-351.

Giovannelli, L; Pitozzi, V; Moretti, S; Boddi, V; Dolara, P. (2006). Seasonal variations of DNA damage in human lymphocytes: Correlation with different environmental variables. Mutat Res-Fundam Mol Mech Mutagen 593: 143-152. http://dx.doi.org/10.1016/j.mrfmmm.2005.07.002

Gonzalez-Pina, R; Escalante-Membrillo, C; Alfaro-Rodriguez, A; Gonzalez-Maciel, A. (2008). Prenatal exposure to ozone disrupts cerebellar monoamine contents in newborn rats. Neurochem Res 33: 912-918. http://dx.doi.org/10.1007/s11064-007-9534-3

Goss, CH; Newsom, SA; Schildcrout, JS; Sheppard, L; Kaufman, JD. (2004). Effect of ambient air pollution on pulmonary exacerbations and lung function in cystic fibrosis. Am J Respir Crit Care Med 169: 816-821.

Gouveia, N; Bremner, SA; Novaes, HMD. (2004). Association between ambient air pollution and birth weight in Sao Paulo, Brazil. J Epidemiol Community Health 58: 11-17.

Greer, JR; Abbey, DE; Burchette, RJ. (1993). Asthma related to occupational and ambient air pollutants in nonsmokers. J Occup Environ Med 35: 909-915.

Guerrero, AL; Dorado-Martinez, C; Rodriguez, A; Pedroza-Rios, K; Borgonio-Perez, G; Rivas-Arancibia, S. (1999). Effects of vitamin E on ozone-induced memory deficits and lipid peroxidation in rats. Neuroreport 10: 1689-1692.

Guevara-Guzmán, R; Arriaga, V; Kendrick, KM; Bernal, C; Vega, X; Mercado-Gómez, OF; Rivas-Arancibia, S. (2009). Estradiol prevents ozone-induced increases in brain lipid peroxidation and impaired social recognition memory in female rats. Neuroscience 159: 940-950. http://dx.doi.org/10.1016/j.neuroscience.2009.01.047

Ha, EH; Hong, YC; Lee, BE; Woo, BH; Schwartz, J; Christiani, DC. (2001). Is air pollution a risk factor for low birth weight in Seoul? Epidemiology 12: 643-648.

Ha, EH; Lee, JT; Kim, H; Hong, YC; Lee. (2003). Infant susceptibility of mortality to air pollution in Seoul, South Korea. Pediatrics 111: 284-290.

Hack, M; Fanaroff, AA. (1999). Outcomes of children of extremely low birth weight and gestational age in the 1990s [Review]. Early Hum Dev 53: 193-218. http://dx.doi.org/10.1016/S0378-3782(98)00052-8

Hajat, S; Armstrong, B; Wilkinson, P; Busby, A; Dolk, H. (2007). Outdoor air pollution and infant mortality: Analysis of daily time-series data in 10 English cities. J Epidemiol Community Health 61: 719-722. http://dx.doi.org/10.1136/jech.2006.053942

Han, SG; Bhoopalan, V; Akinbiyi, T; Gairola, CG; Bhalla, DK. (2011). In utero tobacco smoke exposure alters pulmonary responses of newborn rats to ozone. J Toxicol Environ Health A 74: 668-677. http://dx.doi.org/10.1080/15287394.2011.539133

Hanene, C; Jihene, L; Jame, A; Kamel, H; Agnes, H. (2007). Association of GST genes polymorphisms with asthma in Tunisian children. Mediators Inflamm 19564: 6. http://dx.doi.org/10.1155/2007/19564

Hansen, C; Luben, TJ; Sacks, JD; Olshan, A; Jeffay, S; Strader, L; Perreault, SD. (2010). The effect of ambient air pollution on sperm quality. Environ Health Perspect 118: 203-209. http://dx.doi.org/10.1289/ehp.0901022

Hansen, C; Neller, A; Williams, G; Simpson, R. (2006). Maternal exposure to low levels of ambient air pollution and preterm birth in Brisbane, Australia. BJOG 113: 935-941. http://dx.doi.org/10.1111/j.1471-0528.2006.01010.x

Hansen, C; Neller, A; Williams, G; Simpson, R. (2007). Low levels of ambient air pollution during pregnancy and fetal growth among term neonates in Brisbane, Australia. Environ Res 103: 383-389. http://dx.doi.org/10.1016/j.envres.2006.06.010

Hansen, CA; Barnett, AG; Jalaludin, B; Morgan, G. (2009). Ambient air pollution and birth defects in Brisbane, Australia. PLoS ONE 4: e5408. http://dx.doi.org/10.1371/journal.pone.0005408

Hansen, CA; Barnett, AG; Pritchard, G. (2008). The effect of ambient air pollution during early pregnancy on fetal ultrasonic measurements during mid-pregnancy. Environ Health Perspect 116: 362-369. http://dx.doi.org/10.1289/ehp.10720

Harkema, JR; Catalano, PJ; Hotchkiss, JA. (1997a). Consequences of prolonged inhalation of ozone on F344/N rats: Collaborative studies: Part XII. Atrophy of bone in nasal turbinates (pp. 1-19; discussion 21-16). (ISSN 1041-5505

65-XII). Cambridge, MA: Health Effects Institute.

Harkema, JR; Hotchkiss, JA; Barr, EB; Bennett, CB; Gallup, M; Lee, JK; Basbaum, C. (1999). Long-lasting effects of chronic ozone exposure on rat nasal epithelium. Am J Respir Cell Mol Biol 20: 517-529.

Harkema, JR; Hotchkiss, JA; Griffith, WC. (1997b). Mucous cell metaplasia in rat nasal epithelium after a 20-month exposure to ozone: A morphometric study of epithelial differentiation. Am J Respir Cell Mol Biol 16: 521-530.

Harkema, JR; Morgan, KT; Gross, EA; Catalano, PJ; Griffith, WC. (1994). Consequences of prolonged inhalation of ozone on F344/N rats: Collaborative studies Part VII: Effects on the nasal mucociliary apparatus. Cambridge, MA: Health Effects Institute.

Harkema, JR; Plopper, CG; Hyde, DM; St George, JA; Dungworth, DL. (1987a). Effects of an ambient level of ozone on primate nasal epithelial mucosubstances: quantitative histochemistry. Am J Pathol 127: 90-96.

Harkema, JR; Plopper, CG; Hyde, DM; St George, JA; Wilson, DW; Dungworth, DL. (1987b). Response of the macaque nasal epithelium to ambient levels of ozone: A morphologic and morphometric study of the transitional and respiratory epithelium. Am J Pathol 128: 29-44.

Harkema, JR; Plopper, CG; Hyde, DM; St George, JA; Wilson, DW; Dungworth, DL. (1993). Response of macaque bronchiolar epithelium to ambient concentrations of ozone. Am J Pathol 143: 857-866.

Haro, R; Paz, C. (1993). Effects of ozone exposure during pregnancy on ontogeny of sleep in rats. Neurosci Lett 164: 67-70. http://dx.doi.org/10.1016/0304-3940(93)90859-J

Hassett, C; Mustafa, MG; Coulson, WF; Elashoff, RM. (1985). Murine lung carcinogenesis following exposure to ambient ozone concentrations. J Natl Cancer Inst 75: 771-777.

Herbert, RA; Hailey, JR; Grumbein, S; Chou, BJ; Sills, RC; Haseman, JK; Goehl, T; Miller, RA; Roycroft, JH; Boorman, GA. (1996). Two-year and lifetime toxicity and carcinogenicity studies of ozone in B6C3F1 mice. Toxicol Pathol 24: 539-548.

Himes, BE; Hunninghake, GM; Baurley, JW; Rafaels, NM; Sleiman, P; Strachan, DP; Wilk, JB; Willis-Owen, SAG; Klanderman, B; Lasky-Su, J; Lazarus, R; Murphy, AJ; Soto-Quiros, ME; Avila, L; Beaty, T; Mathias, RA; Ruczinski, I; Barnes, KC; Celedon, JC; Cookson, WOC; Gauderman, WJ; Gilliland, FD; Hakonarson, H; Lange, C; Moffatt, MF; O'Connor, GT; Raby, BA; Silverman, EK; Weiss, ST. (2009). Genome-wide Association Analysis Identifies PDE4D as an Asthma-Susceptibility Gene. Am J Hum Genet 84: 581-593. http://dx.doi.org/10.1016/j.ajhg.2009.04.006

Hoffjan, S; Nicolae, D; Ostrovnaya, I; Roberg, K; Evans, M; Mirel, DB; Steiner, L; Walker, K; Shult, P; Gangnon, RE; Gern, JE; Martinez, FD; Lemanske, RF; Ober, C. (2005). Gene-environment interaction effects on the development of immune responses in the 1st year of life. Am J Hum Genet 76: 696-704. http://dx.doi.org/10.1086/429418

Holgate, ST; Davies, DE; Powell, RM; Howarth, PH; Haitchi, HM; Holloway, JW. (2007). Local genetic and environmental factors in asthma disease pathogenesis: chronicity and persistence mechanisms [Review]. Eur Respir J 29: 793-803. http://dx.doi.org/10.1183/09031936.00087506

Horak, F, Jr; Studnicka, M; Gartner, C; Spengler, JD; Tauber, E; Urbanek, R; Veiter, A; Frischer, T. (2002). Particulate matter and lung function growth in children: a 3-yr follow-up study in Austrian schoolchildren. Eur Respir J 19: 838-845.

Huen, K; Gunn, L; Duramad, P; Jeng, M; Scalf, R; Holland, N. (2006). Application of a geographic information system to explore associations between air pollution and micronucleus frequencies in African American children and adults. Environ Mol Mutagen 47: 236-246. http://dx.doi.org/10.1002/em.20193

Hunter, DJ. (2005). Gene-environment interactions in human diseases [Review]. Nat Rev Genet 6: 287-298. http://dx.doi.org/10.1038/nrg1578

Hutcheon, JA; Platt, RW. (2008). The missing data problem in birth weight percentiles and thresholds for "small-for-gestational-age". Am J Epidemiol 167: 786-792. http://dx.doi.org/10.1093/aje/kwm327

Hwang, BF; Jaakkola, JJ. (2008). Ozone and other air pollutants and the risk of oral clefts. Environ Health Perspect 116: 1411-1415. http://dx.doi.org/10.1289/ehp.11311

Hwang, BF; Jaakkola, JJK; Lee, YL; Lin, YC; Y-LL, G. (2006). Relation between air pollution and allergic rhinitis in Taiwanese schoolchildren. Respir Res 7: 23.

Hwang, BF; Lee, YL; Lin, YC; Jaakkola, JJK; Guo, YL. (2005). Traffic related air pollution as a determinant of asthma among Taiwanese school children. Thorax 60: 467-473.

Hyde, DM; Hubbard, WC; Wong, V; Wu, R; Pinkerton, K; Plopper, CG. (1992). Ozone-induced acute tracheobronchial epithelial injury: relationship to granulocyte emigration in the lung. Am J Respir Cell Mol Biol 6: 481-497.

Hyde, DM; Miller, LA; Schelegle, ES; Fanucchi, MV; Van Winkle, LS; Tyler, NK; Avdalovic, MV; Evans, MJ; Kajekar, R; Buckpitt, AR; Pinkerton, KE; Joad, JP; Gershwin, LJ; Wu, R; Plopper, CG. (2006). Asthma: a comparison of animal models using stereological methods. Eur Respir Rev 15: 122-135. http://dx.doi.org/10.1183/09059180.00010103

Hyde, DM; Plopper, CG; Harkema, JR; St George, JA; Tyler, WS; Dungworth, DL. (1989). Ozone-induced structural changes in monkey respiratory system. In T Schneider; SD Lee; GJR Wolters; LD Grant (Eds.), Atmospheric ozone research and its policy implications: Proceedings of the 3rd US-Dutch International Symposium, Nijmegen, the Netherlands May 9-13, 1988 (pp. 523-532). Amsterdam, The Netherlands: Elsevier Science Publishers.

Ihorst, G; Frischer, T; Horak, F; Schumacher, M; Kopp, M; Forster, J; Mattes, J; Kuehr, J. (2004). Long- and medium-term ozone effects on lung growth including a broad spectrum of exposure. Eur Respir J 23: 292-299.

Iijima, MK; Kobayashi, T. (2004). Nasal allergy-like symptoms aggravated by ozone exposure in a concentration-dependent manner in guinea pigs. Toxicology 199: 73-83. http://dx.doi.org/10.1016/j.tox.2004.01.008

Islam, T; Berhane, K; Mcconnell, R; Gauderman, WJ; Avol, E; Peters, JM; Gilliland, FD. (2009). Glutathione-S-transferase (GST) P1, GSTM1, exercise, ozone and asthma incidence in school children. Thorax 64: 197-202. http://dx.doi.org/10.1136/thx.2008.099366

Islam, T; Gauderman, WJ; Berhane, K; Mcconnell, R; Avol, E; Peters, JM; Gilliland, FD. (2007). The relationship between air pollution, lung function and asthma in adolescents. Thorax 62: 957-963. http://dx.doi.org/10.1136/thx.2007.078964

Islam, T; Mcconnell, R; Gauderman, WJ; Avol, E; Peters, JM; Gilliland, FD. (2008). Ozone, oxidant defense genes and risk of asthma during adolescence. Am J Respir Crit Care Med 177: 388-395. http://dx.doi.org/10.1164/rccm.200706-863OC

Jacquemin, B; Kauffmann, F; Pin, I; Le Moual, N; Bousquet, J; Gormand, F; Just, J; Nadif, R; Pison, C; Vervloet, D; Künzli, N; Siroux, V. (In Press) Air pollution and asthma control in the epidemiological study on the genetics and environment of asthma. J Epidemiol Community Health. http://dx.doi.org/10.1136/jech.2010.130229

Jakab, GJ; Bassett, DJP. (1990). Influenza virus infection, ozone exposure, and fibrogenesis. Am J Respir Crit Care Med 141: 1307-1315.

Jalaludin, B; Mannes, T; Morgan, G; Lincoln, D; Sheppeard, V; Corbett, S. (2007). Impact of ambient air pollution on gestational age is modified by season in Sydney, Australia. Environ Health 6: 16. http://dx.doi.org/10.1186/1476-069X-6-16

Jedlinska-Krakowska, M; Bomba, G; Jakubowski, K; Rotkiewicz, T; Jana, B; Penkowskii, A. (2006). Impact of oxidative stress and supplementation with vitamins E and C on testes morphology in rats. J Reprod Dev 52: 203-209.

Jerrett, M; Burnett, RT; Pope, CA, III; Ito, K; Thurston, G; Krewski, D; Shi, Y; Calle, E; Thun, M. (2009). Long-term ozone exposure and mortality. N Engl J Med 360: 1085-1095. http://dx.doi.org/10.1056/NEJMoa0803894

Jiang, LL; Zhang, YH; Song, GX; Chen, GH; Chen, BH; Zhao, NQ; Kan, HD. (2007). A time series analysis of outdoor air pollution and preterm birth in Shanghai, China. Biomed Environ Sci 20: 426-431.

Joad, JP; Kott, KS; Bric, JM; Peake, JL; Plopper, CG; Schelegle, ES; Gershwin, LJ; Pinkerton, KE. (2006). Structural and functional localization of airway effects from episodic exposure of infant monkeys to allergen and/or ozone. Toxicol Appl Pharmacol 214: 237-243. http://dx.doi.org/10.1016/j.taap.2005.12.012

Joad, JP; Kott, KS; Bric, JM; Schelegle, ES; Gershwin, LJ; Plopper, CG; Peake, JL; Pinkerton, KE. (2008). The effects of inhaled corticosteroids on intrinsic responsiveness and histology of airways from infant monkeys exposed to house dust mite allergen and ozone. Toxicol Appl Pharmacol 226: 153-160. http://dx.doi.org/10.1016/j.taap.2007.09.005

Kajekar, R; Pieczarka, EM; Smiley-Jewell, SM; Schelegle, ES; Fanucchi, MV; Plopper, CG. (2007). Early postnatal exposure to allergen and ozone leads to hyperinnervation of the pulmonary epithelium. Respir Physiol Neurobiol 155: 55-63. http://dx.doi.org/10.1016/j.resp.2006.03.002

Karr, C; Lumley, T; Schreuder, A; Davis, R; Larson, T; Ritz, B; Kaufman, J. (2007). Effects of subchronic and chronic exposure to ambient air pollutants on infant bronchiolitis. Am J Epidemiol 165: 553-560. http://dx.doi.org/10.1093/aje/kwk032

Katre, A; Ballinger, C; Akhter, H; Fanucchi, M; Kim, DK; Postlethwait, E; Liu, RM. (2011). Increased transforming growth factor beta 1 expression mediates ozone-induced airway fibrosis in mice. Inhal Toxicol 23: 486-494. http://dx.doi.org/10.3109/08958378.2011.584919

Kavlock, R; Daston, G; Grabowski, CT. (1979). Studies on the developmental toxicity of ozone. I. Prenatal effects. Toxicol Appl Pharmacol 48: 19-28. http://dx.doi.org/10.1016/S0041-008X(79)80004-6

Kavlock, RJ; Meyer, E; Grabowski, CT. (1980). Studies on the developmental toxicity of ozone: Postnatal effects. Toxicol Lett 5: 3-9. http://dx.doi.org/10.1016/0378-4274(80)90141-1

Kim, MY; Cho, MY. (2009a). Toxicity and carcinogenicity of ozone in combination with 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone and dibutyl phthalate in B6C3F1 mice for 16 and 32 weeks. Biomed Environ Sci 22: 216-222. http://dx.doi.org/10.1016/S0895-3988(09)60048-9

Kim, MY; Cho, MY. (2009b). Tumorigenesis in B6C3F1 mice exposed to ozone in combination with 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone and dietary dibutyl phthalate. Toxicol Ind Health 25: 189-195. http://dx.doi.org/10.1177/0748233709106185

Kinney, PL; Lippmann, M. (2000). Respiratory effects of seasonal exposures to ozone and particles. Arch Environ Occup Health 55: 210-216.

Kleeberger, SR; Peden, D. (2005). Gene-environment interactions in asthma and other respiratory diseases [Review]. Annu Rev Med 56: 383-400. http://dx.doi.org/10.1146/annurev.med.56.062904.144908

Kodavanti, UP; Thomas, R; Ledbetter, AD; Schladweiler, MC; Shannahan, JH; Wallenborn, JG; Lund, AK; Campen, MJ; Butler, EO; Gottipolu, RR; Nyska, A; Richards, JE; Andrews, D; Jaskot, RH; Mckee, J; Kotha, S. R.; Patel, RB; Parianandi, NL. (2011). Vascular and cardiac impairments in rats Inhaling ozone and diesel exhaust particles. Environ Health Perspect 119: 312-318. http://dx.doi.org/10.1289/ehp.1002386

Krewski, D; Jerrett, M; Burnett, RT; Ma, R; Hughes, E; Shi, Y; Turner, MC; 3rd, PA; Thurston, G; Calle, EE; Thun, MJ; Beckerman, B; DeLuca, P; Finkelstein, N; Ito, K; Moore, DK; Newbold, KB; Ramsay, T; Ross, Z; Shin, H; Tempalski, B. (2009). Extended follow-up and spatial analysis of the American Cancer Society study linking particulate air pollution and mortality (pp. 5-114; discussion 115-136). (ISSN 1041-5505

Künzli, N. (2012). Is air pollution of the 20th century a cause of current asthma hospitalisations? [Editorial]. Thorax 67: 2-3. http://dx.doi.org/10.1136/thoraxjnl-2011-200919

Kuo, HW; Lai, JS; Lee, MC; Tai, RC; Lee, MC. (2002). Respiratory effects of air pollutants among asthmatics in central Taiwan. Arch Environ Occup Health 57: 194-200.

Lampl, M; Jeanty, P. (2003). Timing is everything: A reconsideration of fetal growth velocity patterns identifies the importance of individual and sex differences. Am J Hum Biol 15: 667-680. http://dx.doi.org/10.1002/ajhb.10204

Larson, SD; Schelegle, ES; Walby, WF; Gershwin, LJ; Fanuccihi, MV; Evans, MJ; Joad, JP; Tarkington, BK; Hyde, DM; Plopper, CG. (2004). Postnatal remodeling of the neural components of the epithelial-mesenchymal trophic unit in the proximal airways of infant rhesus monkeys exposed to ozone and allergen. Toxicol Appl Pharmacol 194: 211-220. http://dx.doi.org/10.1016/j.taap.2003.09.025

Last, JA; Gelzleichter, TR; Harkema, J; Hawk, S. (1994). Consequences of prolonged inhalation of ozone on Fischer-344/N rats: Collaborative studies. Part I: Content and cross-linking of lung collagen [HEI]. (Research Report 65-I). Cambridge, MA: Health Effects Institute. http://pubs.healtheffects.org/view.php?id=81

Last, JA; Reiser, KM; Tyler, WS; Rucker, RB. (1984). Long-term consequences of exposure to ozone. I. Lung collagen content. Toxicol Appl Pharmacol 72: 111-118.

Last, JA; Warren, DL; Pecquet-Goad, E; Witschi, H. (1987). Modification by ozone of lung tumor development in mice. J Natl Cancer Inst 78: 149-154.

Latzin, P; Röösli, M; Huss, A; Kuehni, CE; Frey, U. (2009). Air pollution during pregnancy and lung function in newborns: A birth cohort study. Eur Respir J 33: 594-603. http://dx.doi.org/10.1183/09031936.00084008

Lee, SJ; Hajat, S; Steer, PJ; Filippi, V. (2008c). A time-series analysis of any short-term effects of meteorological and air pollution factors on preterm births in London, UK. Environ Res 106: 185-194.

Lee, YL; Lin, YC; Lee, YC; Wang, JY; Hsiue, TR; Guo, YL. (2004b). Glutathione S-transferase P1 gene polymorphism and air pollution as interactive risk factors for childhood asthma. Clin Exp Allergy 34: 1707-1713.

Lee, YL; Mcconnell, R; Berhane, K; Gilliland, FD. (2009b). Ambient ozone modifies the effect of tumor necrosis factor G-308A on bronchitic symptoms among children with asthma. Allergy 64: 1342-1348. http://dx.doi.org/10.1111/j.1398-9995.2009.02014.x

Legro, RS; Sauer, MV; Mottla, GL; Richter, KS; Li, X; Dodson, WC; Liao, D. (2010). Effect of air quality on assisted human reproduction. Hum Reprod 25: 1317-1324. http://dx.doi.org/10.1093/humrep/deq021

Li, H; Romieu, I; Sienra-Monge, JJ; Ramirez-Aguilar, M; Estela del Rio-Navarro, B; Kistner, EO; Gjessing, HK; Irma del Carmen, LS; Chiu, GY; London, SJ. (2006a). Genetic polymorphisms in arginase I and II and childhood asthma and atopy. J Allergy Clin Immunol 117: 119126.

Li, YF; Gauderman, WJ; Conti, DV; Lin, PC; Avol, E; Gilliland, FD. (2008). Glutathione S-Transferase P1, Maternal Smoking, and Asthma in Children: A Haplotype-Based Analysis. Environ Health Perspect 116: 409-415.

Lin, CA; Pereira, LAA; Nishioka, DC; Conceicao, GMS; Graga, ALF; Saldiva, PHN. (2004a). Air pollution and neonatal deaths in Sao Paulo, Brazil. Braz J Med Biol Res 37: 765-770.

Lin, CM; Li, CY; Yang, GY; Mao, IF. (2004b). Association between maternal exposure to elevated ambient sulfur dioxide during pregnancy and term low birth weight. Environ Res 96: 41-50. http://dx.doi.org/10.1016/j.envres.2004.03.005

Lin, S. (2010). E-mail correspondence from Shao Lin to Dennis Kotchmar dated December 20, 2010. Available online

Lin, S; Fitzgerald, E; Hwang, SA; Munsie, JP; Stark, A. (1999). Asthma hospitalization rates and socioeconomic status in New York State (1987-1993). J Asthma 36: 239-251.

Lin, S; Liu, X; Le, LH; Hwang, SA. (2008b). Chronic exposure to ambient ozone and asthma hospital admissions among children. Environ Health Perspect 116: 1725-1730. http://dx.doi.org/10.1289/ehp.11184

Linn, WS; Rappaport, EB; Berhane, KT; Bastain, TM; Avol, EL; Gilliland, FD. (2009). Exhaled nitric oxide in a population-based study of southern California schoolchildren. Respir Res 10: 28. http://dx.doi.org/10.1186/1465-9921-10-28

Lipfert, FW; Baty, JD; Miller, JP; Wyzga, RE. (2006a). PM2.5 constituents and related air quality variables as predictors of survival in a cohort of U.S. military veterans. Inhal Toxicol 18: 645-657. http://dx.doi.org/10.1080/08958370600742946

Lipfert, FW; Perry, HM, Jr; Miller, JP; Baty, JD; Wyzga, RE; Carmody, SE. (2000). The Washington University-EPRI veterans' cohort mortality study: Preliminary results. Inhal Toxicol 4: 41-73.

Lipfert, FW; Perry, HM, Jr; Miller, JP; Baty, JD; Wyzga, RE; Carmody, SE. (2003). Air pollution, blood pressure, and their long-term associations with mortality. Inhal Toxicol 15: 493-512.

Lipfert, FW; Wyzga, RE; Baty, JD; Miller, JP. (2006b). Traffic density as a surrogate measure of environmental exposures in studies of air pollution health effects: Long-term mortality in a cohort of US veterans. Atmos Environ 40: 154-169.

Liu, S; Krewski, D; Shi, Y; Chen, Y; Burnett, R. (2007b). Association between maternal exposure to ambient air pollutants during pregnancy and fetal growth restriction. J Expo Sci Environ Epidemiol 17: 426-432. http://dx.doi.org/10.1038/sj.jes.7500503

Loomis, D; Castillejos, M; Gold, DR; McDonnell, W; Borja-Aburto, VH. (1999). Air pollution and infant mortality in Mexico City. Epidemiology 10: 118-123.

López, I; Sánchez, I; Bizarro, P; Acevedo, S; Ustarroz, M; Fortoul, T. (2008). Ultrastructural alterations during embryonic rats' lung development caused by ozone. J Electron Microsc (Tokyo) 57: 19-23. http://dx.doi.org/10.1093/jmicro/dfm033

Maniar-Hew, K; Postlethwait, EM; Fanucchi, MV; Ballinger, CA; Evans, MJ; Harkema, JR; Carey, SA; McDonald, RJ; Bartolucci, AA; Miller, LA. (2011). Postnatal episodic ozone results in persistent attenuation of pulmonary and peripheral blood responses to LPS challenge. Am J Physiol Lung Cell Mol Physiol 300: L462-L471. http://dx.doi.org/10.1152/ajplung.00254.2010

Mannes, T; Jalaludin, B; Morgan, G; Lincoln, D; Sheppeard, V; Corbett, S. (2005). Impact of ambient air pollution on birth weight in Sydney, Australia. Occup Environ Med 62: 524-530. http://dx.doi.org/10.1136/oem.2004.014282

Mariassy, AT; Abraham, WM; Phipps, RJ; Sielczak, MW; Wanner, A. (1990). Effect of ozone on the postnatal development of lamb mucociliary apparatus. J Appl Physiol 68: 2504-2510.

Mariassy, AT; Sielczak, MW; Mccray, MN; Abraham, WM; Wanner, A. (1989). Effects of ozone on lamb tracheal mucosa: Quantitative glycoconjugate histochemistry. Am J Pathol 135: 871-879.

Marshall, E; Harris, G; Wartenberg, D. (2010). Oral cleft defects and maternal exposure to ambient air pollutants in New Jersey. Birth Defects Res A Clin Mol Teratol 88: 205-215. http://dx.doi.org/10.1002/bdra.20650

Martinez, FD. (2007a). Gene-environment interactions in asthma: with apologies to William of Ockham [Review]. Proc Am Thorac Soc 4: 26-31. http://dx.doi.org/10.1513/pats.200607-144JG

Martinez, FD. (2007b). Genes, environments, development and asthma: a reappraisal [Review]. Eur Respir J 29: 179-184. http://dx.doi.org/10.1183/09031936.00087906

Martinez, FD; Wright, AL; Taussig, LM; Holberg, CJ; Halonen, M; Morgan, WJ; Associates, GHM. (1995). Asthma and wheezing in the first six years of life. N Engl J Med 332: 133-138.

McConnell, R; Berhane, K; Gilliland, F; London, SJ; Islam, T; Gauderman, WJ; Avol, E; Margolis, HG; Peters, JM. (2002). Asthma in exercising children exposed to ozone: A cohort study. Lancet 359: 386-391. http://dx.doi.org/10.1016/S0140-6736(02)07597-9

McConnell, R; Berhane, K; Gilliland, F; London, SJ; Vora, H; Avol, E; Gauderman, WJ; Margolis, HG; Lurmann, F; Thomas, DC; Peters, JM. (1999). Air pollution and bronchitic symptoms in southern California children with asthma. Environ Health Perspect 107: 757-760.

McConnell, R; Berhane, K; Gilliland, F; Molitor, J; Thomas, D; Lurmann, F; Avol, E; Gauderman, WJ; Peters, JM. (2003). Prospective study of air pollution and bronchitic symptoms in children with asthma. Am J Respir Crit Care Med 168: 790-797.

McConnell, R; Islam, T; Shankardass, K; Jerrett, M; Lurmann, F; Gilliland, F; Gauderman, J; Avol, E; Kuenzli, N; Yao, L; Peters, J; Berhane, K. (2010). Childhood incident asthma and traffic-related air pollution at home and school. Environ Health Perspect 118: 1021-1026. http://dx.doi.org/10.1289/ehp.0901232

McDonnell, WF; Abbey, DE; Nishino, N; Lebowitz, MD. (1999a). Long-term ambient ozone concentration and the incidence of asthma in nonsmoking adults: the Ahsmog study. Environ Res 80: 110-121.

Meng, YY; Rull, RP; Wilhelm, M; Lombardi, C; Balmes, J; Ritz, B. (2010). Outdoor air pollution and uncontrolled asthma in the San Joaquin Valley, California. J Epidemiol Community Health 64: 142-147. http://dx.doi.org/10.1136/jech.2008.083576

Meng, YY; Wilhelm, M; Rull, RP; English, P; Ritz, B. (2007). Traffic and outdoor air pollution levels near residences and poorly controlled asthma in adults. Ann Allergy Asthma Immunol 98: 455-463.

Miller, LA; Gerriets, JE; Tyler, NK; Abel, K; Schelegle, ES; Plopper, CG; Hyde, DM. (2009). Ozone and allergen exposure during postnatal development alters the frequency and airway distribution of CD25+ cells in infant rhesus monkeys. Toxicol Appl Pharmacol 236: 39-48. http://dx.doi.org/10.1016/j.taap.2008.12.031

Moffatt, RK; Hyde, DM; Plopper, CG; Tyler, WS; Putney, LF. (1987). Ozone-induced adaptive and reactive cellular changes in respiratory bronchioles of Bonnet monkeys. Exp Lung Res 12: 57-74.

Monchaux, G; Morlier, JP; Morin, M; Rochefort, P; Maximilien, R; Tredaniel, J. (1996). Co-carcinogenic effects in rats of combined exposure to radon and ozone. Environ Int 221: S909-S915.

Moore, K; Neugebauer, R; Lurmann, F; Hall, J; Brajer, V; Alcorn, S; Tager, I. (2008). Ambient ozone concentrations cause increased hospitalizations for asthma in children: An 18-year study in Southern California. Environ Health Perspect 116: 1063-1070. http://dx.doi.org/10.1289/ehp.10497

Morello-Frosch, R; Jesdale, BM; Sadd, JL; Pastor, M. (2010). Ambient air pollution exposure and full-term birth weight in California. Environ Health 9: 44. http://dx.doi.org/10.1186/1476-069X-9-44

Morris, CR; Poljakovic, M; Lavrisha, L; Machado, L; Kuypers, FA; Morris, SM, Jr. (2004). Decreased arginine bioavailability and increased serum arginase activity in asthma. Am J Respir Crit Care Med 170: 148-153. http://dx.doi.org/10.1164/rccm.200309-1304OC

Mortimer, K; Neugebauer, R; Lurmann, F; Alcorn, S; Balmes, J; Tager, I. (2008a). Air pollution and pulmonary function in asthmatic children: Effects of prenatal and lifetime exposures. Epidemiology 19: 550-557. http://dx.doi.org/10.1097/EDE.0b013e31816a9dcb

Mortimer, K; Neugebauer, R; Lurmann, F; Alcorn, S; Balmes, J; Tager, I. (2008b). Early-lifetime exposure to air pollution and allergic sensitization in children with asthma. J Asthma 45: 874-881. http://dx.doi.org/10.1080/02770900802195722

Murphy, SL; Xu, JQ; Kochanek, KD. (2012). Deaths: Preliminary data for 2010. In National Vital Statistics Reports. (4). Hyattsville, MD: National Center for Health Statistics. http://www.cdc.gov/nchs/data/nvsr/nvsr60/nvsr60_04.pdf

NHLBI (National Institutes of Health, National Heart Lung and Blood Institute). (2007). Expert panel report 3: guidelines for the diagnosis and management of asthma. (07-4051). Bethesda, MD: National Institute of Health.

NTP (National Toxicology Program). (1994). Toxicology and carcinogenesis: Studies of ozone (CAS No 10028-15-6) and ozone/NNK (CAS No 10028-15-6/64091-91-4) in F344/N rats and B6C3F1 mice (pp. 314). (Technical Report No. 440). Research Triangle Park, NC. http://ntp.niehs.nih.gov/index.cfm?objectid=070A0EBD-081E-B501-E38F640803C3542C

Ober, C. (2005). Perspectives on the past decade of asthma genetics. J Allergy Clin Immunol 116: 274-278. http://dx.doi.org/10.1016/j.jaci.2005.04.039

Oryszczyn, MP; Bouzigon, E; Maccario, J; Siroux, V; Nadif, R; Wright, A; Kauffmann, F. (2007). Interrelationships of quantitative asthma-related phenotypes in the epidemiological study on the genetics and environment of asthma, bronchial hyperresponsiveness, and atopy. J Allergy Clin Immunol 119: 57-63.

Palli, D; Sera, F; Giovannelli, L; Masala, G; Grechi, D; Bendinelli, B; Caini, S; Dolara, P; Saieva, C. (2009). Environmental ozone exposure and oxidative DNA damage in adult residents of Florence, Italy. Environ Pollut 157: 1521-1525. http://dx.doi.org/10.1016/j.envpol.2008.09.011

Parker, JD; Akinbami, LJ; Woodruff, TJ. (2009). Air pollution and childhood respiratory allergies in the United States. Environ Health Perspect 117: 140-147. http://dx.doi.org/10.1289/ehp.11497

Parker, JD; Rich, DQ; Glinianaia, SV; Leem, JH; Wartenberg, D; Bell, ML; Bonzini, M; Brauer, M; Darrow, L; Gehring, U; Gouveia, N; Grillo, P; Ha, E; van den Hooven, EH; Jalaludin, B; Jesdale, BM; Lepeule, J; Morello-Frosch, R; Morgan, GG; Slama, R; Pierik, FH; Pesatori, AC; Sathyanarayana, S; Seo, J; Strickland, M; Tamburic, L; Woodruff, TJ. (2011). The international collaboration on air pollution and pregnancy outcomes: Initial results. Environ Health Perspect 119: 1023-1028. http://dx.doi.org/10.1289/ehp.1002725

Paz, C; Bazan-Perkins, B. (1992). Sleep-wake disorganization in cats exposed to ozone. Neurosci Lett 140: 270-272.

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

Peel, JL; Tolbert, PE; Klein, M; Metzger, KB; Flanders, WD; Knox, T; Mulholland, JA; Ryan, PB; Frumkin, H. (2005). Ambient air pollution and respiratory emergency department visits. Epidemiology 16: 164-174.

Peluso, M; Munnia, A; Hoek, G; Krzyzanowski, M; Veglia, F; Airoldi, L; Autrup, H; Dunning, A; Garte, S; Hainaut, P; Malaveille, C; Gormally, E; Matullo, G; Overvad, K; Raaschou-Nielsen, O; Clavel-Chapelon, F; Linseisen, J; Boeing, H; Trichopoulou, A; Trichopoulos, D; Kaladidi, A; Palli, D; Krogh, V; Tumino, R; Panico, S; Bueno-De-Mesquita, HB; Peeters, PH; Kumle, M; Gonzalez, CA; Martinez, C; Dorronsoro, M; Barricarte, A; Navarro, C; Quiros, JR; Berglund, G; Janzon, L; Jarvholm, B; Day, NE; Key, TJ; Saracci, R; Kaaks, R; Riboli, E; Vineis, P. (2005). DNA adducts and lung cancer risk: A prospective study. Cancer Res 65: 8042-8048. http://dx.doi.org/10.1158/0008-5472.CAN-04-3488

Penard-Morand, C; Charpin, D; Raherison, C; Kopferschmitt, C; Caillaud, D; Lavaud, F; Annesi-Maesano, I. (2005). Long-term exposure to background air pollution related to respiratory and allergic health in schoolchildren. Clin Exp Allergy 35: 1279-1287.

Pereira, FAC; De Assuncao, JV; Saldiva, PHN; Pereira, LAA; Mirra, AP; Braga, ALF. (2005). Influence of air pollution on the incidence of respiratory tract neoplasm. J Air Waste Manag Assoc 55: 83-87.

Pereira, LAA; Loomis, D; Conceicao, GMS; Braga, ALF; Arcas, RM; Kishi, HS; Singer, JM; Bohm, GM; Saldiva, PHN. (1998). Association between air pollution and intrauterine mortality in Sao Paulo, Brazil. Environ Health Perspect 106: 325-329.

Perepu, RS; Garcia, C; Dostal, D; Sethi, R. (2010). Enhanced death signaling in ozone-exposed ischemic-reperfused hearts. Mol Cell Biochem 336: 55-64. http://dx.doi.org/10.1007/s11010-009-0265-4

Peters, JM; Avol, E; Gauderman, WJ; Linn, WS; Navidi, W; London, SJ; Margolis, H; Rappaport, E; Vora, H; Gong, H, Jr; Thomas, DC. (1999a). A study of twelve southern California communities with differing levels and types of air pollution: II. Effects on pulmonary function. Am J Respir Crit Care Med 159: 768-775.

Peters, JM; Avol, E; Navidi, W; London, SJ; Gauderman, WJ; Lurmann, F; Linn, WS; Margolis, H; Rappaport, E; Gong, H, Jr; Thomas, DC. (1999b). A study of twelve southern California communities with differing levels and types of air pollution: I. Prevalence of respiratory morbidity. Am J Respir Crit Care Med 159: 760-767.

Petruzzi, S; De Acetis, L; Chiarotti, F; Sorace, A; Alleva, E. (1999). Limited changes in handedness and morphine reactivity in CD-1 mice after pre- and postnatal ozone exposure. Acta Neurobiol Exp (Wars) 59: 115-122.

Petruzzi, S; Fiore, M; Dell'Omo, G; Bignami, G; Alleva, E. (1995). Medium and long-term behavioral effects in mice of extended gestational exposure to ozone. Neurotoxicol Teratol 17: 463-470.

Pinkerton, KE; Brody, AR; Miller, FJ; Crapo, JD. (1989). Exposure to low levels of ozone results in enhanced pulmonary retention of inhaled asbestos fibers. Am J Respir Crit Care Med 140: 1075-1081.

Pinkerton, KE; Ménache, MG; Plopper, CG. (1995). Consequences of prolonged inhalation of ozone on F344/N rats: Collaborative studies. Part IX: Changes in the tracheobronchial epithelium, pulmonary acinus, and lung antioxidant enzyme activity [HEI] (pp. 41-98). (Research Report 65-IX). Cambridge, MA: Health Effects Institute. http://pubs.healtheffects.org/view.php?id=82

Pinkerton, KE; Weller, BL; Menache, MG; Plopper, CG. (1998). Consequences of prolonged inhalation of ozone on F344/N rats: Collaborative studies: Part XIII. A comparison of changes in the tracheobronchial epithelium and pulmonary acinus in male rats at 3 and 20 months. (HEI Research Report 65). Cambridge, MA: Health Effects Institute.

Plopper, CG; Chu, FP; Haselton, CJ; Peake, J; Wu, J; Pinkerton, KE. (1994). Dose-dependent tolerance to ozone: I. Tracheobronchial epithelial reorganization in rats after 20 months' exposure. Am J Pathol 144: 404-420.

Plopper, CG; Smiley-Jewell, SM; Miller, LA; Fanucchi, MV; Evans, MJ; Buckpitt, AR; Avdalovic, M; Gershwin, LJ; Joad, JP; Kajekar, R; Larson, S; Pinkerton, KE; Van Winkle, LS; Schelegle, ES; Pieczarka, EM; Wu, R; Hyde, DM. (2007). Asthma/allergic airways disease: Does postnatal exposure to environmental toxicants promote airway pathobiology? Toxicol Pathol 35: 97-110. http://dx.doi.org/10.1080/01926230601132030

Pope, CA, III. (2007). Mortality effects of longer term exposures to fine particulate air pollution: Review of recent epidemiological evidence [Review]. Inhal Toxicol 19: 33-38.

Pope, CA, III; Burnett, RT; Thun, MJ; Calle, EE; Krewski, D; Ito, K; Thurston, GD. (2002). Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. JAMA 287: 1132-1141.

Qian, Z; Liao, D; Lin, HM; Whitsel, EA; Rose, KM; Duan, Y. (2005). Lung function and long-term exposure to air pollutants in middle-aged American adults. Arch Environ Occup Health 60: 156-163.

Rage, E; Jacquemin, B; Nadif, R; Oryszczyn, MP; Siroux, V; Aguilera, I; Kauffmann, F; Kunzli, N. (2009a). Total serum IgE levels are associated with ambient ozone concentration in asthmatic adults. Allergy 64: 40-46. http://dx.doi.org/10.1111/j.1398-9995.2008.01800.x

Rage, E; Siroux, V; Kunzli, N; Pin, I; Kauffmann, F. (2009b). Air pollution and asthma severity in adults. Occup Environ Med 66: 182-188. http://dx.doi.org/10.1136/oem.2007.038349

Rahman, I; Biswas, SK; Kode, A. (2006). Oxidant and antioxidant balance in the airways and airway diseases. Eur J Pharmacol 533: 222-239. http://dx.doi.org/10.1016/j.ejphar.2005.12.087

Ramadour, M; Burel, C; Lanteaume, A; Vervloet, D; Charpin, D; Brisse, F; Dutau, H; Charpin, D. (2000). Prevalence of asthma and rhinitis in relation to long-term exposure to gaseous air pollutants. Allergy 55: 1163-1169.

Reiser, KM; Tyler, WS; Hennessy, SM; Dominguez, JJ; Last, JA. (1987). Long-term consequences of exposure to ozone: II. Structural alterations in lung collagen of monkeys. Toxicol Appl Pharmacol 89: 314-322. http://dx.doi.org/10.1016/0041-008X(87)90151-7

Renzetti, G; Silvestre, G; D'Amario, C; Bottini, E; Gloria-Bottini, F; Bottini, N; Auais, A; Perez, MK; Piedimonte, G. (2009). Less air pollution leads to rapid reduction of airway inflammation and improved airway function in asthmatic children. Pediatrics 123: 1051-1058. http://dx.doi.org/10.1542/peds.2008-1153

Ritz, B; Wilhelm, M. (2008). Ambient air pollution and adverse birth outcomes: Methodologic issues in an emerging field. Basic Appl Ecol 102: 182-190.

Ritz, B; Wilhelm, M; Hoggatt, KJ; Ghosh, JK. (2007). Ambient air pollution and preterm birth in the environment and pregnancy outcomes study at the University of California, Los Angeles. Am J Epidemiol 166: 1045-1052.

Ritz, B; Wilhelm, M; Zhao, Y. (2006). Air pollution and infant death in southern California, 1989-2000. Pediatrics 118: 493-502.

Ritz, B; Yu, F. (1999). The effect of ambient carbon monoxide on low birth weight among children born in southern California between 1989 and 1993. Environ Health Perspect 107: 17-25.

Ritz, B; Yu, F; Chapa, G; Fruin, S. (2000). Effect of air pollution on preterm birth among children born in Southern California between 1989 and 1993. Epidemiology 11: 502-511.

Ritz, B; Yu, F; Fruin, S; Chapa, G; Shaw, GM; Harris, JA. (2002). Ambient air pollution and risk of birth defects in Southern California. Am J Epidemiol 155: 17-25.

Rivas-Arancibia, S; Dorado-Martinez, C; Borgonio-Perez, G; Hiriart-Urdanivia, M; Verdugo-Diaz, L; Duran-Vazquez, A; Colin-Baranque, L; Avila-Costa, MR. (2000). Effects of taurine on ozone-induced memory deficits and lipid peroxidation levels in brains of young, mature, and old rats. Environ Res 82: 7-17. http://dx.doi.org/10.1006/enrs.1999.3996

Rivas-Arancibia, S; Guevara-Guzmán, R; López-Vidal, Y; Rodríguez-Martínez, E; Gomes, MZ; Angoa-Pérez, M; Raisman-Vozari, R. (2010). Oxidative stress caused by ozone exposure induces loss of brain repair in the hippocampus of adult rats. Toxicol Sci 113: 187-197. http://dx.doi.org/10.1093/toxsci/kfp252

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

Rojas-Martinez, R; Perez-Padilla, R; Olaiz-Fernandez, G; Mendoza-Alvarado, L; Moreno-Macias, H; Fortoul, T; Mcdonnell, W; Loomis, D; Romieu, I. (2007). Lung function growth in children with long-term exposure to air pollutants in Mexico City. Am J Respir Crit Care Med 176: 377-384. http://dx.doi.org/10.1164/rccm.200510-1678OC

Romero-Velazquez, RM; Alfaro-Rodriguez, A; Gonzalez-Pina, R; Gonzalez-Maciel, A. (2002). Effect of ozone prenatal exposure on postnatal development of cerebellum. Proc West Pharmacol Soc 45: 65-67.

Romero, R; Espinoza, J; Kusanovic, JP; Gotsch, F; Hassan, S; Erez, O; Chaiworapongsa, T; Mazor, M. (2006). The preterm parturition syndrome. BJOG 113: 17-42. http://dx.doi.org/10.1111/j.1471-0528.2006.01120.x

Romieu, I; Ramirez-Aguilar, M; Moreno-Macias, H; Barraza-Villarreal, A; Miller, P; Hernandez-Cadena, L; Carbajal-Arroyo, LA; Hernandez-Avila, M. (2004a). Infant mortality and air pollution: Modifying effect by social class. J Occup Environ Hyg 46: 1210-1216.

Rubes, J; Selevan, SG; Evenson, DP; Zudova, D; Vozdova, M; Zudova, Z; Robbins, WA; Perreault, SD. (2005). Episodic air pollution is associated with increased DNA fragmentation in human sperm without other changes in semen quality. Hum Reprod 20: 2776-2783.

Rubio, C; Paz, C. (2003). Indomethacin reverts sleep disorders produced by ozone exposure in rats. Toxicology 191: 89-96. http://dx.doi.org/10.1016/S0300-483X(03)00245-2

Salam, MT; Islam, T; Gauderman, WJ; Gilliland, FD. (2009). Roles of arginase variants, atopy, and ozone in childhood asthma. J Allergy Clin Immunol 123: 596-602. http://dx.doi.org/10.1016/j.jaci.2008.12.020

Salam, MT; Millstein, J; Li, YF; Lurmann, FW; Margolis, HG; Gilliland, FD. (2005). Birth outcomes and prenatal exposure to ozone, carbon monoxide, and particulate matter: Results from the Children's Health Study. Environ Health Perspect 113: 1638-1644. http://dx.doi.org/10.1289/ehp.8111

Santiago-López, D; Bautista-Martínez, JA; Reyes-Hernandez, CI; Aguilar-Martínez, M; Rivas-Arancibia, S. (2010). Oxidative stress, progressive damage in the substantia nigra and plasma dopamine oxidation, in rats chronically exposed to ozone. Toxicol Lett 197: 193-200. http://dx.doi.org/10.1016/j.toxlet.2010.05.020

Santucci, D; Sorace, A; Francia, N; Aloe, L; Alleva, E. (2006). Prolonged prenatal exposure to low-level ozone affects aggressive behaviour as well as NGF and BDNF levels in the central nervous system of CD-1 mice. Behav Brain Res 166: 124-130. http://dx.doi.org/10.1016/j.bbr.2005.07.032

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

Schmelzer, KR; Wheelock, AM; Dettmer, K; Morin, D; Hammock, BD. (2006). The role of inflammatory mediators in the synergistic toxicity of ozone and 1-nitronaphthalene in rat airways. Environ Health Perspect 114: 1354-1360.

Schöpke, R; Wolfer, DP; Lipp, HP; Leisinger-Trigona, MC. (1991). Swimming navigation and structural variations of the infrapyramidal mossy fibers in the hippocampus of the mouse. Hippocampus 1: 315-328. http://dx.doi.org/10.1002/hipo.450010322

Schroer, KT; Biagini Myers, JM; Ryan, PH; Lemasters, GK; Bernstein, DI; Villareal, M; Lockey, JE; Reponen, T; Grinshpun, S; Khurana Hershey, GK. (2009). Associations between multiple environmental exposures and Glutathione S-Transferase P1 on persistent wheezing in a birth cohort. J Pediatr 154: 401-408, 408.e401. http://dx.doi.org/10.1016/j.jpeds.2008.08.040

Selevan, SG; Borkovec, L; Slott, VL; Zudova, Z; Rubes, J; Evenson, DP; Perreault, SD. (2000). Semen quality and reproductive health of young Czech men exposed to seasonal air pollution. Environ Health Perspect 108: 887-894.

Sharkhuu, T; Doerfler, DL; Copeland, C; Luebke, RW; Gilmour, MI. (2011). Effect of maternal exposure to ozone on reproductive outcome and immune, inflammatory, and allergic responses in the offspring. J Immunotoxicol 8: 183-194. http://dx.doi.org/10.3109/1547691X.2011.568978

Silverman, RA; Ito, K. (2010). Age-related association of fine particles and ozone with severe acute asthma in New York City. J Allergy Clin Immunol 125: 367-373. http://dx.doi.org/10.1016/j.jaci.2009.10.061

Simonian, NA; Coyle, JT. (1996). Oxidative stress in neurodegenerative diseases [Review]. Annu Rev Pharmacol Toxicol 36: 83-106. http://dx.doi.org/10.1146/annurev.pa.36.040196.000503

Slama, R; Darrow, L; Parker, J; Woodruff, TJ; Strickland, M; Nieuwenhuijsen, M; Glinianaia, S; Hoggatt, KJ; Kannan, S; Hurley, F; Kalinka, J; Sram, R; Brauer, M; Wilhelm, M; Heinrich, J; Ritz, B. (2008). Meeting report: Atmospheric pollution and human reproduction. Environ Health Perspect 116: 791-798.

Smith, KR; Jerrett, M; Anderson, HR; Burnett, RT; Stone, V; Derwent, R; Atkinson, RW; Cohen, A; Shonkoff, SB; Krewski, D; Pope, CA, III; Thun, MJ; Thurston, G. (2009a). Public health benefits of strategies to reduce greenhouse-gas emissions: Health implications of short-lived greenhouse pollutants. Lancet 374: 2091-2103. http://dx.doi.org/10.1016/s0140-6736(09)61716-5

Sokol, RZ; Kraft, P; Fowler, IM; Mamet, R; Kim, E; Berhane, KT. (2006). Exposure to environmental ozone alters semen quality. Environ Health Perspect 114: 360-365. http://dx.doi.org/10.1289/ehp.8232

Son, JY; Cho, YS; Lee, JT. (2008). Effects of air pollution on postneonatal infant mortality among firstborn infants in Seoul, Korea: Case-crossover and time-series analyses. Arch Environ Occup Health 63: 108-113. http://dx.doi.org/10.3200/AEOH.63.3.108-113

Sousa, SI; Ferraz, C; Alvim-Ferraz, MC; Martins, FG; Vaz, LG; Pereira, MC. (2011). Spirometric tests to assess the prevalence of childhood asthma at Portuguese rural areas: Influence of exposure to high ozone levels. Environ Int 37: 474-478. http://dx.doi.org/10.1016/j.envint.2010.11.014

Sousa, SIV; Alvim-Ferraz, MCM; Martins, FG; Pereira, MC. (2009). Ozone exposure and its influence on the worsening of childhood asthma. Allergy 64: 1046-1055. http://dx.doi.org/10.1111/j.1398-9995.2009.01946.x

Sousa, SIV; Pereira, MMC; Martins, FG; Alvim-Ferraz, CM. (2008). Identification of regions with high ozone concentrations aiming the impact assessment on childhood asthma. Hum Ecol Risk Assess 14: 610-622. http://dx.doi.org/10.1080/10807030802074147

Sram, RJ; Binkova, B; Rossner, P; Rubes, J; Topinka, J; Dejmek, J. (1999). Adverse reproductive outcomes from exposure to environmental mutagens. Mutat Res-Fundam Mol Mech Mutagen 428: 203-215. http://dx.doi.org/10.1016/S1383-5742(99)00048-4

Stedman, JR; Kent, AJ. (2008). An analysis of the spatial patterns of human health related surface ozone metrics across the UK in 1995, 2003 and 2005. Atmos Environ 42: 1702-1716. http://dx.doi.org/10.1016/j.atmosenv.2007.11.033

Stephens, RJ; Sloan, MF; Groth, DG. (1976). Effects of long-term, low-level exposure of NO2 or O3 on rat lungs. Environ Health Perspect 16: 178-179.

Stockstill, BL; Chang, LY; Menache, MG; Mellick, PW; Mercer, RR; Crapo, JD. (1995). Bronchiolarized metaplasia and interstitial fibrosis in rat lungs chronically exposed to high ambient levels of ozone. Toxicol Appl Pharmacol 134: 251-263. http://dx.doi.org/10.1006/taap.1995.1191

Stokinger, HE. (1962). Effects of air pollution in animals. In AC Stern (Ed.), Air pollution (pp. 282-334). New York, NY: Academic Press.

Strickland, MJ; Klein, M; Correa, A; Reller, MD; Mahle, WT; Riehle-Colarusso, TJ; Botto, LD; Flanders, WD; Mulholland, JA; Siffel, C; Marcus, M; Tolbert, PE. (2009). Ambient air pollution and cardiovascular malformations in Atlanta, Georgia, 1986-2003. Am J Epidemiol 169: 1004-1014. http://dx.doi.org/10.1093/aje/kwp011

Tager, IB; Balmes, J; Lurmann, F; Ngo, L; Alcorn, S; Kunzli, N. (2005). Chronic exposure to ambient ozone and lung function in young adults. Epidemiology 16: 751-759. http://dx.doi.org/10.1097/01.ede.0000183166.68809.b0

Tamer, L; Calikoglu, M; Ates, NA; Yildirim, H; Ercan, B; Saritas, E; Unlu, A; Atik, U. (2004). Glutathione-S-transferase gene polymorphisms (GSTT1, GSTM1, GSTP1) as increased risk factors for asthma. Respirology 9: 493-498. http://dx.doi.org/10.1111/j.1440-1843.2004.00657.x

Tovalin, H; Valverde, M; Morandi, MT; Blanco, S; Whitehead, L; Rojas, E. (2006). DNA damage in outdoor workers occupationally exposed to environmental air pollutants. Occup Environ Med 63: 230-236.

Tran, MU; Weir, AJ; Fanucchi, MV; Rodriguez, AE; Pantle, LM; Smiley-Jewell, SM; Van Winkle, LS; Evans, MJ; Miller, LA; Schelegle, ES; Gershwin, LJ; Hyde, DM; Plopper, CG. (2004). Smooth muscle hypertrophy in distal airways of sensitized infant rhesus monkeys exposed to house dust mite allergen. Clin Exp Allergy 34: 1627-1633. http://dx.doi.org/10.1111/j.1365-2222.2004.02057.x

Tsai, SS; Chen, CC; Hsieh, HJ; Chang, CC; Yang, CY. (2006). Air pollution and postneonatal mortality in a tropical city: Kaohsiung, Taiwan. Inhal Toxicol 18: 185-189.

Tyler, WS; Tyler, NK; Last, JA; Gillespie, MJ; Barstow, TJ. (1988). Comparison of daily and seasonal exposures of young monkeys to ozone. Toxicology 50: 131-144.

U.S. EPA (U.S. Environmental Protection Agency). (1996o). Table 6-19. Alveolar/bronchiolar tumor incidence in B6C3F mice in the National Toxicology Program's chronic ozone study [EPA Report]. In Air quality criteria for ozone and related photochemical oxidants (pp. 116). (EPA/600/P-93/004AF). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (2006b). Air quality criteria for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-05/004AF). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=149923

Van Bree, L; Dormans, JAM, A; Koren, HS; Devlin, RB; Rombout, PJA. (2002). Attenuation and recovery of pulmonary injury in rats following short-term, repeated daily exposure to ozone. Inhal Toxicol 14: 883-900. http://dx.doi.org/10.1080/08958370290084674

Van Winkle, LS; Baker, GL; Chan, JK; Schelegle, ES; Plopper, CG. (2010). Airway mast cells in a rhesus model of childhood allergic airways disease. Toxicol Sci 116: 313-322. http://dx.doi.org/10.1093/toxsci/kfq119

Vanguilder, HD; Freeman, WM. (2011). The hippocampal neuroproteome with aging and cognitive decline: Past progress and future directions. Front Aging Neurosci 3: 8. http://dx.doi.org/10.3389/fnagi.2011.00008

Veninga, TS. (1967). Toxicity of ozone in comparison with ionizing radiation. Strahlentherapie 134: 469-477.

von Mutius, E. (2009). Gene-environment interactions in asthma [Review]. J Allergy Clin Immunol 123: 3-11. http://dx.doi.org/10.1016/j.jaci.2008.10.046

Vrijheid, M; Martinez, D; Manzanares, S; Dadvand, P; Schembari, A; Rankin, J; Nieuwenhuijsen, M. (2011). Ambient air pollution and risk of congenital anomalies: A systematic review and meta-analysis [Review]. Environ Health Perspect 119: 598-606. http://dx.doi.org/10.1289/ehp.1002946

Wang, TN; Ko, YC; Chao, YY; Huang, CC; Lin, RS. (1999). Association between indoor and outdoor air pollution and adolescent asthma from 1995 to 1996 in Taiwan. Environ Res 81: 239-247.

Wang, XY; Hu, W; Tong, S. (2009c). Long-term exposure to gaseous air pollutants and cardio-respiratory mortality in Brisbane, Australia. Geospat Health 3: 257-263.

Wenten, M; Gauderman, WJ; Berhane, K; Lin, PC; Peters, J; Gilliland, FD. (2009). Functional variants in the catalase and myeloperoxidase genes, ambient air pollution, and respiratory-related school absences: An example of epistasis in gene-environment interactions. Am J Epidemiol 170: 1494-1501. http://dx.doi.org/10.1093/aje/kwp310

Wilhelm, M; Ritz, B. (2005). Local variations in CO and particulate air pollution and adverse birth outcomes in Los Angeles County, California, USA. Environ Health Perspect 113: 1212-1221. http://dx.doi.org/10.1289/ehp.7751

Witschi, H. (1991). Effects of oxygen and ozone on mouse lung tumorigenesis [Review]. Exp Lung Res 17: 473-483. http://dx.doi.org/10.3109/01902149109064433

Witschi, H; Espiritu, I; Pinkerton, KE; Murphy, K; Maronpot, RR. (1999). Ozone carcinogenesis revisited. Toxicol Sci 52: 162-167.

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

Wollmann, HA. (1998). Intrauterine growth restriction: Definition and etiology. Horm Res 49: 1-6.

Wood, AM; Harrison, RM; Semple, S; Ayres, JG; Stockley, RA. (2009). Outdoor air pollution is associated with disease severity in a1-antitrypsin deficiency. Eur Respir J 34: 346-353. http://dx.doi.org/10.1183/09031936.00087908

Woodruff, TJ; Darrow, LA; Parker, JD. (2008). Air pollution and postneonatal infant mortality in the United States, 1999-2002. Environ Health Perspect 116: 110-115. http://dx.doi.org/10.1289/ehp.10370

Woodruff, TJ; Parker, JD; Adams, K; Bell, ML; Gehring, U; Glinianaia, S; Ha, EH; Jalaludin, B; Slama, R. (2010). International Collaboration on Air Pollution and Pregnancy Outcomes (ICAPPO). Int J Environ Res Public Health 7: 2638-2652. http://dx.doi.org/10.3390/ijerph7062638

Woodruff, TJ; Parker, JD; Darrow, LA; Slama, R; Bell, ML; Choi, H; Glinianaia, S; Hoggatt, KJ; Karr, CJ; Lobdell, DT; Wilhelm, M. (2009). Methodological issues in studies of air pollution and reproductive health. Environ Res 109: 311-320. http://dx.doi.org/10.1016/j.envres.2008.12.012

Yang, CY; Hsieh, HJ; Tsai, SS; Wu, TN; Chiu, HF. (2006). Correlation between air pollution and postneonatal mortality in a subtropical city: Taipei, Taiwan. J Toxicol Environ Health A 69: 2033-2040. http://dx.doi.org/10.1080/15287390600746181

Zanobetti, A; Bind, MAC; Schwartz, J. (2008). Particulate air pollution and survival in a COPD cohort. Environ Health Perspect 7: 48. http://dx.doi.org/10.1186/1476-069X-7-48

Zanobetti, A; Schwartz, J. (2007). Particulate air pollution, progression, and survival after myocardial infarction. Environ Health Perspect 115: 769-775. http://dx.doi.org/10.1289/ehp.9201

Zanobetti, A; Schwartz, J. (2011). Ozone and survival in four cohorts with potentially predisposing diseases. Am J Respir Crit Care Med 184: 836-841. http://dx.doi.org/10.1164/rccm.201102-0227OC

Zelac, RE; Cromroy, HL; Bolch, WE, Jr; Dunavant, BG; Bevis, HA. (1971a). Inhaled ozone as a mutagen: I Chromosome aberrations induced in Chinese hamster lymphocytes. Environ Res 4: 262-282.

Zelac, RE; Cromroy, HL; Bolch, WE, Jr; Dunavant, BG; Bevis, HA. (1971b). Inhaled ozone as a mutagen: II Effect on the frequency of chromosome aberrations observed in irradiated Chinese hamsters. Environ Res 4: 325-342.

# 8 POPULATIONS POTENTIALLY AT INCREASED RISK FOR OZONE-RELATED HEALTH EFFECTS

Interindividual variation in human responses to air pollution exposure can result in some groups being at increased risk for detrimental effects in response to ambient exposure to an air pollutant. The NAAQS are intended to provide an adequate margin of safety for both the population as a whole and those potentially at increased risk for health effects in response to ambient air pollution[1]. To facilitate the identification of populations and lifestages at greater risk for air pollutant related health effects, studies have evaluated factors that may contribute to the susceptibility and/or vulnerability of an individual to air pollutants. The definitions of susceptibility and vulnerability have been found to vary across studies, but in most instances "susceptibility" refers to biological or intrinsic factors (e.g., lifestage, sex, pre-existing disease/conditions) while "vulnerability" refers to non-biological or extrinsic factors (e.g., socioeconomic status [SES]) (U.S. EPA, 2010c, 2009d). In some cases, the terms "at-risk" and "sensitive" populations have been used to encompass these concepts more generally. The main goal of this evaluation is to identify and understand those factors that result in a population or lifestage being at increased risk of an air pollutant-related health effect, not to categorize the factors. To this end, previous ISAs and reviews (Sacks et al., 2011; U.S. EPA, 2010c, 2009d) have used "susceptible populations" to encompass these various factors. In this chapter, "at-risk" is the all-encompassing term used for groups with specific factors that increase the risk of an air pollutant (e.g., $O_3$)-related health effect in a population.

Individuals, and ultimately populations, could experience increased risk for air pollutant induced health effects in multiple different ways. A group with intrinsically increased risk would have some factor(s) that increases risk for an effect through a biological mechanism. In general, people in this category would have a steeper concentration-risk relationship, compared to those not in the category. Potential factors that are often considered intrinsic include genetic background and sex. A group of people could also have extrinsically increased risk, which would be through an external, non-biological factor. Examples of extrinsic factors include SES and diet.

In addition, some groups are at increased risk due to differential exposure, which can encompass multiple forms. This includes increased risk due to increased internal dose at a given exposure concentration. For example, individuals may have a greater dose of delivered pollutant because of their breathing pattern. This group would include persons who work outdoors or exercise outdoors. Some outdoor workers could also have greater exposure (concentration x time), regardless of the delivered dose and this greater exposure may increase the risk of $O_3$-related health effects.

[1] The legislative history of section 109 indicates that a primary standard is to be set at "the maximum permissible ambient air level … which will protect the health of any [sensitive] group of the population," and that for this purpose "… reference should be made to a representative sample of persons comprising the sensitive group rather than to a single person in such a group." [S. Rep. No. 91- 1196, 91st Cong., 2d Sess. 10 (1970)].

Finally, there are those who might be placed at increased risk for experiencing a greater exposure, and therefore increased risk of health effects, by being exposed at a higher concentration. For example, groups of people exposed to higher air pollutant concentrations due to less availability/use of home air conditioners (i.e., more open windows on high $O_3$ days) or close proximity to known sources of air pollution.

Some factors described above are multifaceted. For example, SES may affect access to medical care, which itself may contribute to the presence of pre-existing diseases and conditions considered as intrinsic factors. Additionally, children tend to spend more time outdoors at higher levels of activity than adults, which leads to increased intake dose and exposure, but they also have biological (i.e., intrinsic) differences when compared to adults.

The emphasis of this chapter is to identify and understand the factors that potentially increase the risk of $O_3$-related health effects, regardless of whether the increased risk is due to intrinsic factors, extrinsic factors, increased dose, increased exposure, or a combination. The following sections examine factors that potentially lead to increased risk of $O_3$-related health effects and characterize the overall weight of evidence for each factor. Most of the factors are related to greater health effects given a specific dose but there is also discussion of increased internal dose and/or exposure at a given concentration integrated throughout the sections (i.e., lifestage, outdoor workers, and air conditioning use).

### Approach to Classifying Potential At-Risk Factors

To identify factors that potentially lead to some populations being at greater risk to air pollutant related health effects, the evidence across relevant scientific disciplines (i.e., exposure sciences, dosimetry, controlled human exposure, toxicology, and epidemiology) was evaluated. In this systematic approach, the collective evidence is used to examine coherence of effects across disciplines and determine biological plausibility. By first focusing on studies that conduct stratified analyses (i.e., epidemiologic or controlled human exposure) it is possible to identify factors that may result in some populations being at greater risk of an air pollutant related health effect. These types of studies allow for an evaluation of populations exposed to similar air pollutant (e.g., $O_3$) concentrations within the same study design. Experimental studies also provide important lines of evidence in the evaluation of factors that may lead to increased risk of an air pollutant related-health effect. Toxicological studies conducted using animal models of disease and controlled human exposure studies that examine individuals with underlying disease or genetic polymorphisms may provide evidence to inform whether a population is at increased risk of an air pollutant related health effect in the absence of stratified epidemiologic analyses. Additionally these studies can provide support for coherence with the health effects observed in epidemiologic studies as well as an understanding of biological plausibility. Information on factors that may result in increased risk of $O_3$-related health effects can also be obtained from studies that examine exposure differences between populations. The collective results across the scientific

disciplines comprise the overall weight of evidence that is used to determine whether a specific factor results in a population being at increased risk of an air pollutant related health effect.

Building on the causal framework discussed in detail in the Preamble and used throughout the ISA, conclusions are made regarding the strength of evidence, based on the evaluation and synthesis across scientific disciplines, for each factor that may contribute to increased risk of an $O_3$-related health effect. The conclusions were drawn while considering the "Aspects to Aid in Judging Causality" discussed in Table 1 of the Preamble. The categories considered for evaluating the potential increased risk of an air pollutant-related health effect are "adequate evidence," "suggestive evidence," "inadequate evidence," and "evidence of no effect." They are described in more detail in Table 8-1.

**Table 8-1 Classification of Evidence for Potential At-Risk Factors.**

| | Health Effects |
|---|---|
| Adequate evidence | There is substantial, consistent evidence within a discipline to conclude that a factor results in a population or lifestage being at increased risk of air pollutant-related health effect(s) relative to some reference population or lifestage. Where applicable this includes coherence across disciplines. Evidence includes multiple high-quality studies. |
| Suggestive evidence | The collective evidence suggests that a factor results in a population or lifestage being at increased risk of an air pollutant-related health effect relative to some reference population or lifestage, but the evidence is limited due to some inconsistency within a discipline or, where applicable, a lack of coherence across disciplines. |
| Inadequate evidence | The collective evidence is inadequate to determine if a factor results in a population or lifestage being at increased risk of an air pollutant-related health effect relative to some reference population or lifestage. The available studies are of insufficient quantity, quality, consistency and/or statistical power to permit a conclusion to be drawn. |
| Evidence of no effect | There is substantial, consistent evidence within a discipline to conclude that a factor does not result in a population or lifestage being at increased risk of air pollutant-related health effect(s) relative to some reference population or lifestage. Where applicable this includes coherence across disciplines. Evidence includes multiple high-quality studies. |

This chapter evaluates the various factors indicated in the literature that may result in a population being at increased risk of an $O_3$-related health effect. For further detail on the epidemiologic, controlled human exposure, and toxicological studies included in this chapter, see Chapters 5, 6, and 7.

## 8.1 Genetic Factors

The potential effects of air pollution on individuals with specific genetic characteristics have been examined; studies often target polymorphisms in already identified candidate susceptibility genes or in genes whose protein products are thought to be involved in the biological mechanism underlying the health effect of an air pollutant (Sacks et al., 2011). As a result, multiple studies that examined the effect of short- and long-term $O_3$ exposure on respiratory function have focused on whether various gene profiles lead to an increased risk of $O_3$-related health effects.

For more details on the function and mode of action of the genetic factors discussed in this section, see Section 5.4.2.1. Additionally, a limited number of toxicological studies have examined the joint effects of nutrition and genetics. Details on these toxicological studies of nutrition and genetics can be found in Section 5.4.2.3.

Multiple genes, including glutathione S-transferase Mu 1 (GSTM1) and tumor necrosis factor-α (TNF-α) were evaluated in the 2006 $O_3$ AQCD and found to have a "potential role… in the innate susceptibility to $O_3$" (U.S. EPA, 2006b). Epidemiologic, controlled human exposure, and toxicological studies performed since the 2006 $O_3$ AQCD have continued to examine the roles of GSTM1 and TNF-α in modifying $O_3$-related health effects and have examined other gene variants that may also increase risk. Due to small sample sizes, many controlled human exposure studies are limited in their ability to test genes with low frequency minor alleles and therefore, some genes important for $O_3$-related health effects may not have been examined in these types of studies. A summary of effect measure modification findings from epidemiologic and controlled human exposure studies discussed in this section is included in Table 8-2 and from animal toxicology studies in Table 8-3.

Epidemiologic studies that examined the effects of short-term exposure to $O_3$ on lung function included analyses of potential gene-environment interactions. Romieu et al. (2006) reported an association between $O_3$ and respiratory symptoms that were larger among children with GSTM1 null or glutathione S-transferase P 1 (GSTP1) Val/Val genotypes compared with children with GSTM1 positive or GSTP1 Ile/Ile or Ile/Val genotypes, respectively. However, results suggested that $O_3$-associated decreases in lung function may be greater among children with GSTP1 Ile/Ile or Ile/Val compared to GSTP1 Val/Val. Alexeeff et al. (2008) reported greater $O_3$-related decreases in lung function among GSTP1 Val/Val adults than those with GSTP1 Ile/Ile or GSTP1 Ile/Val genotypes. In addition, they detected greater $O_3$-associated decreases in lung function for adults with long GT dinucleotide repeats in heme-oxygenase-1 (HMOX1) promoters.

## Table 8-2 Summaries of results from epidemiologic and controlled human exposures studies of modification by genetic variants.

| Gene variant | Comparison group | Health outcome /population | Effect modification of association for the gene variant | Reference |
|---|---|---|---|---|
| GSTM1 null | GSTM1 positive | Respiratory symptoms among asthmatic children | ↑ | Romieu et al. (2006) |
| GSTP1 Val/Val | GSTP1 Ile/Ile or Ile/Val | Respiratory symptoms among asthmatic children | ↑ | |
| GSTP1 Ile/Ile or Ile/Val | GSTP1 Val/Val | Lung function among asthmatic children | ↓ | |
| GSTP1 Ile/Val or Val/Val | GSTP1 Ile/Ile | Lung function among adults | ↓ | Alexeeff et al. (2008) |
| HMOX1 S/L or L/L | HMOX1 S/S | Lung function among adults | ↓ | |
| NQO1 wildtype and GSTM1 null | Other combinations | Lung function among healthy adults with exercise | ↓ | Bergamaschi et al. (2001) |
| NQO1 wildtype and GSTM1 null | Other combinations | Lung function among mild-to-moderate asthmatics with moderate exercise | = | Vagaggini et al. (2010) |
| NQO1 wildtype and GSTM1 null | Other combinations | Inflammatory responses among mild-to-moderate asthmatics with moderate exercise | = | |
| GSTM1 null | GSTM1 positive | Lung function among healthy adults with intermittent moderate exercise | = | Kim et al. (2011) |
| GSTM1 null | GSTM1 positive | Inflammatory responses among healthy adults with intermittent moderate exercise | = | |
| GSTM1 null | GSTM1 positive | Lung function among asthmatic children | ↓ | Romieu et al. (2004b) |
| GSTM1 null | GSTM1 positive | Lung function among healthy adults with intermittent moderate exercise | = | Alexis et al. (2009) |
| GSTM1 null | GSTM1 positive | Inflammatory changes among healthy adults with intermittent moderate exercise | ↑ | |

Several controlled human exposure studies have reported that genetic polymorphisms of antioxidant enzymes may modulate pulmonary function and inflammatory responses to $O_3$ challenge. Healthy carriers of NAD(P)H quinone oxidoreductase 1 (NQO1) wild type (wt) in combination with GSTM1 null genotype had greater decreases in lung function parameters with exposure to $O_3$ (Bergamaschi et al., 2001). Vagaggini et al. (2010) exposed mild-to-moderate asthmatics to $O_3$ during moderate exercise. In subjects with NQO1 wt and GSTM1 null, there was no evidence of changes in lung function or inflammatory responses to $O_3$. Kim et al. (2011) also recently conducted a study among young adults, about half of whom were GSTM1-null and half of whom were GSTM1-sufficient. They detected no difference in the $FEV_1$ responses to $O_3$ exposure by GSTM1 genotype and did not examine NQO1. In another study that examined GSTM1 but not NQO1, asthmatic children with GSTM1 null genotype (Romieu et al., 2004b) were reported to have greater decreases in lung function in relation to $O_3$ exposure. Additionally, supplementation with antioxidants (Vitamins C and E) had a slightly more beneficial effect among GSTM1 null children (for more on modification by diet, see Section 8.4.1).

In a study of healthy volunteers with GSTM1 sufficient and GSTM1 null genotypes exposed to $O_3$ with exercise, Alexis et al. (2009) found genotype effects on inflammatory responses but not lung function responses to $O_3$. At 4 hours post-$O_3$ exposure, individuals with either GSTM1 genotype had statistically significant increases in sputum neutrophils with a tendency for a greater increase in GSTM1 sufficient than GSTM1 nulls. At 24 hours postexposure, neutrophils had returned to baseline levels in the GSTM1 sufficient individuals. In the GSTM1 null subjects, neutrophil levels increased from 4 to 24 hours and were significantly greater than both baseline levels and levels at 24 hours in the GSTM1 sufficient individuals. In addition, $O_3$ exposure increased the expression of the surface marker CD14 in airway neutrophils of GSTM1 null subjects compared with GSTM1 sufficient subjects. CD14 and TLR4 are co-receptors for endotoxin, and signaling through this innate immune pathway has been shown to be important for a number of biological responses to $O_3$ exposure in toxicological studies (Garantziotis et al., 2010; Hollingsworth et al., 2010; Hollingsworth et al., 2004; Kleeberger et al., 2000). Alexis et al. (2009) also demonstrated decreased numbers of airway macrophages at 4 and 24 hours following $O_3$ exposure in GSTM1 sufficient subjects. Airway macrophages in GSTM1 null subjects were greater in number and found to have greater oxidative burst and phagocytic capability following $O_3$ exposure than those of GSTM1 sufficient subjects. Airway macrophages and dendritic cells from GSTM1 null subjects exposed to $O_3$ expressed higher levels of the surface marker HLA-DR; again suggesting activation of the innate immune system. Since there was no FA control in the Alexis et al. (2009) study, effects of the exposure other than $O_3$ cannot be ruled out. In general, the findings between these studies are inconsistent. It is possible that different genes may be important for different phenotypes. Additional studies, which include appropriate controls, are needed to clarify the influence of genetic polymorphisms on $O_3$ responsiveness in humans.

## Table 8-3 Summaries of results from animal toxicology studies of modification by genetic variants.

| Gene variant | Reference[a] | Exposure | Health outcome /population |
|---|---|---|---|
| Tlr4 | Hollingsworth et al. (2004); Kleeberger et al. (2000) | 0.3 ppm, 72 hours<br>0.3 ppm, 72 hours<br>2.0 ppm, 3 hours | Decreased hyperpermeability<br>No genotype difference in hyperpermeability, BALF cells, or AHR at 0.3ppm. Reduced AHR at 2.0ppm. |
| | Williams et al. (2007b) | 0.3 ppm, 3-24 hours | Decreased AHR. Reduced inflammation at 3 hours |
| Tlr2 | Williams et al. (2007b) | 0.3 ppm, 3-24 hours | Decreased inflammation and AHR |
| MyD88 | Williams et al. (2007b) | 0.3 ppm, 3-24 hours | Decreased inflammation, hyperpermeability, and AHR |
| Tnfr1/Tnfr2 | Cho et al. (2001) | 0.3 ppm, 3-48 hours | Decreased BALF cells, neutrophilia and lung damage. No genotype difference in hyperpermeability. |
| | Cho et al. (2007) | 2.0 ppm, 3 hours | Reduced AHR |
| Nfkb | Cho et al. (2007) | 0.3 ppm, 6-48 hours | Decreased inflammation, hyperpermeability, and lung damage |
| Jnk | Cho et al. (2007) | 0.3 ppm, 6-48 hours | Decreased inflammation, hyperpermeability, and lung damage |
| Il6 | Johnston et al. (2005b) | 0.3 ppm, 3-72 hours | Decreased neutrophilia and hyperpermeability, reduced soluble TNFRs, no effect on AHR |
| | | 2.0 ppm, 3 hours | Reduced neutrophilia and soluble TNFR2 and MIP-2 |
| Il10 | Backus et al. (2010) | 0.3 ppm, 24-72 hours | Increased inflammation |
| Marco | Dahl et al. (2007) | 0.3 ppm, 48 hours | Increased inflammation, 8-isoprostane, and hyperpermeability |
| Nos2 | Kleeberger et al. (2001) | 0.3 ppm, 72 hours | Decreased hyperpermeability, no effect on BALF cells |
| | Fakhrzadeh et al. (2002) | 0.8 ppm, 3 hours | Reduced AM nitric oxide, reactive nitrogen species, and superoxide anion, decreased $PGE_2$, increased COX expression, decreased hyperpermeability and BALF cells |
| | Kenyon et al. (2002) | 1.0 ppm, 8 h/night for 3 nights | Increased hyperpermeability, neutrophilia, MMP-9 activity, and protein nitration products |
| Hsp70 | Bauer et al. (2011) | 0.3 ppm, 6-72 hours | Decreased hyperpermeability and inflammation |
| NQO1 | Voynow et al. (2009) | 1.0 ppm, 3 hours | Reduced inflammation and AHR |
| Csb | Kooter et al. (2007) | 0.8 ppm, 8 hours | Decreased TNF-α in BALF. No genotype difference in neutrophilia or lung damage. |
| Mmp9 | Yoon et al. (2007) | 0.3 ppm 6-72 hours | Increased hyperpermeability, neutrophilia, inflammation, and lung damage |
| CD44 | Garantziotis et al. (2009) | 2.0 ppm, 3 hours | Decreased AHR |
| Cxcr2 | Johnston et al. (2005) | 1.0 ppm, 3 hours | Reduced neutrophilia, lung injury, and AHR. No change in chemokine expression or hyperpermeability |
| Il13 | Williams et al. (2008b) | 3.0 ppm, 3 hours | Reduced AHR, BALF cells, and neutrophilia |

[a]The table includes animal toxicology studies where responses are assessed after gene deletion.

In general, toxicological studies have reported differences in cardiac and respiratory effects after $O_3$ exposure among different mouse strains, which alludes to differential risk among individuals due to genetic variability (Tankersley et al., 2010; Chuang et al., 2009; Hamade and Tankersley, 2009; Hamade et al., 2008). Thus strains of mice which are prone to or resistant to $O_3$-induced effects have been used to systematically identify candidate genes that may increase risk of $O_3$-related health effects. Genome wide linkage analyses have identified quantitative trait loci for $O_3$-induced lung inflammation and hyperpermeability on chromosome 17 (Kleeberger et al., 1997) and chromosome 4 (Kleeberger et al., 2000), respectively, using recombinant inbred strains of mice. More specifically, these studies found that TNF (protein product is the inflammatory cytokine TNF-α) and Tlr4 (protein product is TLR4, involved in endotoxin responses) were candidate susceptibility genes (Kleeberger et al., 2000; Kleeberger et al., 1997). The TNF receptors 1 and 2 have also been found to play a role in injury, inflammation, and airway hyperreactivity in studies of $O_3$-exposed knockout mice (Cho et al., 2007; Cho et al., 2001) through NF-κB and MAPK/AP-1 (Jnk) signaling pathways (Cho et al., 2007). In addition to Tlr4, other innate immune pattern recognition signaling pathway genes, including Tlr2 and Myd88, appear to be important in responses to $O_3$, as demonstrated by Williams et al. (2007b). A role for the inflammatory cytokine IL-6 has been demonstrated in gene-deficient mice with respect to inflammation and injury, but not AHR (Johnston et al., 2005b; Yu et al., 2002). Other studies have demonstrated a key role for CXCR2, the chemokine receptor for the neutrophil chemokines KC and MIP-2, (Johnston et al., 2005a) and CD44, the major receptor for the extracellular matrix component hyaluronan (Garantziotis et al., 2009) in $O_3$-mediated AHR. Mice deficient in IL-10, an anti-inflammatory cytokine, demonstrated increased pulmonary inflammation in response to $O_3$ exposure (Backus et al., 2010). Thus genes related to innate immune signaling and pro- and anti-inflammatory genes are important for $O_3$-induced responses.

Altered $O_3$ responses between mouse strains could be due to genetic variability in nuclear factor erythroid 2-related factor 2 (Nrf-2), suggesting a role for genetic differences in altering the formation of ROS (Hamade et al., 2010). Additionally, some studies have reported $O_3$-related effects to vary by Inf-1 and Inf-2 quantitative trait loci (Tankersley and Kleeberger, 1994) and a gene coding for Clara cell secretory protein (CCSP) (Broeckaert et al., 2003; Wattiez et al., 2003). Other investigations in inbred mouse strains found that differences in expression of certain proteins, such as CCSP (Broeckaert et al., 2003) and MARCO (Dahl et al., 2007), are responsible for phenotypic characteristics, such as epithelial permeability and scavenging of oxidized lipids, respectively, which confer sensitivity to $O_3$.

Nitric oxide (NO), derived from activated macrophages, is produced upon exposure to $O_3$ and is thought to participate in lung damage. Mice deficient in the gene for inducible nitric oxide synthase (NOS2/NOSII/iNOS) are partially protected against lung injury (Kleeberger et al., 2001), and it appears that $O_3$-induced iNOS expression is tied to the TLR4 pathway described above. Similarly, iNOS deficient mice do not produce reactive nitrogen intermediates after $O_3$ exposure, in contrast to their wild-type counterparts, and also produce less PGE2 comparatively (Fakhrzadeh et

al., 2002). These gene-deficient mice were protected from $O_3$-induced lung injury and inflammation. In contrast, another study using a similar exposure concentration but longer duration of exposure found that iNOS deficient mice were more at risk of $O_3$-induced lung damage (Kenyon et al., 2002). Therefore, the role of iNOS in mediating the response to $O_3$ exposure is likely dependent on the exposure concentration and duration.

Voynow et al. (2009) have shown that NQO1 deficient mice, like their human counterparts, are resistant to $O_3$-induced AHR and inflammation. NQO1 catalyzes the reduction of quinones to hydroquinones, and is capable of both protective detoxification reactions and redox cycling reactions resulting in the generation of reactive oxygen species. Reduced production of inflammatory mediators and cells and blunted AHR were observed in NQO1 null mice after exposure to $O_3$. These results correlated with those from in vitro experiments in which human bronchial epithelial cells treated with an NQO1 inhibitor exhibited reduced inflammatory responses to exposure to $O_3$. This study may provide biological plausibility for the increased biomarkers of oxidative stress and increased pulmonary function decrements observed in $O_3$-exposed individuals bearing both the wild-type NQO1 gene and the null GSTM1 gene (Bergamaschi et al., 2001). Deletion of the gene for MMP9 also conferred protection against $O_3$-induced airways inflammation and injury (Yoon et al., 2007).

The role of TNF-α signaling in $O_3$-induced responses has been previously established through depletion experiments, but a more recent toxicological study investigated the effects of combined $O_3$ and PM exposure in transgenic TNF overexpressing mice. Kumarathasan et al. (2005) found that subtle effects of these pollutants were difficult to identify in the midst of the severe pathological changes caused by constitutive TNF-α overexpression. However, there was evidence that TNF transgenic mice were at increased risk of $O_3$/PM-induced oxidative stress, and they exhibited elevation of a serum creatine kinase after pollutant exposure, which may suggest potential systemic or cardiac related effects. Differential risk of $O_3$ among inbred strains of animals does not seem to be dose dependent since absorption of $^{18}O$ in various strains of mice did not correlate with resistance or sensitivity (Vancza et al., 2009).

Defects in DNA repair mechanisms may also confer increased risk of $O_3$-related health effects. Cockayne syndrome, a rare autosomal recessive disorder in humans, is characterized by UV sensitivity abnormalities, neurological abnormalities, and premature aging. The same genetic defect in mice ($Csb^{-/-}$) makes them sensitive to oxidative stressors, including $O_3$. Kooter et al. (2007) demonstrated that $Csb^{-/-}$ mice produced significantly more TNF-α after exposure to $O_3$ than their wild-type counterparts. However, there were no statistically significant differences in other markers of inflammation or lung injury between the two strains of mice.

Overall, for variants in multiple genes there is adequate evidence for involvement in populations being more at-risk than others to the effects of $O_3$ exposure on health. Controlled human exposure and epidemiologic studies have reported evidence of $O_3$-related increases in respiratory symptoms or decreases in lung function with variants

including GSTM1, GSTP1, HMOX1, and NQO1. NQO1 deficient mice were found to be resistant to $O_3$-induced AHR and inflammation, providing biological plausibility for results of studies in humans. Additionally, studies of rodents have identified a number of other genes that may affect $O_3$-related health outcomes, including genes related to innate immune signaling and pro- and anti-inflammatory genes, which have not been investigated in human studies.

## 8.2 Pre-existing Disease/Conditions

Individuals with certain pre-existing diseases are likely to constitute an at-risk population. This may be the result of individuals with a pre-existing disease/condition having less reserve than healthy individuals, so although the absolute change may be the same, the health consequences are different. Previous $O_3$ AQCDs concluded that some people with pre-existing pulmonary disease, especially asthma, are among those at increased risk of an $O_3$-related health effect. Extensive toxicological evidence indicates that altered physiological, morphological and biochemical states typical of respiratory diseases may render people at risk of an additional oxidative burden induced by $O_3$ exposure. In addition, a number of epidemiologic studies found that some individuals with respiratory diseases are at increased risk of $O_3$-related effects. The majority of the studies identified in previous AQCDs focused on whether pre-existing respiratory diseases result in increased risk of $O_3$-related health effects, with a limited number of studies examining other pre-existing diseases, such as cardiovascular.

Studies identified since the completion of the 2006 $O_3$ AQCD that examined whether pre-existing diseases and conditions lead to increased risk of $O_3$-induced health effects were identified and are summarized below. Table 8-4 displays the prevalence rates of some of these conditions categorized by age and region among adults in the U.S. population; data for children, when available, are presented within the following sections. Substantial proportions of the U.S. population are affected by these conditions and therefore may represent potentially large at-risk populations. While these diseases and conditions represent biological or intrinsic factors that could lead to increased risk, the pathways to their development may have intrinsic or extrinsic origins.

**Table 8-4 Prevalence of respiratory diseases, cardiovascular diseases, and diabetes among adults by age and region in the U.S.**

| | Adults | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | N (in thousands) | Age | | | | Region | | | |
| Chronic Disease/Condition | | 18-44 | 45-64 | 65-74 | 75+ | North east | Mid west | South | West |
| Respiratory Diseases | | | | | | | | | |
| Asthma[a] | 16,380 | 7.2 | 7.5 | 7.8 | 6.4 | 7.7 | 8.0 | 5.9 | 8.4 |
| COPD | | | | | | | | | |
| Chronic Bronchitis | 9,832 | 3.2 | 5.5 | 5.9 | 5.3 | 3.4 | 4.8 | 5.2 | 2.9 |
| Emphysema | 3,789 | 0.2 | 2.0 | 5.7 | 5.0 | 1.2 | 1.9 | 1.9 | 1.3 |
| Cardiovascular Diseases | | | | | | | | | |
| All Heart Disease | 26,628 | 4.6 | 12.3 | 26.7 | 39.2 | 11.3 | 12.7 | 12.2 | 9.9 |
| Coronary Heart Disease | 14,428 | 1.1 | 6.7 | 16.9 | 26.7 | 5.7 | 6.5 | 7.3 | 4.9 |
| Hypertension | 56,159 | 8.7 | 32.5 | 54.4 | 61.1 | 22.9 | 24.1 | 27.1 | 20.6 |
| Diabetes | 18,651 | 2.3 | 12.1 | 20.4 | 17.3 | 4.5 | 7.6 | 9.0 | 7.7 |

[a]Asthma prevalence is reported for "still has asthma."

Source: Pleis et al. (2009); National Center for Health Statistics.

### 8.2.1 Influenza/Infections

Recent studies have indicated that underlying infections may increase the risk of $O_3$-related health effects because $O_3$ exposure likely impairs host defenses, which may increase the body's response to an infectious agent. However, there is little epidemiologic or experimental evidence that infection or influenza itself renders an individual at greater risk of an $O_3$-induced health effect. A study of hospitalizations in Hong Kong reported that increased levels of influenza intensity resulted in increased excess risk of respiratory disease hospitalizations related to $O_3$ exposure (Wong et al., 2009). In addition, a study of lung function in asthmatic children reported decreases in lung function with increased short-term $O_3$ exposure for those with upper respiratory infections but not for those without infections (Lewis et al., 2005). Toxicological studies provide biological plausibility for the increase in $O_3$-induced health effects observed in epidemiologic studies that examined infections by way of studies that demonstrated that exposure to 0.08 ppm $O_3$ increased streptococcus-induced mortality, regardless of whether $O_3$ exposure preceded or followed infection (Miller et al., 1978; Coffin and Gardner, 1972; Coffin et al., 1967). Overall, the epidemiologic and experimental evidence supports the potential for increased risk to be conferred by an infection but the number of studies is limited. There have only been a few epidemiologic studies and these studies examine

different outcomes (respiratory-related hospital admissions or lung function) and different modifiers (influenza or respiratory infection). In some of the toxicological studies, the $O_3$ exposure came before the infection. Therefore, evidence is inadequate to determine if influenza/infections increase the risk of $O_3$-related health effects.

### 8.2.2 Asthma

Previous $O_3$ AQCDs identified individuals with asthma as a population at increased risk of $O_3$-related health effects. Within the U.S., approximately 7.3% of adults have reported currently having asthma (Pleis et al., 2009), and 9.5% of children have reported currently having asthma (Bloom et al., 2008). For more detailed prevalence by age, see Table 8-5.

**Table 8-5 Prevalence of asthma by age in the U.S.**

| Age (years) | N (in thousands) | Percent |
|---|---|---|
| 0-4 | 1,276 | 6.2 |
| 5-11 | 3,159 | 11.2 |
| 12-17 | 2,518 | 10.2 |
| 18-44 | 7,949 | 7.2 |
| 45-64 | 5,768 | 7.5 |
| 65-74 | 1,548 | 7.8 |
| 75+ | 1,116 | 6.4 |

[a]Asthma prevalence is reported for "still has asthma."

Source: Statistics for adults: Pleis et al. (2009); statistics for children: Bloom et al. (2008); National Center for Health Statistics.

Multiple epidemiologic studies included within this ISA have evaluated the potential for increased risk of $O_3$-related health effects among individuals with asthma. A study of lifeguards in Texas reported decreased lung function with short-term $O_3$ exposure among both individuals with and without asthma, however, the decrease was greater among those with asthma (Thaller et al., 2008). A Mexican study of children ages 6-14 detected an association between short-term $O_3$ exposure and wheeze, cough, and bronchodilator use among asthmatics but not non-asthmatics, although this may have been the result of a small non-asthmatic population (Escamilla-Nuñez et al., 2008). A study of modification by airway hyperresponsiveness (AHR) (a condition common among asthmatics) reported greater short-term $O_3$-associated decreases in lung function in elderly individuals with AHR, especially among those who were obese (Alexeeff et al., 2007). However, no evidence for increased risk was found in a study performed among children in Mexico City that examined the effect of short-term $O_3$ exposure on respiratory health (Barraza-Villarreal et al., 2008). In this study, a positive association was reported for airway inflammation among asthmatic children, but the observed association was

similar in magnitude to that of non-asthmatics. Similarly, a study of children in California reported an association between $O_3$ concentration and exhaled nitric oxide fraction (FeNO) that persisted both among children with and without asthma as well as those with and without respiratory allergy (Berhane et al., 2011). Finally, Khatri et al. (2009) found no association between short-term $O_3$ exposure and altered lung function for either asthmatic or non-asthmatic adults, but did note a decrease in lung function among individuals with allergies.

Evidence for difference in effects among asthmatics has been observed in studies that examined the association between $O_3$ exposure and altered lung function by asthma medication use. A study of children with asthma living in Detroit reported a greater association between short-term $O_3$ and lung function for corticosteroid users compared with noncorticosteroid users (Lewis et al., 2005). Conversely, another study of children found decreased lung function among noncorticosteroid users compared to corticosteroid users, although in this study, a large proportion of non-users were considered to be persistent asthmatics (Hernández-Cadena et al., 2009). Lung function was not related to short-term $O_3$ exposure among corticosteroid users and non-users in a study taking place among children during the winter months in Canada (Liu et al., 2009a). Additionally, a study of airway inflammation among individuals aged 12-65 years old reported a counterintuitive inverse association with $O_3$ of similar magnitude for all groups of corticosteroid users and non-users (Qian et al., 2009).

Controlled human exposure studies that have examined the effects of $O_3$ on individuals with asthma and healthy controls are limited. Based on studies reviewed in the 1996 and 2006 $O_3$ AQCDs, subjects with asthma appeared to be at least as sensitive to acute effects of $O_3$ in terms of $FEV_1$ and inflammatory responses as healthy non-asthmatic subjects. For instance, Horstman et al. (1995) observed that mild-to-moderate asthmatics, on average, experienced double the $O_3$-induced $FEV_1$ decrement of healthy subjects (19% versus 10%, respectively, $p = 0.04$). Moreover, a statistically significant positive correlation between $FEV_1$ responses to $O_3$ exposure and baseline lung function was observed in individuals with asthma, i.e., responses increased with severity of disease. Kreit et al. (1989) performed a short duration study in which asthmatics also showed a considerably larger average $O_3$-induced $FEV_1$ decrement than the healthy controls (25% versus 16%, respectively) following exposure to $O_3$ with moderate-heavy exercise. Alexis et al. (2000) and Jorres et al. (1996) also reported a tendency for slightly greater $FEV_1$ decrements in asthmatics than healthy subjects. Minimal evidence exists suggesting that individuals with asthma have smaller $O_3$-induced $FEV_1$ decrements than healthy subjects (3% versus 8%, respectively) (Mudway et al., 2001). However, the asthmatics in that study also tended to be older than the healthy subjects, which could partially explain their lesser response since $FEV_1$ responses to $O_3$ exposure diminish with age. Individuals with asthma also had more neutrophils in the BALF (18 hours postexposure) than similarly exposed healthy individuals (Peden et al., 1997; Scannell et al., 1996; Basha et al., 1994). Furthermore, a study examining the effects of $O_3$ on individuals with atopic asthma and healthy controls reported that greater numbers of neutrophils, higher levels of cytokines and hyaluronan, and greater expression of macrophage

cell-surface markers were observed in induced sputum of atopic asthmatics compared with healthy controls (Hernandez et al., 2010). Differences in $O_3$-induced epithelial cytokine expression were noted in bronchial biopsy samples from asthmatics and healthy controls (Bosson et al., 2003). Cell-surface marker and cytokine expression results, and the presence of hyaluronan, are consistent with $O_3$ having greater effects on innate and adaptive immunity in these asthmatic individuals (see Section 5.4.2.2). In addition, studies have demonstrated that $O_3$ exposure leads to increased bronchial reactivity to inhaled allergens in mild allergic asthmatics (Kehrl et al., 1999; Jorres et al., 1996) and to the influx of eosinophils in individuals with pre-existing allergic disease (Vagaggini et al., 2002; Peden et al., 1995). Taken together, these results point to several mechanistic pathways which could account for the increased risk of $O_3$–related health effects in subjects with asthma (see Section 5.4.2.2).

Toxicological studies provide biological plausibility for greater effects of $O_3$ among those with asthma or AHR. In animal toxicological studies, an asthmatic phenotype is modeled by allergic sensitization of the respiratory tract. Many of the studies that provide evidence that $O_3$ exposure is an inducer of AHR and remodeling utilize these types of animal models. For example, a series of experiments in infant rhesus monkeys have shown these effects, but only in monkeys sensitized to house dust mite allergen (Fanucchi et al., 2006; Joad et al., 2006; Schelegle et al., 2003). Similarly, Funabashi et al. (2004) demonstrated changes in pulmonary function in mice exposed to $O_3$, and Wagner et al. (2007) demonstrated enhanced inflammatory responses in rats exposed to $O_3$, but only in animals sensitized to allergen. In general, it is the combined effects of $O_3$ and allergic sensitization which result in measurable effects on pulmonary function. In a bleomycin induced pulmonary fibrosis model, exposure to 250 ppb $O_3$ for 5 days increased pulmonary inflammation and fibrosis, along with the frequency of bronchopneumonia in rats (Oyarzún et al., 2005). Thus, short-term exposure to $O_3$ may enhance damage in a previously injured lung.

In the 2006 $O_3$ AQCD, the potential for individuals with asthma to have greater risk of $O_3$-related health effects was supported by a number of controlled human exposure studies, evidence from toxicological studies, and a limited number of epidemiologic studies. Overall, in the recent epidemiologic literature some, but not all, studies report greater risk of health effects among individuals with asthma. Studies examining effect measure modification of the relationship between short-term $O_3$ exposure and altered lung function by corticosteroid use provided limited and inconsistent evidence of $O_3$-related health effects. Additionally, recent studies of behavioral responses have found that studies do not take into account individual behavioral adaptations to forecasted air pollution levels (such as avoidance and reduced time outdoors), which may underestimate the observed associations in studies that examined the effect of $O_3$ exposure on respiratory health (Neidell and Kinney, 2010). This could explain some inconsistency observed among recent epidemiologic studies. The evidence from controlled human exposure studies provides support for increased decrements in $FEV_1$ and greater inflammatory responses to $O_3$ in individuals with asthma than in healthy individuals without a history of asthma. These studies are often performed among individuals with mild asthma and therefore it is possible that individuals with severe asthma may have an

even greater risk of $O_3$-related health effects. The collective evidence for increased risk of $O_3$-related health effects among individuals with asthma from controlled human exposure studies is supported by recent toxicological studies which provide biological plausibility for heightened risk of asthmatics to respiratory effects due to $O_3$ exposure. Evidence indicating $O_3$-induced respiratory effects among individuals with asthma is further supported by additional studies of $O_3$-related respiratory effects (Section 6.2). Overall, there is adequate evidence for asthmatics to be an at-risk population based on the substantial, consistent evidence among controlled human exposure studies and coherence from epidemiologic and toxicological studies.

### 8.2.3 Chronic Obstructive Pulmonary Disease (COPD)

In the U.S. over 4% of adults report having chronic bronchitis and almost 2% report having emphysema, both of which are classified as COPD (Pleis et al., 2009).

A recent study reported no association between $O_3$ exposure and lung function regardless of whether the study participant had COPD or other pre-existing diseases (asthma or IHD) (Lagorio et al., 2006).

Peel et al. (2007) found that individuals with COPD were at increased risk of cardiovascular ED visits in response to short-term $O_3$ exposure compared to healthy individuals in Atlanta, GA. The authors reported that short-term $O_3$ exposure was associated with higher odds of an emergency department (ED) visit for peripheral and cerebrovascular disease among individuals with COPD compared to individuals without COPD. However, pre-existing COPD did not increase the odds of hospitalization for all CVD outcomes (i.e., IHD, dysrhythmia, or congestive heart failure). In an additional study performed in Taiwan, individuals with and without COPD had higher odds of congestive heart failure associated with $O_3$ exposure on warm days (Lee et al., 2008a). As discussed in Section 6.3, most studies reported no overall association between $O_3$ concentration and CVD morbidity.

Recent epidemiologic evidence indicates that persons with COPD may have increased risk of $O_3$-related cardiovascular effects, but little information is available on whether COPD leads to an increased risk of $O_3$-induced respiratory effects. Overall, this small number of studies provides inadequate evidence to determine whether COPD results in increased risk of $O_3$-related health effects.

### 8.2.4 Cardiovascular Disease (CVD)

Cardiovascular disease has become increasingly prevalent in the U.S., with about 12% of adults reporting a diagnosis of heart disease (Table 8-4). A high prevalence of other cardiovascular-related conditions has also been observed, such as hypertension which is prevalent among approximately 24% of adults. In the 2006 $O_3$ AQCD, little evidence was available regarding whether pre-existing CVD

contributed to increased risk of $O_3$-related health effects. Recent epidemiologic studies have examined cardiovascular-related diseases as modifiers of the $O_3$-outcome associations; however, no recent evidence is available from controlled human exposure studies or toxicological studies.

Peel et al. (2007) compared the associations between short-term $O_3$ exposure and cardiovascular ED visits in Atlanta, GA among multiple comorbid conditions. The authors found no evidence of increased risk of cardiovascular ED visits in individuals previously diagnosed with dysrhythmia, congestive heart failure, or hypertension compared to healthy individuals. Similarly, a study in France examined the association between $O_3$ concentrations and ischemic cerebrovascular events (ICVE) and myocardial infarction (MI) and the influence of multiple vascular risk factors on any observed associations (Henrotin et al., 2010). The association between $O_3$ exposure and ICVE was elevated for individuals with multiple risk factors, specifically individuals with diabetes or hypertension. For the association between $O_3$ and MI, increased odds were apparent only for those with hypercholesterolemia. In a study conducted in Taiwan, a positive association was observed for $O_3$ on warm days and congestive heart failure hospital admissions, but the association did not differ between individuals with/without hypertension or with/without dysrhythmia (Lee et al., 2008a). Another study in Taiwan reported that the association between $O_3$ levels and ED visits for arrhythmias were greater on warm days among those with congestive heart failure compared to those without congestive heart failure; however, the estimate and 95% CIs for those without congestive heart failure is completely contained within the 95% CI of those with congestive heart failure (Chiu and Yang, 2009).

Although not studied extensively, a study has examined the increased risk of $O_3$-related changes in blood markers for individuals with CVD. There was a greater association between $O_3$ exposure and some, but not all, blood inflammatory markers among individuals with a history of CVD (Liao et al., 2005). Liao et al. (2005) found that increased fibrinogen was positively associated with short-term $O_3$ exposure but this association was present only among individuals with a history of CVD. No association was observed among those without a history of CVD. However, for another biomarker (vWF), CVD status did not modify the positive association with short-term $O_3$ exposure (Liao et al., 2005).

Mortality studies provide some evidence for a potential increase in $O_3$-induced mortality in individuals with pre-existing atrial fibrillation and atherosclerosis. In a study of 48 U.S. cities, increased risk of mortality with short-term $O_3$ exposure was observed only among individuals with secondary atrial fibrillation (Medina-Ramón and Schwartz, 2008). No association was observed for short-term $O_3$ exposure and mortality in a study of individuals with diabetes with or without CVD prior to death; however, there was some evidence of increased risk of mortality during the warm season if individuals had diabetes and atherosclerosis compared to only having diabetes (Goldberg et al., 2006).

Finally, although not extensively examined, a study explored whether a pre-existing CVD increased the risk of an $O_3$-induced respiratory effect. Lagorio et al. (2006)

examined the effect of $O_3$ exposure on lung function among participants with a variety of pre-existing diseases, including IHD. No association was observed regardless of whether the participant had IHD.

Overall, most short-term exposure studies did not report increased $O_3$-related cardiovascular morbidity for individuals with pre-existing CVD. However, as discussed in Section 6.3, most studies reported no overall association between $O_3$ concentration and CV morbidity. Thus, it is likely the association would be null regardless of the stratification. A limited number of studies examined whether cardiovascular disease modifies the association between $O_3$ and respiratory effects. There was some evidence that cardiovascular disease increases the risk of $O_3$-related mortality but again the number of studies was limited. Currently, evidence is inadequate to classify pre-existing CVD as a potential at-risk factor for $O_3$-related health effects. Future research among those with CVD compared to those without will increase the understanding of potential increased risk of $O_3$-related health effects among this group.

### 8.2.5 Diabetes

The literature has not extensively examined whether individuals with diabetes (about 8% of U.S. adults) are potentially at increased risk of $O_3$-related health effects. In a study of short-term $O_3$ exposure and cardiovascular ED visits in Atlanta, GA, no association was observed for individuals with or without diabetes (Peel et al., 2007). A similar study conducted in Taiwan reported a positive association between $O_3$ exposure on warm days and hospital admissions for congestive heart failure; however, no modification of the association by diabetes was observed (Lee et al., 2008a). Finally, in a study of $O_3$ exposure and ED visits for arrhythmia in Taiwan, there was no evidence of effect measure modification by diabetes on warm or cool days (Chiu and Yang, 2009). Currently, the limited number of epidemiologic studies as well as the lack of controlled human exposure or toxicological studies provides inadequate evidence to indicate whether diabetes results in a potentially increased risk of $O_3$-related health effects.

### 8.2.6 Hyperthyroidism

Hyperthyroidism has been identified in toxicological studies as a potential factor that may lead to increased risk of $O_3$-related health effects but has not yet been explored in epidemiologic or controlled human exposure studies. Lung damage and inflammation due to oxidative stress may be modulated by thyroid hormones. Compared to controls, hyperthyroid rats exhibited elevated levels of BAL neutrophils and albumin after a 4-hour exposure to $O_3$, indicating $O_3$-induced inflammation and damage. Hyperthyroidism did not affect production of reactive oxygen or nitrogen species, but BAL phospholipids were increased, indicating greater activation of Type II cells and surfactant protein production compared to normal rats (Huffman et al.,

2006). Thus, this study provides some underlying evidence, which suggests that individuals with hyperthyroidism may represent an at-risk population; however, overall the lack of additional studies provides inadequate evidence to determine whether hyperthyroidism results in potentially increased risk of $O_3$-related health effects.

## 8.3 Sociodemographic Factors

### 8.3.1 Lifestage

The 1996 and 2006 $O_3$ AQCDs identified children, especially those with asthma, and older adults as at-risk populations. These previous AQCDs reported clinical (controlled human exposure) evidence that children have greater spirometric responses to $O_3$ than middle-aged and older adults (U.S. EPA, 1996a). Similar results were observed for symptomatic responses and $O_3$ exposure. Among older adults, most studies reported in the 2006 $O_3$ AQCD reported greater effects of short-term $O_3$ exposure and mortality compared to other age groups (U.S. EPA, 2006b). Evidence published since the 2006 $O_3$ AQCD, summarized below, further supports these findings.

#### 8.3.1.1 Children

The 2000 Census reported that 28.6% of the U.S. population was under 20 years of age, with 14.1% under the age of 10 (SSDAN CensusScope, 2010a). Children's respiratory systems are undergoing lung growth until about 18-20 years of age and are therefore thought to be intrinsically more at risk for $O_3$-induced damage (U.S. EPA, 2006b). It is generally recognized that children spend more time outdoors than adults, and therefore would be expected to have higher exposure to $O_3$ than adults. The ventilation rates also vary between children and adults, particularly during moderate/heavy activity. Children aged 11 years and older and adults have higher absolute ventilation rates than children aged 1 -11 years. However, children have higher ventilation rates relative to their lung volumes, which tends to increase dose normalized to lung surface area. Exercise intensity has a substantial effect on ventilation rate, with high intensity activities resulting in nearly double the ventilation rate during moderate activity among children and those adults less than 31 years of age. For more information on time spent outdoors and ventilation rate differences by age group, see Section 4.4.1.

The 1996 $O_3$ AQCD, reported clinical evidence that children, adolescents, and young adults (<18 years of age) appear, on average, to have nearly equivalent spirometric responses to $O_3$ exposure, but have greater responses than middle-aged and older adults (U.S. EPA, 1996a). Symptomatic responses (e.g., cough, shortness of breath,

pain on deep inspiration) to $O_3$ exposure, however, appear to increase with age until early adulthood and then gradually decrease with increasing age (U.S. EPA, 1996a). For subjects aged 18-36 years, McDonnell et al. (1999b) reported that symptom responses from $O_3$ exposure also decrease with increasing age. Complete lung growth and development is not achieved until 18-20 years of age in women and the early 20s for men; pulmonary function is at its maximum during this time as well. Additionally, PBPK modeling reported lung regional extraction of $O_3$ to be higher in infants compared to adults. This is thought to be due to the smaller nasal and pulmonary regions' surface area in children under the age of 5 years compared to the total airway surface area observed in adults (Sarangapani et al., 2003).

Recent epidemiologic studies have examined different age groups and their risk to $O_3$-related respiratory hospital admissions and ED visits. A study in Cyprus of short-term $O_3$ concentrations and respiratory hospital admissions detected possible effect measure modification by age with a larger association among individuals <15 years of age compared with those >15 years of age. However, this difference was only apparent with a 2-day lag (Middleton et al., 2008). Similarly, a Canadian study of asthma-ED visits reported the strongest $O_3$-related associations among 5 to 14 year-olds compared to the other age groups (ages examined 0-75+) (Villeneuve et al., 2007). Greater $O_3$-associated risk in asthma-related ED visits were also reported among children (<15 years) as compared to adults (15 to 64 years) in a study from Finland (Halonen et al., 2009). A study of New York City hospital admissions demonstrated an increase in the association between $O_3$ exposure and asthma-related hospital admissions for 6 to 18 year-olds compared to those <6 years old and those >18 years old (Silverman and Ito, 2010). When examining long-term $O_3$ exposure and asthma hospital admissions among children, associations were determined to be larger among children 1 to 2 years old compared to children 2 to 6 years old (Lin et al., 2008b). A few studies reported positive associations among both children and adults and no modification of the effect by age. A study performed in Hong Kong examined $O_3$ exposure and asthma-related hospital admissions for ages 0 to14, 15 to 65, and >65 (Ko et al., 2007). The researchers reported that the association was greater among the 0 to 14 and 14 to 65 age groups compared to the >65 age group. Another study looking at asthma-related ED visits and $O_3$ exposure in Maine reported positive associations for all age groups (ages 2 to 65) (Paulu and Smith, 2008). Effects of $O_3$ exposure on asthma hospitalizations among both children and adults (<18 and ≥ 18 years old) were demonstrated in a study in Washington, but only children (<18 years of age) had statistically significant results at lag day 0, which the authors wrote, "suggests that children are more immediately responsive to adverse effects of $O_3$ exposure" (Mar and Koenig, 2009).

The evidence reported in epidemiologic studies is supported by recent toxicological studies which observed $O_3$-induced health effects in immature animals. Early life exposures of multiple species of laboratory animals, including infant monkeys, resulted in changes in conducting airways at the cellular, functional, ultra-structural, and morphological levels. Carey et al. (2007) conducted a study of $O_3$ exposure in infant rhesus macaques, whose respiratory tract closely resembles that of humans. Monkeys were exposed either acutely for 5 days to 0.5 ppm $O_3$, or episodically for 5

biweekly cycles alternating 5 days of 0.5 ppm $O_3$ with 9 days of filtered air, designed to mimic human exposure (70 days total). All monkeys acutely exposed to $O_3$ had moderate to marked necrotizing rhinitis, with focal regions of epithelial exfoliation, numerous infiltrating neutrophils, and some eosinophils. The distribution, character, and severity of lesions in episodically exposed infant monkeys were similar to that of acutely exposed animals. Neither exposure protocol for the infant monkeys produced mucous cell metaplasia proximal to the lesions, an adaptation observed in adult monkeys exposed continuously to 0.3 ppm $O_3$ in another study (Harkema et al., 1987a). Functional (increased airway resistance and responsiveness with antigen + $O_3$ co-exposure) and cellular changes in conducting airways (increased numbers of inflammatory eosinophils) were common manifestations of exposure to $O_3$ among both the adult and infant monkeys (Plopper et al., 2007). In addition, the lung structure of the conducting airways in the infant monkeys was stunted by $O_3$ and this aberrant development was persistent 6 months postexposure. This developmental endpoint was not, of course, studied in the adult monkey experiments (Fanucchi et al., 2006). Thus, some functional and biochemical effects were similar between the infant and adult monkeys exposed to $O_3$, but because the study designs did not include concentration-response experiments, it is not possible to determine whether the infant monkeys were more at risk for the effects of $O_3$.

Similarly, rat fetuses exposed to $O_3$ in utero had ultrastructural changes in bronchiolar epithelium when examined near the end of gestation (López et al., 2008). In addition, exposure of mice to mixtures of air pollutants early in development affected pup lung cytokine levels (TNF, IL-1, KC, IL-6, and MCP-1) (Auten et al., 2009). In utero exposure of animals to PM augmented $O_3$-induced airway hyper-reactivity in these pups as juveniles.

Age may affect the inflammatory response to $O_3$ exposure. In comparing neonatal mice to adult mice, increased bronchoalveolar lavage (BAL) neutrophils were observed in four strains of neonates 24 hours after exposure to 0.8 ppm $O_3$ for 5 hours (Vancza et al., 2009). Three of these strains also exhibited increased BAL protein, although the two endpoints were not necessarily consistently correlated in a given strain. In some strains, however, adults were responsive, indicating a strain-age interaction. Measurement of $^{18}O$ determined that the observed strain- and age-dependent differences were not due to absorbed $O_3$ dose. Using electron microscopy, Bils (1970) studied the lungs of mice of different ages (4 days or 1 to 2 months) exposed to 0.6 to 1.3 ppm $O_3$ for 6 to 7 h/day for 1 to 2 days and noted swelling of the alveolar epithelial lining cells without intra-alveolar edema. Swelling of endothelial cells and occasional breaks in the basement membrane were observed. These effects were most evident in younger mice exposed for 2 days. Toxicological studies reported that the difference in effects among younger lifestage test animals may be due to age-related changes in endogenous antioxidants and sensitivity to oxidative stress. A recent study demonstrated that 0.25 ppm $O_3$ exposure differentially altered expression of metalloproteinases in the skin of young (8 weeks old) and aged (18 months old) mice, indicating age-related variations in risk of oxidative stress (Fortino et al., 2007). Valacchi et al. (2007) found that aged mice had more Vitamin E in their plasma but less in their lungs compared to young mice,

which may affect their pulmonary antioxidant defenses. Servais et al. (2005) found higher levels of oxidative damage indicators in immature (3 weeks old) and aged (20 months old) rats compared to adult rats, the latter which were relatively resistant to an intermittent 7-day exposure to 0.5 ppm $O_3$. Immature rats exhibited a higher ventilation rate, which may have increased exposure. Additionally, a series of toxicological studies reported an association between $O_3$ exposure and bradycardia that was present among young but not older mice (Hamade et al., 2010; Tankersley et al., 2010; Hamade and Tankersley, 2009; Hamade et al., 2008). Regression analysis revealed an interaction between age and strain on heart rate, which implies that aging may affect heart rate differently among mouse strains (Tankersley et al., 2010). The authors proposed that the genetic differences between the mice strains could be altering the formation of ROS, which tends to increase with age, thus modulating the changes in cardiopulmonary physiology after $O_3$ exposure.

The previous and recent human clinical (controlled human exposure) and toxicological studies reported evidence of increased risk from $O_3$ exposure for younger ages, which provides coherence and biological plausibility for the findings from epidemiologic studies. Although there was some inconsistency, generally, the epidemiologic studies reported larger associations for respiratory hospital admissions and ED visits for children than adults. The interpretation of these studies is limited by the lack of consistency in comparison age groups and outcomes examined. Toxicological studies observed $O_3$-induced health effects in immature animals, including infant monkeys, though the effects were not consistently greater in young animals than adults. However, overall, the epidemiologic, controlled human exposure, and toxicological studies provide substantial and consistent evidence within and across disciplines. Therefore, there is adequate evidence to conclude that children are at increased risk of $O_3$-related health effects.

### 8.3.1.2 Older Adults

Older adults may be at greater risk of health effects associated with $O_3$ exposure through a variety of intrinsic pathways. In addition, older adults may differ in their exposure and internal dose. Older adults spend slightly more time outdoors than adults aged 18-64 years. Older adults also have somewhat lower ventilation rates than adults aged 31 - less than 61 years. For more information on time spent outdoors and ventilation rate differences by age group, see Section 4.4.1. The gradual decline in physiological processes that occur with aging may lead to increased risk of $O_3$-related health effects (U.S. EPA, 2006a). Respiratory symptom responses to $O_3$ exposure appears to increase with age until early adulthood and then gradually decrease with increasing age (U.S. EPA, 1996a), which may put older adults at increased risk by withstanding continued $O_3$ exposure and thus not seeking relief and avoiding exposure. In addition, older adults, in general, have a higher prevalence of pre-existing diseases, with the exception of asthma, compared to younger age groups and this may also lead to increased risk of $O_3$-related health effects (see Table 8-4 that gives pre-existing rates by age).With the number of older Americans increasing

in upcoming years (estimated to increase from 12.4% of the U.S. population to 19.7% between 2000 to 2030, which is approximately 35 million and 71.5 million individuals, respectively) this group represents a large population potentially at risk of $O_3$-related health effects (SSDAN CensusScope, 2010a; U.S. Census Bureau, 2010).

The majority of recent studies reported greater effects of short-term $O_3$ exposure and mortality among older adults, which is consistent with the findings of the 2006 $O_3$ AQCD. A study conducted in 48 cities across the U.S. reported larger effects among adults ≥ 65 years old compared to those <65 years (Medina-Ramón and Schwartz, 2008). Further investigation of this study population revealed a trend of $O_3$-related mortality risk that gets larger with increasing age starting at age 50 (Zanobetti and Schwartz, 2008a). A study of 7 urban centers in Chile reported similar results, with greater effects in adults ≥ 65 years old, however the effects were smaller among those ≥ 85 years old compared to those in the 75 to 84 years old age range (Cakmak et al., 2007). More recently, a study conducted in the same area reported similar associations between $O_3$ exposure and mortality in adults aged <64 years old and 65 to 74 years old, but the risk was increased among older age groups (Cakmak et al., 2011). A study performed in China reported greater effects in populations ≥ 45 years old (compared to 5 to 44 year-olds), with statistically significant effects present only among those ≥ 65 years old (Kan et al., 2008). An Italian study reported higher risk of all-cause mortality associated with increased $O_3$ concentrations among individuals ≥ 85 years old as compared to those 35 to 84 years old. Those 65 to 74 and 75 to 84 years old did not show a greater increase in risk compared to those aged 35 to 64 years (Stafoggia et al., 2010). The Air Pollution and Health: A European and North American Approach (APHENA) project examined the association between $O_3$ exposure and mortality for those <75 and ≥ 75 years of age. In Canada, the associations for all-cause and cardiovascular mortality were greater among those ≥ 75 years old in the summer-only and all-year analyses. Age groups were not compared in the analysis for respiratory mortality in Canada. In the U.S., the association for all-cause mortality was slightly greater for those <75 years of age compared to those ≥ 75 years old in summer-only analyses. No consistent pattern was observed for CVD mortality. In Europe, slightly larger associations for all-cause mortality were observed in those <75 years old in all-year and summer-only analyses. Larger associations were reported among those <75years for CVD mortality in all-year analyses, but the reverse was true for summer-only analyses (Katsouyanni et al., 2009).

Multiple epidemiologic studies of $O_3$ exposure and hospital admissions were stratified by age groups. A positive association was reported between short-term $O_3$ exposure and respiratory hospital admissions for adults ≥ 65 years old but not for those adults aged 15 to 64 years (Halonen et al., 2009). In the same study, no association was observed between $O_3$ concentration and respiratory mortality among those ≥ 65 years old or those 15 to 64 years old; however, an inverse association between $O_3$ concentration and cardiovascular mortality was present among individuals ≥ 65 years old but not among individuals <65 years old. This inverse association among those ≥ 65 years old persisted when examining hospital

admissions for coronary heart disease. A study of CVD-related hospital visits in Bangkok, Thailand reported an increase in percent change for hospital visits with previous day and cumulative 2-day $O_3$ levels among those ≥ 65 years old, whereas no association was present for individuals less than 65 years of age (Buadong et al., 2009). No association was observed for current day or cumulative 3-day averages in any age group. A study examining $O_3$ and hospital admissions for CVD-related health effects reported no association for individuals aged 15 to 64 or individuals aged ≥ 65 years, although one lag-time did show an inverse effect for coronary heart disease among elderly that was not present among 15 to 64 year-olds (Halonen et al., 2009). However, as discussed in the section on CVD hospital admissions (Section 6.3.2.7), results were inconsistent and often null so it is plausible that no association would be observed regardless of age. No modification by age (40 to 64 year-olds versus >64 years old) was observed in a study from Brazil examining $O_3$ levels and COPD ED visits (Arbex et al., 2009).

Biological plausibility for differences by age is provided by toxicological studies. Ozone exposure resulted in an increase in left ventricular chamber dimensions at end diastole (LVEDD) in young and old mice, whereas decreases in left ventricular posterior wall thickness at end systole (PWTES) were only observed among older mice (Tankersley et al., 2010). Other toxicological studies also indicate increased risk in older animals for additional endpoints, including neurological and immune. The hippocampus, one of the main regions affected by age-related neurodegenerative diseases, may be more sensitive to oxidative damage in aged rats. In a study of young (47 days) and aged (900 days) rats exposed to 1 ppm $O_3$ for 4 hours, $O_3$-induced lipid peroxidation occurred to a greater extent in the striatum of young rats, whereas it was highest in the hippocampus in aged rats (Rivas-Arancibia et al., 2000). In young mice, healing of skin wounds is not significantly affected by $O_3$ exposure (Lim et al., 2006). However, exposure to 0.5 ppm $O_3$ for 6 h/day significantly delays wound closure in aged mice.

Although some outcomes reported mixed findings regarding an increase in risk for older adults, recent epidemiologic studies report consistent positive associations between short-term $O_3$ exposure and mortality in older adults. The evidence from mortality studies is consistent with the results reported in the 2006 $O_3$ AQCD and is supported by toxicological studies providing biological plausibility for increased risk of effects in older adults. Also, older adults may be experiencing increased exposure compared to younger adults due to time spend outdoors and withstanding exposures. Overall, adequate evidence is available indicating that older adults are at increased risk of $O_3$-related health effects based on the substantial and consistent evidence within epidemiologic studies on $O_3$ exposure and mortality and the coherence with toxicological studies.

### 8.3.2 Sex

The distribution of males and females in the U.S. is similar. In 2000, 49.1% of the U.S. population was male and 50.9% were female. However, this distribution does vary by age with a greater prevalence of females ≥ 65 years old compared to males (SSDAN CensusScope, 2010a). The 2006 $O_3$ AQCD did not report evidence of differences between the sexes in health responses to $O_3$ exposure (U.S. EPA, 2006b). Recent epidemiologic studies have evaluated the effects of short-term and long-term exposure to $O_3$ on multiple health endpoints stratified by sex.

A study in Maine that examined short-term $O_3$ concentrations and asthma ED visits detected greater effects among males ages 2 to14 years and among females ages 15 to 34 years compared to males and females in the same age groups (no difference was detected for males and females aged 35 to 64) (Paulu and Smith, 2008). A Canadian study reported no associations between short-term $O_3$ and respiratory infection hospital admissions for either boys or girls under the age of 15 (Lin et al., 2005), whereas another Canadian study reported a slightly higher but non-statistically significant increase in respiratory hospital admissions for males (mean ages 47.6 to 69.0 years) (Cakmak et al., 2006b). A recent study from Hong Kong examining individuals of all ages reported no effect measure modification by sex for overall respiratory disease hospital admissions, but did detect a greater excess risk of hospital admissions for COPD among females compared to males (Wong et al., 2009). Similarly a study in Brazil found higher effect estimates for COPD ED visits among females compared to males (Arbex et al., 2009). Higher levels of respiratory hospital admissions with greater $O_3$ concentrations was also observed for females in a study of individuals living in Cyprus (Middleton et al., 2008). A study of lung function unrelated to hospital admissions and ED visits was conducted among lifeguards in Texas and reported decreased lung function with increased $O_3$ exposure among females but not males (Thaller et al., 2008). This study included individuals aged 16 to 27 years, and the majority of participants were male. A New York study found no evidence of effect measure modification of the association between long-term $O_3$ exposure and asthma hospital admissions among males and females between 1 and 6 years old (Lin et al., 2008b).

In addition to examining the potential modification of $O_3$ associations with respiratory outcomes by sex, studies also examined cardiovascular-related outcomes specifically hospital admissions and ED visits. All of these studies reported no effect modification by sex with some studies reporting null associations for both males and females (Wong et al., 2009; Middleton et al., 2008; Villeneuve et al., 2006a) and one study reporting a positive associations for both sexes (Cakmak et al., 2006a). A French study examining the associations between $O_3$ concentrations and risk of ischemic strokes (not limited to ED visits or hospital admissions) reported no association for either males or females with lags of 0, 2, or 3 days (Henrotin et al., 2007). A positive association was reported for males with a lag of 1 day, but this association was null for females. The authors noted that men in the study had much higher rates of current and former smoking than women (67.4% versus 9.3%). Additionally, cardiovascular hospital admissions and ED visits overall have

demonstrated inconsistent and null results (Section 6.3.2.7). The lack of effect measure modification by sex may be indicative of the lack of association, not the lack of an effect by sex.

A biomarker study investigating the effects of $O_3$ concentrations on high-sensitivity C-reactive protein (hs-CRP), fibrinogen, and white blood cell (WBC) count, reported observations for various lag times ranging from 0 to 7 days (Steinvil et al., 2008). Most of the associations were null for males and females although one association between $O_3$ and fibrinogen was positive for males and null for females (lag day 4); however, this positive association was null or negative when other pollutants were included in the model. One study examining correlations between $O_3$ levels and oxidative DNA damage examined results stratified by sex. In this study Palli et al. (2009) reported stronger correlations for males than females, both during short-term exposure (less than 30 days) and long-term exposure (0-90 days). However, the authors commented that this difference could have been partially explained by different distributions of exposure to traffic pollution at work.

A few studies have examined the association between short-term $O_3$ concentrations and mortality stratified by sex and, in contrast with studies of other endpoints, were more consistent in reporting elevated risks among females. These studies, conducted in the U.S. (Medina-Ramón and Schwartz, 2008), Italy (Stafoggia et al., 2010), and Asia (Kan et al., 2008), reported larger effect estimates in females compared to males. In the U.S. study, the elevated risk of mortality among females was greater specifically among those ≥ 60 years old (Medina-Ramón and Schwartz, 2008). However, a recent study in Chile reported similar associations between $O_3$ exposure and mortality among both men and women (Cakmak et al., 2011). A long-term $O_3$ exposure study of respiratory mortality stratified their results by sex and reported relative risks of 1.01 (95% CI: 0.99, 1.04) for males and 1.04 (95% CIs 1.03, 1.07) for females (Jerrett et al., 2009).

Experimental research provided a further understanding of the underlying mechanisms that may explain a possible differential risk in $O_3$-related health effects among males and females. Several studies have suggested that physiological differences between sexes may predispose females to greater effects from $O_3$. In females, lower plasma and nasal lavage fluid (NLF) levels of uric acid (most prevalent antioxidant), the initial defense mechanism of $O_3$ neutralization, may be a contributing factor (Housley et al., 1996). Consequently, reduced absorption of $O_3$ in the upper airways of females may promote its deeper penetration. Dosimetric measurements have shown that the absorption distribution of $O_3$ is independent of sex when absorption is normalized to anatomical dead space (Bush et al., 1996). Thus, a differential removal of $O_3$ by uric acid seems to be minimal. In general, the physiologic response of young healthy females to $O_3$ exposure appears comparable to the response of young males (Hazucha et al., 2003). A few studies have examined changes in $O_3$ responses during various menstrual cycle phases. Lung function response to $O_3$ was enhanced during the follicular phase of the menstrual cycle compared to the luteal phase in a small study of women (Fox et al., 1993). However, Seal et al. (1996) later reported no effect of menstrual cycle phase in their analysis of

responses from 150 women, but conceded that the methods used by Fox et al. (1993) more precisely defined the menstrual cycle phase. Another study also reported no difference in responses among females during the follicular and luteal phases of their cycle (Weinmann et al., 1995c). Additionally, in this study the responses in women were comparable to those reported for men in the study. In a toxicological study, small differences in effects by sex were seen in adult mice with respect to pulmonary inflammation and injury after a 5-h exposure to 0.8 ppm $O_3$, and although adult females were generally more at risk, these differences were strain-dependent, with some strains exhibiting greater risk in males (Vancza et al., 2009). The most obvious sex difference was apparent in lactating females, which incurred the greatest lung injury or inflammation among several of the strains.

Overall, results have varied, with recent evidence for increased risk for $O_3$-related health effects present for females in some studies and males in other studies. Most studies examining the associations $O_3$ and mortality report females to be at greater risk than males, but minimal evidence is available regarding a difference between the sexes for other outcomes. Inconsistent findings were reported on whether effect measure modification exists by sex for respiratory and cardiovascular hospital admissions and ED visits, although there is some indication that females are at increased risk of $O_3$-related respiratory hospital admissions and ED visits. While $O_3$-related effects may occur in both men and women, there is suggestive evidence exists indicating that females are at potentially increased risk of $O_3$-related health effects as there are consistent findings among epidemiologic studies of mortality.

### 8.3.3 Socioeconomic Status

SES is often represented by personal or neighborhood SES, which is comprised of a variety of components such as educational attainment, household income, health insurance status, and other such factors. SES is often indicative of such things as access to healthcare, quality of housing, and pollution gradient to which people are exposed. One or a combination of these components could modify the risk of $O_3$-related health effects. Based on the 2000 Census data, 12.4% of Americans live in poverty (poverty threshold for a family of four was $17,463) (SSDAN CensusScope, 2010c). Although included below, studies stratifying by SES that are conducted outside the U.S. may not be comparable to those studies from within the United States. Having low SES in another country may be different than having low SES in the U.S. based on SES definitions, population composition, and/or conditions in that country.

Multiple epidemiologic studies have reported individuals of low SES to have increased risk for the effects of short-term $O_3$ exposure on respiratory hospital admissions and ED visits. In New York State, larger associations between long-term $O_3$ exposure and asthma hospital admissions were observed among children of mothers who did not graduate from high school, whose births were covered by Medicaid/self-paid, or who were living in poor neighborhoods compared to children

whose mothers graduated from high school, whose births were covered by other insurance, or who were not living in poor neighborhoods, respectively (Lin et al., 2008b). In addition, a study conducted across 10 cities in Canada found the largest association between $O_3$ exposure and respiratory hospital admissions was among those with an educational level less than grade 9, but no consistent trend in the effect was seen across quartiles of income (Cakmak et al., 2006b). A Canadian study reported inverse effects of $O_3$ on respiratory hospital admissions and ED visits for all levels of SES, measured by average census tract household income (Burra et al., 2009). A study performed in Korea examined the association between $O_3$ concentrations and asthma hospital admissions and reported larger effect estimates in areas of moderate and low SES compared with areas of high SES (SES was based on average regional insurance rates) (Lee et al., 2006).

The examination of the potential effects of SES on $O_3$-related cardiovascular health effects is relatively limited. A study conducted in Canada reported the association between short-term $O_3$ and ED visits for cardiac disease by quartiles of neighborhood-level education and income. No effect measure modification was apparent for either measure of SES (Cakmak et al., 2006a). However, this may be due to the lack of association present between $O_3$ and ED visits for cardiac disease regardless of SES.

Several studies were conducted that examined the modification of the relationship between short-term $O_3$ concentrations and mortality by SES. A U.S. multicity study reported that communities with a higher proportion of the population unemployed had higher $O_3$-related mortality effect estimates (Bell and Dominici, 2008). A study in seven urban centers in Chile reported on modification of the association between $O_3$ exposure and mortality using multiple SES markers (Cakmak et al., 2011). Increased risk was observed among the categories of low SES for all measures (personal educational attainment, personal occupation, community income level). Additionally, the APHENA study, which examined the association between $O_3$ and mortality by percentage unemployed, reported a higher percent change in mortality with increased percent unemployed but this varied across the regions included in the study (U.S., Canada, Europe) (Katsouyanni et al., 2009). A Chinese study reported that the greatest effects between $O_3$ concentrations and mortality at lag day 0 were among individuals living in areas of high social deprivation (i.e., low SES), but this association was not consistent across lag days (at other lag times, the middle social deprivation index category had the greatest association) (Wong et al., 2008). However, another study in Asia comparing low to high educational attainment populations reported no evidence of greater mortality effects (total, CVD, or respiratory) (Kan et al., 2008). Additionally, a study in Italy reported no difference in risk of mortality among census-block level derived income levels (Stafoggia et al., 2010). A study of infant mortality in Mexico reported no association between $O_3$ concentrations and infant mortality among any of the three levels of SES determined using a socioeconomic index based on residential areas (Romieu et al., 2004a). Another study in Mexico reported a positive association between $O_3$ levels at lag 0 and respiratory-related infant mortality in only the low SES group (determined based on education, income, and household conditions across residential areas), but no

association was observed in any of the SES groups with other lags (Carbajal-Arroyo et al., 2011).

Studies of $O_3$ concentrations and reproductive outcomes have also examined associations by SES levels. A study in California reported greater decreases in birth weight associated with full pregnancy $O_3$ concentration for those with neighborhood poverty levels of at least 7% compared with those in neighborhoods with less than 7% poverty (the authors do not provide information on how categories of the SES variable were determined) (Morello-Frosch et al., 2010). No dose response was apparent and those with neighborhood poverty levels of 7-21% had greater decreases observed for the association than those living in areas with poverty rates of at least 22%. An Australian study reported an inverse association between $O_3$ exposure during days 31-60 of gestation and abdominal circumference during gestation (Hansen et al., 2008). The interaction with SES (area-level measured socioeconomic disadvantage) was examined and although the inverse association remained statistically significant in only the highest SES quartile, there were large confidence interval overlaps among estimates for each quartile so no difference in the association for the quartiles was apparent.

Evidence from a controlled human exposure study that examined $O_3$ effects on lung function does not provide support for greater $O_3$-related health effects in individuals of lower SES. In a follow-up study on modification by race, Seal et al. (1996) reported that, of three SES categories, individuals in the middle SES category showed greater concentration-dependent decline in percent-predicted $FEV_1$ (4-5% at 400 ppb $O_3$) than in low and high SES groups. The authors did not have an "immediately clear" explanation for this finding and controlled human exposure studies are typically not designed to answer questions about SES.

Overall, most studies of individuals have reported that individuals with low SES and those living in neighborhoods with low SES are more at risk for $O_3$-related health effects, resulting in increased risk of respiratory hospital admissions and ED visits. Inconsistent results have been observed in the few studies examining effect modification of associations between $O_3$ exposure and mortality and reproductive outcomes. Also, a controlled human exposure study does not support evidence of increased risk of respiratory morbidity among individuals with lower SES. Overall, evidence is suggestive of SES as a factor affecting risk of $O_3$-related health outcomes based on collective evidence from epidemiologic studies of respiratory hospital admissions but inconsistency among epidemiologic studies of mortality and reproductive outcomes. Further studies are needed to confirm this relationship, especially in populations within the U.S.

### 8.3.4 Race/Ethnicity

Based on the 2000 Census, 69.1% of the U.S. population identified as non-Hispanic whites. Approximately 12.1% of people reported their race/ethnicity as non-Hispanic black and 12.6% reported being Hispanic (SSDAN CensusScope, 2010b).

Only a few studies examined the associations between short-term $O_3$ concentrations and mortality and reported higher effect estimates among blacks (Medina-Ramón and Schwartz, 2008) and among communities with larger proportions of blacks (Bell and Dominici, 2008). Another study examined long-term exposure to $O_3$ concentrations and asthma hospital admissions among children in New York State. These authors reported no statistically significant difference in the odds of asthma hospital admissions for blacks compared to other races but did detect higher odds for Hispanics compared to non-Hispanics (Lin et al., 2008b).

Additionally, recent epidemiologic studies have stratified by race when examining the association between $O_3$ concentration and birth outcomes. A study conducted in Atlanta, GA reported decreases in birth weight with increased third trimester $O_3$ concentrations among Hispanics but not among non-Hispanic whites (Darrow et al., 2011b). A California study reported that the greatest decrease in birth weight associated with full pregnancy $O_3$ concentration was among non-Hispanic whites (Morello-Frosch et al., 2010). This inverse association was also apparent, although not as strong, for Hispanics and non-Hispanic blacks. Increased birth weight was associated with higher $O_3$ exposure among non-Hispanic Asians and Pacific Islanders but these results were not statistically significant.

Similar to the epidemiologic studies, a controlled human exposure study suggested differences in lung function responses by race (Seal et al., 1993). The independent effects of sex-race group and $O_3$ concentration on lung function were positive, but the interaction between sex-race group and $O_3$ concentration was not statistically significant. The findings indicated some overall difference between the sex-race groups that was independent of $O_3$ concentration (the concentration-response curves for the four sex-race groups are parallel). In a multiple comparison procedure on data collapsed across all $O_3$ concentrations for each sex-race group, both black men and black women had larger decrements in $FEV_1$ than did white men. The authors noted that the $O_3$ dose per unit of lung tissue would be greater in blacks and females than whites and males, respectively. That this difference in tissue dose might have affected responses to $O_3$ cannot be ruled out. The college students recruited for the Seal et al. (1993) study were probably from better educated and more SES advantaged families, thus reducing potential for these variables to be confounding factors. Que et al. (2011) also examined pulmonary responses to $O_3$ exposure in blacks of African American ancestry and in whites. On average, the black males experienced the greatest decrements in $FEV_1$ following $O_3$ exposure. This decrease was larger than the decrement observed among black females, white males, and white females.

Overall, the results of recent studies indicate that there may be race-related increase in risk of $O_3$-related health effects for some outcomes, although the overall understanding of potential effect measure modification by race is limited by the small number of studies. Additionally, these results may be confounded by other factors, such as SES. Overall, evidence is inadequate to determine if $O_3$-related health effects vary by race because of the insufficient quantity of studies and lack of consistency within disciplines.

## 8.4 Behavioral and Other Factors

### 8.4.1 Diet

Diet was not examined as a factor potentially affecting risk in previous $O_3$ AQCDs, but recent studies have examined modification of the association between $O_3$ and health effects by dietary factors. Because $O_3$ mediates some of its toxic effects through oxidative stress, the antioxidant status of an individual is an important factor that may contribute to increased risk of $O_3$-related health effects. Supplementation with Vitamins C and E has been investigated in a number of studies as a means of inhibiting $O_3$-mediated damage.

Epidemiologic studies have examined effect measure modification by diet and found evidence that certain dietary components are related to the effect $O_3$ has on respiratory outcomes. In a recent study the effects of fruit/vegetable intake and Mediterranean diet was examined (Romieu et al., 2009). Increases in these food patterns, which have been noted for their high Vitamins C and E and omega-3 fatty acid content, protected against $O_3$-related decreases in lung function among children living in Mexico City. Another study examined supplementation of the diets of asthmatic children in Mexico with Vitamins C and E (Sienra-Monge et al., 2004). Associations were detected between short-term $O_3$ exposure and nasal airway inflammation among children in the placebo group but not in those receiving the supplementation. The authors concluded that "Vitamin C and E supplementation above the minimum dietary requirement in asthmatic children with a low intake of Vitamin E might provide some protection against the nasal acute inflammatory response to ozone."

The epidemiologic evidence is supported by controlled human exposure studies, which have shown that the first line of defense against oxidative stress is antioxidants-rich extracellular lining fluid (ELF) which scavenge free radicals and limit lipid peroxidation. Exposure to $O_3$ depletes the antioxidant level in nasal ELF probably due to scrubbing of $O_3$ (Mudway et al., 1999a); however, the concentration and the activity of antioxidant enzymes either in ELF or plasma do not appear to be related to $O_3$ responsiveness (e.g., pulmonary function and inflammation) (Samet et al., 2001; Avissar et al., 2000; Blomberg et al., 1999). Carefully controlled studies of dietary antioxidant supplementation have demonstrated some protective effects of α-tocopherol (a form of Vitamin E) and ascorbate (Vitamin C) on spirometric measures of lung function after $O_3$ exposure but not on the intensity of subjective symptoms and inflammatory response including cell recruitment, activation and a release of mediators (Samet et al., 2001; Trenga et al., 2001). Dietary antioxidants have also afforded partial protection to asthmatics by attenuating postexposure bronchial hyperresponsiveness (Trenga et al., 2001).

Toxicological studies provide evidence of biological plausibility to the epidemiologic and controlled human exposure studies. Wagner et al. (2009); (2007) found reductions in $O_3$-exacerbated nasal allergy responses in rats with γ-tocopherol

treatment (a form of Vitamin E). $O_3$-induced inflammation and mucus production were also inhibited by γ-tocopherol. Supplementation with Vitamins C and E partially ameliorated inflammation, oxidative stress, and airway hyperresponsiveness in guinea pigs exposed subchronically to 0.12 ppm $O_3$ ppm (Chhabra et al., 2010). Inconsistent results were observed in other toxicological studies of Vitamin C deficiency and $O_3$-induced responses. Guinea pigs deficient in Vitamin C displayed only minimal injury and inflammation after exposure to $O_3$ (Kodavanti et al., 1995). A recent study in mice demonstrated a protective effect of β-carotene in the skin, where it limited the production of proinflammatory markers and indicators of oxidative stress induced by $O_3$ exposure (Valacchi et al., 2009). Deficiency of Vitamin A, which has a role in regulating the maintenance and repair of the epithelial layer, particularly in the lung, appears to enhance the risk of $O_3$-induced lung injury (Paquette et al., 1996). Differentially susceptible mouse strains that were fed a Vitamin A sufficient diet were observed to have different tissue concentrations of the vitamin, potentially contributing to their respective differences in $O_3$-related outcomes. In addition to the studies of antioxidants, one toxicological study examined protein deficiency. Protein deficiency alters the levels of enzymes and chemicals in the brain of rats involved with redox status; exposure to 0.75 ppm $O_3$ has been shown to differentially affect $Na^+/K^+$ ATPase, glutathione, and lipid peroxidation, depending on the nutritional status of the animal, but the significance of these changes is unclear (Calderón Guzmán et al., 2006). There may be a protective effect of overall dietary restriction with respect to lung injury, possibly related to increased Vitamin C in the lung surface fluid (Kari et al., 1997).

There is adequate evidence that individuals with reduced intake of Vitamins E and C are at risk for $O_3$-related health effects based on substantial, consistent evidence both within and among disciplines. The evidence from epidemiologic studies is supported by controlled human exposure and toxicological studies.

### 8.4.2 Obesity

Obesity, defined as a BMI of 30 $kg/m^2$ or greater, is an issue of increasing importance in the U.S., with self-reported rates of obesity of 26.7% in 2009, up from 19.8% in 2000 (Sherry et al., 2010). BMI may affect $O_3$-related health effects through multiple avenues, such as, inflammation in the body, increased pre-existing disease, and poor diet. Increased risk of PM-related health effects have been observed among obese individuals compared with non-obese individuals [2009 PM ISA (U.S. EPA, 2009d)]

A few studies have been performed examining the association between BMI and $O_3$-related changes in lung function. An epidemiologic study reported decreased lung function with increased short-term $O_3$ exposure for both obese and non-obese subjects; however, the magnitude of the reduction in lung function was greater for those subjects who were obese (Alexeeff et al., 2007). Further decrements in lung function were noted for obese individuals with AHR. Controlled human exposure

studies have also detected differential effects of $O_3$ exposure on lung function for individuals with varying BMIs. In a retrospective analysis of data from 541 healthy, nonsmoking, white males between the ages of 18-35 years from 15 studies conducted at the U.S. EPA Human Studies Facility in Chapel Hill, North Carolina, McDonnell et al. (2010) found that increased body mass index (BMI) was found to be associated with enhanced $FEV_1$ responses. The BMI effect was of the same order of magnitude but in the opposite direction of the age effect whereby $FEV_1$ responses diminish with increasing age. In a similar analysis, Bennett et al. (2007) found enhanced $FEV_1$ decrements following $O_3$ exposure with increasing BMI in a group of healthy, nonsmoking, women (BMI range 15.7 to 33.4), but not among healthy, nonsmoking men (BMI range 19.1 to 32.9). In the women, greater $O_3$-induced $FEV_1$ decrements were seen in individuals that were overweight/obese (BMI >25) compared to normal weight (BMI from 18.5 to 25), and in normal weight compared to underweight (BMI <18.5). Even disregarding the five underweight women, a greater $O_3$ response in the overweight/obese category (BMI >25) was observed compared with the normal weight group (BMI from 18.5 to 24.9).

Studies in genetically and dietarily obese mice have shown enhanced pulmonary inflammation and injury with acute $O_3$ exposure (Johnston et al., 2008; Shore, 2007). However, a recent study found that obese mice are actually resistant to $O_3$-induced pulmonary injury and inflammation and reduced lung compliance following longer exposures (72 hours) at lower concentrations (0.3 ppm $O_3$), regardless of whether obesity was genetic- or diet-induced (Shore et al., 2009).

Multiple epidemiologic, controlled human exposure, and toxicological studies have reported suggestive evidence for increased $O_3$-related respiratory health effects among obese individuals. Future research of the effect modification of the relationship between $O_3$ and other health-related outcomes besides respiratory health effects by BMI and studies examining the role of physical conditioning will advance understanding of obesity as a factor potentially increasing an individual's risk.

### 8.4.3 Smoking

Previous $O_3$ AQCDs have concluded that smoking does not increase the risk of $O_3$-related health effects; in fact, in controlled human exposure studies, smokers have been found to be less responsive to $O_3$ than non-smokers. Data from recent interviews conducted as part of the 2008 National Health Interview Survey (NHIS) (Pleis et al., 2009) have shown the rate of smoking among adults ≥ 18 years old to be approximately 20% in the United States. Approximately 21% of individuals surveyed were identified as former smokers.

Baccarelli et al. (2007) performed a study of $O_3$ concentrations and plasma homocysteine levels (a risk factor for vascular disease). They found no interaction of smoking (smokers versus non-smokers) for the associations between $O_3$ concentrations and plasma homocysteine levels. Another study examined the association between $O_3$ and resting heart rate and also reported no interaction with

smoking status (current smokers versus current non-smokers) (Ruidavets et al., 2005a).

A study examining correlations between $O_3$ levels and oxidative DNA damage examined results stratified by current versus never and former smokers (Palli et al., 2009). Ozone was positively associated with DNA damage for short-term and long-term exposures among never/former smokers. For current smokers, short-term $O_3$ concentrations were inversely associated with DNA damage; however, the number of current smokers in the study was small (n = 12).

The findings of Palli et al. (2009) were consistent with those from controlled human exposure studies that have confirmed that smokers are less responsive to $O_3$ exposure than non-smokers. Spirometric and plethysmographic pulmonary function decline, nonspecific AHR, and inflammatory responses of smokers to $O_3$ exposure were all weaker than those reported for non-smokers. Similarly, the time course of development and recovery from these effects, as well as their reproducibility, was not different from non-smokers. Chronic airway inflammation with desensitization of bronchial nerve endings and an increased production of mucus may plausibly explain the pseudo-protective effect of smoking (Frampton et al., 1997a; Torres et al., 1997).

These findings for smoking are consistent with the conclusions from previous AQCDs. An epidemiologic study of $O_3$-associated DNA damage reported smokers to be less at risk for $O_3$-related health effects. In addition, both epidemiologic studies of short-term exposure and CVD outcomes found no evidence of effect measure modification by smoking. No toxicological studies provide biological support for $O_3$-related effects. Overall, evidence of potential differences in $O_3$-related health effects by smoking status is inadequate due to insufficient coherence and a limited number of studies.

### 8.4.4 Outdoor Workers

Studies included in the 2006 $O_3$ AQCD reported that individuals who participate in outdoor activities or work outside to be a population at increased risk based on consistently reported associations between $O_3$ exposure and respiratory health outcomes in these groups (U.S. EPA, 2006b). Outdoor workers are exposed to ambient $O_3$ concentrations for a greater period of time than individuals who spend their days indoors. As discussed in Section 4.3.3 of this ISA, outdoor workers sampled during the work shift had a higher ratio of personal exposure to fixed-site monitor concentrations than health clinic workers who spent most of their time indoors. Additionally, an increase in dose to the lower airways is possible during outdoor exercise due to both increases in the amount of air breathed (i.e., minute ventilation) and a shift from nasal to oronasal breathing (Sawyer et al., 2007; Nodelman and Ultman, 1999; Hu et al., 1994). For further discussion of the association between $FEV_1$ responses to $O_3$ exposure and minute ventilation, refer to Section 6.2.3.1 of the 2006 $O_3$ AQCD. A recent study has explored the potential effect measure modification of $O_3$ exposure and DNA damage by indoor/outdoor

workplace (Tovalin et al., 2006). In a study of indoor and outdoor workers in Mexico, individuals who worked outdoors in Mexico City had a slight association between $O_3$ exposure and DNA damage (measured by comet tail length assay), whereas no association was observed for indoor workers. However, workers in another Mexican city, Puebla, demonstrated no association between $O_3$ levels and DNA damage, regardless of whether they worked indoors or outdoors.

Previous studies have shown that increased exposure to $O_3$ due to outdoor work leads to increased risk of $O_3$-related health effects, specifically decrements in lung function (U.S. EPA, 2006b). Additionally, outdoor workers may be an at-risk population due to their increased dose and exposure to $O_3$. Recent evidence from a stratified analysis does not indicate that increased $O_3$ exposure due to outdoor work leads to DNA damage. However, the strong evidence from the 2006 $O_3$ AQCD which demonstrated increased exposure, dose, and ultimately risk of $O_3$-related health effects in this population supports that there is adequate evidence available to indicate that increased exposure to $O_3$ through outdoor work increases the risk of $O_3$-related health effects.

### 8.4.5 Air Conditioning Use

Air conditioning use is an important indicator of $O_3$ exposure, as use of central air conditioning will limit exposure to $O_3$ by blocking the penetration of $O_3$ into the indoor environment and lack of air conditioning may be linked to increased exposure by use of open windows (see Section 4.3.2). Air conditioning use is a difficult effect measure modifier to examine in epidemiologic studies because it is often estimated using regional prevalence data and may not reflect individual-level use. More generally, air conditioning prevalence is associated with temperature of a region; those areas with higher temperatures have a greater prevalence of households with air conditioning. Therefore, not having air conditioning is not necessarily indicative of higher $O_3$ exposure. Despite these limitations, a few studies have examined effect measure modification by prevalence of air conditioning use in an area. Studies examining multiple cities across the U.S. have assessed whether associations between $O_3$ concentrations and hospital admissions and mortality varied among areas with high and low prevalence of air conditioning. Medina-Ramon et al. (2006) conducted a study during the warm season and observed a greater association between $O_3$ levels and pneumonia-hospital admissions among areas with a lower proportion of households having central air conditioning compared to areas with a larger proportion of households with air conditioning. However, a similar observation was not observed when examining COPD hospital admissions complicating the interpretation of the results from this study. Bell and Dominici (2008) found evidence of increased risk of $O_3$-related mortality in areas with a lower prevalence of central air conditioning in a study of 98 U.S. communities. Conversely, Medina-Ramón and Schwartz (2008) found that among individuals with atrial fibrillation, a lower risk of mortality was observed for areas with a lower prevalence of central air conditioning.

The limited number of studies that examined whether air conditioning use modifies the association between $O_3$ exposure and health has not provided consistent evidence across health endpoints. Therefore, the limited and inconsistent results across epidemiologic studies and the regional variation of air conditioning use has provided inadequate evidence to determine whether a lower prevalence of air conditioning use leads to a potential increased risk of $O_3$-related health effects.

## 8.5 Summary

In this section, epidemiologic, controlled human exposure, and toxicological studies have been evaluated and indicate that various factors may lead to increased risk of $O_3$-related health effects (Table 8-6).

The populations and lifestages identified in this section that have "adequate" evidence for increased $O_3$-related health effects are individuals with certain genotypes, individuals with asthma, younger and older age groups, individuals with reduced intake of certain nutrients, and outdoor workers, based on consistency in findings across studies and evidence of coherence in results from different scientific disciplines. Multiple genetic variants have been observed in epidemiologic and controlled human exposure studies to affect the risk of $O_3$-related respiratory outcomes and support is provided by toxicological studies of genetic factors. Asthma as a factor affecting risk was supported by controlled human exposure and toxicological studies, as well as some evidence from epidemiologic studies. Generally, studies of age groups reported positive associations for respiratory hospital admissions and ED visits among children. Biological plausibility for this increased risk is supported by toxicological and controlled human exposure research. Also, children have higher exposure and dose due to increased time spent outdoors and ventilation rate. Most studies comparing age groups reported greater effects of short-term $O_3$ exposure on mortality among older adults, although studies of other health outcomes had inconsistent findings regarding whether older adults were at increased risk. Older adults may also withstand greater $O_3$ exposure and not seek relief as quickly as younger adults. Multiple epidemiologic, controlled human exposure, and toxicological studies reported that reduced Vitamins E and C intake are associated with risk of $O_3$-related health effects. Previous studies have shown that increased exposure to $O_3$ due to outdoor work leads to an increased risk of $O_3$-related health effects and it is clear that outdoor workers have higher exposures, and possibly greater internal doses, of $O_3$, which may lead to increased risk of $O_3$-related health effects.

**Table 8-6 Summary of evidence for potential increased risk of $O_3$-related health effects.**

| Evidence Classification | Potential At Risk Factor |
|---|---|
| Adequate evidence | Genetic factors (Section 8.1)<br>Asthma (Section 8.2.2)<br>Children (Section 8.3.1.1)<br>Older adults (Section 8.3.1.2)<br>Diet (Section 8.4.1)<br>Outdoor workers (Section 8.4.4) |
| Suggestive evidence | Sex (Section 8.3.2)<br>SES (Section 8.3.3)<br>Obesity (Section 8.4.2) |
| Inadequate evidence | Influenza/Infection (Section 8.2.1)<br>COPD (Section 8.2.3)<br>CVD (Section 8.2.4)<br>Diabetes (Section 8.2.5)<br>Hyperthyroidism (Section 8.2.6)<br>Race/ethnicity (Section 8.3.4)<br>Smoking (Section 8.4.3)<br>Air conditioning use (Section 8.4.5) |
| Evidence of no effect | -- |

In some cases, it is difficult to determine a factor that results in potentially increased risk of effects. For example, previous assessments have included controlled human exposure studies in which some healthy individuals demonstrate greater $O_3$-related health effects compared to other healthy individuals. Intersubject variability has been observed for lung function decrements, symptomatic responses, pulmonary inflammation, AHR, and altered epithelial permeability in healthy adults exposed to $O_3$ (Que et al., 2011; Holz et al., 2005; McDonnell, 1996). These responses to $O_3$ exposure in healthy individuals tend to be reproducible within a given individual over a period of several months indicating differences in the intrinsic responsiveness (Holz et al., 2005; Hazucha et al., 2003; Holz et al., 1999; McDonnell et al., 1985c). In addition, there is the possibility of attenuation. In controlled human exposure and toxicological studies, pre-exposure to $O_3$ was observed to lead to a dampening of some responses following subsequent exposure to $O_3$ (for more details see Sections 5.4.2.5 and 6.2.1.1).

Limitations include the challenge of evaluating effect measure modification in epidemiologic studies with widespread populations with variation in numerous factors. For a number of the factors described below, there are few available studies. Also, some factors are inconsistent across studies, both in regards to the categorization of the variable and its measurement in the studies. Many toxicological and controlled human exposure studies are the only ones that have examined certain factors and therefore have not been replicated. In considering epidemiologic studies conducted in other countries, it is possible that those populations may differ in SES

or other demographic indicators, thus limiting generalizability to a U.S. population. Additionally, many epidemiologic studies that stratify by factors of interest have small sample sizes, which can decrease precision of effect estimates and make drawing conclusions difficult.

These challenges and limitations in evaluating the factors that can increase risk for experiencing $O_3$-related health effects may contribute to conclusions that evidence for some factors, such as sex, SES, and obesity provided "suggestive" evidence of potentially increased risk. In addition, for a number of factors listed in Table 8-6 the evidence was inadequate to draw conclusions about potential increase in risk of effects. Overall, the factors most strongly supported as contributing to increased risk of $O_3$-related effects among various populations and lifestages were related to genetic factors, asthma, age group (children and older adults), dietary factors, and working outdoors.

## References


Alexeeff, SE; Litonjua, AA; Suh, H; Sparrow, D; Vokonas, PS; Schwartz, J. (2007). Ozone exposure and lung function: Effect modified by obesity and airways hyperresponsiveness in the VA Normative Aging Study. Chest 132: 1890-1897. http://dx.doi.org/10.1378/chest.07-1126

Alexeeff, SE; Litonjua, AA; Wright, RO; Baccarelli, A; Suh, H; Sparrow, D; Vokonas, PS; Schwartz, J. (2008). Ozone exposure, antioxidant genes, and lung function in an elderly cohort: VA Normative Aging Study. Occup Environ Med 65: 736-742. http://dx.doi.org/10.1136/oem.2007.035253

Alexis, N; Urch, B; Tarlo, S; Corey, P; Pengelly, D; O'Byrne, P; Silverman, F. (2000). Cyclooxygenase metabolites play a different role in ozone-induced pulmonary function decline in asthmatics compared to normals. Inhal Toxicol 12: 1205-1224.

Alexis, NE; Zhou, H; Lay, JC; Harris, B; Hernandez, ML; Lu, TS; Bromberg, PA; Diaz-Sanchez, D; Devlin, RB; Kleeberger, SR; Peden, DB. (2009). The glutathione-S-transferase Mu 1 null genotype modulates ozone-induced airway inflammation in human subjects. J Allergy Clin Immunol 124: 1222-1228. http://dx.doi.org/10.1016/j.jaci.2009.07.036

Arbex, AM; de Souza Conceicao, GM; Perez Cendon, S; Arbex, FF; Lopes, AC; Providello Moyses, E; Santiago, SL; Saldiva, PHN; Pereira, LAA; Ferreira Braga, AL. (2009). Urban air pollution and COPD-related emergency room visits. J Epidemiol Community Health 966: 777-783. http://dx.doi.org/10.1136/jech.2008.078360

Auten, RL; Potts, EN; Mason, SN; Fischer, B; Huang, Y; Foster, WM. (2009). Maternal exposure to particulate matter increases postnatal ozone-induced airway hyperreactivity in juvenile mice. Am J Respir Crit Care Med 180: 1218-1226. http://dx.doi.org/10.1164/rccm.200901-0116OC

Avissar, NE; Reed, CK; Cox, C; Frampton, MW; Finkelstein, JN. (2000). Ozone, but not nitrogen dioxide, exposure decreases glutathione peroxidases in epithelial lining fluid of human lung. Am J Respir Crit Care Med 162: 1342-1347.

Baccarelli, A; Zanobetti, A; Martinelli, I; Grillo, P; Hou, L; Lanzani, G; Mannucci, PM; Bertazzi, PA; Schwartz, J. (2007). Air pollution, smoking, and plasma homocysteine. Environ Health Perspect 115: 176-181. http://dx.doi.org/10.1289/ehp.9517

Backus, GS; Howden, R; Fostel, J; Bauer, AK; Cho, HY; Marzec, J; Peden, DB; Kleeberger, SR. (2010). Protective role of interleukin-10 in ozone-induced pulmonary inflammation. Environ Health Perspect 118: 1721-1727. http://dx.doi.org/10.1289/ehp.1002182

Barraza-Villarreal, A; Sunyer, J; Hernandez-Cadena, L; Escamilla-Nunez, MC; Sienra-Monge, JJ; Ramirez-Aguilar, M; Cortez-Lugo, M; Holguin, F; Diaz-Sanchez, D; Olin, AC; Romieu, I. (2008). Air pollution, airway inflammation, and lung function in a cohort study of Mexico City schoolchildren. Environ Health Perspect 116: 832-838. http://dx.doi.org/10.1289/ehp.10926

Basha, MA; Gross, KB; Gwizdala, CJ; Haidar, AH; Popovich, J, Jr. (1994). Bronchoalveolar lavage neutrophilia in asthmatic and healthy volunteers after controlled exposure to ozone and filtered purified air. Chest 106: 1757-1765.

Bauer, AK; Rondini, EA; Hummel, KA; Degraff, LM; Walker, C; Jedlicka, AE; Kleeberger, SR. (2011). Identification of candidate genes downstream of TLR4 signaling after ozone exposure in mice: A role for heat shock protein 70. Environ Health Perspect 119: 1091-1097. http://dx.doi.org/10.1289/ehp.1003326

Bell, ML; Dominici, F. (2008). Effect modification by community characteristics on the short-term effects of ozone exposure and mortality in 98 US communities. Am J Epidemiol 167: 986-997. http://dx.doi.org/10.1093/aje/kwm396

Bennett, WD; Hazucha, MJ; Folinsbee, LJ; Bromberg, PA; Kissling, GE; London, SJ. (2007). Acute pulmonary function response to ozone in young adults as a function of body mass index. Inhal Toxicol 19: 1147-1154. http://dx.doi.org/10.1080/08958370701665475

Bergamaschi, E; De Palma, G; Mozzoni, P; Vanni, S; Vettori, MV; Broeckaert, F; Bernard, A; Mutti, A. (2001). Polymorphism of quinone-metabolizing enzymes and susceptibility to ozone-induced acute effects. Am J Respir Crit Care Med 163: 1426-1431.

Berhane, K; Zhang, Y; Linn, WS; Rappaport, EB; Bastain, TM; Salam, MT; Islam, T; Lurmann, F; Gilliland, FD. (2011). The effect of ambient air pollution on exhaled nitric oxide in the Children's Health Study. Eur Respir J 37: 1029-1036. http://dx.doi.org/10.1183/09031936.00081410

Bils, RF. (1970). Ultrastructural alterations of alveolar tissue of mice: III. Ozone. Arch Environ Health 20: 468-480.

Blomberg, A; Mudway, IS; Nordenhall, C; Hedenstrom, H; Kelly, FJ; Frew, AJ; Holgate, ST; Sandstrom, T. (1999). Ozone-induced lung function decrements do not correlate with early airway inflammatory or antioxidant responses. Eur Respir J 13: 1418-1428.

Bloom, B; Cohen, RA; Freeman, G. (2008). Summary health statistics for U.S. children: National Health Interview Survey, 2008 (pp. 90). Washington, DC: National Center for Health Statistics.

Bosson, J; Stenfors, N; Bucht, A; Helleday, R; Pourazar, J; Holgate, ST; Kelly, FJ; Sandstrom, T; Wilson, S; Frew, AJ; Blomberg, A. (2003). Ozone-induced bronchial epithelial cytokine expression differs between healthy and asthmatic subjects. Clin Exp Allergy 33: 777-782.

Broeckaert, F; Clippe, A; Wattiez, R; Falmagne, P; Bernard, A. (2003). Lung hyperpermeability, Clara-cell secretory potein (CC16), and susceptibility to ozone of five inbred strains of mice. Inhal Toxicol 15: 1209-1230.

Buadong, D; Jinsart, W; Funatagawa, I; Karita, K; Yano, E. (2009). Association between PM10 and O3 levels and hospital visits for cardiovascular diseases in Bangkok, Thailand. J Epidemiol 19: 182-188. http://dx.doi.org/10.2188/jea.JE20080047

Burra, TA; Moineddin, R; Agha, MM; Glazier, RH. (2009). Social disadvantage, air pollution, and asthma physician visits in Toronto, Canada. Environ Res 109: 567-574. http://dx.doi.org/10.1016/j.envres.2009.03.004

Bush, ML; Asplund, PT; Miles, KA; Ben-Jebria, A; Ultman, JS. (1996). Longitudinal distribution of O3 absorption in the lung: gender differences and intersubject variability. J Appl Physiol 81: 1651-1657.

Cakmak, S; Dales, RE; Angelica Rubio, M; Blanco Vidal, C. (2011). The risk of dying on days of higher air pollution among the socially disadvantaged elderly. Environ Res 111: 388-393. http://dx.doi.org/10.1016/j.envres.2011.01.003

Cakmak, S; Dales, RE; Judek, S. (2006a). Do gender, education, and income modify the effect of air pollution gases on cardiac disease? J Occup Environ Med 48: 89-94. http://dx.doi.org/10.1097/01.jom.0000184878.11956.4b

Cakmak, S; Dales, RE; Judek, S. (2006b). Respiratory health effects of air pollution gases: Modification by education and income. Arch Environ Occup Health 61: 5-10. http://dx.doi.org/10.3200/AEOH.61.1.5-10

Cakmak, S; Dales, RE; Vidal, CB. (2007). Air pollution and mortality in Chile: Susceptibility among the elderly. Environ Health Perspect 115: 524-527.

Calderón Guzmán, D; Barragan Mejia, G; Hernandez Garcia, E; Juarez Olguin, H. (2006). Effect of nutritional status and ozone exposure on some biomarkers of oxidative stress in rat brain regions. Nutr Cancer 55: 195-200. http://dx.doi.org/10.1207/s15327914nc5502_11

Carbajal-Arroyo, L; Miranda-Soberanis, V; Medina-Ramón, M; Rojas-Bracho, L; Tzintzun, G; Solís-Gutiérrez, P; Méndez-Ramírez, I; Hurtado-Díaz, M; Schwartz, J; Romieu, I. (2011). Effect of PM10 and O3 on infant mortality among residents in the Mexico City Metropolitan Area: A case-crossover analysis, 19972005. J Epidemiol Community Health 65: 715-721. http://dx.doi.org/10.1136/jech.2009.101212

Carey, SA; Minard, KR; Trease, LL; Wagner, JG; Garcia, GJ; Ballinger, CA; Kimbell, JS; Plopper, CG; Corley, RA; Postlethwait, EM; Harkema, JR. (2007). Three-dimensional mapping of ozone-induced injury in the nasal airways of monkeys using magnetic resonance imaging and morphometric techniques. Toxicol Pathol 35: 27-40. http://dx.doi.org/10.1080/01926230601072343

Chhabra, SK; Yasir, A; Chaudhry, K; Shah, B. (2010). Effect of ozone on response to ovalbumin & its modulation by vitamins C & E in sensitized guinea pigs. Indian J Med Res 132: 87-93.

Chiu, HF; Yang, CY. (2009). Air pollution and emergency room visits for arrhythmias: Are there potentially sensitive groups? J Toxicol Environ Health A 72: 817-823. http://dx.doi.org/10.1080/15287390902800405

Cho, HY; Morgan, DL; Bauer, AK; Kleeberger, SR. (2007). Signal transduction pathways of tumor necrosis factor--mediated lung injury induced by ozone in mice. Am J Respir Crit Care Med 175: 829-839. http://dx.doi.org/10.1164/rccm.200509-1527OC

Cho, HY; Zhang, LY; Kleeberger, SR. (2001). Ozone-induced lung inflammation and hyperreactivity are mediated via tumor necrosis factor-alpha receptors. Am J Physiol 280: L537-L546.

Chuang, GC; Yang, Z; Westbrook, DG; Pompilius, M; Ballinger, CA; White, RC; Krzywanski, DM; Postlethwait, EM; Ballinger, SW. (2009). Pulmonary ozone exposure induces vascular dysfunction, mitochondrial damage, and atherogenesis. Am J Physiol Lung Cell Mol Physiol 297: L209-L216. http://dx.doi.org/10.1152/ajplung.00102.2009

Coffin, DL; Blommer, EJ; Gardner, DE; Holzman, R. (1967). Effect of air pollution on alteration of susceptibility to pulmonary infection (pp. 71-80). (AMRL-TR-67-200). Cincinnati, OH: Aerospace Medical Research Laboratories.

Coffin, DL; Gardner, DE. (1972). Interaction of biological agents and chemical air pollutants. Ann Occup Hyg 15: 219-234.

Dahl, M; Bauer, AK; Arredouani, M; Soininen, R; Tryggvason, K; Kleeberger, SR; Kobzik, L. (2007). Protection against inhaled oxidants through scavenging of oxidized lipids by macrophage receptors MARCO and SR-AI/II. J Clin Invest 117: 757-764. http://dx.doi.org/10.1172/JCI29968

Darrow, LA; Klein, M; Strickland, MJ; Mulholland, JA; Tolbert, PE. (2011b). Ambient air pollution and birth weight in full-term infants in Atlanta, 1994-2004. Environ Health Perspect 119: 731-737. http://dx.doi.org/10.1289/ehp.1002785

Escamilla-Nuñez, MC; Barraza-Villarreal, A; Hernandez-Cadena, L; Moreno-Macias, H; Ramirez-Aguilar, M; Sienra-Monge, JJ; Cortez-Lugo, M; Texcalac, JL; del Rio-Navarro, B; Romieu, I. (2008). Traffic-related air pollution and respiratory symptoms among asthmatic children, resident in Mexico City: The EVA cohort study. Respir Res 9: 74. http://dx.doi.org/10.1186/1465-9921-9-74

Fakhrzadeh, L; Laskin, JD; Laskin, DL. (2002). Deficiency in inducible nitric oxide synthase protects mice from ozone-induced lung inflammation and tissue injury. Am J Respir Cell Mol Biol 26: 413-419.

Fanucchi, MV; Plopper, CG; Evans, MJ; Hyde, DM; Van Winkle, LS; Gershwin, LJ; Schelegle, ES. (2006). Cyclic exposure to ozone alters distal airway development in infant rhesus monkeys. Am J Physiol Lung Cell Mol Physiol 291: L644-L650. http://dx.doi.org/10.1152/ajplung.00027.2006

Fortino, V; Maioli, E; Torricelli, C; Davis, P; Valacchi, G. (2007). Cutaneous MMPs are differently modulated by environmental stressors in old and young mice. Toxicol Lett 173: 73-79. http://dx.doi.org/10.1016/j.toxlet.2007.06.004

Fox, SD; Adams, WC; Brookes, KA; Lasley, BL. (1993). Enhanced response to ozone exposure during the follicular phase of the menstrual cycle. Environ Health Perspect 101: 242-244.

Frampton, MW; Morrow, PE; Torres, A; Cox, C; Voter, KZ; Utell, MJ; Gibb, FR; Speers, DM. (1997a). Ozone responsiveness in smokers and nonsmokers. Am J Respir Crit Care Med 155: 116-121.

Funabashi, H; Shima, M; Kuwaki, T; Hiroshima, K; Kuriyama, T. (2004). Effects of repeated ozone exposure on pulmonary function and bronchial responsiveness in mice sensitized with ovalbumin. Toxicology 204: 75-83. http://dx.doi.org/10.1016/j.tox.2004.06.047

Garantziotis, S; Li, Z; Potts, EN; Kimata, K; Zhuo, L; Morgan, DL; Savani, RC; Noble, PW; Foster, WM; Schwartz, DA; Hollingsworth, JW. (2009). Hyaluronan mediates ozone-induced airway hyperresponsiveness in mice. J Biol Chem 284: 11309-11317. http://dx.doi.org/10.1074/jbc.M802400200

Garantziotis, S; Li, Z; Potts, EN; Lindsey, JY; Stober, VP; Polosukhin, VV; Blackwell, TS; Schwartz, DA; Foster, WM; Hollingsworth, JW. (2010). TLR4 is necessary for hyaluronan-mediated airway hyperresponsiveness after ozone inhalation. Am J Respir Crit Care Med 181: 666-675. http://dx.doi.org/10.1164/rccm.200903-0381OC

Goldberg, MS; Burnett, RT; Yale, JF; Valois, MF; Brook, JR. (2006). Associations between ambient air pollution and daily mortality among persons with diabetes and cardiovascular disease. Environ Res 100: 255-267.

Halonen, JI; Lanki, T; Tiittanen, P; Niemi, JV; Loh, M; Pekkanen, J. (2009). Ozone and cause-specific cardiorespiratory morbidity and mortality. J Epidemiol Community Health 64: 814-820. http://dx.doi.org/10.1136/jech.2009.087106

Hamade, AK; Misra, V; Rabold, R; Tankersley, CG. (2010). Age-related changes in cardiac and respiratory adaptation to acute ozone and carbon black exposures: Interstrain variation in mice. Inhal Toxicol 22: 84-94. http://dx.doi.org/10.3109/08958378.2010.503974

Hamade, AK; Rabold, R; Tankersley, CG. (2008). Adverse cardiovascular effects with acute particulate matter and ozone exposures: Interstrain variation in mice. Environ Health Perspect 116: 1033-1039. http://dx.doi.org/10.1289/ehp.10689

Hamade, AK; Tankersley, CG. (2009). Interstrain variation in cardiac and respiratory adaptation to repeated ozone and particulate matter exposures. Am J Physiol Regul Integr Comp Physiol 296: R1202-R1215. http://dx.doi.org/10.1152/ajpregu.90808.2008

Hansen, CA; Barnett, AG; Pritchard, G. (2008). The effect of ambient air pollution during early pregnancy on fetal ultrasonic measurements during mid-pregnancy. Environ Health Perspect 116: 362-369. http://dx.doi.org/10.1289/ehp.10720

Harkema, JR; Plopper, CG; Hyde, DM; St George, JA; Dungworth, DL. (1987a). Effects of an ambient level of ozone on primate nasal epithelial mucosubstances: quantitative histochemistry. Am J Pathol 127: 90-96.

Hazucha, MJ; Folinsbee, LJ; Bromberg, PA. (2003). Distribution and reproducibility of spirometric response to ozone by gender and age. J Appl Physiol 95: 1917-1925.

Henrotin, JB; Besancenot, JP; Bejot, Y; Giroud, M. (2007). Short-term effects of ozone air pollution on ischaemic stroke occurrence: A case-crossover analysis from a 10-year population-based study in Dijon, France. Occup Environ Med 64: 439-445. http://dx.doi.org/10.1136/oem.2006.029306

Henrotin, JB; Zeller, M; Lorgis, L; Cottin, Y; Giroud, M; Béjot, Y. (2010). Evidence of the role of short-term exposure to ozone on ischaemic cerebral and cardiac events: The Dijon Vascular Project (DIVA). Heart 96: 1990-1996. http://dx.doi.org/10.1136/hrt.2010.200337

Hernández-Cadena, L; Holguin, F; Barraza-Villarreal, A; Del Río-Navarro, BE; Sienra-Monge, JJ; Romieu, I. (2009). Increased levels of outdoor air pollutants are associated with reduced bronchodilation in children with asthma. Chest 136: 1529-1536. http://dx.doi.org/10.1378/chest.08-1463

Hernandez, ML; Lay, JC; Harris, B; Esther, CR; Brickey, WJ; Bromberg, PA; Diaz-Sanchez, D; Devlin, RB; Kleeberger, SR; Alexis, NE; Peden, DB. (2010). Atopic asthmatic subjects but not atopic subjects without asthma have enhanced inflammatory response to ozone. J Allergy Clin Immunol 126: 537-544. http://dx.doi.org/10.1016/j.jaci.2010.06.043

Hollingsworth, JW; Cook, DN; Brass, DM; Walker, JKL; Morgan, DL; Foster, WM; Schwartz, DA. (2004). The role of Toll-like receptor 4 in environmental airway injury in mice. Am J Respir Crit Care Med 170: 126-132. http://dx.doi.org/10.1164/rccm.200311-1499OC

Hollingsworth, JW; Free, ME; Li, Z; Andrews, LN; Nakano, H; Cook, DN. (2010). Ozone activates pulmonary dendritic cells and promotes allergic sensitization through a Toll-like receptor 4-dependent mechanism [Letter]. J Allergy Clin Immunol 125: 1167-1170. http://dx.doi.org/10.1016/j.jaci.2010.03.001

Holz, O; Jorres, RA; Timm, P; Mucke, M; Richter, K; Koschyk, S; Magnussen, H. (1999). Ozone-induced airway inflammatory changes differ between individuals and are reproducible. Am J Respir Crit Care Med 159: 776-784.

Holz, O; Tal-Singer, R; Kanniess, F; Simpson, KJ; Gibson, A; Vessey, RSJ; Janicki, S; Magnussen, H; Jorres, RA; Richter, K. (2005). Validation of the human ozone challenge model as a tool for assessing anti-inflammatory drugs in early development. J Clin Pharmacol 45: 498-503.

Horstman, DH; Ball, BA; Brown, J; Gerrity, T; Folinsbee, LJ. (1995). Comparison of pulmonary responses of asthmatic and nonasthmatic subjects performing light exercise while exposed to a low level of ozone. Toxicol Ind Health 11: 369-385.

Housley, DG; Eccles, R; Richards, RJ. (1996). Gender difference in the concentration of the antioxidant uric acid in human nasal lavage. Acta Otolaryngol 116: 751-754.

Hu, SC; Ben-Jebria, A; Ultman, JS. (1994). Longitudinal distribution of ozone absorption in the lung: Effects of respiratory flow. J Appl Physiol 77: 574-583.

Huffman, LJ; Beighley, CM; Frazer, DG; Mckinney, WG; Porter, DW. (2006). Increased susceptibility of the lungs of hyperthyroid rats to oxidant injury: Specificity of effects. Toxicology 225: 119-127. http://dx.doi.org/10.1016/j.tox.2006.05.008

Jerrett, M; Burnett, RT; Pope, CA, III; Ito, K; Thurston, G; Krewski, D; Shi, Y; Calle, E; Thun, M. (2009). Long-term ozone exposure and mortality. N Engl J Med 360: 1085-1095. http://dx.doi.org/10.1056/NEJMoa0803894

Joad, JP; Kott, KS; Bric, JM; Peake, JL; Plopper, CG; Schelegle, ES; Gershwin, LJ; Pinkerton, KE. (2006). Structural and functional localization of airway effects from episodic exposure of infant monkeys to allergen and/or ozone. Toxicol Appl Pharmacol 214: 237-243. http://dx.doi.org/10.1016/j.taap.2005.12.012

Johnston, RA; Mizgerd, JP; Shore, SA. (2005a). CXCR2 is essential for maximal neutrophil recruitment and methacholine responsiveness after ozone exposure. Am J Physiol Lung Cell Mol Physiol 288: L61-L67. http://dx.doi.org/10.1152/ajplung.00101.2004 00101.2004

Johnston, RA; Schwartzman, IN; Flynt, L; Shore, SA. (2005b). Role of interleukin-6 in murine airway responses to ozone. Am J Physiol Lung Cell Mol Physiol 288: L390-L397. http://dx.doi.org/10.1152/ajplung.00007.2004

Johnston, RA; Theman, TA; Lu, FL; Terry, RD; Williams, ES; Shore, SA. (2008). Diet-induced obesity causes innate airway hyperresponsiveness to methacholine and enhances ozone-induced pulmonary inflammation. J Appl Psychol 104: 1727-1735. http://dx.doi.org/10.1152/japplphysiol.00075.2008

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

Kan, H; London, SJ; Chen, G; Zhang, Y; Song, G; Zhao, N; Jiang, L; Chen, B. (2008). Season, sex, age, and education as modifiers of the effects of outdoor air pollution on daily mortality in Shanghai, China: The Public Health and Air Pollution in Asia (PAPA) Study. Environ Health Perspect 116: 1183-1188. http://dx.doi.org/10.1289/ehp.10851

Kari, F; Hatch, G; Slade, R; Crissman, K; Simeonova, PP; Luster, M. (1997). Dietary restriction mitigates ozone-induced lung inflammation in rats: A role for endogenous antioxidants. Am J Respir Cell Mol Biol 17: 740-747.

Katsouyanni, K; Samet, JM; Anderson, HR; Atkinson, R; Le Tertre, A; Medina, S; Samoli, E; Touloumi, G; Burnett, RT; Krewski, D; Ramsay, T; Dominici, F; Peng, RD; Schwartz, J; Zanobetti, A. (2009). Air pollution and health: A European and North American approach (APHENA). (Research Report 142). Boston, MA: Health Effects Institute. http://pubs.healtheffects.org/view.php?id=327

Kehrl, HR; Peden, DB; Ball, BA; Folinsbee, LJ; Horstman, DH. (1999). Increased specific airway reactivity of persons with mild allergic asthma after 7.6 hours of exposure to 0.16 ppm ozone. J Allergy Clin Immunol 104: 1198-1204.

Kenyon, NJ; Van Der Vliet, A; Schock, BC; Okamoto, T; McGrew, GM; Last, JA. (2002). Susceptibility to ozone-induced acute lung injury in iNOS-deficient mice. Am J Physiol 282: L540-L545.

Khatri, SB; Holguin, FC; Ryan, PB; Mannino, D; Erzurum, SC; Teague, WG. (2009). Association of ambient ozone exposure with airway inflammation and allergy in adults with asthma. J Asthma 46: 777-785. http://dx.doi.org/10.1080/02770900902779284

Kim, CS; Alexis, NE; Rappold, AG; Kehrl, H; Hazucha, MJ; Lay, JC; Schmitt, MT; Case, M; Devlin, RB; Peden, DB; Diaz-Sanchez, D. (2011). Lung function and inflammatory responses in healthy young adults exposed to 0.06 ppm ozone for 6.6 hours. Am J Respir Crit Care Med 183: 1215-1221. http://dx.doi.org/10.1164/rccm.201011-1813OC

Kleeberger, SR; Levitt, RC; Zhang, LY; Longphre, M; Harkema, J; Jedlicka, A; Eleff, SM; DiSilvestre, D; Holroyd, KJ. (1997). Linkage analysis of susceptibility to ozone-induced lung inflammation in inbred mice. Nat Genet 17: 475-478.

Kleeberger, SR; Reddy, S; Zhang, LY; Jedlicka, AE. (2000). Genetic susceptibility to ozone-induced lung hyperpermeability: Role of toll-like receptor 4. Am J Respir Cell Mol Biol 22: 620-627.

Kleeberger, SR; Reddy, SP; Zhang, LY; Cho, HY; Jedlicka, AE. (2001). Toll-like receptor 4 mediates ozone-induced murine lung hyperpermeability via inducible nitric oxide synthase. Am J Physiol 280: L326-L333.

Ko, FWS; Tam, W; Wong, TW; Lai, CKW. (2007). Effects of air pollution on asthma hospitalization rates in different age groups in Hong Kong. Clin Exp Allergy 37: 1312-1319. http://dx.doi.org/10.1111/j.1365-2222.2007.02791.x

Kodavanti, UP; Costa, DL; Dreher, KL; Crissman, K; Hatch, GE. (1995). Ozone-induced tissue injury and changes in antioxidant homeostasis in normal and ascorbate-deficient guinea pigs. Biochem Pharmacol 50: 243-251. http://dx.doi.org/10.1016/0006-2952(95)00122-G

Kooter, IM; Pennings, JL; Fokkens, PH; Leseman, DL; Boere, AJ; Gerlofs-Nijland, ME; Cassee, FR; Schalk, JA; Orzechowski, TJ; Schaap, MM; Breit, TM; Dormans, JA; van Oostrom, CT; de Vries, A; van Steeg, H. (2007). Ozone induces clear cellular and molecular responses in the mouse lung independently of the transcription-coupled repair status. J Appl Physiol 102: 1185-1192. http://dx.doi.org/10.1152/japplphysiol.00796.2006

Kreit, JW; Gross, KB; Moore, TB; Lorenzen, TJ; D'Arcy, J; Eschenbacher, WL. (1989). Ozone-induced changes in pulmonary function and bronchial responsiveness in asthmatics. J Appl Physiol 66: 217-222.

Kumarathasan, P; Blais, E; Goegan, P; Yagminas, A; Guenette, J; Adamson, IY; Crapo, JD; Mason, RJ; Vincent, R. (2005). 90-day repeated inhalation exposure of surfactant Protein-C/tumor necrosis factor-alpha, (SP-C/TNF-alpha) transgenic mice to air pollutants. Int J Toxicol 24: 59-67.

Lagorio, S; Forastiere, F; Pistelli, R; Iavarone, I; Michelozzi, P; Fano, V; Marconi, A; Ziemacki, G; Ostro, BD. (2006). Air pollution and lung function among susceptible adult subjects: A panel study. Environ Health 5: 11. http://dx.doi.org/10.1186/1476-069X-5-11

Lee, IM; Tsai, SS; Ho, CK; Chiu, HF; Wu, TN; Yang, CY. (2008a). Air pollution and hospital admissions for congestive heart failure: Are there potentially sensitive groups? Environ Res 108: 348-353. http://dx.doi.org/10.1016/j.envres.2008.07.024

Lee, JT; Son, JY; Kim, H; Kim, SY. (2006). Effect of air pollution on asthma-related hospital admissions for children by socioeconomic status associated with area of residence. Arch Environ Occup Health 61: 123-130.

Lewis, TC; Robins, TG; Dvonch, JT; Keeler, GJ; Yip, FY; Mentz, GB; Lin, X; Parker, EA; Israel, BA; Gonzalez, L; Hill, Y. (2005). Air pollution-associated changes in lung function among asthmatic children in Detroit. Environ Health Perspect 113: 1068-1075.

Liao, D; Heiss, G; Chinchilli, VM; Duan, Y; Folsom, AR; Lin, HM; Salomaa, V. (2005). Association of criteria pollutants with plasma hemostatic/inflammatory markers: A population-based study. J Expo Sci Environ Epidemiol 15: 319-328.

Lim, Y; Phung, AD; Corbacho, AM; Aung, HH; Maioli, E; Reznick, AZ; Cross, CE; Davis, PA; Valacchi, G. (2006). Modulation of cutaneous wound healing by ozone: Differences between young and aged mice. Toxicol Lett 160: 127-134. http://dx.doi.org/10.1016/j.toxlet.2005.06.013

Lin, M; Stieb, DM; Chen, Y. (2005). Coarse particulate matter and hospitalization for respiratory infections in children younger than 15 years in Toronto: A case-crossover analysis. Pediatrics 116: 235-240.

Lin, S; Liu, X; Le, LH; Hwang, SA. (2008b). Chronic exposure to ambient ozone and asthma hospital admissions among children. Environ Health Perspect 116: 1725-1730. http://dx.doi.org/10.1289/ehp.11184

Liu, L; Poon, R; Chen, L; Frescura, AM; Montuschi, P; Ciabattoni, G; Wheeler, A; Dales, R. (2009a). Acute effects of air pollution on pulmonary function, airway inflammation, and oxidative stress in asthmatic children. Environ Health Perspect 117: 668-674. http://dx.doi.org/10.1289/ehp11813

López, I; Sánchez, I; Bizarro, P; Acevedo, S; Ustarroz, M; Fortoul, T. (2008). Ultrastructural alterations during embryonic rats' lung development caused by ozone. J Electron Microsc (Tokyo) 57: 19-23. http://dx.doi.org/10.1093/jmicro/dfm033

Mar, TF; Koenig, JQ. (2009). Relationship between visits to emergency departments for asthma and ozone exposure in greater Seattle, Washington. Ann Allergy Asthma Immunol 103: 474-479. http://dx.doi.org/10.1016/S1081-1206(10)60263-3

McDonnell, WF. (1996). Individual variability in human lung function responses to ozone exposure. Environ Toxicol Pharmacol 2: 171-175.

McDonnell, WF, III; Horstman, DH; Abdul-Salaam, S; House, DE. (1985c). Reproducibility of individual responses to ozone exposure. Am Rev Respir Dis 131: 36-40.

McDonnell, WF; Stewart, PW; Smith, MV. (2010). Prediction of ozone-induced lung function responses in humans. Inhal Toxicol 22: 160-168. http://dx.doi.org/10.3109/08958370903089557

McDonnell, WF; Stewart, PW; Smith, MV; Pan, WK; Pan, J. (1999b). Ozone-induced respiratory symptoms: Exposure-response models and association with lung function. Eur Respir J 14: 845-853.

Medina-Ramón, M; Schwartz, J. (2008). Who is more vulnerable to die from ozone air pollution? Epidemiology 19: 672-679. http://dx.doi.org/10.1097/EDE.0b013e3181773476

Medina-Ramon, M; Zanobetti, A; Schwartz, J. (2006). The effect of ozone and PM10 on hospital admissions for pneumonia and chronic obstructive pulmonary disease: A national multicity study. Am J Epidemiol 163: 579-588. http://dx.doi.org/10.1093/aje/kwj078

Middleton, N; Yiallouros, P; Kleanthous, S; Kolokotroni, O; Schwartz, J; Dockery, DW; Demokritou, P; Koutrakis, P. (2008). A 10-year time-series analysis of respiratory and cardiovascular morbidity in Nicosia, Cyprus: The effect of short-term changes in air pollution and dust storms. Environ Health 7: 39.

Miller, FJ; Illing, JW; Gardner, DE. (1978). Effect of urban ozone levels on laboratory-induced respiratory infections. Toxicol Lett 2: 163-169.

Morello-Frosch, R; Jesdale, BM; Sadd, JL; Pastor, M. (2010). Ambient air pollution exposure and full-term birth weight in California. Environ Health 9: 44. http://dx.doi.org/10.1186/1476-069X-9-44

Mudway, IS; Blomberg, A; Frew, AJ; Holgate, ST; Sandstrom, T; Kelly, FJ. (1999a). Antioxidant consumption and repletion kinetics in nasal lavage fluid following exposure of healthy human volunteers to ozone. Eur Respir J 13: 1429-1438.

Mudway, IS; Stenfors, N; Blomberg, A; Helleday, R; Dunster, C; Marklund, SL; Frew, AJ; Sandstrom, T; Kelly, FJ. (2001). Differences in basal airway antioxidant concentrations are not predictive of individual responsiveness to ozone: A comparison of healthy and mild asthmatic subjects. Free Radic Biol Med 31: 962-974.

Neidell, M; Kinney, PL. (2010). Estimates of the association between ozone and asthma hospitalizations that account for behavioral responses to air quality information. Environ Sci Pol 13: 97-103. http://dx.doi.org/10.1016/j.envsci.2009.12.006

Nodelman, V; Ultman, JS. (1999). Longitudinal distribution of chlorine absorption in human airways: A comparison to ozone absorption. J Appl Physiol 87: 2073-2080.

Oyarzún, M; Dussaubat, N; González, S. (2005). Effect of 0.25 ppm ozone exposure on pulmonary damage induced by bleomycin. Biol Res 38: 353-358.

Palli, D; Sera, F; Giovannelli, L; Masala, G; Grechi, D; Bendinelli, B; Caini, S; Dolara, P; Saieva, C. (2009). Environmental ozone exposure and oxidative DNA damage in adult residents of Florence, Italy. Environ Pollut 157: 1521-1525. http://dx.doi.org/10.1016/j.envpol.2008.09.011

Paquette, NC; Zhang, LY; Ellis, WA; Scott, AL; Kleeberger, SR. (1996). Vitamin A deficiency enhances ozone-induced lung injury. Am J Physiol 270: L475-L482.

Paulu, C; Smith, AE. (2008). Tracking associations between ambient ozone and asthma-related emergency department visits using case-crossover analysis. J Public Health Manag Pract 14: 581-591.

Peden, DB; Boehlecke, B; Horstman, D; Devlin, R. (1997). Prolonged acute exposure to 0.16 ppm ozone induces eosinophilic airway inflammation in asthmatic subjects with allergies. J Allergy Clin Immunol 100: 802-808.

Peden, DB; Setzer, RW, Jr; Devlin, RB. (1995). Ozone exposure has both a priming effect on allergen-induced responses and an intrinsic inflammatory action in the nasal airways of perennially allergic asthmatics. Am J Respir Crit Care Med 151: 1336-1345.

Peel, JL; Metzger, KB; Klein, M; Flanders, WD; Mulholland, JA; Tolbert, PE. (2007). Ambient air pollution and cardiovascular emergency department visits in potentially sensitive groups. Am J Epidemiol 165: 625-633. http://dx.doi.org/10.1093/aje/kwk051

Pleis, JR; Lucas, JW; Ward, BW. (2009). Summary health statistics for U.S. adults: National Health Interview Survey, 2008. (DHHS 2010-1570). Hyattsville, MD: National Center for Health Statistics. http://www.cdc.gov/nchs/data/series/sr_10/sr10_242.pdf

Plopper, CG; Smiley-Jewell, SM; Miller, LA; Fanucchi, MV; Evans, MJ; Buckpitt, AR; Avdalovic, M; Gershwin, LJ; Joad, JP; Kajekar, R; Larson, S; Pinkerton, KE; Van Winkle, LS; Schelegle, ES; Pieczarka, EM; Wu, R; Hyde, DM. (2007). Asthma/allergic airways disease: Does postnatal exposure to environmental toxicants promote airway pathobiology? Toxicol Pathol 35: 97-110. http://dx.doi.org/10.1080/01926230601132030

Qian, Z; Lin, HM; Chinchilli, VM; Lehman, EB; Duan, Y; Craig, TJ; Wilson, WE; Liao, D; Lazarus, SC; Bascom, R. (2009). Interaction of ambient air pollution with asthma medication on exhaled nitric oxide among asthmatics. Arch Environ Occup Health 64: 168-176. http://dx.doi.org/10.1080/19338240903240616

Que, LG; Stiles, JV; Sundy, JS; Foster, WM. (2011). Pulmonary function, bronchial reactivity, and epithelial permeability are response phenotypes to ozone and develop differentially in healthy humans. J Appl Physiol 111: 679-687. http://dx.doi.org/10.1152/japplphysiol.00337.2011

Rivas-Arancibia, S; Dorado-Martinez, C; Borgonio-Perez, G; Hiriart-Urdanivia, M; Verdugo-Diaz, L; Duran-Vazquez, A; Colin-Baranque, L; Avila-Costa, MR. (2000). Effects of taurine on ozone-induced memory deficits and lipid peroxidation levels in brains of young, mature, and old rats. Environ Res 82: 7-17. http://dx.doi.org/10.1006/enrs.1999.3996

Romieu, I; Barraza-Villarreal, A; Escamilla-Núñez, C; Texcalac-Sangrador, JL; Hernandez-Cadena, L; Díaz-Sánchez, D; De Batlle, J; Del Rio-Navarro, BE. (2009). Dietary intake, lung function and airway inflammation in Mexico City school children exposed to air pollutants. Respir Res 10: 122.

Romieu, I; Ramirez-Aguilar, M; Moreno-Macias, H; Barraza-Villarreal, A; Miller, P; Hernandez-Cadena, L; Carbajal-Arroyo, LA; Hernandez-Avila, M. (2004a). Infant mortality and air pollution: Modifying effect by social class. J Occup Environ Hyg 46: 1210-1216.

Romieu, I; Ramirez-Aguilar, M; Sienra-Monge, JJ; Moreno-Macias, H; Del Rio-Navarro, BE; David, G; Marzec, J; Hernandez-Avila, M; London, S. (2006). GSTM1 and GSTP1 and respiratory health in asthmatic children exposed to ozone. Eur Respir J 28: 953-959. http://dx.doi.org/10.1183/09031936.06.00114905

Romieu, I; Sienra-Monge, JJ; Ramirez-Aguilar, M; Moreno-Macias, H; Reyes-Ruiz, NI; Estela del Rio-Navarro, B; Hernandez-Avila, M; London, SJ. (2004b). Genetic polymorphism of GSTM1 and antioxidant supplementation influence lung function in relation to ozone exposure in asthmatic children in Mexico City. Thorax 59: 8-10.

Ruidavets, JB; Cassadou, S; Cournot, M; Bataille, V; Meybeck, M; Ferrieres, J. (2005a). Increased resting heart rate with pollutants in a population based study. J Epidemiol Community Health 59: 685-693.

Sacks, JD; Stanek, LW; Luben, TJ; Johns, DO; Buckley, BJ; Brown, JS; Ross, M. (2011). Particulate-matter induced health effects: Who is susceptible? Environ Health Perspect 119: 446-454. http://dx.doi.org/10.1289/ehp.1002255

Samet, JM; Hatch, GE; Horstman, D; Steck-Scott, S; Arab, L; Bromberg, PA; Levine, M; McDonnell, WF; Devlin, RB. (2001). Effect of antioxidant supplementation on ozone-induced lung injury in human subjects. Am J Respir Crit Care Med 164: 819-825.

Sarangapani, R; Gentry, PR; Covington, TR; Teeguarden, JG; Clewell HJ, III. (2003). Evaluation of the potential impact of age- and gender-specific lung morphology and ventilation rate on the dosimetry of vapors. Inhal Toxicol 15: 987-1016. http://dx.doi.org/10.1080/08958370390226350

Sawyer, K; Brown, J; HazuchaM; Bennett, WD. (2007). The effect of exercise on nasal uptake of ozone in healthy human adults. J Appl Physiol 102: 1380-1386. http://dx.doi.org/10.1152/japplphysiol.00269.2006

Scannell, C; Chen, L; Aris, RM; Tager, I; Christian, D; Ferrando, R; Welch, B; Kelly, T; Balmes, JR. (1996). Greater ozone-induced inflammatory responses in subjects with asthma. Am J Respir Crit Care Med 154: 24-29.

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

Seal, E, Jr; McDonnell, WF; House, DE. (1996). Effects of age, socioeconomic status, and menstrual cycle on pulmonary response to ozone. Arch Environ Occup Health 51: 132-137.

Seal, E, Jr; McDonnell, WF; House, DE; Salaam, SA; Dewitt, PJ; Butler, SO; Green, J; Raggio, L. (1993). The pulmonary response of white and black adults to six concentrations of ozone. Am J Respir Crit Care Med 147: 804-810.

Servais, S; Boussouar, A; Molnar, A; Douki, T; Pequignot, JM; Favier, R. (2005). Age-related sensitivity to lung oxidative stress during ozone exposure. Free Radic Res 39: 305-316. http://dx.doi.org/10.1080/10715760400011098

Sherry, B; Blanck, HM; Galuska, DA; Pan, L; Dietz, WH; Balluz, L. (2010). Vital signs: State-specific obesity prevalence among adults - United States, 2009. MMWR Recomm Rep 59: 951-955.

Shore, SA. (2007). Obesity and asthma: Lessons from animal models [Review]. J Appl Physiol 102: 516-528. http://dx.doi.org/10.1152/japplphysiol.00847.2006

Shore, SA; Lang, JE; Kasahara, DI; Lu, FL; Verbout, NG; Si, H; Williams, ES; Terry, RD; Lee, A; Johnston, RA. (2009). Pulmonary responses to subacute ozone exposure in obese vs. lean mice. J Appl Physiol 107: 1445-1452. http://dx.doi.org/10.1152/japplphysiol.00456.2009

Sienra-Monge, JJ; Ramirez-Aguilar, M; Moreno-Macias, H; Reyes-Ruiz, NI; Del Rio-Navarro, BE; Ruiz-Navarro, MX; Hatch, G; Crissman, K; Slade, R; Devlin, RB; Romieu, I. (2004). Antioxidant supplementation and nasal inflammatory responses among young asthmatics exposed to high levels of ozone. Clin Exp Immunol 138: 317-322. http://dx.doi.org/10.1111/j.1365-2249.2004.02606.x

Silverman, RA; Ito, K. (2010). Age-related association of fine particles and ozone with severe acute asthma in New York City. J Allergy Clin Immunol 125: 367-373. http://dx.doi.org/10.1016/j.jaci.2009.10.061

SSDAN CensusScope (Social Science Data Analysis Network, CensusScope). (2010a). United States: Age distribution [Database]. Ann Arbor, Michigan: Social Science Data Analysis Network. Retrieved from http://www.censusscope.org/us/chart_age.html

SSDAN CensusScope (Social Science Data Analysis Network, CensusScope). (2010b). United States: Population by race [Database]. Ann Arbor, Michigan. Retrieved from http://www.censusscope.org/us/chart_race.html

SSDAN CensusScope (Social Science Data Analysis Network, CensusScope). (2010c). United States: Poverty by age [Database]. Ann Arbor, Michigan. Retrieved from http://www.censusscope.org/us/chart_poverty.html

Stafoggia, M; Forastiere, F; Faustini, A; Biggeri, A; Bisanti, L; Cadum, E; Cernigliaro, A; Mallone, S; Pandolfi, P; Serinelli, M; Tessari, R; Vigotti, MA; Perucci, CA. (2010). Susceptibility factors to ozone-related mortality: A population-based case-crossover analysis. Am J Respir Crit Care Med 182: 376-384. http://dx.doi.org/10.1164/rccm.200908-1269OC

Steinvil, A; Kordova-Biezuner, L; Shapira, I; Berliner, S; Rogowski, O. (2008). Short-term exposure to air pollution and inflammation-sensitive biomarkers. Environ Res 106: 51-61. http://dx.doi.org/10.1016/j.envres.2007.08.006

Tankersley, CG; Kleeberger, SR. (1994). Ozone-induced inflammation and altered ventilation in genetically susceptible mice: A comparison of acute and subacute exposures. Toxicol Lett 72: 279-289.

Tankersley, CG; Peng, RD; Bedga, D; Gabrielson, K; Champion, HC. (2010). Variation in echocardiographic and cardiac hemodynamic effects of PM and ozone inhalation exposure in strains related to Nppa and Npr1 gene knock-out mice. Inhal Toxicol 22: 695-707. http://dx.doi.org/10.3109/08958378.2010.487549

Thaller, EI; Petronella, SA; Hochman, D; Howard, S; Chhikara, RS; Brooks, EG. (2008). Moderate increases in ambient PM2.5 and ozone are associated with lung function decreases in beach lifeguards. J Occup Environ Med 50: 202-211. http://dx.doi.org/10.1097/JOM.0b013e31816386b4

Torres, A; Utell, MJ; Morow, PE; Voter, KZ; Whitin, JC; Cox, C; Looney, RJ; Speers, DM; Tsai, Y; Frampton, MW. (1997). Airway inflammation in smokers and nonsmokers with varying responsiveness to ozone. Am J Respir Crit Care Med 156: 728-736.

Tovalin, H; Valverde, M; Morandi, MT; Blanco, S; Whitehead, L; Rojas, E. (2006). DNA damage in outdoor workers occupationally exposed to environmental air pollutants. Occup Environ Med 63: 230-236.

Trenga, CA; Koenig, JQ; Williams, PV. (2001). Dietary antioxidants and ozone-induced bronchial hyperresponsiveness in adults with asthma. Arch Environ Occup Health 56: 242-249.

U.S. Census Bureau. (2010). U.S. population projections [Database]. Retrieved from http://www.census.gov/population/www/projections/projectionsagesex.html

U.S. EPA (U.S. Environmental Protection Agency). (1996a). Air quality criteria for ozone and related photochemical oxidants [EPA Report]. (EPA/600/P-93/004AF). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (2006a). Aging and toxic response: Issues relevant to risk assessment [EPA Report]. (EPA/600/P-03/004A). Washington, DC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=156648

U.S. EPA (U.S. Environmental Protection Agency). (2006b). Air quality criteria for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-05/004AF). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=149923

U.S. EPA (U.S. Environmental Protection Agency). (2009d). Integrated science assessment for particulate matter [EPA Report]. (EPA/600/R-08/139F). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=216546

U.S. EPA (U.S. Environmental Protection Agency). (2010c). Integrated science assessment for carbon monoxide [EPA Report]. (EPA/600/R-09/019F). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=218686

Vagaggini, B; Bartoli, MLE; Cianchetti, S; Costa, F; Bacci, E; Dente, FL; Di Franco, A; Malagrino, L; Paggiaro, P. (2010). Increase in markers of airway inflammation after ozone exposure can be observed also in stable treated asthmatics with minimal functional response to ozone. Respir Res 11: 5. http://dx.doi.org/10.1186/1465-9921-11-5

Vagaggini, B; Taccola, M; Clanchetti, S; Carnevali, S; Bartoli, ML; Bacci, E; Dente, FL; Di Franco, A; Giannini, D; Paggiaro, PL. (2002). Ozone exposure increases eosinophilic airway response induced by previous allergen challenge. Am J Respir Crit Care Med 166: 1073-1077.

Valacchi, G; Pecorelli, A; Mencarelli, M; Maioli, E; Davis, PA. (2009). Beta-carotene prevents ozone-induced proinflammatory markers in murine skin. Toxicol Ind Health 25: 241-247. http://dx.doi.org/10.1177/0748233709103030

Valacchi, G; Vasu, VT; Yokohama, W; Corbacho, AM; Phung, A; Lim, Y; Aung, HH; Cross, CE; Davis, PA. (2007). Lung vitamin E transport processes are affected by both age and environmental oxidants in mice. Toxicol Appl Pharmacol 222: 227-234. http://dx.doi.org/10.1016/j.taap.2007.04.010

Vancza, EM; Galdanes, K; Gunnison, A; Hatch, G; Gordon, T. (2009). Age, strain, and gender as factors for increased sensitivity of the mouse lung to inhaled ozone. Toxicol Sci 107: 535-543. http://dx.doi.org/10.1093/toxsci/kfn253

Villeneuve, PJ; Chen, L; Rowe, BH; Coates, F. (2007). Outdoor air pollution and emergency department visits for asthma among children and adults: A case-crossover study in northern Alberta, Canada. Environ Health Global Access Sci Source 6: 40. http://dx.doi.org/10.1186/1476-069X-6-40

Villeneuve, PJ; Chen, L; Stieb, D; Rowe, BH. (2006a). Associations between outdoor air pollution and emergency department visits for stroke in Edmonton, Canada. Eur J Epidemiol 21: 689-700. http://dx.doi.org/10.1007/s10654-006-9050-9

Voynow, JA; Fischer, BM; Zheng, S; Potts, EN; Grover, AR; Jaiswal, AK; Ghio, AJ; Foster, WM. (2009). NAD(P)H quinone oxidoreductase 1 is essential for ozone-induced oxidative stress in mice and humans. Am J Respir Cell Mol Biol 41: 107-113. http://dx.doi.org/10.1165/rcmb.2008-0381OC

Wagner, JG; Harkema, JR; Jiang, Q; Illek, B; Ames, BN; Peden, DB. (2009). Gamma-tocopherol attenuates ozone-induced exacerbation of allergic rhinosinusitis in rats. Toxicol Pathol 37: 481-491. http://dx.doi.org/10.1177/0192623309335630

Wagner, JG; Jiang, Q; Harkema, JR; Illek, B; Patel, DD; Ames, BN; Peden, DB. (2007). Ozone enhancement of lower airway allergic inflammation is prevented by gamma-tocopherol. Free Radic Biol Med 43: 1176-1188. http://dx.doi.org/10.1016/j.freeradbiomed.2007.07.013

Wattiez, R; Noel-Georis, I; Cruyt, C; Broeckaert, F; Bernard, A; Falmagne, P. (2003). Susceptibility to oxidative stress: proteomic analysis of bronchoalveolar lavage from ozone-sensitive and ozone-resistant strains of mice. Proteomics 3: 658-665. http://dx.doi.org/10.1002/pmic.200300417

Weinmann, GG; Weidenbach-Gerbase, M; Foster, WM; Zacur, H; Frank, R. (1995c). Evidence for ozone-induced small-airway dysfunction: Lack of menstrual-cycle and gender effects. Am J Respir Crit Care Med 152: 988-996.

Williams, AS; Leung, SY; Nath, P; Khorasani, NM; Bhavsar, P; Issa, R; Mitchell, JA; Adcock, IM; Chung, KF. (2007b). Role of TLR2, TLR4, and MyD88 in murine ozone-induced airway hyperresponsiveness and neutrophilia. J Appl Physiol 103: 1189-1195. http://dx.doi.org/10.1152/japplphysiol.00172.2007

Williams, AS; Nath, P; Leung, SY; Khorasani, N; Mckenzie, ANJ; Adcock, IM; Chung, KF. (2008b). Modulation of ozone-induced airway hyperresponsiveness and inflammation by interleukin-13. Eur Respir J 32: 571-578. http://dx.doi.org/10.1183/09031936.00121607

Wong, CM; Ou, CQ; Chan, KP; Chau, YK; Thach, TQ; Yang, L; Chung, RY; Thomas, GN; Peiris, JS; Wong, TW; Hedley, AJ; Lam, TH. (2008). The effects of air pollution on mortality in socially deprived urban areas in Hong Kong, China. Environ Health Perspect 116: 1189-1194. http://dx.doi.org/10.1289/ehp.10850

Wong, CM; Yang, L; Thach, TQ; Chau, PY; Chan, KP; Thomas, GN; Lam, TH; Wong, TW; Hedley, AJ; Peiris, JS. (2009). Modification by influenza on health effects of air pollution in Hong Kong. Environ Health Perspect 117: 248-253. http://dx.doi.org/10.1289/ehp.11605

Yoon, HK; Cho, HY; Kleeberger, SR. (2007). Protective role of matrix metalloproteinase-9 in ozone-induced airway inflammation. Environ Health Perspect 115: 1557-1563. http://dx.doi.org/10.1289/ehp.10289

Yu, M; Zheng, X; Witschi, H; Pinkerton, KE. (2002). The role of interleukin-6 in pulmonary inflammation and injury induced by exposure to environmental air pollutants. Toxicol Sci 68: 488-497.

Zanobetti, A; Schwartz, J. (2008a). Is there adaptation in the ozone mortality relationship: A multi-city case-crossover analysis. Environ Health 7: 22. http://dx.doi.org/10.1186/1476-069X-7-22

# 9 ENVIRONMENTAL EFFECTS: OZONE EFFECTS ON VEGETATION AND ECOSYSTEMS

## 9.1 Introduction

This chapter synthesizes and evaluates the relevant science to help form the scientific foundation for the review of a vegetation- and ecologically-based secondary NAAQS for $O_3$. The secondary NAAQS are based on welfare effects. The Clean Air Act (CAA) definition of welfare effects includes, but is not limited to, effects on soils, water, wildlife, vegetation, visibility, weather, and climate, as well as effects on materials, economic values, and personal comfort and well-being. The effects of $O_3$ as a greenhouse gas and its direct effects on climate are discussed in Chapter 10 of this document.

The intent of the ISA, according to the CAA, is to "accurately reflect the latest scientific knowledge expected from the presence of [a] pollutant in ambient air" (42 U.S.C.7408 and 42 U.S.C.7409. This chapter of the ISA includes scientific research from biogeochemistry, soil science, plant physiology, and ecology conducted at multiple levels of biological organization (e.g., molecular, organ, organism, population, community, ecosystem). Key information and judgments formerly found in the AQCDs regarding $O_3$ effects on vegetation and ecosystems are found in this chapter. This chapter of the $O_3$ ISA serves to update and revise Chapter 9 and AX9 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b).

Numerous studies of the effects of $O_3$ on vegetation and ecosystems were reviewed in the 2006 $O_3$ AQCD. That document concluded that the effects of ambient $O_3$ on vegetation and ecosystems appear to be widespread across the U.S., and experimental studies demonstrated plausible mechanisms for these effects. Ozone effect studies published from 2005 to July 2011 are reviewed in this document in the context of the previous $O_3$ AQCDs. From 2005 to 2011, some areas have had very little new research published and the reader is referred back to sections of the 2006 $O_3$ AQCD for a more comprehensive discussion of those subjects. This chapter is focused on studies of vegetation and ecosystems that occur in the U.S. and that provide information on endpoints or processes most relevant to the review of the secondary standard. Many studies have been published about vegetation and ecosystems outside of the U.S. and North America, largely in Europe and Asia. This document includes discussion of studies of vegetation and ecosystems outside of North America only if those studies contribute to the general understanding of $O_3$ effects across species and ecosystems. For example, studies outside North America are discussed that consider physiological and biochemical processes that contribute to the understanding of effects of $O_3$ across species. Also, ecosystem studies outside of North America that contribute to the understanding of $O_3$ effects on general ecosystem processes are discussed in the chapter.

Sections of this chapter first discuss exposure methods, followed by effects on vegetation and ecosystems at various levels of biological organization and ends with policy-relevant discussions of exposure indices and exposure-response. Figure 9-1 is a simplified illustrative diagram of the major endpoints $O_3$ may affect. First, Section 9.2 presents a brief overview of various methodologies that have been, and continue to be, central to quantifying $O_3$ effects on vegetation (see AX9.1 of the 2006 $O_3$ AQCD for more detailed discussion) (U.S. EPA, 2006b). Section 9.3 through Section 9.4 begin with a discussion of effects at the cellular and subcellular level followed by consideration of the $O_3$ effects on plant and ecosystem processes (Figure 9-1).
In Section 9.3 research is reviewed from the molecular to the biochemical and physiological levels in impacted plants, offering insight into the mode of action of $O_3$. Section 9.4 provides a review of the effects of $O_3$ exposure on major endpoints at the whole plant scale including growth, reproduction, visible foliar injury and leaf gas exchange in woody and herbaceous plants in the U.S., as well as a brief discussion of $O_3$ effects on agricultural crop yield and quality. Section 9.4 also integrates the effects of $O_3$ on individual plants in a discussion of available research for assessing the effect of $O_3$ on ecosystems, along with available studies that could inform assessments of various ecosystem services (see Section 9.4.1.2).
The development of indices of $O_3$ exposure and dose modeling is discussed in Section 9.5. Finally, exposure-response relationships for a number of tree species, native vegetation, and crop species and cultivars are reviewed, tabulated, and compared in Section 9.6 to form the basis for an assessment of the potential risk to vegetation from current ambient levels of $O_3$.

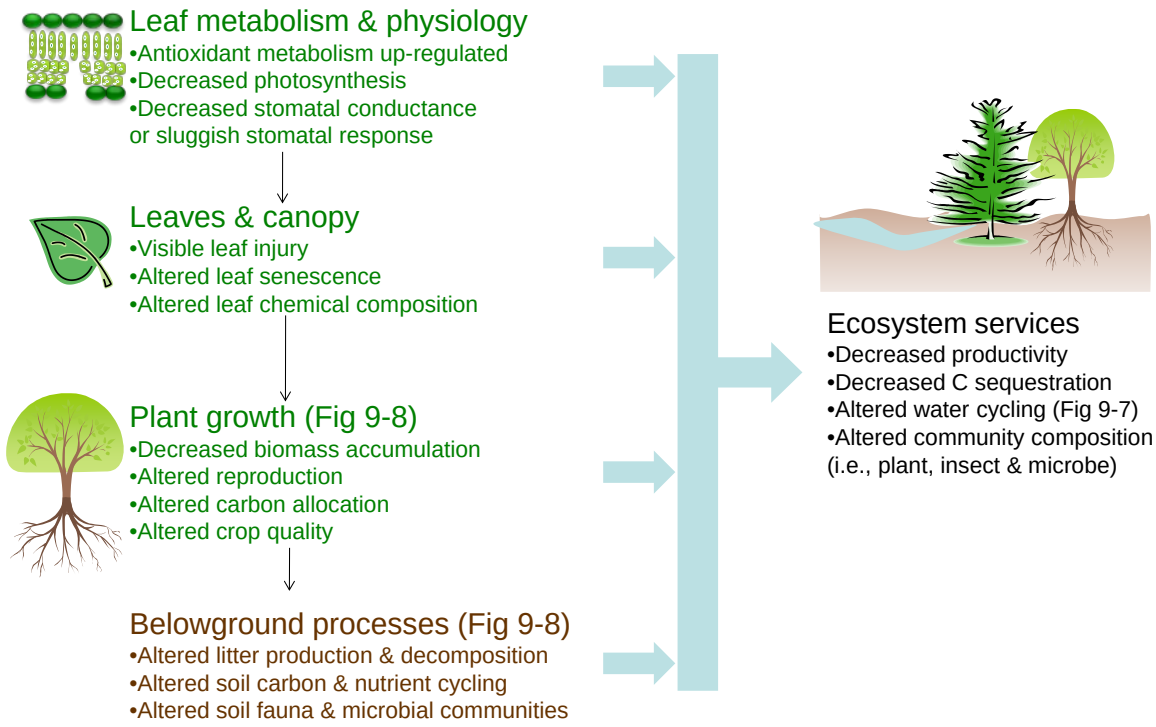



**Figure 9-1 An illustrative diagram of the major endpoints that $O_3$ may affect in plants and ecosystems.**

## 9.2 Experimental Exposure Methodologies

### 9.2.1 Introduction

A variety of methods for studying plant response to $O_3$ exposures have been developed over the last several decades. The majority of methodologies currently used have been discussed in detail in the 1996 $O_3$ AQCD (U.S. EPA, 1996a) and 2006 $O_3$ AQCD (U.S. EPA, 2006b). This section will serve as a short overview of the methodologies and the reader is referred to the previous $O_3$ AQCDs for more in-depth discussion.

### 9.2.2 "Indoor," Controlled Environment, and Greenhouse Chambers

The earliest experimental investigations of the effects of $O_3$ on plants utilized simple glass or plastic-covered chambers, often located within greenhouses, into which a flow of $O_3$-enriched air or oxygen could be passed to provide the exposure. The types, shapes, styles, materials of construction, and locations of these chambers have been numerous. Hogsett et al. (1987a) have summarized the construction and performance of more elaborate and better instrumented chambers since the 1960s, including those installed in greenhouses (with or without some control of temperature and light intensity).

One greenhouse chamber approach that continues to yield useful information on the relationships of $O_3$ uptake to both physiological and growth effects employs continuous stirred tank reactors (CSTRs) first described by Heck et al. (1978). Although originally developed to permit mass-balance studies of $O_3$ flux to plants, their use has more recently widened to include short-term physiological and growth studies of $O_3 \times CO_2$ interactions (Loats and Rebbeck, 1999; Reinert et al., 1997; Rao et al., 1995; Reinert and Ho, 1995; Heagle et al., 1994a), and validation of visible foliar injury on a variety of plant species (Kline et al., 2009; Orendovici et al., 2003). In many cases, supplementary lighting and temperature control of the surrounding structure have been used to control or modify the environmental conditions (Heagle et al., 1994a).

Many investigations have utilized commercially available controlled environment chambers and walk-in rooms adapted to permit the introduction of a flow of $O_3$ into the controlled air-volume. Such chambers continue to find use in genetic screening and in physiological and biochemical studies aimed primarily at improving the understanding of modes of action. For example, some of the studies of the $O_3$ responses of common plantain (*Plantago major*) populations have been conducted in controlled environment chambers (Whitfield et al., 1996; Reiling and Davison, 1994).

More recently, some researchers have been interested in attempting to investigate direct $O_3$ effects on reproductive processes, separate from the effects on vegetative processes (Black et al., 2010). For this purpose, controlled exposure systems have been employed to expose the reproductive structures of annual plants to gaseous pollutants independently of the vegetative component (Black et al., 2010; Stewart et al., 1996).

### 9.2.3 Field Chambers

In general, field chamber studies are dominated by the use of various versions of the open top chamber (OTC) design, first described by Heagle et al. (1973) and Mandl et al. (1973). The OTC method continues to be a widely used technique in the U.S. and Europe for exposing plants to varying levels of $O_3$. Most of the new information confirms earlier conclusions and provides additional support for OTC use in

assessing plant species and in developing exposure-response relationships. Chambers are generally ~3 meters in diameter with 2.5 meter-high walls. Hogsett et al. (1987b) described in detail many of the various modifications to the original OTC designs that appeared subsequently, e.g., the use of larger chambers for exposing small trees (Kats et al., 1985) or grapevines (Mandl et al., 1989), the addition of a conical baffle at the top to improve ventilation (Kats et al., 1976), a frustum at the top to reduce ambient air incursions, and a plastic rain-cap to exclude precipitation (Hogsett et al., 1985). All versions of OTCs included the discharge of air via ports in annular ducting or interiorly perforated double-layered walls at the base of the chambers to provide turbulent mixing and the upward mass flow of air.

Chambered systems, including OTCs, have several advantages. For instance, they can provide a range of treatment levels including charcoal-filtered (CF), clean-air control, and several above ambient concentrations for $O_3$ experiments. Depending on experimental intent, a replicated, clean-air control treatment is an essential component in many experimental designs. The OTC can provide a consistent, definable exposure because of the constant wind speed and delivery systems. Statistically robust concentration-response (C-R) functions can be developed using such systems for evaluating the implications of various alternative air quality scenarios on vegetation response. Nonetheless, there are several characteristics of the OTC design and operation that can lead to exposures that might differ from those experienced by plants in the field. First, the OTC plants are subjected to constant air flow turbulence, which, by lowering the boundary layer resistance to diffusion, may result in increased uptake. This may lead to an overestimation of effects relative to areas with less turbulence (Krupa et al., 1995; Legge et al., 1995). However, other research has found that OTC's may slightly change vapor pressure deficit (VPD) in a way that may decrease the uptake of $O_3$ into leaves (Piikki et al., 2008a). As with all methods that expose vegetation to modified $O_3$ concentrations in chambers, OTCs create internal environments that differ from ambient air. This so-called "chamber effect" refers to the modification of microclimatic variables, including reduced and uneven light intensity, uneven rainfall, constant wind speed, reduced dew formation, and increased air temperatures (Fuhrer, 1994; Manning and Krupa, 1992). However, in at least one case where canopy resistance was quantified in OTCs and in the field, it was determined that gaseous pollutant exposure to crops in OTCs was similar to that which would have occurred at the same concentration in the field (Unsworth et al., 1984a, b). Because of the standardized methodology and protocols used in National Crop Loss Assessment Network (NCLAN) and other programs, the database can be assumed to be internally consistent.

While it is clear that OTCs can alter some aspects of the microenvironment and plant growth, it is important to establish whether or not these differences affect the relative response of a plant to $O_3$. As noted in the 1996 $O_3$ AQCD (U.S. EPA, 1996a), evidence from a number of comparative studies of OTCs and other exposure systems suggested that responses were essentially the same regardless of exposure system used and chamber effects did not significantly affect response. In studies that included exposure to ambient concentrations of $O_3$ in both OTCs, and open-air, chamberless control plots, responses in the OTCs were the same as in open-air plots.

Examples include studies of tolerant and sensitive white clover clones (*Trifolium repens*) to ambient $O_3$ in greenhouse, open top, and ambient plots (Heagle et al., 1996), Black Cherry (*Prunus serotina*) (Neufeld et al., 1995), and three species of conifers (Neufeld et al., 2000). Experimental comparisons between exposure methodologies are reviewed in Section 9.2.6.

Another type of field chamber called a "terracosm" has been developed and used in recent studies (Lee et al., 2009a). Concern over the need to establish realistic plant-litter-soil relationships as a prerequisite to studies of the effects of $O_3$ and $CO_2$ enrichment on ponderosa pine (*Pinus ponderosa*) seedlings led Tingey et al. (1996) to develop closed, partially environmentally controlled, sun-lit chambers ("terracosms") incorporating lysimeters (1 meter deep) containing forest soil in which the appropriate horizon structure was retained.

Other researchers have recently published studies using another type of out-door chamber called recirculating Outdoor Plant Environment Chambers (OPECs) (Flowers et al., 2007). These closed chambers are approximately 2.44 meters ×1.52 meters with a growth volume of approximately 3.7 $m^3$ in each chamber. These chambers admit 90% of full sunlight and control temperature, humidity and vapor pressure (Fiscus et al., 1999).

### 9.2.4 Plume and FACE-Type Systems

Plume systems are chamberless exposure facilities in which the atmosphere surrounding plants in the field is modified by the injection of pollutant gas into the air above or around them from multiple orifices spaced to permit diffusion and turbulence, so as to establish relatively homogeneous conditions as the individual plumes disperse and mix with the ambient air. They can only be used to increase the $O_3$ levels in the ambient air.

The most common plume system used in the U.S. is a modification of the free-air carbon dioxide/ozone enrichment (FACE) system (Hendrey et al., 1999; Hendrey and Kimball, 1994). Although originally designed to provide chamberless field facilities for studying the $CO_2$ effects of climate change, FACE systems have been adapted to include the dispensing of $O_3$ (Karnosky et al., 1999). This method has been employed in Illinois (SoyFACE) to study soybeans (Morgan et al., 2004; Rogers et al., 2004) and in Wisconsin (Aspen FACE) to study trembling aspen (*Populus tremuloides*), birch (*Betula papyrifera*) and maple (*Acer saccharum*) (Karnosky et al., 1999). Volk et al. (2003) described a similar system for exposing grasslands that uses 7-m diameter plots. Another similar FACE system has been used in Finland (Saviranta et al., 2010; Oksanen, 2003).

The FACE systems in the U.S. discharge the pollutant gas ($O_3$ and/or $CO_2$) through orifices spaced along an annular ring (or torus) or at different heights on a ring of vertical pipes. Computer-controlled feedback from the monitoring of gas concentration regulates the feed rate of enriched air to the dispersion pipes. Feedback

of wind speed and directional information ensures that the discharges only occur upwind of the treatment plots, and that discharge is restricted or closed down during periods of low wind speed or calm conditions. The diameter of the arrays and their height (25-30 meters) in some FACE systems requires large throughputs of enriched air per plot, particularly in forest tree systems. The cost of the throughputs tends to limit the number of enrichment treatments, although Hendrey et al. (1999) argued that the cost on an enriched volume basis is comparable to that of chamber systems.

A different FACE-type facility has been developed for the Kranzberg Ozone Fumigation Experiment (KROFEX) in Germany beginning in 2000 (Nunn et al., 2002; Werner and Fabian, 2002). The experiment aims to study the effects of $O_3$ on mature stands of beech (*Fagus sylvatica*) and spruce (*Picea abies*) trees in a system that functions independently of wind direction. The enrichment of a large volume of the ambient air immediately above the canopy takes place via orifices in vertical tubes suspended from a horizontal grid supported above the canopy.

Although plume systems make virtually none of the modifications to the physical environment that are inevitable with chambers, their successful use depends on selecting the appropriate numbers, sizes, and orientations of the discharge orifices to avoid "hot-spots" resulting from the direct impingement of jets of pollutant-enriched air on plant foliage (Werner and Fabian, 2002). Because mixing is unassisted and completely dependent on wind turbulence and diffusion, local gradients are inevitable especially in large-scale systems. FACE systems have provisions for shutting down under low wind speed or calm conditions and for an experimental area that is usually defined within a generous border in order to strive for homogeneity of the exposure concentrations within the treatment area. They are also dependent upon continuous computer-controlled feedback of the $O_3$ concentrations in the mixed treated air and of the meteorological conditions. Plume and FACE systems also are unable to reduce $O_3$ levels below ambient in areas where $O_3$ concentrations are phytotoxic.

### 9.2.5 Ambient Gradients

Ambient $O_3$ gradients that occur in the U.S. hold potential for the examination of plant responses over multiple levels of exposure. However, few such gradients can be found that meet the rigorous statistical requirements for comparable site characteristics such as soil type, temperature, rainfall, radiation, and aspect (Manning and Krupa, 1992); although with small plants, soil variability can be avoided by the use of plants in large pots. The use of soil monoliths transported to various locations along natural $O_3$ gradients is another possible approach to overcome differences in soils; however, this approach is also limited to small plants.

Studies in the 1970s used the natural gradients occurring in southern California to assess yield losses of alfalfa and tomato (Oshima et al., 1977; Oshima et al., 1976). A transect study of the impact of $O_3$ on the growth of white clover and barley in the U.K. was confounded by differences in the concurrent gradients of $SO_2$ and $NO_2$

pollution (Ashmore et al., 1988). Studies of forest tree species in national parks in the eastern U.S. (Winner et al., 1989) revealed increasing gradients of $O_3$ and visible foliar injury with increased elevation.

Several studies have used the San Bernardino Mountains Gradient Study in southern California to study the effects of $O_3$ and N deposition on forests dominated by ponderosa and Jeffrey pine (Jones and Paine, 2006; Arbaugh et al., 2003; Grulke, 1999; U.S. EPA, 1977). However, it is difficult to separate the effects of N and $O_3$ in some instances in these studies (Arbaugh et al., 2003). An $O_3$ gradient in Wisconsin has been used to study foliar injury in a series of trembling aspen clones (*Populus tremuloides*) differing in $O_3$ sensitivity (Maňkovská et al., 2005; Karnosky et al., 1999). Also in the Midwest, an east-west $O_3$ gradient around southern Lake Michigan was used to look at growth and visible foliar injury in (*P. serotina*) and common milkweed (*Asclepias syriaca*) (Bennett et al., 2006).

More recently, studies have been published that have used natural gradients to study a variety of endpoints and species. For example, Gregg et al. (2003) studied cottonwood (*Populus deltoides*) saplings grown in an urban to rural gradient of $O_3$ by using seven locations in the New York City area. The secondary nature of the reactions of $O_3$ formation and $NO_X$ titration reactions within the city center resulted in significantly higher cumulative $O_3$ exposures in more rural sites. Potential modifying factors such as soil composition, moisture, or temperature were either controlled or accounted for in analysis. As shown in Section 9.6.3.3, the response of this species to $O_3$ exposure was much stronger than most species. The natural gradient exposures were reproduced in parallel using OTCs, and yielded similar results. Also, the U.S. Forest Service - Forest Inventory and Analysis (FIA) program uses large-scale $O_3$ exposure patterns across the continental U.S. to study occurrences of foliar injury due to $O_3$ exposure (Smith et al., 2003) (Section 9.4.2). Finally, McLaughlin et al. (2007a; 2007b) used spatial and temporal $O_3$ gradients to study forest growth and water use in the southern Appalachians. These studies found varying $O_3$ exposures between years and between sites.

### 9.2.6 Comparative Studies

All experimental approaches used to expose plants to $O_3$ have strengths and weaknesses. One potential weakness of laboratory, greenhouse, or field chamber studies is the potential effect of the chamber on micrometeorology. In contrast, plume, FACE and gradient systems are limited by the very small number of possible exposure levels (almost always no more than two), small replication and the inability to reduce $O_3$ levels below ambient. In general, experiments that aim at characterizing the effect of a single variable, e.g., exposure to $O_3$, must not only manipulate the levels of that variable, but also control potentially interacting variables and confounders, or else account for them. However, while increasing control of environmental variables makes it easier to discern the effect of the variable of interest, it must be balanced with the ability to extend conclusions to natural,

non-experimental settings. More naturalistic exposure systems, on the other hand, let interacting factors vary freely, resulting in greater unexplainable variability. The various exposure methodologies used with $O_3$ vary in the balance each strikes between control of environmental inputs, closeness to the natural environment, noisiness of the response data, and ability to make general inferences.

Studies have examined the comparability of results obtained though the various exposure methodologies. As noted in the 1996 $O_3$ AQCD, evidence from the comparative studies of OTCs and from closed chamber and $O_3$-exclusion exposure systems on the growth of alfalfa (*Medicago sativa*) by Olszyk et al. (1986) suggested that, since significant differences were found for fewer than 10% of the growth parameters measured, the responses were, in general, essentially the same regardless of exposure system used, and chamber effects did not significantly affect response. Heagle et al. (1988) concluded: "Although chamber effects on yield are common, there are no results showing that this will result in a changed yield response to $O_3$." A study of the effects of an enclosure examined the responses of tolerant and sensitive white clover clones (*Trifolium repens*) to ambient $O_3$ in a greenhouse, open-top chamber, and ambient (no chamber) plots (Heagle et al., 1996). For individual harvests, greenhouse $O_3$ exposure reduced the forage weight of the sensitive clone 7 to 23% more than in OTCs. However, the response in OTCs was the same as in ambient plots. Several studies have shown very similar response of yield to $O_3$ for plants grown in pots or in the ground, suggesting that even such a significant change in environment does not alter the proportional response to $O_3$, providing that the plants are well watered (Heagle et al., 1983; Heagle, 1979).

A few recent studies have compared results of $O_3$ experiments between OTCs, FACE experiments, and gradient studies. For example, a series of studies undertaken at Aspen FACE (Isebrands et al., 2001; Isebrands et al., 2000) showed that $O_3$ symptom expression was generally similar in OTCs, FACE, and ambient $O_3$ gradient sites, and supported the previously observed variation among trembling aspen clones using OTCs (Maňkovská et al., 2005; Karnosky et al., 1999). In the SoyFACE experiment in Illinois, soybean (Pioneer 93B15 cultivar) yield loss data from a two-year study was published (Morgan et al., 2006). This cultivar is a recent selection and, like most modern cultivars, has been selected under an already high current $O_3$ exposure. It was found to have average sensitivity to $O_3$ compared to 22 other cultivars tested at SoyFACE. In this experiment, ambient hourly $O_3$ concentrations were increased by approximately 20% and measured yields were decreased by 15% in 2002 as a result of the increased $O_3$ exposure (Morgan et al., 2006). To compare these results to chamber studies, Morgan et al. (2006) calculated the expected yield loss from a linear relationship constructed from chamber data using seven-hour seasonal averages (Ashmore, 2002). They calculated an 8% expected yield loss from the 2002 $O_3$ exposure using that linear relationship. As reported in Section 9.2.5, Gregg et al. (2006, 2003) found similar $O_3$ effects on cottonwood sapling biomass growth along an ambient $O_3$ gradient in the New York City area and a parallel OTC study.

Finally, EPA conducted comparisons of exposure-response model predictions based on OTC studies, and more recent FACE observations. These comparisons include

yield of annual crops, and biomass growth of trees. They are presented in Section 9.6.3 of this document.

## 9.3 Mechanisms Governing Vegetation Response to Ozone

### 9.3.1 Introduction

This section focuses on the effects of $O_3$ stress on plants and their responses to that stress on the molecular, biochemical and physiological levels. First, the pathway of $O_3$ uptake into the leaf and the initial chemical reactions occurring in the substomatal cavity and apoplast will be described (Section 9.3.2); additionally, direct effects of $O_3$ on the stomatal apparatus will be discussed. Once $O_3$ has entered the substomatal cavity and apoplast, the cell initiates rapid changes in signaling pathways and gene expression that have been measured in $O_3$-treated plants. The next section focuses on changes in gene and protein expression measured in plants exposed to $O_3$, with particular emphasis on results from transcriptome (all RNA molecules produced in a cell) and proteome (all proteins produced in a cell) analyses (Section 9.3.3.2). Subsequently, the role of phytohormones such as salicylic acid (SA), ethylene (ET), jasmonic acid (JA), and abscisic acid (ABA) and their interactions in both signal transduction processes and in determining plant response to $O_3$ is discussed in Section 9.3.3.3. After $O_3$ uptake, some plants can respond to the oxidative stress with detoxification to minimize damage. These mechanisms of detoxification, with particular emphasis on antioxidant enzymes and metabolites, are reviewed in Section 9.3.4. The next section focuses on changes in primary and secondary metabolism in plants exposed to $O_3$, looking at photosynthesis, respiration and several secondary metabolites, some of which may also act as antioxidants and protect the plant from oxidative stress (Section 9.3.5). For many of these topics, information from the 2006 $O_3$ AQCD (U.S. EPA, 2006b) has been summarized, as this information is still valid and supported by more recent findings. For other topics, such as genomics and proteomics, which have arisen due to the availability of new technologies, the information is based solely on new publications with no reference to the 2006 $O_3$ AQCD.

As Section 9.3 focuses on mechanisms underlying effects of $O_3$ on plants and their response to it, the conditions that are used to study these mechanisms do not always reflect conditions that a plant may be exposed to in an agricultural setting or natural ecosystem. The goal of many of these studies is to generate an $O_3$ effect in a relatively short period of time and not always to simulate ambient $O_3$ exposures. Therefore, plants are often exposed to unrealistically high $O_3$ concentrations for several hours or days (acute exposure), and only in some cases to ambient or slightly elevated $O_3$ concentrations for longer time periods (chronic exposure). Additionally, the plant species utilized in these studies are often not agriculturally important or commonly found as part of natural ecosystems. Model organisms such as *Arabidopsis thaliana* are used frequently as they are easy to work with, and mutants

or transgenic plants are easy to develop or have already been developed. Furthermore, the Arabidopsis genome has been sequenced, and much is known about the molecular basis of many biochemical and cellular processes.

Many of the studies described in this section focus on changes in the expression of genes in $O_3$-treated plants. Some very recent studies utilizing proteomics techniques have evaluated changes in protein expression for large numbers of proteins in $O_3$ treated plants, and the findings from these studies support the previous results regarding changes in gene expression studies as a result of $O_3$ exposure. The next step in the process is to determine the implications of the measured changes occurring at the cellular level to whole plants and ecosystems, which is an important topic of study which has not been widely addressed.

The most noteworthy new body of research since the 2006 $O_3$ AQCD is on the understanding of molecular mechanisms underlying how plants are affected by $O_3$; many of the recent studies reviewed here focus on changes in gene expression in plants exposed to elevated $O_3$. The findings summarized in the 2006 $O_3$ AQCD included decreases in transcript levels of photosynthesis associated genes, and increases in transcript levels of genes encoding for pathogenesis-related proteins, enzymes needed for ethylene synthesis, antioxidant enzymes and defense genes such as phenylalanine ammonia lyase in plants exposed to $O_3$. These findings have been supported by the new studies, and the advent of new technologies has allowed for a more comprehensive understanding of the mechanisms governing how plants are affected by $O_3$.

In summary, these new studies have increased knowledge of the molecular, biochemical and cellular mechanisms occurring in plants in response to $O_3$ by often using artificial exposure conditions and model organisms. This information adds to the understanding of the basic biology of how plants are affected by oxidative stress in the absence of any other potential stressors. The results of these studies provide important insights, even though they may not always directly translate into effects observed in other plants under more realistic exposure conditions.

### 9.3.2 Ozone Uptake into the Leaf

Appendix AX9.2.3 of the 2006 $O_3$ AQCD clearly described the process by which $O_3$ enters plant leaves through open stomata (U.S. EPA, 2006b). This information continues to be valid and is only summarized here.

Ozone moves from the atmosphere above the canopy boundary layer into the canopy primarily by turbulent air flow. Canopy conductance is controlled by the complexity of the canopy architecture. Within the canopy, $O_3$ is adsorbed onto surfaces as well as being absorbed into the leaves. Absorption into leaves is controlled by leaf boundary layer and stomatal conductance, which together determine leaf conductance (Figure 9-2, Panel A). Other factors that may also limit uptake include the size of the stomatal aperture and the reactions of $O_3$ with biogenically-emitted

hydrocarbons (U.S. EPA, 2006b; Kurpius and Goldstein, 2003). Stomata provide the principal pathway for $O_3$ to enter and affect plants (Massman and Grantz, 1995; Fuentes et al., 1992; Reich, 1987; Leuning et al., 1979). Ozone moves into the leaf interior by diffusing through open stomata, and environmental conditions which promote high rates of gas exchange will favor the uptake of the pollutant by the leaf (Figure 9-2, Panel B). Once inside the substomatal cavity, $O_3$ is thought to rapidly react with the aqueous apoplast to form breakdown products known as reactive oxygen species (ROS), such as hydrogen peroxide ($H_2O_2$), superoxide ($O_2^-$), hydroxyl radicals ($HO^{\cdot}$) and peroxy radicals ($HO_2^{\bullet}$) (Figure 9-3). Hydrogen peroxide is not only a toxic breakdown product of $O_3$, but has been shown to function as a signaling molecule, which is activated in response to both biotic and abiotic stressors. The role of $H_2O_2$ in signaling was described in detail in the 2006 $O_3$ AQCD. Additional organic molecules present in the apoplast or cell wall, such as those containing double bonds or sulfhydryls that are sensitive to oxidation, could also be converted to oxygenated molecules after interacting with $O_3$ (Figure 9-4). These reactions are not only pH dependent, but are also influenced by the presence of other molecules in the apoplast (U.S. EPA, 2006b). The 2006 $O_3$ AQCD provided a comprehensive summary of these possible interactions of $O_3$ with other biomolecules (U.S. EPA, 2006b). It is in the apoplast that initial detoxification reactions by antioxidant metabolites and enzymes take place, and these initial reactions are critical to reduce concentrations of the oxidative breakdown products of $O_3$; these reactions are described in more detail in Section 9.3.4 of this document.

#### 9.3.2.1 Changes in Stomatal Function

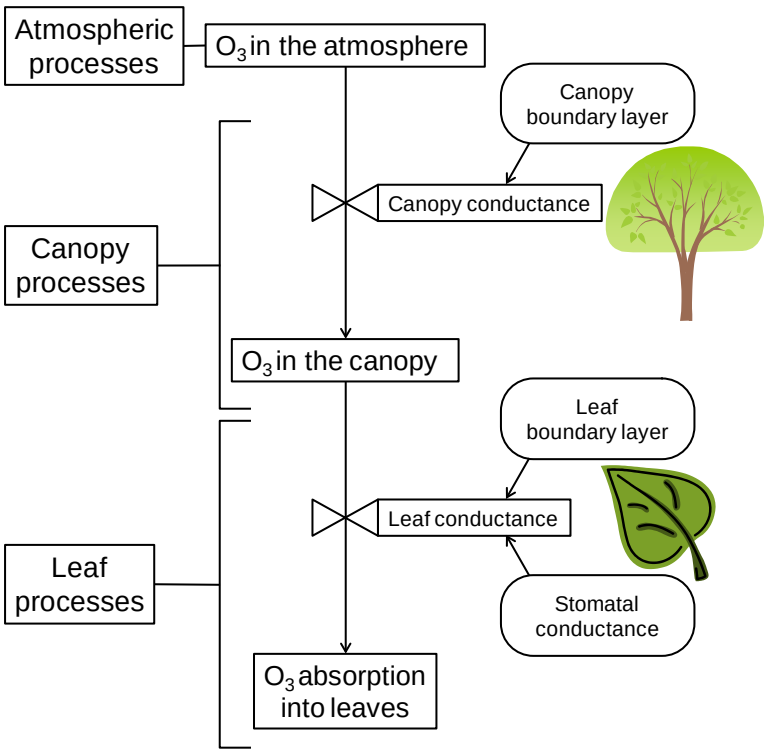
Ozone-induced changes in stomatal conductance have been reviewed in detail in previous $O_3$ AQCDs. The findings summarized in these documents demonstrate that stomatal conductance is often reduced in plants exposed to $O_3$, resulting either from a direct impact of $O_3$ on the stomatal complex which causes closure, or as a response to increasing $CO_2$ concentrations in the substomatal cavity as carbon fixation is reduced. Although the nature of these effects depends upon many different factors, including the plant species, concentration and duration of the $O_3$ exposure, and prevailing meteorological conditions, stomatal conductance is often negatively affected by plant exposure to $O_3$ (Wittig et al., 2007). Decreases in conductance have been shown to result from direct as well as indirect effects on stomata (Wittig et al., 2007). However, some recent studies have reported increased conductance in response to $O_3$ exposure, suggesting partial stomatal dysfunction (Paoletti and Grulke, 2010).

Results from the use of Arabidopsis mutants and new technologies, which allow for analysis of guard cell function in whole plants rather than in isolated guard cells or epidermal peels, suggest that $O_3$ may also have a direct impact on stomatal guard cells, leading to alterations in stomatal conductance. The use of a new simultaneous $O_3$ exposure/gas exchange device has demonstrated that exposure of Arabidopsis ecotypes Col-0 and Ler to 150 ppb $O_3$ resulted in a 60-70% decline in stomatal

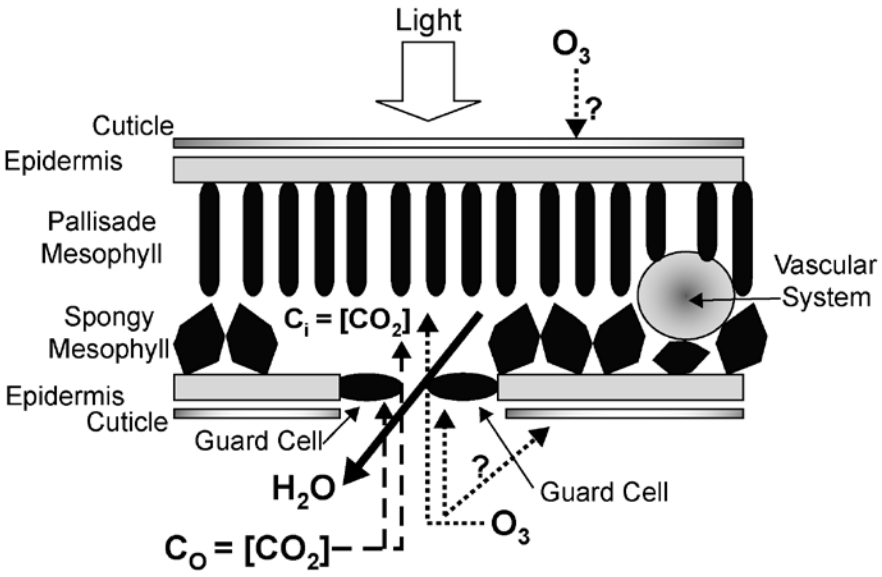
conductance within 9-12 minutes of beginning the exposure. Twenty to thirty minutes later, stomatal conductance had returned to its initial value, even with continuing exposure to $O_3$, indicating a rapid direct effect of $O_3$ on stomatal function (Kollist et al., 2007). This transient decrease in stomatal conductance was not observed in the abscisic acid insensitive (ABI2) Arabidopsis mutant. As the ABI2 protein is thought to regulate the signal transduction process involved in stomatal response downstream of ROS production, the authors suggest that the transient decrease in stomatal conductance in the Col-0 and Ler ecotypes results from the biological action of ROS in transducing signals, rather than direct physical damage to guard cells by ROS (Kollist et al., 2007). This rapid transient decrease in stomatal conductance was also not observed when exposing the Arabidopsis mutant slac1 (slow anion channel-associated 1) to 200 ppb $O_3$ (Vahisalu et al., 2008). The SLAC1 protein was shown to be essential for guard cell slow anion channel functioning and for stomatal closure in response to $O_3$. Based on additional studies using a variety of Arabidopsis mutants impaired in various aspects of stomatal function, Vahisalu et al. (2008) suggest that the presence of ROS in the guard cell apoplast (formed either by $O_3$ breakdown or through ROS production from NADPH oxidase activity) leads to the activation of a signaling pathway in the guard cells, which includes SLAC1, and results in stomatal closure.

A review by McAinsh et al. (2002) discusses the role of calcium as a part of the signal transduction pathway involved in regulating stomatal responses to pollutant stress. A number of studies in this review provide some evidence that exposure to $O_3$ increases the cytosolic free calcium concentration ($[Ca^{2+}]$cyt) in guard cells, which may result in an inhibition of the plasma membrane inward-rectifying $K^+$ channels in guard cells, which allow for the $K^+$ uptake needed for stomatal opening (McAinsh et al., 2002; Torsethaugen et al., 1999). This would compromise the ability of the stomata to respond to various stimuli, including light, $CO_2$ concentration and drought. Pei et al. (2000) reported that the presence of $H_2O_2$ activated $Ca^{2+}$ -permeable channels, which mediate increases in $[Ca^{2+}]$cyt in guard cell plasma membranes of Arabidopsis. They also determined that abscisic acid (ABA) induced $H_2O_2$ production in guard cells, leading to ABA-induced stomatal closure via activation of the membrane $Ca^{2+}$ channels. Therefore, it is possible that $H_2O_2$, a byproduct of $O_3$ breakdown in the apoplast, could disrupt the $Ca^{2+}$-ABA signaling pathway that is involved in regulating stomatal responses (McAinsh et al., 2002). The studies described here provide some evidence to suggest that $O_3$ and its breakdown products can directly affect stomatal functioning by impacting the signal transduction pathways which regulate guard cells. Stomatal sluggishness has been described as a delay in stomatal response to changing environmental conditions in sensitive species exposed to higher concentrations and/or longer-term $O_3$ exposures (Paoletti and Grulke, 2010, 2005; McAinsh et al., 2002). It is possible that the signaling pathways described above could be involved in mediating this stomatal sluggishness in some plant species under certain $O_3$ exposure conditions (Paoletti and Grulke, 2005; McAinsh et al., 2002).

**A.**

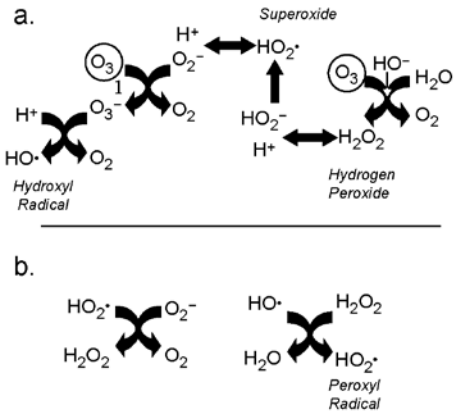



**B.**



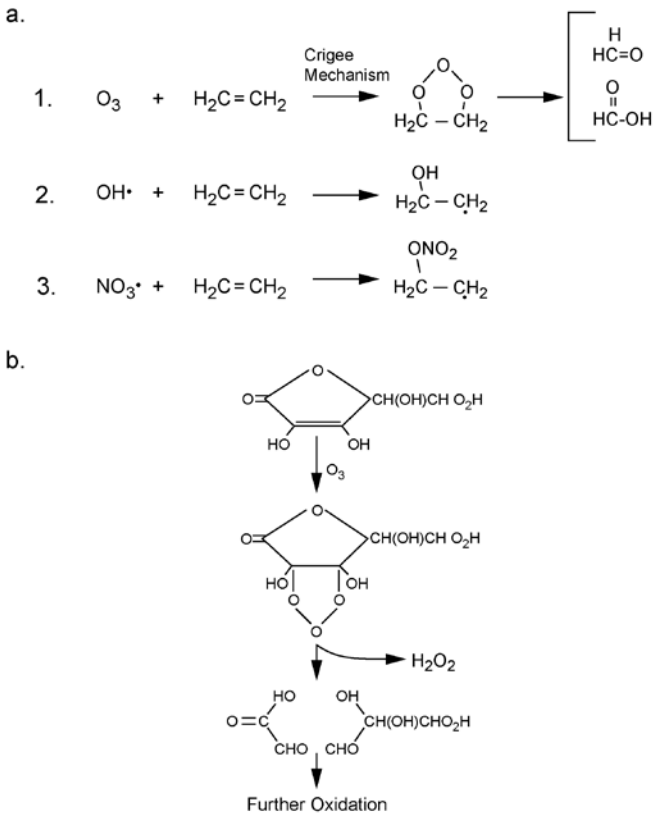

Note: While details among species vary, the general overview remains the same. Light that drives photosynthesis generally falls upon the upper (adaxial) leaf surface. Carbon dioxide (CO) and $O_3$ enter through the stomata, while water vapor exits through the stomata (transpiration). Stomata are usually on the lower (abaxial) leaf surface, but may occur on the upper leaf surface in some species.

**Figure 9-2 Ozone uptake from the atmosphere (A), and The anatomy of a dicot leaf (B).**




Note: (a) Ozone reacts at the double bonds to form carbonyl groups. (b) Under certain circumstances, peroxides are generated.

## Figure 9-3 Possible reactions of $O_3$ within water.




Note: (a) The typical Crigee mechanism is shown in which several reaction paths from the initial product are shown. (b) Typical reaction of ascorbic acid with $O_3$.

Source: Adapted from Mudd et al. (1996).

## Figure 9-4 The Crigee mechanism of $O_3$ attack of a double bond.

## 9.3.3 Cellular to Systemic Responses

### 9.3.3.1 Ozone Signal Transduction

New technologies allowing for large-scale analysis of oxidative stress-induced changes in gene expression have facilitated the study of signal transduction processes associated with plant response to $O_3$ exposure. Many of these studies have been conducted using Arabidopsis or tobacco plants, for which a variety of mutants are available and/or which can be easily genetically modified to generate either loss-of-function or over-expressing genotypes. Several comprehensive review articles provide an overview of what is known of $O_3$-induced signal transduction processes and how they may help to explain differential sensitivity of plants to the pollutant (Ludwikow and Sadowski, 2008; Baier et al., 2005; Kangasjarvi et al., 2005). Additionally, analysis of several studies of transcriptome changes has also allowed for the compilation of these data to determine an initial time-course for $O_3$-induced activation of various signaling compounds (Kangasjarvi et al., 2005).

Some of the earliest events that occur in plants exposed to $O_3$ have been described in the guard cells of stomata. Reactive oxygen species were observed in the chloroplasts of guard cells in the $O_3$ tolerant Col-0 *Arabidopsis thaliana* ecotype plants within 5 minutes of plant exposure to 350 ppb $O_3$ (Joo et al., 2005). Reactive oxygen species from the breakdown of $O_3$ in the apoplast are believed to activate GTPases (G-proteins), which, in turn, activate several intracellular sources of ROS, including ROS derived from the chloroplasts. G-proteins are also believed to play a role in activating membrane-bound NADPH oxidases to produce ROS and, as a result, propagate the oxidative burst to neighboring cells (Joo et al., 2005). Therefore, G-proteins are recognized as important molecules involved in plant responses to $O_3$ and may play a role in the initiation of signal transduction mechanisms resulting from the presence of ROS in the apoplast (Kangasjarvi et al., 2005; Booker et al., 2004a).

A change in the redox state of the plant may contribute to initiating the process of signaling of oxidative stress in plants. Disulfide-thiol conversions in proteins and the redox state of the glutathione pool may be important components of redox and signal transduction (Foyer and Noctor, 2005a, b).

Calcium ($Ca^{2+}$) has also been implicated in the transduction of signals to the nucleus in response to oxidative stress. The influx of $Ca^{2+}$ from the apoplast into the cell occurs early during plant exposure to $O_3$, and it is thought to play a role in regulating the activity of protein kinases, which are discussed below (Baier et al., 2005; Hamel et al., 2005). Calcium channel blockers inhibited $O_3$-induced activation of protein kinases in tobacco suspension cells exposed to 500 ppb $O_3$ for 10 minutes, indicating that the opening of $Ca^{2+}$ channels is an important upstream signaling event or that the (as yet unknown) upstream process has a requirement for $Ca^{2+}$ (Samuel et al., 2000).

Further transmission of information regarding the presence of ROS to the nucleus involves mitogen-activated protein kinases (MAPKs), which phosphorylate proteins

and activate various cellular responses (Hamel et al., 2005). Mitogen-activated protein kinases are induced in several different plant species in response to $O_3$ exposure, including tobacco (Samuel et al., 2005), Arabidopsis (Ludwikow et al., 2004), the shrub *Phillyrea latifolia* (Paolacci et al., 2007) and poplar (Hamel et al., 2005). Disruption of these signal transduction pathways by over-expressing or suppressing MAPK activity in different Arabidopsis and tobacco lines resulted in increased plant sensitivity to $O_3$ (Miles et al., 2005; Samuel and Ellis, 2002). Additionally, greater $O_3$ tolerance of several Arabidopsis ecotypes was correlated with greater upregulation of MAPK signaling pathways upon $O_3$ exposure than in more sensitive Arabidopsis ecotypes (Li et al., 2006b; Mahalingam et al., 2006; Overmyer et al., 2005), indicating that determination of plant sensitivity and plant response to $O_3$ may, in part, be determined not only by whether these pathways are turned on, but also by the magnitude of the signals moving through these communication channels.

In conclusion, experimental evidence suggests that there are likely several different mechanisms by which signaling as part of plant response to $O_3$ or its breakdown products is initiated. These mechanisms may vary by species or developmental stage of the plant, or may co-exist and be activated by different exposure conditions. Calcium and protein kinases are likely involved in relaying information to the nucleus and other cellular compartments as a first step in determining whether and how the plant will respond to the stress.

#### 9.3.3.2 Gene and Protein Expression Changes in Response to Ozone

The advent of DNA microarray technology has allowed for the study of gene expression in cells on a large scale. Rather than assessing changes in gene expression of individual genes, DNA microarrays facilitate the evaluation of entire transcriptomes, providing a comprehensive picture of simultaneous alterations in gene expression. In addition, these studies have provided more insight into the complex interactions between molecules, how those interactions lead to the communication of information in the cell (or between neighboring cells), and which role these interactions play in determining tolerance or sensitivity and how a plant may respond to stresses such as $O_3$ (Ludwikow and Sadowski, 2008). Transcriptome analysis of $O_3$-treated plants has been performed in several species, including *Arabidopsis thaliana* (Li et al., 2006b; Tosti et al., 2006; Heidenreich et al., 2005; Mahalingam et al., 2005; Tamaoki et al., 2003), pepper (*Capsicum annuum)* (Lee and Yun, 2006), clover (*Medicago truncatula*) (Puckette et al., 2008), *Phillyrea latifolia* (Paolacci et al., 2007), poplar (Street et al., 2011), and European beech (*Fagus sylvatica*) (Olbrich et al., 2010; Olbrich et al., 2009; Olbrich et al., 2005). In some cases, researchers compared transcriptomes of two or more cultivars, ecotypes or mutants that differed in their sensitivity to $O_3$ (Puckette et al., 2008; Rizzo et al., 2007; Lee and Yun, 2006; Li et al., 2006b; Tamaoki et al., 2003). Species, $O_3$ exposure conditions (concentration, duration of exposure) and sampling times varied

considerably in these studies. However, functional classification of the genes that were either upregulated or downregulated by plant exposure to $O_3$ exhibited common trends. Genes involved in plant defense, signaling and those associated with the synthesis of plant hormones and secondary metabolism were generally upregulated, while those related to photosynthesis and general metabolism were typically downregulated in $O_3$-treated plants (Puckette et al., 2008; Lee and Yun, 2006; Li et al., 2006b; Tosti et al., 2006; Olbrich et al., 2005; Tamaoki et al., 2003).

Analysis of the transcriptome has been used to evaluate differences in gene expression between sensitive and tolerant plants in response to $O_3$ exposure. In pepper, 67% of the 180 genes studied that were affected by $O_3$ were differentially regulated in the sensitive and tolerant cultivars. At both 0 hours and 48 hours after a 3-day exposure at 150 ppb, $O_3$ responsive genes were either upregulated or downregulated more markedly in the sensitive than in the tolerant cultivar (Lee and Yun, 2006). Transcriptome analysis also revealed differences in timing and magnitude of changes in gene expression between sensitive and tolerant clovers. Acute exposure (300 ppb $O_3$ for 6 hours) led to the production of an oxidative burst in both clovers (Puckette et al., 2008). However, the sensitive-Jemalong cultivar exhibited a sustained ROS burst and a concomitant downregulation of defense response genes at 12 hours after the onset of exposure, while the tolerant JE 154 accession showed much more rapid and large-scale transcriptome changes than the Jemalong cultivar (Puckette et al., 2008).

Arabidopsis ecotypes WS and Col-0 were exposed to 1.2 × ambient $O_3$ concentrations for 8-12 days at the SoyFACE site (Li et al., 2006b). The sensitive WS ecotype showed a far greater number of changes in gene expression in response to this low-level $O_3$ exposure than the tolerant Col-0 ecotype. In a different study, exposure of the WS ecotype to 300 ppb $O_3$ for 6 hours showed a rapid induction of genes leading to cell death, such as proteases, and downregulation or inactivation of cell signaling genes, demonstrating an ineffective defense response in this $O_3$ sensitive ecotype (Mahalingam et al., 2006).

The temporal response of plants to $O_3$ exposure was evaluated in the Arabidopsis Col-0 ecotype during a 6-hour exposure at 350 ppb $O_3$ and for 6 hours after the exposure was completed. Results of this study, shown in Figure 9-5 indicate that genes associated with signal transduction and regulation of transcription were in the class of early upregulated genes, while genes associated with redox homeostasis and defense/stress response were in the class of late upregulated genes (Mahalingam et al., 2005).

A few studies have been conducted to evaluate transcriptome changes in response to longer term chronic $O_3$ exposures in woody plant species. Longer term exposures resulted in the upregulation of genes associated with secondary metabolites, including isoprenoids, polyamines and phenylpropanoids in 2-year-old seedlings of the Mediterranean shrub *Phillyrea latifolia* exposed to 110 ppb $O_3$ for 90 days (Paolacci et al., 2007). In 3-year-old European beech saplings exposed to $O_3$ for 20 months (with monthly average twice ambient $O_3$ concentrations ranging from 11 to 80 ppb), $O_3$-induced changes in gene transcription were similar to those observed

for herbaceous species (Olbrich et al., 2009). Genes encoding proteins associated with plant stress response, including ethylene biosynthesis, pathogenesis-related proteins and enzymes detoxifying ROS, were upregulated. Some genes associated with primary metabolism, cell structure, cell division and cell growth were reduced (Olbrich et al., 2009). In a similar study using adult European beech trees, it was determined that the magnitude of the transcriptional changes described above was far greater in the saplings than in the adult trees exposed to the same $O_3$ concentrations for the same time period (Olbrich et al., 2010).

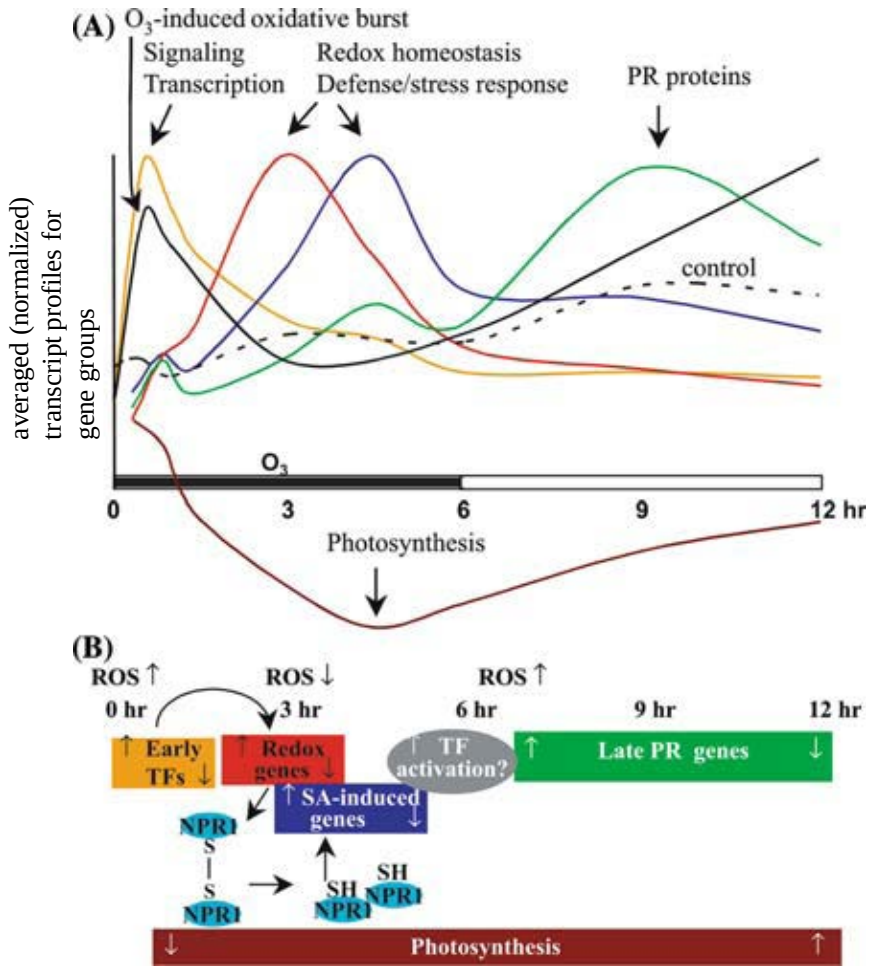
The results from transcriptome studies described above have been substantiated by results from proteome analysis in rice, poplar, European beech, wheat, and soybean. Exposure of soybean to 120 ppb $O_3$ for 12 hours/day for 3 days in growth chambers resulted in decreases in the quantity of proteins associated with photosynthesis, while proteins involved with antioxidant defense and carbon metabolism increased (Ahsan et al., 2010). Young poplar plants exposed to 120 ppb $O_3$ in a growth chamber for 35 days also showed significant changes in proteins involved in carbon metabolism (Bohler et al., 2007). Declines in enzymes associated with carbon fixation, the Calvin cycle and photosystem II were measured, while ascorbate peroxidase and enzymes associated with glucose catabolism increased in abundance. In another study to determine the impacts of $O_3$ on both developing and fully expanded poplar leaves, young poplars were exposed to 120 ppb $O_3$ for 13 hours/day for up to 28 days (Bohler et al., 2010). Impacts on protein quantity only occurred after the plants had been exposed to $O_3$ for 14 days, and at this point in time, several Calvin cycle enzymes were reduced in quantity, while the effects on the light reactions appeared later, at 21 days after beginning treatment. Some of the antioxidant enzymes increased in abundance with $O_3$ treatment, while others (ascorbate peroxidase) did not. In relationship to leaf expansion, it was shown that $O_3$ did not affect protein quantity until leaves had reached full expansion, after about 7 days (Bohler et al., 2010).

Two-week-old rice seedlings exposed to varying levels of $O_3$ (4, 40, 80, 120 ppb) in a growth chamber for 9 days showed reductions in quantities of proteins associated with photosynthesis and energy metabolism, and increases in some antioxidant and defense related proteins (Feng et al., 2008a). A subsequent study of $O_3$-treated rice seedlings (exposed to 200 ppb $O_3$ for 24 hours) focusing on the integration of transcriptomics and proteomics, supported and further enhanced these results (Cho et al., 2008). The authors found that of the 22,000 genes analyzed from the rice genome, 1,535 were differentially regulated by $O_3$. Those differentially regulated genes were functionally categorized as transcription factors, MAPK cascades, those encoding for enzymes involved in the synthesis of jasmonic acid (JA), ethylene (ET), shikimate, tryptophan and lignin, and those involved in glycolysis, the citric acid cycle, oxidative respiration and photosynthesis. The authors determined that the proteome and metabolome (all small molecule metabolites in a cell) analysis supported the results of the transcriptome changes described above (Cho et al., 2008). This type of study, which ties together results from changes in gene expression, protein quantity and activity, and metabolite levels, provides the most

complete picture of the molecular and biochemical changes occurring in plants exposed to a stressor such as $O_3$.

Sarkar et al. (2010) compared proteomes of two cultivars of wheat grown in OTCs at several $O_3$ concentrations, including filtered air, ambient $O_3$ (mean concentration 47 ppb), ambient + 10 ppb and ambient + 20 ppb for 5 hours/day for 50 days. Declines in the rate of photosynthesis and stomatal conductance were related to decreases in proteins involved in carbon fixation and electron transport and increased proteolysis of photosynthetic proteins such as the large subunit of ribulose-1,6-bisphosphate carboxylase/oxygenase (Rubisco). Enzymes that take part in energy metabolism, such as ATP synthesis, were also downregulated, while defense/stress related proteins were upregulated in $O_3$-treated plants. In comparing the two wheat cultivars, Sarkar et al. (2010) found that while the qualitative changes in protein expression between the two cultivars were similar, the magnitude of these changes differed between the sensitive and tolerant wheat cultivars. Greater foliar injury and a smaller decline in stomatal conductance was observed in the sensitive cultivar as compared to the more tolerant cultivar, along with greater losses in photosynthetic enzymes and higher quantities of antioxidant enzymes. Results from a three-year exposure of European beech saplings to elevated $O_3$ (AOT40 value was 52.6 ppm-h for 2006, when trees were sampled) supported the results from the short-term exposure studies described above (Kerner et al., 2011). The $O_3$ treatment of the saplings resulted in reductions in enzymes associated with the Calvin cycle, which could lead to reduced carbon fixation. Enzymes associated with carbon metabolism/catabolism were increased, and quantities of starch and sucrose were reduced in response to the $O_3$ treatment in these trees, indicating a potential impact of $O_3$ on overall carbon metabolism in long-term exposure conditions (Kerner et al., 2011).

Transcriptome and proteome studies have provided valuable information about $O_3$ effects on plants. These studies allow for simultaneous analysis of changes in the expression patterns of many different genes and proteins, and also provide information on how these molecules might interact with one another as a result of plant exposure to oxidative stress. Gene and protein expression patterns generally differ between $O_3$-sensitive and tolerant plants, which could result from differential uptake or detoxification of $O_3$ or from differential regulation of the transcriptome and proteome.




Note: (A) Temporal profile of the oxidative stress response to $O_3$. The biphasic $O_3$-induced oxidative burst is represented in black, with the ROS (reactive oxygen species) control measurements shown as a broken line. Average transcript profiles are shown for early upregulated genes (yellow, peaks at 0.5-1 hours), and the 3 hours (blue), 4.5 hours (red) and 9-12 hours (green) late upregulated genes and for the downregulated genes coding for photosynthesis proteins (brown). PR = pathogenesis related. (B) Diagrammatic representation of redox regulation of the oxidative stress response. TF = transcription factor; SA = salicylic acid.

Source: Reprinted with permission of Springer (Mahalingam et al., 2005).

**Figure 9-5 Composite diagram of major themes in the temporal evolution of the genetic response to $O_3$ stress.**

All of these studies describe common trends for changes in gene and protein expression which occur in a variety of plant species exposed to $O_3$. While genes associated with carbon assimilation and general metabolism are typically downregulated, genes associated with signaling, catabolism, and defense are upregulated. The magnitude of these changes in gene and protein expression appears to be related to plant species, age and their sensitivity or tolerance to $O_3$.

### 9.3.3.3 Role of Phytohormones in Plant Response to Ozone

Many studies of $O_3$ effects on plants have analyzed the importance of plant hormones such as SA, ET and JA in determining plant response to $O_3$. The 2006 $O_3$ AQCD documents the $O_3$-induced production of ET and its role in promoting the formation of leaf lesions. Transcriptome analysis and the use of a variety of mutants have allowed for further elucidation of the complex interactions between SA, ET, JA and the role of abscisic acid (ABA) in mediating plant response to $O_3$ (Ludwikow and Sadowski, 2008). In addition to their roles in signaling pathways, phytohormones also appear to regulate, and be regulated by, the MAPK signaling cascades described previously. Most evidence suggests that while ET and SA are needed to develop $O_3$-induced leaf lesions, JA acts antagonistically to SA and ET to limit the lesions (Figure 9-6) (Kangasjarvi et al., 2005).

The rapid production of ET in $O_3$ treated plants has been described in many plant species and has been further characterized through the use of a variety of mutants that either over-produce or are insensitive to ET. Production of stress ET in $O_3$-treated plants, which is thought to be part of a wounding response, was found to be correlated to the degree of injury development in leaves (U.S. EPA, 2006b). More recent studies have supported these conclusions and have also focused on the interactions occurring between several oxidative-stress induced phytohormones. Yoshida et al. (2009) determined that ET likely amplifies the oxidative signal generated by ROS, thereby promoting lesion formation. By analyzing the $O_3$-induced transcriptome of several Arabidopsis mutants of the Col-0 ecotype, Tamaoki et al. (2003) determined that at 12 hours after initiating the $O_3$ exposure (200 ppb for 12 hours), the ET and JA signaling pathways were the main pathways used to activate plant defense responses, with a lesser role for SA. The authors also demonstrated that low levels of ET production could stimulate the expression of defense genes, rather than promoting cell death which occurs when ET production is high. Tosti et al. (2006) supported these findings by showing that plant exposure to $O_3$ not only results in activation of the biosynthetic pathways of ET, JA and SA, but also increases the expression of genes related to the signal transduction pathways of these phytohormones in $O_3$-treated Arabidopsis plants (300 ppb $O_3$ for 6 hours). Conversely, in the $O_3$ sensitive Ws ecotype, its sensitivity may, in part, be due to intrinsically high ET levels leading to SA accumulation, and the high ET and SA may act to repress JA-associated genes, which would serve to inhibit the spread of lesions (Mahalingam et al., 2006). Ogawa et al. (2005) found that increases in SA in $O_3$-treated plants leads to the formation of leaf lesions in tobacco plants exposed to 200 ppb $O_3$ for 6 hours. Furthermore, in transgenic tobacco plants with reduced levels of ET production in response to $O_3$ exposure, several genes encoding for enzymes in the biosynthetic pathway of SA were suppressed, suggesting that SA levels are, in part, controlled by ET in the presence of $O_3$.

Exposure of the Arabidopsis mutant rcd1 to acute doses of $O_3$ (250 ppb $O_3$ for 8 hours/day for 3 days) resulted in programmed cell death (PCD) and the formation of leaf lesions (Overmyer et al., 2000). They determined that the observed induction of ET synthesis promotes cell death, and that ET perception and signaling are

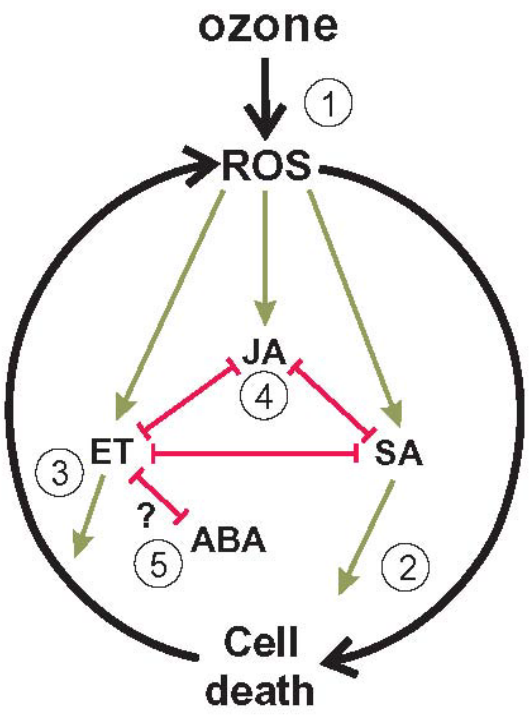
required for the accumulation of superoxide, which leads to cell death and propagation of lesions. Jasmonic acid, conversely, contains the spread of leaf lesions (Overmyer et al., 2000). Transcriptome analysis of several Arabidopsis mutants, which are insensitive to SA, ET and JA, exposed to 12-h of 200 ppb $O_3$ showed that approximately 78 of the upregulated genes measured in this study were controlled by ET and JA signaling pathways, while SA signaling pathways were suggested to antagonize ET and JA pathways (Tamaoki et al., 2003). In a subsequent transcriptome study on the Col-0 ecotype exposed to 150 ppb $O_3$ for 48-h, JA and ET synthesis were downregulated, while SA was upregulated in $O_3$-treated plants. In cotton plants exposed to a range of $O_3$ concentrations (0-120 ppb) and methyl jasmonate (MeJA), Grantz et al. (2010b) determined that exogenous applications of MeJA did not protect plants from chronic $O_3$ exposure.

Abscisic acid has been investigated for its role in regulating stomatal aperture and also for its contribution to signaling pathways in the plant. The role of ABA and the interaction between ABA and $H_2O_2$ in $O_3$-induced stomatal closure was described in the 2006 $O_3$ AQCD. It was determined that the presence of $H_2O_2$, which is formed from $O_3$ degradation, increases the sensitivity of guard cells to ABA and, therefore, more readily results in stomatal closure. More recently, it was determined that synthesis of ABA was induced in $O_3$-treated Arabidopsis plants (250-350 ppb $O_3$ for 6 hours), with a more pronounced induction in the $O_3$ sensitive rcd3 mutant as compared to the wildtype Col-0 (Overmyer et al., 2008). The rcd3 mutant also exhibited a lack of $O_3$-induced stomatal closure, and the RCD3 protein has been shown to be required for slow anion channels (Overmyer et al., 2008). Ludwikow et al. (2009) used Arabidopsis ABI1td mutants, in which a key negative regulator of ABA action (abscisic acid insensitive1 protein phosphatase 2C) has been knocked out, to examine $O_3$ responsive genes in this mutant compared to the Arabidopsis Col-0. Results of this study indicate a role for ABI1 in negatively regulating the synthesis of both ABA and ET in $O_3$-treated plants (350 ppb $O_3$ for 9 hours). Additionally, ABI1 may stimulate JA-related gene expression, providing evidence for an antagonistic interaction between ABA and JA signaling pathways (Ludwikow et al., 2009).

Nitric oxide (NO) has also been shown to play a role in regulating gene expression in plants in response to $O_3$ exposure. However, little is known to date about NO and its role in the complex interactions of molecules in response to $O_3$. Exposure of tobacco to $O_3$ (150 ppb for 5 hours) stimulated NO and NO-dependent ET production, while NO production itself did not depend on the presence of ET (Ederli et al., 2006). Analysis of $O_3$-treated Arabidopsis indicated the possibility of a dual role for NO in the initiation of cell death and later lesion containment (Ahlfors et al., 2009).

While much work remains to be done to better elucidate what determines plant sensitivity and response to $O_3$, it is clear that the mechanism for response to $O_3$ and signal transduction is very complex. Many of the phytohormones and other signaling molecules thought to be involved in these processes are interactive and depend upon a variety of other factors, which could be either internal or external to the plant. This results in a highly dynamic and complex system, capable of resulting in a spectrum

of plant sensitivity to oxidative stress and generating a variety of plant responses to that stress.




Note: Ozone-derived radicals induce endogenous ROS production (1) which results in salicylic acid (SA) accumulation and programmed cell death; (2) Cell death triggers ethylene (ET) production, which is required for the continuing ROS production responsible for the propagation of cell death; (3) Jasmonates counteract the progression of the cycle by antagonizing the cell death promoting function of SA and ET; (4) Abscisic acid (ABA) antagonizes ET function in many situations and might also have this role in $O_3$-induced cell death; (5) Mutually antagonistic interactions between ET, SA and jasmonic acid (JA) are indicated with red bars.

Source: Reprinted with permission of Blackwell Publishing Ltd. (Kangasjarvi et al., 2005).

**Figure 9-6 The oxidative cell death cycle.**

### 9.3.4 Detoxification

#### 9.3.4.1 Overview of Ozone-induced Defense Mechanisms

Plants are exposed to an oxidizing environment on a continual basis, and many reactions that are part of the basic metabolic processes, such as photosynthesis and respiration, generate ROS. As a result, there is an extensive and complex mechanism in place to detoxify these oxidizing radicals, including both enzymes and metabolites, which are located in several locations in the cell and also in the apoplast of the cell. As $O_3$ enters the leaf through open stomata, the first point of contact of

$O_3$ with the plant is likely in the apoplast, where it breaks down to form oxidizing radicals such as $H_2O_2$, $O_2^-$, HO· and $HO_2$. Another source of oxidizing radicals is an oxidative burst, generated by a membrane-bound NADPH oxidase enzyme, which is recognized as an integral component of the plant's defense system against pathogens (Schraudner et al., 1998). Antioxidant metabolites and enzymes located in the apoplast are thought to form a first line of defense by detoxifying $O_3$ and/or the ROS that are formed as breakdown products of $O_3$ (Section 9.3.2). However, even with the presence of several antioxidants, including ascorbate, the redox buffering capacity of the apoplast is far less than that of the cytoplasm, as it lacks the regeneration systems necessary to retain a reduced pool of antioxidants (Foyer and Noctor, 2005b).

Redox homeostasis is regulated by the presence of a pool of antioxidants, which are typically found in a reduced state and detoxify ROS produced by oxidases or electron transport components. As ROS increase due to environmental stress such as $O_3$, it is unclear whether the antioxidant pool can maintain its reduced state (Foyer and Noctor, 2005b). As such, not only the quantity and types of antioxidant enzymes and metabolites present, but also the cellular ability to regenerate those antioxidants are important considerations in mechanisms of plant tolerance to oxidative stress (Dizengremel et al., 2008). Molecules such as glutathione (GSH), thioredoxins and NADPH play very important roles in this regeneration process; additionally, it has been hypothesized that alterations in carbon metabolism would be necessary to supply the needed reducing power for antioxidant regeneration (Dizengremel et al., 2008).

#### 9.3.4.2 Role of Antioxidants in Plant Defense Responses

Ascorbate has been the focus of many different studies as an antioxidant metabolite that protects plants from exposure to $O_3$. It is found in several cellular locations, including the chloroplast, the cytosol and the apoplast (Noctor and Foyer, 1998). Ascorbate is synthesized in the cell and transported to the apoplast. Apoplastic ascorbate can be oxidized to dehydroascorbate (DHA) with exposure to $O_3$ and is then transported back to the cytoplasm. Here, DHA is reduced to ascorbate by the enzyme dehydroascorbate reductase (DHAR) and reduced GSH, which is part of the ascorbate-glutathione cycle (Noctor and Foyer, 1998). Many studies have focused on evaluating whether ascorbate is the primary determining factor in differential sensitivity of plants to $O_3$. An evaluation of several species of wildflowers in Great Smoky Mountains National Park showed a correlation between higher quantities of reduced apoplastic ascorbate and lower levels of foliar injury from $O_3$ exposure in a field study on tall milkweed plants (*Asclepsias exaltata* L.) (Burkey et al., 2006; Souza et al., 2006). Cheng et al. (2007) exposed two soybean cultivars to elevated $O_3$ (77 ppb) and filtered air for 7 hours/day for 6 days. The differences in sensitivity between the two cultivars could not be explained by differential $O_3$ uptake or by the fraction of reduced ascorbate present in the apoplast. However, total antioxidant capacity of the apoplast was 2-fold higher in the tolerant Essex cultivar as compared to the sensitive Forrest cultivar, indicating that there may be other compounds in the

leaf apoplast that scavenge ROS. D'Haese et al. (2005) exposed the NC-S (sensitive) and NC-R (resistant) clones of white clover (*Trifolium repens*) to 60 ppb $O_3$ for 7 hours/day for 5 days in environmental chambers. Surprisingly, the NC-S clone had a higher constitutive concentration of apoplastic ascorbate with a higher redox status than the NC-R clone. However, the redox status of symplastic GSH was higher in NC-R, even though the concentration of GSH was not higher than in NC-S. In addition, total symplastic antioxidative capacity was not a determining factor in differential sensitivity between these two clones. Severino et al. (2007) also examined the role of antioxidants in the differential sensitivity of the two white clover clones by growing them in the field for a growing season and then exposing them to elevated $O_3$ (100 ppb for 8 hours/day for 10 days) in OTC at the end of the field season. The NC-R clone had greater quantities of total ascorbate and total antioxidants than the NC-S clone at the end of the experiment. In snap bean, plants of the $O_3$ tolerant Provider cultivar had greater total ascorbate and more ascorbate in the apoplast than the sensitive S156 cultivar after exposure to 71 ppb $O_3$ for 10 days in OTC (Burkey et al., 2003). While most of the apoplastic ascorbate was in the oxidized form, the ratio of reduced ascorbate to total ascorbate was higher in Provider than S156, indicating that Provider is better able to maintain this ratio to maximize plant protection from oxidative stress. Exposure of two wheat varieties to ambient (7-h average 44 ppb $O_3$) and elevated (7-h average 56 ppb $O_3$) $O_3$ for 60 days in open-air field conditions showed higher concentrations of reduced ascorbate in the apoplast in the tolerant Y16 variety than the more sensitive Y2 variety, however no varietal differences were seen in the decrease in reduced ascorbate quantity in response to $O_3$ exposure (Feng et al., 2010). To evaluate whether $O_3$ affected apoplastic concentrations of ascorbic acid and phenolic compounds, wildtype *Arabidopsis thaliana* (Col-0, Ler-0) and null mutants lacking sinapoyl and flavonol glycosides were exposed to either 125 or 175 ppb $O_3$ for up to 2 days. The authors determined that ascorbic acid, which was found in very low quantities in the reduced form, and the phenolic compounds did not play an important role in protecting plants from $O_3$ injury (Booker et al., 2012). While there is much evidence that supports an important role for ascorbate, particularly apoplastic ascorbate, in protecting plants from oxidative stressors such as $O_3$, it is also clear that there is much variation in the importance of ascorbate for different plant species and differing exposure conditions. Additionally, the work of several authors suggests that there may be other compounds in the apoplast which have the capacity to act as antioxidants.

While the quantities of antioxidant metabolites such as ascorbate are an important indicator of plant tolerance to $O_3$, the ability of the plant to recycle oxidized ascorbate efficiently also plays a large role in determining the plant's ability to effectively protect itself from sustained exposure to oxidative stress. Tobacco plants over-expressing DHAR were better protected from exposure to either chronic (100 ppb $O_3$ 4 hours/day for 30 days) or acute (200 ppb $O_3$ for 2 hours) $O_3$ conditions than control plants and those with reduced expression of DHAR (Chen and Gallie, 2005). The DHAR over-expressing plants exhibited an increase in guard cell ascorbic acid, leading to a decrease in stomatal responsiveness to $O_3$ and an increase in stomatal conductance and $O_3$ uptake. Despite this, the presence of higher

levels of ascorbic acid led to a lower oxidative load and a higher level of photosynthetic activity in the DHAR over-expressing plants (Chen and Gallie, 2005). A subsequent study with tobacco plants over-expressing DHAR confirmed some of these results. Levels of ascorbic acid were higher in the transgenic tobacco plants, and they exhibited greater tolerance to $O_3$ exposure (200 ppb $O_3$) as demonstrated by higher photosynthetic rates in the transgenic plants as compared to the control plants (Eltayeb et al., 2006). Over-expression of monodehydroascorbate reductase (MDAR) in tobacco plants also showed enhanced stress tolerance in response to $O_3$ exposure (200 ppb $O_3$), with higher rates of photosynthesis and higher levels of reduced ascorbic acid as compared to controls (Eltayeb et al., 2007). Results of these studies demonstrate the importance of ascorbic acid as a detoxification mechanism in some plant species, and also emphasize that the recycling of oxidized ascorbate to maintain a reduced pool of ascorbate is a factor in determining plant tolerance to oxidative stress.

The roles of other antioxidant metabolites and enzymes, including GSH, catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD), were comprehensively reviewed in the 2006 $O_3$ AQCD. Based on the review of the literature, no conclusive and consistent effects of $O_3$ on the quantity of GSH and CAT could be identified. Both apoplastic and cytosolic POD activity increased in response to $O_3$ exposure, while various isoforms of SOD showed inconsistent changes in quantity in response to $O_3$. Additional studies have been conducted to further elucidate the roles of these antioxidant enzymes and metabolites in protecting plants from oxidative stress. Superoxide dismutase and POD activities were measured in both the tolerant Bel B and sensitive Bel W3 tobacco cultivars exposed to ambient $O_3$ concentrations for 2 weeks 3 times throughout a growing season (Borowiak et al., 2009). In this study, SOD and POD activity, including that of several different isoforms, increased in both the sensitive and tolerant tobacco cultivars with exposure to $O_3$, however the isoenzyme composition for POD differed between the sensitive and tolerant tobacco cultivars (Borowiak et al., 2009) Tulip poplar (*Liriodendron tulipifera*) trees exposed to increasing $O_3$ concentrations (from 100 to 300 ppb $O_3$ during a 2-week period) showed increases in activities of SOD, ascorbate peroxidase (APX), glutathione reductase (GR), MDAR, DHAR, CAT and POD in the 2-week period, although individual enzyme activities increased at different times during the 2-week period (Ryang et al., 2009).

Longer, chronic $O_3$ exposures in trees revealed increases in SOD and APX activity in *Quercus mongolica* after 45 days of plant exposure to 80 ppb $O_3$, which were followed by declines in the activities and quantities of these enzymes after 75 days of exposure (Yan et al., 2010). Similarly, activities of SOD, APX, DHAR, MDAR, and GR increased in *Gingko biloba* trees during the first 50 days of exposure to 80 ppb $O_3$, followed by decreases in activity below control values after 50 days of exposure (He et al., 2006). Soybean plants exposed to 70 or 100 ppb $O_3$ for 4 hours/day over the course of a growing season showed elevated POD activity and a decrease in CAT activity at 40 and 60 days after germination (Singh et al., 2010a).

Antioxidant enzymes and metabolites have been shown to play an important role in determining plant tolerance to $O_3$ and mediating plant responses to $O_3$. However, there is also some evidence to suggest that the direct reaction of ascorbate with $O_3$ could lead to the formation of secondary toxicants, such as peroxy compounds, which may act upon signal transduction pathways and modulate plant response to $O_3$ (Sandermann, 2008). Therefore, the role of ascorbate and other antioxidants and their interaction with other plant responses to $O_3$, such as the activation of signal transduction pathways, is likely far more complex than is currently understood.

## 9.3.5 Effects on Primary and Secondary Metabolism

### 9.3.5.1 Light and Dark Reactions of Photosynthesis

Declines in the rate of photosynthesis in $O_3$-treated plants have been documented for many different plant species (Booker et al., 2009; Wittig et al., 2007; U.S. EPA, 2006b). The 2006 $O_3$ AQCD described the mechanism by which plant exposure to $O_3$ reduces carboxylation capacity, and the more recent scientific literature confirms these findings. While several measures of the light reactions of photosynthesis are sensitive to exposure to $O_3$ (see below), photosynthetic carbon assimilation is generally considered to be more affected by pollutant exposure, resulting in an overall decline in photosynthesis (Guidi and Degl'lnnocenti, 2008; Heath, 2008; Fiscus et al., 2005). Loss of carbon assimilation capacity has been shown to result from declines in the quantity and activity of Rubisco (Calatayud et al., 2010; Goumenaki et al., 2010; Singh et al., 2009; Bagard et al., 2008; Calatayud et al., 2007a; Crous et al., 2006). Experimental evidence suggests that both decreases in Rubisco synthesis and enhanced degradation of the protein contribute to the measured reduction in its quantity. Additionally, the reduction in Rubisco quantity has been associated with the $O_3$-induced oxidative modification of the enzyme, which is evidenced by the increases in carbonyl groups on the protein after plant exposure to $O_3$ (U.S. EPA, 2006b). Reduced carbon assimilation has been linked to reductions in biomass and yield (Wang et al., 2009b; He et al., 2007; Novak et al., 2007; Gregg et al., 2006; Keutgen et al., 2005). Recent studies evaluating $O_3$ induced changes in the transcriptome and proteome of several different species confirm these findings. Levels of mRNA for rbcS (the gene that encodes the small subunit [SSU] of the RuBisCO protein [ribulose-1,5-bisphosphate carboxylase/oxygenase, a major stromal enzyme involved in carbon fixation by plants]) declined in European beech saplings exposed to 300 ppb $O_3$ for 8 hours/day for up to 26 days (Olbrich et al., 2005). Similar declines in rbcS mRNA were also measured in the beech saplings in a free air exposure system over a course of two growing seasons (Olbrich et al., 2009). Proteomics studies have also confirmed the effects of $O_3$ on proteins involved in carbon assimilation. Reductions in quantities of the small and large subunit (rbcL) of Rubisco and Rubisco activase were measured in soybean plants exposed to 120 ppb $O_3$ for 3 days in growth chambers (Ahsan et al., 2010). Exposure of young poplar trees to 120 ppb $O_3$ for 35 days in exposure chambers resulted in reductions of

Rubisco, Rubisco activase, and up to 24 isoforms of Calvin cycle enzymes, most of which play a role in regenerating the $CO_2$ acceptor molecule, ribulose-1,5-bisphosphate (Bohler et al., 2007). Reductions in protein quantity of both the small and large subunit of Rubisco were seen in wheat plants exposed to ambient (average concentration 47.3 ppb $O_3$) and elevated $O_3$ (ambient + 10 or 20 ppb $O_3$) in open-top chambers for 5 hours/day for 50 days (Sarkar et al., 2010). Lettuce plants exposed to 100 ppb $O_3$ in growth chambers for 8 hours/day for 3 weeks also showed reductions in transcript and protein levels of the small and large subunits of Rubisco and Rubisco activase (Goumenaki et al., 2010).

Reductions in photosynthesis are not only related to declines in the quantity of Rubisco, but also of its activity level. The maximum carboxylation rate ($V_{cmax}$) has been shown to decline in plants species exposed to $O_3$, including lettuce (Goumenaki et al., 2010), white clover (Crous et al., 2006), young poplar trees (Bagard et al., 2008) and evergreen deciduous shrubs (Calatayud et al., 2010). While a significant proportion of the reduction in $V_{cmax}$ is caused by declines in the quantity of Rubisco, other contributors to changes in $V_{cmax}$ result from reductions in the quantity and activity of Rubisco activase, an enzyme which prepares Rubisco for carbamylation by accelerating the release of bound sugar phosphates. Reductions in Rubisco activase quantity have been observed in several studies evaluating the effects of $O_3$ on the proteomes of poplar (Bohler et al., 2007), European beech (Kerner et al., 2011) and soybean (Ahsan et al., 2010).

In addition to impacts on carbon assimilation, the deleterious effects of $O_3$ on the photosynthetic light reactions have received more attention in recent years. Chlorophyll fluorescence provides a useful measure of changes to the photosynthetic process from exposure to oxidative stress. Decreases in the Fv/Fm ratio (a measure of the maximum efficiency of Photosystem II) in dark adapted leaves indicate a decline in the efficiency of the PSII photosystems and a concomitant increase in non-photochemical quenching (Guidi and Degl'lnnocenti, 2008; Scebba et al., 2006). Changes in these parameters have been correlated to differential sensitivity of plants to the pollutant. In a study to evaluate the response of 4 maple species to $O_3$ (exposed to an 8-h avg of 51 ppb for ambient and 79 ppb for elevated treatment in OTC), the 2 species which were most sensitive based on visible injury and declines in $CO_2$ assimilation also showed the greatest decreases in Fv/Fm in symptomatic leaves. In asymptomatic leaves, $CO_2$ assimilation decreased significantly but there was no significant decline in Fv/Fm (Calatayud et al., 2007a). Degl'Innocenti et al. (2007) measured significant decreases in Fv/Fm in young and symptomatic leaves of a resistant tomato genotype (line 93.1033/1) in response to $O_3$ exposure (150 ppb $O_3$ for 3 hours in a growth chamber), but only minor decreases in asymptomatic leaves with no associated changes in net photosynthetic rate. In the $O_3$ sensitive tomato cultivar Cuor Di Bue, the Fv/Fm ratio did not change, while the photosynthetic rate declined significantly in asymptomatic leaves (Degl'Innocenti et al., 2007). In two soybean cultivars, Fv/Fm also declined significantly with plant exposure to $O_3$ (Singh et al., 2009). It appears that in asymptomatic leaves, photoinhibition, as indicated by a decrease in Fv/Fm, is not the main reason for a decline in photosynthesis.

An evaluation of photosynthetic parameters of two white clover (*Trifolium repens* cv. Regal) clones that differ in their $O_3$ sensitivity revealed that $O_3$ (40-110 ppb $O_3$ for 7 hours/day for 5 days) increased the coefficient of non-photochemical quenching ($q_{NP}$) in both the resistant (NC-R) and sensitive (NC-S) clones, however $q_{NP}$ was significantly lower for the sensitive clone (Crous et al., 2006). Sensitive *Acer* clones had a lower coefficient of non-photochemical quenching, while exposure to $O_3$ increased $q_{NP}$ in both sensitive and tolerant clones (Calatayud et al., 2007a). While exposure to $O_3$ also increased $q_{NP}$ in tomato, there were no differences in the coefficient of photochemical quenching between cultivars thought to be differentially sensitive to $O_3$ (Degl'Innocenti et al., 2007). Higher $q_{NP}$ as a result of exposure to $O_3$ indicates a reduction in the proportion of absorbed light energy being used to drive photochemistry. A lower coefficient of non-photochemical quenching in $O_3$ sensitive plants could indicate increased vulnerability to ROS generated during exposure to oxidative stress (Crous et al., 2006).

Most of the research on $O_3$ effects on photosynthesis has focused on C3 (Calvin cycle) plants because C4 (Hatch-Slack) plants have lower stomatal conductance and are, therefore, thought to be less sensitive to $O_3$ stress. However, some studies have been conducted to evaluate the effects of $O_3$ on C4 photosynthesis. In older maize leaves, Leitao et al. (2007c; 2007a) found that the activity, quantity and transcript levels of both Rubisco and phosphoenolpyruvate carboxylase (PEPc) decreased as a function of rising $O_3$ concentration. In younger maize leaves, the quantity, activity, and transcript levels of the carboxylases were either increased or unaffected in plants exposed to 40 ppb $O_3$ for 7 hours/day for 28-33 days, but decreased at 80 ppb (Leitao et al., 2007b; Leitao et al., 2007c). In another study, Grantz et al. (2009) reported that $O_3$ exposures (4, 58, and 114 ppb, 12-h mean) decreased sugarcane biomass production by more than one third and allocation to roots by more than two thirds.

#### 9.3.5.2 Respiration and Dark Respiration

While much research emphasis regarding $O_3$ effects on plants has focused on the negative impacts on carbon assimilation, other studies have measured impacts on catabolic pathways such as shoot respiration and photorespiration. Generally, shoot respiration has been found to increase in plants exposed to $O_3$. Bean plants exposed to ambient (average 12-h mean 43 ppb) and twice ambient (average 12-h mean 80 ppb) $O_3$ showed increases in respiration. When mathematically partitioned, the maintenance coefficient of respiration was significantly increased in $O_3$ treated plants, while the growth coefficient of respiration was not affected (Amthor, 1988). Loblolly pines were exposed to ambient (12-h daily mean was 45 ppb) and twice ambient (12-h daily mean was 86 ppb) $O_3$ for 12 hours/day for approximately seven months per year for 3 and 4 years. While photosynthetic activity declined with the age of the needles and increasing $O_3$ concentration, enzymes associated with respiration showed higher levels of activity with increasing $O_3$ concentration (Dizengremel et al., 1994). In their review on the role of metabolic changes in plant redox status after $O_3$ exposure, Dizengremel et al. (2009) summarized multiple

studies in which several different tree species were exposed to $O_3$ concentrations ranging from ambient to 200 ppb $O_3$ for at least several weeks. In all cases, the activity of enzymes, including phosphofructokinase, pyruvate kinase and fumarase, which are part of several catabolic pathways, were increased in $O_3$ treated plants.

Photorespiration is a light-stimulated process which consumes $O_2$ and releases $CO_2$. While it has been regarded as a wasteful process, more recent evidence suggests that it may play a role in photoprotection during photosynthesis (Bagard et al., 2008). The few studies that have been conducted on $O_3$ effects on photorespiration suggest that rates of photorespiration decline concomitantly with rates of photosynthesis. Soybean plants were exposed to ambient (daily averages 43-58 ppb) and 1.5 ambient $O_3$ (daily averages 63-83 ppb) $O_3$ in OTCs for 12 hours/day for 4 months. Rates of photosynthesis and photorespiration and photorespiratory enzyme activity declined only at the end of the growing season and did not appear to be very sensitive to $O_3$ exposure (Booker et al., 1997). Young hybrid poplars exposed to 120 ppb $O_3$ for 13 hours/day for 35 days in phytotron chambers showed that effects on photorespiration and photosynthesis were dependent upon the developmental stage of the leaf. While young leaves were not impacted, reductions in photosynthesis and photorespiration were measured in fully expanded leaves (Bagard et al., 2008).

#### 9.3.5.3 Secondary Metabolism

Transcriptome analysis of Arabidopsis plants has revealed modulation of several genes involved in plant secondary metabolism (Ludwikow and Sadowski, 2008). Phenylalanine ammonia lyase (PAL) has been the focus of many studies involving plant exposure to $O_3$ due to its importance in linking the phenylpropanoid pathway of plant secondary metabolism to primary metabolism in the form of the shikimate pathway. Genes encoding several enzymes of the phenylpropanoid pathway and lignin biosynthesis were upregulated in transcriptome analysis of Arabidopsis plants (Col-0) exposed to 350 ppb $O_3$ for 6 hours, while 2 genes involved in flavonoid biosynthesis were downregulated (Ludwikow et al., 2004). Exposure of Arabidopsis (Col-0) to lower $O_3$ concentrations (150 ppb for 8 hours/day for 2 days) resulted in the induction of 11 transcripts involved in flavonoid synthesis. In their exposure of 2-year-old Mediterranean shrub *Phillyrea latifolia* to 110 ppb $O_3$ for 90 days, Paolacci et al. (2007) identified four clones that were upregulated and corresponded to genes involved in the synthesis of secondary metabolites, such as isoprenoids, polyamines and phenylpropanoids. Upregulation of genes involved in isoprene synthesis was also observed in *Medicago trunculata* exposed to 300 ppb $O_3$ for 6 hours, while genes encoding enzymes of the flavonoid synthesis pathway were either upregulated or downregulated (Puckette et al., 2008). Exposure of red clover to 1.5 × ambient $O_3$ (average concentrations of 32.4 ppb) for up to 9 weeks in an open field exposure system resulted in increases in leaf total phenolic content. However, the types of phenolics that were increased in response to $O_3$ exposure differed depending upon the developmental stage of the plant. While almost all of the 31 different phenolic compounds measured increased in quantity initially during the

exposure, after 3 weeks the quantity of isoflavones decreased while other phenolics increased (Saviranta et al., 2010). Exposure of beech saplings to ambient and 2 × ambient $O_3$ concentrations over 2 growing seasons resulted in the induction of several enzymes which contribute to lignin formation, while enzymes involved in flavonoid biosynthesis were downregulated (Olbrich et al., 2009). Exposure of tobacco Bel W3 to 160 ppb $O_3$ for 5 hours showed upregulation of almost all genes encoding for enzymes which are part of the prechorismate pathway (Janzik et al., 2005). Isoprenoids can serve as antioxidant compounds in plants exposed to oxidative stress (Paolacci et al., 2007).

The prechorismate pathway is the pathway leading to the formation of chorismate, a precursor to the formation of the aromatic amino acids tryptophan, tyrosine and phenylalanine. These amino acids are precursors for the formation of many secondary aromatic compounds, and, therefore, the prechorismate pathway represents a branch-point in the regulation of metabolites into either primary or secondary metabolism (Janzik et al., 2005). Exposure of the $O_3$ sensitive Bel W3 tobacco cultivar at 160 ppb for 5 hours showed an increase in transcript levels of most of the genes encoding enzymes of the prechorismate pathway. However, shikimate kinase (SK) did not show any change in transcript levels and only one of three isoforms of DAHPS (3-deoxy-D-arabino-heptulosonat-7-phosphate synthase), the first enzyme in this pathway, was induced by $O_3$ exposure (Janzik et al., 2005). Differential induction of DAHPS isoforms was also observed in European beech after 40 days of exposure to 150-190 ppb $O_3$. At this time point in the beech experiment, transcript levels of shikimate pathway enzymes, including SK, were generally strongly induced after an only weak initial induction after the first 40 days of exposure. Both soluble and cell-wall bound phenolic metabolites showed only minimal increases in response to $O_3$ for the duration of the exposure period (Alonso et al., 2007). Total leaf phenolics decreased with leaf age in *Populus nigra* exposed to 80 ppb $O_3$ for 12 hours/day for 14 days. Ozone increased the concentration of total leaf phenolics in newly expanded leaves, with the greatest increases occurring in compounds such as quercitin glycoside, which has a high antioxidant capacity (Fares et al., 2010b). While several phenylpropanoid pathway enzymes were induced in two poplar clones exposed to 60 ppb $O_3$ for 5 hours/day for 15 days, the degree of induction differed between the two clones. In the tolerant I-214 clone, PAL activity increased 9-fold in $O_3$-treated plants as compared to controls, while there was no significant difference in PAL activity in the sensitive Eridano clone (Di Baccio et al., 2008).

Polyamines such as putrescine, spermidine and spermine play a variety of roles in plants and have been implicated in plant defense responses to both abiotic and biotic stresses. They exist in both a free form and conjugated to hydroxycinnamic acids. Investigations on the role of polyamines have found that levels of putrescine increase in response to oxidative stress. This increase stems largely from the increase in the activity of arginine decarboxylase (ADC), a key enzyme in the synthesis of putrescine (Groppa and Benavides, 2008). Langebartels et al. (1991) described differences in putrescine accumulation in $O_3$-treated tobacco plants exposed to several $O_3$ concentrations, ranging from 0-400 ppb for 5-7 hours. A large and rapid

increase in putrescine occurred in the tolerant Bel B cultivar and only a small increase in the sensitive Bel W3 cultivar, which occurred only after the formation of necrotic leaf lesions. Van Buuren et al. (2002) further examined the role of polyamines in these two tobacco cultivars during an acute (130 ppb $O_3$ for 7-h in a growth chamber) exposure. They found that while free putrescine accumulated in undamaged tissue of both cultivars, conjugated putrescine predominantly accumulated in tissues undergoing cell death after plant exposure to $O_3$ (van Buuren et al., 2002). The authors suggest that while free putrescine may not play a role in conferring tolerance in the Bel B cultivar, conjugated putrescine may play a role in $O_3$-induced programmed cell death in Bel W3 plants.

Isoprene is emitted by some plant species and represents the predominant biogenic source of hydrocarbon emissions in the atmosphere (Guenther et al., 2006). In the atmosphere, the oxidation of isoprene by hydroxyl radicals can enhance $O_3$ formation in the presence of $NO_X$, thereby impacting the $O_3$ concentration that plants are exposed to. While isoprene emission varies widely between species, it has been proposed to stabilize membranes and provide those plant species that produce it with a mechanism of thermotolerance (Sharkey et al., 2008). It has also been suggested that isoprene may act as an antioxidant compound to scavenge $O_3$ (Loreto and Velikova, 2001). Recent studies using a variety of plant species have shown conflicting results in trying to understand the effects of $O_3$ on isoprene emission. Exposure to acute doses of $O_3$ (300 ppb for 3-h) in detached leaves of *Phragmites australis* resulted in stimulation of isoprene emissions (Velikova et al., 2005). Similar increases in isoprene emissions were measured in *Populus nigra* after exposure to 100 ppb $O_3$ for 5 days continuously (Fares et al., 2008). Isoprene emission in attached leaves of *Populus alba*, which were exposed to 150 ppb $O_3$ for 11 hours/day for 30 days inside cuvettes, was inhibited, while isoprene emission and transcript levels of isoprene synthase mRNA were increased in the leaves exposed to ambient $O_3$ (40 ppb), which were located above the leaves enclosed in the exposure cuvettes (Fares et al., 2006). Exposure of 2 genotypes of hybrid poplar to 120 ppb $O_3$ for 6 hours/day for 8 days resulted in a significant reduction in isoprene emission in the $O_3$-sensitive but not the tolerant genotype (Ryan et al., 2009). Similarly, $O_3$ treatment (80 ppb 12 hours/day for 14 days) of *Populus nigra* showed that isoprene emission was reduced in the treated plants relative to the control plants (Fares et al., 2010b). Based on results of this and other studies, Fares et al. (2010b) concluded that the isoprenoid pathway may be induced in plants exposed to acute $O_3$ doses, while at lower doses isoprene emission may be inhibited. Vickers et al. (2009) developed transgenic tobacco plants with the isoprene synthase gene from *Populus alba* and exposed them to 120 ppb $O_3$ for 6 hours/day for 2 days. They determined that the wildtype plants showed significantly more $O_3$ damage, including the development of leaf lesions and a decline in photosynthetic rates, than the transgenic, isoprene-emitting plants. Transgenic plants also accumulated less $H_2O_2$ and had lower levels of lipid peroxidation following exposure to $O_3$ than the wildtype plants (Vickers et al., 2009). These results indicate that isoprene may have a protective role for plants exposed to oxidative stress.

### 9.3.6 Summary

The results of recent studies on the effects of $O_3$ stress on plants support and strengthen those reported in the 2006 $O_3$ AQCD. The most significant new body of evidence since the 2006 $O_3$ AQCD comes from research on molecular mechanisms of the biochemical and physiological changes observed in many plant species in response to $O_3$ exposure. Recent studies have employed new techniques, such as those used in evaluating transcriptomes and proteomes to perform very comprehensive analyses of changes in gene transcription and protein expression in plants exposed to $O_3$. These newer molecular studies not only provide very important information regarding the many mechanisms of plant responses to $O_3$, they also allow for the analysis of interactions between various biochemical pathways which are induced in response to $O_3$. However, many of these studies have been conducted in artificial conditions with model plants, which are typically exposed to very high, short doses of $O_3$. Therefore, additional work remains to elucidate whether these plant responses are transferable to other plant species exposed to more realistic ambient conditions.

Ozone is taken up into leaves through open stomata. Once inside the substomatal cavity, $O_3$ is thought to rapidly react with the aqueous layer surrounding the cell (apoplast) to form breakdown products such as hydrogen peroxide ($H_2O_2$), superoxide ($O_2^-$), hydroxyl radicals ($HO^\cdot$) and peroxy radicals ($HO_2^\cdot$). Experimental evidence suggests that mitogen-activated protein kinases and calcium are important components of the signal transduction pathways, which communicate signals to the nucleus and lead to changes in gene expression in response to $O_3$. It is probable that there are multiple signal transduction pathways, and their activation may depend upon the plant species, its developmental stage and/or $O_3$ exposure conditions. Initiation of signal transduction pathways in $O_3$ treated plants has also been observed in stomatal guard cells. Reductions in stomatal conductance have been described for many plant species exposed to $O_3$. Some recent studies have also reported sluggish stomatal responses and increased stomatal conductance in some situations. New experimental evidence suggests that these effects on stomates may be due not only to a decrease in carboxylation efficiency, but also to a direct impact of $O_3$ on stomatal guard cell function, leading to a changes in stomatal conductance.

Alterations in gene transcription that have been observed in $O_3$-treated plants are now evaluated more comprehensively using DNA microarray studies, which measure changes in the entire transcriptome rather than measuring the transcript levels of individual genes. These studies have demonstrated very consistent trends, even though $O_3$ exposure conditions (concentration, duration of exposure), plant species and sampling times vary significantly. Genes involved in plant defense, signaling, and those associated with the synthesis of plant hormones and secondary metabolism are generally upregulated in plants exposed to $O_3$, while those related to photosynthesis and general metabolism are typically downregulated. Proteome studies support these results by demonstrating concomitant increases or decreases in the proteins encoded by these genes. Transcriptome analysis has also illuminated the complex interactions that exist between several different phytohormones and how

they modulate plant sensitivity and response to $O_3$. Experimental evidence suggests that while ethylene and salicylic acid are needed to develop $O_3$-induced leaf lesions, jasmonic acid acts antagonistically to ethylene and salicylic acid to limit the spread of the lesions. Abscisic acid, in addition to its role in regulating stomatal aperture, may also act antagonistically to the jasmonic acid signaling pathway. Changes in the quantity and activity of these phytohormones and the interactions between them reveal some of the complexity of plant responses to an oxidative stressor such as $O_3$.

Another critical area of interest is to better understand and quantify the capacity of the plant to detoxify oxygen radicals using antioxidant metabolites, such as ascorbate and glutathione, and the enzymes that regenerate them. Ascorbate remains an important focus of research, and, due to its location in the apoplast in addition to other cellular compartments, it is regarded as a first line of defense against oxygen radicals formed in the apoplast. Most studies demonstrate that antioxidant metabolites and enzymes increase in quantity and activity in plants exposed to $O_3$, indicating that they play an important role in protecting plants from oxidative stress. However, attempts to quantify the detoxification capacity of plants have remained unsuccessful, as high quantities of antioxidant metabolites and enzymes do not always translate into greater protection of the plant. Considerable variation exists between plant species, different developmental stages, and the environmental and $O_3$ exposure conditions which plants are exposed to.

As indicated earlier, the described alterations in transcript levels of genes correlate with observed changes quantity and activity of the enzymes and metabolites involved in primary and secondary metabolism. In addition to the generalized upregulation of the antioxidant defense system, photosynthesis typically declines in $O_3$ treated plants. Declines in C fixation due to reductions in quantity and activity of Rubisco were extensively described in the 2006 $O_3$ AQCD. More recent studies support these results and indicate that declines in Rubisco activity may also result from reductions in Rubisco activase enzyme quantity. Other studies, which have focused on the light reactions of photosynthesis, demonstrate that plant exposure to $O_3$ results in declines in electron transport efficiency and a decreased capacity to quench oxidizing radicals. Therefore, the overall declines in photosynthesis observed in $O_3$-treated plants likely result from combined impacts on stomatal conductance, carbon fixation and the light reactions. While photosynthesis generally declines in plants exposed to $O_3$, catabolic pathways such as respiration have been shown to increase. It has been hypothesized that increased respiration may result from greater energy needs for defense and repair. Secondary metabolism is generally upregulated in a variety of species exposed to $O_3$ as a part of a generalized plant defense mechanism. Some secondary metabolites, such as flavonoids and polyamines, are of particular interest as they are known to have antioxidant properties. The combination of decreases in C assimilation and increases in catabolism and the production of secondary metabolites would negatively impact plants by decreasing the energy available for growth and reproduction.

## 9.4 Nature of Effects on Vegetation and Ecosystems

### 9.4.1 Introduction

Ambient $O_3$ concentrations have long been known to cause visible symptoms, decreases in photosynthetic rates, decreases in growth and yield of plants as well as many other effects on ecosystems (U.S. EPA, 2006b, 1996c, 1986, 1978a). Numerous studies have related $O_3$ exposure to plant responses, with most effort focused on the yield of crops and the growth of tree seedlings. Many experiments exposed individual plants grown in pots or soil under controlled conditions to known concentrations of $O_3$ for a segment of daylight hours for some portion of the plant's life span. Information in this section also goes beyond individual plant-scale responses to consider effects at the broader ecosystem scale, including effects related to ecosystem services.

This section will focus mainly on studies published since the release of the 2006 $O_3$ AQCD. However, because much $O_3$ research was conducted prior to the 2006 $O_3$ AQCD, the present discussion of vegetation and ecosystem response to $O_3$ exposure is largely based on the conclusions of the 1978, 1986, 1996, and 2006 $O_3$ AQCDs.

#### 9.4.1.1 Ecosystem Scale, Function, and Structure

Information presented in this section was collected at multiple spatial scales or levels of biological organization, ranging from the physiology of a given species to population, community, and ecosystem investigations. An ecological population is a group of individuals of the same species and a community is an assemblage of populations of different species interacting with one another that inhabit an area. For this assessment, "ecosystem" is defined as the interactive system formed from all living organisms and their abiotic (physical and chemical) environment within a given area (IPCC, 2007a). The boundaries of what could be called an ecosystem are somewhat arbitrary, depending on the focus of interest or study. Thus, the extent of an ecosystem may range from very small spatial scales or levels of biological organization to, ultimately, the entire Earth (IPCC, 2007a). All ecosystems, regardless of size or complexity, have interactions and physical exchanges between biota and abiotic factors, this includes both structural (e.g., soil type and food web trophic levels) and functional (e.g., energy flow, decomposition, nitrification) attributes.

Ecosystems can be described, in part, by their structure, i.e., the number and type of species present. Structure may refer to a variety of measurements including the species richness, abundance, community composition and biodiversity as well as landscape attributes. Competition among and within species and their tolerance to environmental stressors are key elements of survivorship. When environmental conditions are shifted, for example, by the presence of anthropogenic air pollution,

these competitive relationships may change and tolerance to stress may be exceeded. Ecosystems may also be defined on a functional basis. “Function” refers to the suite of processes and interactions among the ecosystem components and their environment that involve nutrient and energy flow as well as other attributes including water dynamics and the flux of trace gases. Plants, via such processes as photosynthesis, respiration, C allocation, nutrient uptake and evaporation, affect energy flow, C, nutrient cycling and water cycling. The energy accumulated and stored by vegetation (via photosynthetic C capture) is available to other organisms. Energy moves from one organism to another through food webs, until it is ultimately released as heat. Nutrients and water can be recycled. Air pollution alters the function of ecosystems when elemental cycles or the energy flow are altered. This alteration can also be manifested in changes in the biotic composition of ecosystems.

There are at least three levels of ecosystem response to pollutants: (1) the individual organism and its environment; (2) the population and its environment; and (3) the biological community composed of many species and their environment (Billings, 1978). Individual organisms within a population vary in their ability to withstand the stress of environmental change. The response of individual organisms within a population is based on their genetic constitution, stage of growth at time of exposure to stress, and the microhabitat in which they are growing (Levine and Pinto, 1998). The stress range within which organisms can exist and function determines the ability of the population to survive.

#### 9.4.1.2 Ecosystem Services

Ecosystem structure and function may be translated into ecosystem services. Ecosystem services are the benefits people obtain from ecosystems (UNEP, 2003). Ecosystems provide many goods and services that are of vital importance for the functioning of the biosphere and provide the basis for the delivery of tangible benefits to human society. Hassan et al. (2005) define these benefits to include supporting, provisioning, regulating, and cultural services:

- Supporting services are necessary for the production of all other ecosystem services. Some examples include biomass production, production of atmospheric $O_2$, soil formation and retention, nutrient cycling, water cycling, and provisioning of habitat. Biodiversity is a supporting service that is increasingly recognized to sustain many of the goods and services that humans enjoy from ecosystems. These provide a basis for three higher-level categories of services.
- Provisioning services, such as products (Gitay et al., 2001), i.e., food (including game, roots, seeds, nuts and other fruit, spices, fodder), water, fiber (including wood, textiles), and medicinal and cosmetic products (such as aromatic plants, pigments).
- Regulating services that are of paramount importance for human society such as (1) C sequestration, (2) climate and water regulation, (3) protection from

natural hazards such as floods, avalanches, or rock-fall, (4) water and air purification, and (5) disease and pest regulation.

- Cultural services that satisfy human spiritual and aesthetic appreciation of ecosystems and their components including recreational and other nonmaterial benefits.

In the sections that follow, available information on individual, population and community response to $O_3$ will be discussed. Effects of $O_3$ on productivity and C sequestration, water cycling, below-ground processes, competition and biodiversity, and insects and wildlife are considered below and in the context of ecosystem services where appropriate.

### 9.4.2 Visible Foliar Injury and Biomonitoring

Visible foliar injury resulting from exposure to $O_3$ has been well characterized and documented over several decades on many tree, shrub, herbaceous, and crop species (U.S. EPA, 2006b, 1996b, 1984, 1978a). Visible foliar injury symptoms are considered diagnostic as they have been verified experimentally in exposure-response studies, using exposure methodologies such as CSTRs, OTCs, and free-air fumigation (see Section 9.2 for more detail on exposure methodologies). Several pictorial atlases and guides have been published, providing details on diagnosis and identification of $O_3$-induced visible foliar injury on many plant species throughout North America (Flagler, 1998; NAPAP, 1987) and Europe (Innes et al., 2001; Sánchez et al., 2001). Typical visible injury symptoms on broad-leaved plants include: stippling, flecking, surface bleaching, bifacial necrosis, pigmentation (e.g., bronzing), chlorosis, and/or premature senescence. Typical visible injury symptoms for conifers include: chlorotic banding, tip burn, flecking, chlorotic mottling, and/or premature senescence of needles. Although common patterns of injury develop within a species, these foliar lesions can vary considerably between and within taxonomic groups. Furthermore, the degree and extent of visible foliar injury development varies from year to year and site to site (Smith, 2012; Orendovici-Best et al., 2008; Chappelka et al., 2007; Smith et al., 2003), even among co-members of a population exposed to similar $O_3$ levels, due to the influence of co-occurring environmental and genetic factors (Souza et al., 2006; Chappelka et al., 2003; Somers et al., 1998). Nevertheless, Chappelka et al. (2007) reported that the average incidence of $O_3$-induced foliar injury was 73% on milkweed observed in the Great Smoky Mountains National Park in the years 1992-1996.

Although the majority of $O_3$-induced visible foliar injury occurrence has been observed on seedlings and small plants, many studies have reported visible injury of mature coniferous trees, primarily in the western U.S. (Arbaugh et al., 1998) and to mature deciduous trees in eastern North America (Schaub et al., 2005; Vollenweider et al., 2003; Chappelka et al., 1999a; Chappelka et al., 1999b; Somers et al., 1998; Hildebrand et al., 1996).

It is important to note that visible foliar injury occurs only when sensitive plants are exposed to elevated $O_3$ concentrations in a predisposing environment. A major modifying factor for $O_3$-induced visible foliar injury is the amount of soil moisture available to a plant during the year that the visible foliar injury is being assessed. This is because lack of soil moisture generally decreases stomatal conductance of plants and, therefore, limits the amount of $O_3$ entering the leaf that can cause injury (Matyssek et al., 2006; Panek, 2004; Grulke et al., 2003a; Panek and Goldstein, 2001; Temple et al., 1992; Temple et al., 1988). Consequently, many studies have shown that dry periods in local areas tend to decrease the incidence and severity of $O_3$-induced visible foliar injury; therefore, the incidence of visible foliar injury is not always higher in years and areas with higher $O_3$, especially with co-occurring drought (Smith, 2012; Smith et al., 2003). Other factors such as leaf age influence the severity of symptom expression with older leaves showing greater injury severity as a result of greater seasonal exposure (Zhang et al., 2010a).

Although visible injury is a valuable indicator of the presence of phytotoxic concentrations of $O_3$ in ambient air, it is not always a reliable indicator of other negative effects on vegetation. The significance of $O_3$ injury at the leaf and whole plant levels depends on how much of the total leaf area of the plant has been affected, as well as the plant's age, size, developmental stage, and degree of functional redundancy among the existing leaf area. Previous $O_3$ AQCDs have noted the difficulty in relating visible foliar injury symptoms to other vegetation effects such as individual plant growth, stand growth, or ecosystem characteristics (U.S. EPA, 2006b, 1996b). As a result, it is not presently possible to determine, with consistency across species and environments, what degree of injury at the leaf level has significance to the vigor of the whole plant. However, in some cases, visible foliar symptoms have been correlated with decreased vegetative growth (Somers et al., 1998; Karnosky et al., 1996; Peterson et al., 1987; Benoit et al., 1982) and with impaired reproductive function (Chappelka, 2002; Black et al., 2000). Conversely, the lack of visible injury does not always indicate a lack of phytotoxic concentrations of $O_3$ or a lack of non-visible $O_3$ effects (Gregg et al., 2006, 2003).

#### 9.4.2.1 Biomonitoring

The use of biological indicators to detect phytotoxic levels of $O_3$ is a longstanding and effective methodology (Chappelka and Samuelson, 1998; Manning and Krupa, 1992). A plant bioindicator can be defined as a vascular or nonvascular plant exhibiting a typical and verifiable response when exposed to a plant stress such as an air pollutant (Manning, 2003). To be considered a good indicator species, plants must (1) exhibit a distinct, verified response; (2) have few or no confounding disease or pest problems; and (3) exhibit genetic stability (U.S. EPA, 2006b). Such sensitive plants can be used to detect the presence of a specific air pollutant such as $O_3$ in the ambient air at a specific location or region and, as a result of the magnitude of their response, provide unique information regarding specific ambient air quality. Bioindicators can be either introduced sentinels, such as the widely used tobacco

(*Nicotiana tabacum*) variety Bel W3 (Calatayud et al., 2007b; Laffray et al., 2007; Nali et al., 2007; Gombert et al., 2006; Kostka-Rick and Hahn, 2005; Heggestad, 1991) or detectors, which are sensitive native plant species (Chappelka et al., 2007; Souza et al., 2006). The approach is especially useful in areas where $O_3$ monitors are not operated (Manning, 2003). For example, in remote wilderness areas where instrument monitoring is generally not available, the use of bioindicator surveys in conjunction with the use of passive samplers (Krupa et al., 2001) may be a useful methodology (Manning, 2003). However, it requires expertise in recognizing those signs and symptoms uniquely attributable to exposure to $O_3$ as well as in their quantitative assessment.

Since the 2006 $O_3$ AQCD, new sensitive plant species have been identified from field surveys and verified in controlled exposure studies (Kline et al., 2009; Kline et al., 2008). Several multiple-year field surveys have also been conducted at National Wildlife Refuges in Maine, Michigan, New Jersey, and South Carolina (Davis, 2009, 2007a, b; Davis and Orendovici, 2006).

The USDA Forest Service through the Forest Health Monitoring Program (FHM) (1990 - 2001) and currently the Forest Inventory and Analysis (FIA) Program has been collecting data regarding the incidence and severity of visible foliar injury on a variety of $O_3$ sensitive plant species throughout the U.S. (Smith, 2012; Coulston et al., 2003; Smith et al., 2003). The plots where these data are taken are known as biosites. These biosites are located throughout the country and analysis of visible foliar injury within these sites follows a set of established protocols. For more details, see http://www.nrs.fs.fed.us/fia/topics/ozone/ (USDA, 2011). The network has provided evidence of $O_3$ concentrations high enough to induce visible symptoms on sensitive vegetation. From repeated observations and measurements made over a number of years, specific patterns of areas experiencing visible $O_3$ injury symptoms can be identified. (Coulston et al., 2003) used information gathered over a 6-year period (1994-1999) from the network to identify several species that were sensitive to $O_3$ over entire regions, including sweetgum (*Liquidambar styraciflua*), loblolly pine (*Pinus taeda*), and black cherry (*P. serotina*). A recent paper by Smith et al. (2012) reported trends in foliar $O_3$ injury in the northeast and north central U.S. within the biomonitoring network over a 16-year period (1994-2009). The results showed that incidence and severity of foliar injury were dependent upon local site conditions (i.e., soil moisture availability) and $O_3$ exposure. Overall, there was a declining trend in the incidence of foliar injury as peak $O_3$ concentrations declined. Nevertheless, moderate $O_3$ exposures continued to cause foliar injury at sites throughout the region.

In a study of the west coast of the U.S, Campbell et al. (2007) reported $O_3$ injury in 25-37% of biosites in California forested ecosystems from 2000-2005.

A study by Kohut et al. (2007) assessed the estimated risk of $O_3$-induced visible foliar injury on bioindicator plants (NPS, 2006) in 244 national parks in support of the National Park Service's Vital Signs Monitoring Network (NPS, 2007). The risk assessment was based on a simple model relating response to the interaction of species, level of $O_3$ exposure, and exposure environment. Kohut et al. (2007)

concluded that the estimated risk of visible foliar injury was high in 65 parks (27%), moderate in 46 parks (19%), and low in 131 parks (54%). Some of the well-known parks with a high risk of $O_3$-induced visible foliar injury include Gettysburg, Valley Forge, Delaware Water Gap, Cape Cod, Fire Island, Antietam, Harpers Ferry, Manassas, Wolf Trap Farm Park, Mammoth Cave, Shiloh, Sleeping Bear Dunes, Great Smoky Mountains, Joshua Tree, Sequoia and Kings Canyon, and Yosemite.

Lichens have also long been used as biomonitors of air pollution effects on forest health (Nash, 2008). It has been suspected, based on field surveys in the San Bernardino Mountains surrounding the Los Angeles air basin, that declines in lichen diversity and abundance were correlated with measured $O_3$ gradients (Gül et al., 2011). Several recent studies in North America (Geiser and Neitlich, 2007; Gombert et al., 2006; Jovan and McCune, 2006) and Europe (Nali et al., 2007; Gombert et al., 2006) have used lichens as biomonitors of atmospheric deposition (e.g., N and S) and $O_3$ exposure. Nali et al. (2007) found that epiphytic lichen biodiversity was not related to $O_3$ geographical distribution. In addition, a recent study by Riddell et al. (2010) found that lichen species, *Ramalina menziesii*, showed no decline in physiological response to low and moderate concentrations of $O_3$ and may not be a good indicator for $O_3$ pollution. Mosses have also been used as biomonitors of air pollution; however, there remains a knowledge gap in the understanding of the effects of $O_3$ on mosses as there has been very little information available on this topic in recent years.

### 9.4.2.2 Summary

Visible foliar injury resulting from exposure to $O_3$ has been well characterized and documented over several decades of research on many tree, shrub, herbaceous, and crop species (U.S. EPA, 2006b, 1996b, 1984, 1978a). Ozone-induced visible foliar injury symptoms on certain bioindicator plant species are considered diagnostic as they have been verified experimentally in exposure-response studies, using exposure methodologies such as continuous stirred tank reactors (CSTRs), OTCs, and free-air fumigation. Experimental evidence has clearly established a consistent association of visible injury with $O_3$ exposure, with greater exposure often resulting in greater and more prevalent injury. Since the 2006 $O_3$ AQCD, results of several multi-year field surveys of $O_3$-induced visible foliar injury at National Wildlife Refuges in Maine, Michigan, New Jersey, and South Carolina have been published. New sensitive species showing visible foliar injury continue to be identified from field surveys and verified in controlled exposure studies.

The use of biological indicators in field surveys to detect phytotoxic levels of $O_3$ is a longstanding and effective methodology. The USDA Forest Service through the Forest Health Monitoring (FHM) Program (1990-2001) and currently the Forest Inventory and Analysis (FIA) Program has been collecting data regarding the incidence and severity of visible foliar injury on a variety of $O_3$ sensitive plant species throughout the United States. The network has provided evidence that $O_3$

concentrations were high enough to induce visible symptoms on sensitive vegetation. From repeated observations and measurements made over a number of years, specific patterns of areas experiencing visible $O_3$ injury symptoms can be identified. As noted in the preceding section, a study of 244 national parks indicated that the estimated risk of visible foliar injury was high in 65 parks (27%), moderate in 46 parks (19%), and low in 131 parks (54%).

Evidence is sufficient to conclude that there **is a causal relationship between ambient $O_3$ exposure and the occurrence of $O_3$-induced visible foliar injury on sensitive vegetation across the U.S.**

### 9.4.3 Growth, Productivity and Carbon Storage in Natural Ecosystems

Ambient $O_3$ concentrations have long been known to cause decreases in photosynthetic rates, decreases in growth, and decreases in yield (U.S. EPA, 2006b, 1996c, 1986, 1978a). The $O_3$-induced damages at the plant scale may translate to damages at the stand, then ecosystem scales, and cause changes in productivity and C storage. This section focuses on the responses of C cycling to seasonal or multi-year exposures to $O_3$ at levels of organization ranging from individual plants to ecosystems. Quantitative responses include changes in plant growth, plant biomass allocation, ecosystem production and ecosystem C sequestration. Most information available on plant-scale responses was obtained from studies that used a single species, especially tree seedlings and crops, while some used mixtures of herbaceous species. Ecosystem changes are difficult to evaluate in natural settings, due to the complexity of interactions, the number of potential confounders, and the large spatial and temporal scales. The discussion of ecosystem effects focuses on new studies at the large-scale FACE experiments and on ecological model simulations.

#### 9.4.3.1 Plant Growth and Biomass Allocation

The previous $O_3$ AQCDs concluded that there is strong evidence that exposure to $O_3$ decreases photosynthesis and growth in numerous plant species (U.S. EPA, 2006b, 1996b, 1984, 1978a). Studies published since the last review support those conclusions and are summarized below.

In general, research conducted over several decades has indicated that exposure to $O_3$ alters stomatal conductance and reduces photosynthesis in a wide variety of plant species. In a review of more than 55 studies, Wittig et al. (2007) reported that current $O_3$ concentrations in the northern hemisphere are decreasing stomatal conductance (13%) and photosynthesis (11%) across tree species. It was also found that younger trees (<4 years) were affected less by $O_3$ than older trees. Further, the authors also found that decreases in photosynthesis are consistent with the cumulative uptake of $O_3$ into the leaf. In contrast, several studies reported that $O_3$ exposure may result in loss of stomatal control, incomplete stomatal closure at night and a decoupling of

photosynthesis and stomatal conductance, which may have implications for whole-plant water use (Section 9.4.5).

In a recently published meta-analysis, Wittig et al. (2009) quantitatively compiled peer reviewed studies from the past 40 years on the effect of current and future $O_3$ exposures on the physiology and growth of forest species. They found that current ambient $O_3$ concentrations as reported in those studies significantly decreased annual total biomass growth (7%) across 263 studies. The authors calculated the ambient $O_3$ concentrations across these studies to average 40 ppb. This average was calculated across the duration of each study and there were therefore many hourly exposures well above 40 ppb. The decreased growth effect was reported to be greater (11 to 17%) in elevated $O_3$ exposures (97 ppb) (Wittig et al., 2009). This meta-analysis demonstrates the coherence of $O_3$ effects across numerous studies and species that used a variety of experimental techniques, and these results support the conclusion of the previous AQCD that exposure to $O_3$ decreases plant growth.

In two companion papers, McLauglin et al. (2007a; 2007b) investigated the effects of ambient $O_3$ on tree growth and hydrology at forest sites in the southern Appalachian Mountains. The authors reported that the cumulative effects of ambient levels of $O_3$ decreased seasonal stem growth by 30-50% for most tree species in a high $O_3$ year in comparison to a low $O_3$ year (McLaughlin et al., 2007a). The authors also reported that high ambient $O_3$ concentrations can increase whole-tree water use and in turn reduce late-season streamflow (McLaughlin et al., 2007b); see Section 9.4.5 for more on water cycling.

Since the 2006 $O_3$ AQCD, several recent studies have reported results from the Aspen FACE "free air" $O_3$ and $CO_2$ exposure experiment in Wisconsin (Darbah et al., 2008; Riikonen et al., 2008; Darbah et al., 2007; Kubiske et al., 2007; Kubiske et al., 2006; King et al., 2005). At the Aspen FACE site, single-species and two-species stands of trees were grown in 12, 30-m diameter rings corresponding to three replications of a full factorial arrangement of two levels each of $CO_2$ and $O_3$ exposure. Over the first seven years of stand development, Kubiske et al. (2006) observed that elevated $O_3$ decreased tree heights, diameters, and main stem volumes in the aspen community by 11, 16, and 20%, respectively. In addition, Kubiske et al. (2007) reported that elevated $O_3$ may change intra- and inter-species competition. For example, $O_3$ treatments increased the rate of conversion from a mixed aspen-birch community to a birch dominated community. In a comparison presented in Section 9.6.3 of this document, EPA found that effects on biomass accumulation in aspen during the first seven years closely agreed with the exposure-response function based on data from earlier OTC experiments.

Several studies at the Aspen FACE site also considered other growth-related effects of elevated $O_3$. Darbah et al. (2008; 2007) reported that $O_3$ treatments decreased paper birch seed weight and seed germination and that this would likely lead to a negative impact of regeneration for that species. Riikonen et al. (2008) found that elevated $O_3$ decreased the amount of starch in birch buds by 16%, and reduced aspen bud size, which may have been related to the observed delay in spring leaf development. The results suggest that elevated $O_3$ concentrations have the potential

to alter C metabolism of overwintering buds, which may have carry-over effects in the subsequent growing season (Riikonen et al., 2008).

Effects on growth of understory vegetation were also investigated at Aspen FACE. Bandeff et al. (2006) found that the effects of elevated $CO_2$ and $O_3$ on understory species composition, total and individual species biomass, N content, and $^{15}N$ recovery were a result of overstory community responses to those treatments; however, the lack of apparent direct $O_3$ treatment effects may have been due to high variability in the data. Total understory biomass increased with increasing light and was greatest under the open canopy of the aspen/maple community, as well as the more open canopy of the elevated $O_3$ treatments (Bandeff et al., 2006). Similarly, data from a study by Awmack et al. (2007) suggest that elevated $CO_2$ and $O_3$ may have indirect growth effects on red (*Trifolium pratense*) and white (*Trifolium repens*) clover in the understory via overstory community effects; however, no direct effects of elevated $O_3$ were observed.

Overall, the studies at the Aspen FACE experiment are consistent with many of the OTC studies that were evaluated in previous $O_3$ AQCDs demonstrating that $O_3$ exposure decreases growth in numerous plant species. These results strengthen the understanding of $O_3$ effects on forests and demonstrate the relevance of the knowledge gained from trees grown in open-top chamber studies.

For some annual species, particularly crops, the relevant measurement for an assessment of the risk of $O_3$ exposure is yield or growth, e.g., production of grain or biomass. For plants grown in mixtures such as hayfields, and natural or semi-natural grasslands (including native nonagricultural species), affected factors other than production of biomass may be important. Such endpoints include biodiversity or species composition, and effects on those endpoints may be indirect, resulting, for example, from competitive interactions among plants in mixed-species communities. Most of the available data on non-crop herbaceous species are for grasslands, with many of the recent studies conducted in Europe. See Section 9.4.7.2 for a review of the recent literature on $O_3$ effects on competition and biodiversity in grasslands.

### Root growth

Although $O_3$ does not penetrate soil, it could alter root development by decreasing C assimilation via photosynthesis leading to less C allocation to the roots (Andersen, 2003). The response of root development to $O_3$ exposure depends on available photosynthate within the plant and could vary over time. Many biotic and abiotic factors, such as community dynamics and drought stress, have been found to alter root development under elevated $O_3$. Generally, there is clear evidence that $O_3$ reduces C allocation to roots; however, results of a few recent individual studies have shown negative (Jones et al., 2010), non-significant (Andersen et al., 2010; Phillips et al., 2009) and positive effects (Pregitzer et al., 2008; Grebenc and Kraigher, 2007) on root biomass and root: shoot ratio.

An earlier study at the Aspen FACE experiment found that elevated $O_3$ reduced coarse root and fine roots biomass in young stands of paper birch and trembling aspen (King et al., 2001). However, this reduction disappeared several years later. Ozone significantly increased fine-root production (<1.0 mm) in the aspen community (Pregitzer et al., 2008). This increase in fine root production was due to changes in community composition, such as better survival of the $O_3$-tolerant aspen genotype, birch, and maple, rather than changes in C allocation at the individual tree level (Pregitzer et al., 2008; Zak et al., 2007). In an adult European beech/Norway spruce forest in Germany, drought was found to nullify the $O_3$-driven stimulation of fine root growth. Ozone stimulated fine-root production of beech during the humid year, but had no significant impact on fine root production in the dry year (Matyssek et al., 2010; Nikolova et al., 2010).

Using a non-destructive method, Vollsnes et al. (2010) studied the in vivo root development of subterranean clover (*Trifolium subterraneum*) before, during and after short-term $O_3$ exposure. It was found that $O_3$ reduced root tip formation, root elongation, the total root length, and the ratios between below- and above-ground growth within one week after exposure. Those effects persisted for up to three weeks; however, biomass and biomass ratios were not significantly altered at the harvest five weeks after exposure.

Several recent meta-analyses have generally indicated that $O_3$ reduced C allocated to roots. In one meta-analysis, Grantz et al. (2006) estimated the effect of $O_3$ on the root:shoot allometric coefficient (k), the ratio between the relative growth rate of the root and shoot. The results showed that $O_3$ reduced the root:shoot allometric coefficient by 5.6%, and the largest decline of the root:shoot allometric coefficient was observed in slow-growing plants. In another meta-analysis including 263 publications, Wittig et al. (2009) found that current $O_3$ exposure had no significant impacts on root biomass and root:shoot ratio when compared to pre-industrial $O_3$ exposure. However, if $O_3$ concentrations rose to 81-101 ppb (projected $O_3$ levels in 2100), both root biomass and root:shoot ratio were found to significantly decrease. Gymnosperms and angiosperms differed in their responses, with gymnosperms being less sensitive to elevated $O_3$. In two other meta-analyses, Wang et al. (2010) found elevated $O_3$ reduced biomass allocation to roots by 8.3% at ambient $CO_2$ and 6.0% at elevated $CO_2$, and Morgan et al. (2003) found $O_3$ reduced root dry weight of soybean.

#### 9.4.3.2 Summary

The previous $O_3$ AQCDs concluded that there is strong and consistent evidence that ambient concentrations of $O_3$ decrease photosynthesis and growth in numerous plant species across the United States . Studies published since the last review continue to support that conclusion.

The meta-analyses by Wittig et al. (2009; 2007) demonstrate the coherence of $O_3$ effects on plant photosynthesis and growth across numerous studies and species

using a variety of experimental techniques. Furthermore, recent meta-analyses have generally indicated that $O_3$ reduced C allocation to roots (Wittig et al., 2009; Grantz et al., 2006). Since the 2006 $O_3$ AQCD, several studies were published based on the Aspen FACE experiment using "free air," $O_3$, and $CO_2$ exposures in a planted forest in Wisconsin. Overall, the studies at the Aspen FACE experiment were consistent with many of the open-top chamber (OTC) studies that were the foundation of previous $O_3$ NAAQS reviews. These results strengthen the understanding of $O_3$ effects on forests and demonstrate the relevance of the knowledge gained from trees grown in open-top chamber studies.

Evidence is sufficient to conclude that there **is a causal relationship between ambient $O_3$ exposure and reduced growth of native woody and herbaceous vegetation.**

### 9.4.3.3 Reproduction

Studies during recent decades have demonstrated $O_3$ effects on various stages of plant reproduction. The impacts of $O_3$ on reproductive development, as reviewed by Black et al. (2000), can occur by influencing (1) age at which flowering occurs, particularly in long-lived trees that often have long juvenile periods of early growth without flower and seed production; (2) flower bud initiation and development; (3) pollen germination and pollen tube growth; (4) seed, fruit, or cone yields; and (5) seed quality (Table 9-1) (U.S. EPA, 2006b). Several recent studies since the 2006 $O_3$ AQCD further demonstrate the effects of $O_3$ on reproductive processes in herbaceous and woody plant species. Although there have been documented effects of $O_3$ on reproductive processes, a knowledge gap still exists pertaining to the exact mechanism of these responses.

Ramo et al. (2007) exposed several meadow species to elevated $O_3$ (40-50 ppb) and $CO_2$ (+100 ppm), both individually and combined, over three growing seasons in ground-planted mesocosms, using OTCs. Elevated $O_3$ delayed flowering of *Campanula rotundifolia* and *Vicia cracca*. Ozone also reduced the overall number of produced flowers and decreased fresh weight of individual *Fragaria vesca* berries.

Black et al. (2007) exposed *Brassica campestris* to 70 ppb for two days during late vegetative growth or ten days during most of the vegetative phase. The two-day exposure had no effect on growth or reproductive characteristics, while the 10 day exposure reduced vegetative growth and reproductive site number on the terminal raceme, emphasizing the importance of exposure duration and timing. Mature seed number and weight per pod were unaffected due to reduced seed abortion, suggesting that, although $O_3$ affected reproductive processes, indeterminate species such as *B. campestris* possess enough compensatory flexibility to avoid reduced seed production (Black et al., 2007).

In the determinate species, *Plantago major*, Black et al. (2010) found that $O_3$ may have direct effects on reproductive development in populations of differing

sensitivity. Only the first flowering spike was exposed to 120 ppb $O_3$ for 7 hours per day on 9 successive days (corresponding to flower development) while the leaves and second spike were exposed to charcoal-filtered air. Exposure of the first spike to $O_3$ affected seed number per capsule on both spikes even though spike two was not exposed. The combined seed weight of spikes one and two was increased by 19% in the two resistant populations, suggesting an overcompensation for injury, whereas, a decrease of 21% was observed in the most sensitive population (Black et al., 2010). The question remains as to whether these effects are true direct $O_3$-induced effects or compensatory responses.

Studies by Darbah et al. (2008; 2007) of paper birch (*Betula papyrifera*) trees at the Aspen FACE site in Rhinelander, WI investigated the effects of elevated $O_3$ and/or $CO_2$ on reproductive fitness. Elevated $O_3$ increased flowering, but decreased seed weight and germination success rate of seeds from the exposed trees. These results suggest that $O_3$ can dramatically affect flowering, seed production, and seed quality of paper birch, ultimately affecting its reproductive fitness (Darbah et al., 2008; Darbah et al., 2007).

**Table 9-1 Ozone effects on plant reproductive processes.**

| Species | Condition Measures | References |
|---|---|---|
| *Apocynum androsaemifolium* (spreading dogbane) | Flowering time | Bergweiler et al. (1999) |
| *Buddleia davidii* (butterfly bush) | Flowering time | Findley et al. (1997) |
| *Rubus cuneifolius* (sand blackberry) | Pollen germination | Chappelka et al. (2002) |
| *Plantago major* (plantain) | Pollen tube elongation | Stewart et al. (1998) |
| *Fragaria* × *ananassa* (cultivated strawberry) | Fruit yield | Drogoudi and Ashmore (2001; 2000) |
| *Plantago major* (plantain) | Seed yield | Lyons and Barnes (1998); Pearson et al. (1996); Reiling and Davison (1992); Whitfield et al. (1997) |
| Understory herbs | Seed yield | Harward and Treshow (1975) |

Source: Derived from Table AX9-22 of the 2006 $O_3$ AQCD.

### 9.4.3.4 Ecosystem Productivity and Carbon Sequestration

During the previous NAAQS review, there were limited studies that investigated the effect of $O_3$ exposure on ecosystem productivity and C sequestration. Recent studies from long-term FACE experiments provide more evidence of the association of $O_3$ exposure and changes in productivity at the ecosystem level of organization. In addition to experimental studies, model studies also assessed the impact of $O_3$ exposure on productivity and C sequestration from stand to global scales.

In this section productivity of ecosystems is expressed in different ways depending on the model or the measurements of a study. The most common metric of productivity is Gross Primary Productivity. Gross Primary Productivity (GPP) is total carbon that enters the ecosystem through photosynthesis by plants. Plants return a larger portion of this carbon back to the atmosphere through respiration from roots and aboveground portions of plants ($R_{plant}$). Net primary production (NPP) is the difference between total carbon gain (GPP) and carbon loss through $R_{plant}$. Net ecosystem production (NEP) is the difference between NPP and carbon loss through heterotrophic respiration ($R_{het}$) (mostly decomposition of dead organic matter) (Lambers et al., 1998). Similarly net ecosystem exchange (NEE) is the net flux of carbon between the land and the atmosphere, typically measured using eddy covariance techniques. Positive values of NEE usually refer to carbon released to the atmosphere (i.e., a source), and negative values refer to carbon uptake (i.e., a sink). Other studies have calculated net carbon exchange (NCE). NCE is defined as NPP minus $R_{het}$, $E_c$ (the carbon emission during the conversion of natural ecosystems to agriculture) and $E_p$ (the sum of carbon emission from the decomposition of agricultural products). For natural vegetation, $E_c$ and $E_p$ are equal to 0, so NCE is equal NEP (Felzer et al., 2005). In general, modeling studies take into account the effect of $O_3$ on C fixation of a system and there is generally not an effect on $R_{plant}$, $R_{het}$, $E_c$ or $E_p$. Therefore, decreases in GPP, NPP, NEP, NEE and NCE indicate a general decrease in productivity of an ecosystem.

Two types of models are most often used to study the ecological consequences of $O_3$ exposure: (1) single plant growth models such as TREGRO (Tree Growth Model) and PnET–II (Photosynthetic EvapoTranspiration-II model) (Hogsett et al., 2008; Martin et al., 2001; Ollinger et al., 1997b), and (2) process-based ecosystem models such as PnET-CN, Dynamic Land Ecosystem Model (DLEM), Terrestrial Ecosystem Model (TEM), or Met Office Surface Exchange Scheme - Top-down Representation of Interactive Foliage and Flora Including Dynamics (MOSES-TRIFFID) (Felzer et al., 2009; Ren et al., 2007b; Sitch et al., 2007; Ollinger et al., 2002) (Table 9-2). In these models, carbon uptake is simulated through photosynthesis (TREGRO, PnET –II, PnET- CN, DLEM and MOSES-TRIFFID) or gross primary production (TEM). Photosynthesis rate at leaf level is modeled by a function of stomatal conductance and other parameters in TREGRO, PnET –II, PnET- CN, DLEM and MOSES-TRIFFID. Photosynthesis at canopy level is calculated by summing either photosynthesis of different leaf types (TREGRO, DLEM, and MOSES-TRIFFID) or photosynthesis of different canopy layers (PnET –II, PnET- CN). The detrimental effect of $O_3$ on plant growth is often simulated by multiplying photosynthesis rate by a coefficient that is dependent on stomatal conductance and cumulative $O_3$ uptake (Table 9-2). Different plant functional groups (PFGs, such as deciduous trees, coniferous trees or crops) show different responses to $O_3$ exposure. PnET-II, PnET-CN, TEM, DLEM and MOSES-TRIFFID estimate this difference by modifying net photosynthesis with coefficients that represent the $O_3$ induced fractional reduction of photosynthesis for each functional group. The coefficients used in PnET-II, PnET-CN, TEM, DLEM are derived from the functions of $O_3$ exposure (AOT40) versus photosynthesis reduction from Reich et al. (1987) and Tjoelker et al. (1995). The coefficients used in MOSES-TRIFFID are derived from the $O_3$ dose-

photosynthesis response function from Pleijel et al. (2004a) and Karlsson et al. (2004), where $O_3$ dose is estimated by a metric named CUOt (cumulative stomatal uptake of $O_3$). The $O_3$ threshold of CUOt is 1.6 nmol/$m^2$/sec for woody PFT and 5 nmol/$m^2$/sec for grass PFT, and is different from AOT40, which has an $O_3$ threshold level of 40 ppb for all PFTs. Experimental and model studies on ecosystem productivity and C sequestration at the forest stand scale as well as regional and global scales are reviewed in the following section.

## Table 9-2 Comparison of models used to simulate the ecological consequences of $O_3$ exposure.

| Model | Model feature | Carbon uptake | Ozone effect | Reference |
|---|---|---|---|---|
| **TREGRO** | Hourly or daily step, single plant model simulating vegetation growth process | Leaf: leaf photosynthesis is a function of stomatal conductance, mesophyll conductance and the gradient of $CO_2$ from atmosphere to the mesophyll cells<br>Canopy: Leaf is divided into different ages. The canopy photosynthesis rate is the sum of the photosynthesis of all foliage groups | The effect of $O_3$ on photosynthesis is simulated by reducing mesophyll conductance, and increasing respiration. The degree of $O_3$ damage is determined by ambient $O_3$ exposure, and the threshold $O_3$ concentration below which $O_3$ does not affect mesophyll conductance and respiration | Hogsett et al. (2008); Weinstein et al. (2005); Tingey et al. (2004) |
| **PnET-II and PnET - CN** | PnET-II: Monthly time-step, single plant model<br>PnET –CN: Monthly time-step, ecosystem model | Leaf: Maximum photosynthesis rate is determined by a function of foliar N concentration, and stomatal conductance is determined by a function of the actual rate of the photosynthesis.<br>Canopy: canopy is divided into multiple, even-mass layers and photosynthesis is simulated by a multilayered canopy submodel | The effect of $O_3$ on photosynthesis is simulated by an equation of stomatal conductance and $O_3$ dose (AOT40). The model assumes that photosynthesis and stomatal conductance remain coupled under $O_3$ exposure, with a reduction in photosynthesis for a given month causing a proportion reduction in stomatal conductance. | Ollinger et al. (2002; 1997b); Pan et al. (2009) |
| **TEM** | Monthly time-step, ecosystem model | Ecosystem: TEM is run at a 0.5*0.5 degree resolution. Each grid cell is classified by vegetation type and soil texture, and vegetation and detritus are assumed to distribute homogeneously within grid cells. Carbon flows into ecosystem via gross primary production, which is a function of maximum rate of assimilation, photosynthetically active radiation, the leaf area relative to the maximum annual leaf area, mean monthly air temperate, and nitrogen availability. | The direct $O_3$ reduction on GPP is simulated by multiplying GPP by $f(O_3)t$, where $f(O_3)t$ is determined by evapotranspiration, mean stomatal conductance, ambient AOT40, and $O_3$ response coefficient empirically derived from previous publications. | Felzer et al. (2005; 2004) |
| **DLEM** | Daily time-step ecosystem model | Leaf: photosynthesis is a function of 6 parameters: photosynthetic photon flux density, stomatal conductance, daytime temperature, the atmospheric $CO_2$ concentration, the leaf N content and the length of daytime.<br>Canopy: Photosynthetic rates for sunlit leaf and shaded leaf scale up to the canopy level by multiplying the estimated leaf area index<br>Ecosystem: GPP is the sum of gross C fixation of different plant function groups | The detrimental effect of $O_3$ is simulated by multiplying the rate of photosynthesis by $O_3$eff, where $O_3$eff is a function of stomatal conductance, ambient AOT40, and $O_3$ sensitive coefficient. Ozone's indirect effect on stomatal conductance is also simulated, with a reduction in photosynthesis for a given month causing a reduction in stomatal conductance, and therefore canopy conductance. | Ren et al. (2007b; 2007a); Zhang et al. (2007a) |

| Model | Model feature | Carbon uptake | Ozone effect | Reference |
|---|---|---|---|---|
| **MOSES-TRIFFID** | 30 minute time-step, dynamic global vegetation model | Leaf: photosynthesis is a function of environmental and leaf parameters and stomatal conductance; Stomatal conductance is a function of the concentration of $CO_2$ and $H_2O$ in air at the leaf surface and the current rate of photosynthesis of the leaf<br>Canopy: Photosynthetic rates scale up to the canopy level by multiplying a function of leaf area index and PAR extinction coefficient<br>Ecosystem: GPP is the sum of gross C fixation of different plant function groups | The effect of $O_3$ is simulated by multiplying the rate of photosynthesis by F, where F depends upon stomatal conductance, $O_3$ exposure, a critical threshold for $O_3$ damage, and $O_3$ sensitive coefficient (functional type dependent) | Sitch et al. (2007) |

### Local scale

Both experimental and modeling studies have provided new information on effects of $O_3$ exposure at the stand or site level, i.e., at the local scale. The above- and below-ground biomass and net primary production (NPP) were measured at the Aspen FACE site after 7 years of $O_3$ exposure. Elevated $O_3$ caused 23, 13 and 14% reductions in total biomass relative to the control in the aspen, aspen–birch and aspen–maple communities, respectively (King et al., 2005). At the Kranzberg Forest FACE experiment in Germany, $O_3$ reduced annual volume growth by 9.5 $m^3$/ha in a mixed mature stand of Norway spruce and European beech (Pretzsch et al., 2010). At the grassland FACE experiment at Alp Flix, Switzerland, $O_3$ reduced the seasonal mean rates of ecosystem respiration and GPP by 8%, but had no significant impacts on aboveground dry matter productivity or growing season net ecosystem production (NEP) (Volk et al., 2011). Ozone also altered C accumulation and turnover in soil, as discussed in Section 9.4.6.

Changes in forest stand productivity under elevated $O_3$ were assessed by several model studies. TREGRO (Table 9-2) has been widely used to simulate the effects of $O_3$ on the growth of several species in different regions in the United States. Hogsett et al. (2008) used TREGRO to evaluate the effectiveness of various forms and levels of air quality standards for protecting tree growth in the San Bernardino Mountains of California. They found that $O_3$ exposures at the Crestline site resulted in a mean 20.9% biomass reduction from 1980 to 1985 and 10.3% biomass reduction from 1995 to 2000, compared to the ‘‘background’’ $O_3$ concentrations ($O_3$ concentration in Crook County, Oregon). The level of vegetation protection projected was different depending on the air quality scenarios under consideration. Specifically, when air quality was simulated to just meet the California 8-h average maximum of 70 ppb and the maximum 3 months 12-h SUM06 of 25 ppm-h, annual growth reductions were limited to 1% or less, while air quality that just met a previous NAAQS (the

2nd-highest 1-h max [125 ppb]) resulted in 6-7% annual reduction in growth, resulting in the least protection relative to background $O_3$ (Hogsett et al., 2008).

ZELIG is a forest succession gap model, and has been used to evaluate the dynamics of natural stand succession. Combining TREGRO with ZELIG, Weinstein et al. (2005) simulated the effects of different $O_3$ levels (0.5, 1.5, 1.75, and 2 times [×] ambient) on the growth and competitive interactions of white fir and ponderosa pine at three sites in California: Lassen National Park, Yosemite National Park, and Crestline. Their results suggested that $O_3$ had little impact on white fir, but greatly reduced the growth of ponderosa pine. If current $O_3$ concentrations continue over the next century, ambient $O_3$ exposure (SUM06 of 110 ppm-h) at Crestline was predicted to decrease individual tree C budget by 10% and decrease ponderosa pine abundance by 16%. Effects at Lassen National Park and Yosemite National Park sites were found to be smaller because of lower $O_3$ exposure levels (Weinstein et al., 2005).

To evaluate the influence of interspecies competition on $O_3$ effects, the linked TREGRO and ZELIG modeling system was used to predict the effects of $O_3$ over 100 years on the basal area of species in a *Liriodendron tulipifera*-dominated forest in the Great Smoky Mountains National Park (Weinstein et al., 2001). Ambient $O_3$ was predicted to decrease individual tree C budget by 28% and reduce the basal area of *L. tulipifera* by 10%, whereas a 1.5×-ambient exposure was predicted to cause a 42% decrease in the individual tree C budget and a 30% reduction in basal area. Individual tree C balance for *Acer rubrum* decreased 14% and 23% under ambient and 1.5×-ambient exposure, respectively. *Prunus serotina* was predicted to have less than a 2% decrease in tree C balance in all scenarios, but its basal area was greatly altered by the $O_3$ effects on the other tree species. Basal area of *A. rubrum* and *P. serotina* was predicted to increase for some years, but then decrease by up to 30%, depending on the scenario. The authors cautioned that the simulation results were heavily dependent on the assumption that only three of ten species studied could directly respond $O_3$ exposure and the rest of the species only indirectly responded through competitive interactions. Very different predictions of stand dynamics may have been simulated if more species could be parameterized to directly respond to $O_3$ exposure.

Some results from models that include competitive interactions between tree species, such as the linked TREGRO and ZELIG modeling system, may differ from empirical modeling based on short-term single-species $O_3$ exposure experiments. Single species experiments were often performed on tree seedlings for one to three years in open top chambers (OTCs) and indoor chambers (see Section 9.2). For example, OTC-based experiments that were used to create $O_3$ concentration-response relationships (discussed in Section 9.6.2) were the basis of estimated tree seedling biomass loss reported in studies by Hogsett et al. (1997) and in the 2007 EPA Staff Paper (2007b). These illustrative biomass loss analyses covered one or two years of $O_3$ exposure based on historical monitoring that was interpolated across regions of the United States. In contrast, competition models, such as the linked TREGRO and ZELIG modeling system, use empirical data to parameterize the simulation of growth

from a seedling into mature trees in competition with other trees (Weinstein et al., 2005; Weinstein et al., 2001). These simulations may be run for 100 years or more and have modeled annual exposures $O_3$ across those years. Complicated competitive interactions emerge across many decades of the simulation. For example, long-term competition simulations can take into account competition for space, light, water, tree longevity, disturbance, shade tolerance as well as the differential effects of $O_3$ on each species. As a result, a particular species may appear to grow poorly under $O_3$ exposure in short-term seedling studies, but may grow relatively well under long-term model scenarios with competition added to the analysis. It is important to note that both of these approaches provide useful information about the long and short term affects of $O_3$ exposure on trees forest stands. However, it is very difficult to validate the results of the long-term simulation of the effects of $O_3$ on forest composition.

The effects of $O_3$ on stand productivity and dynamics were also studied by other tree growth or stand models, such as ECOPHYS, INTRASTAND and LINKAGES. ECOPHYS is a functional-structural tree growth model. The model used the linear relationship between the maximum capacity of carboxylation and $O_3$ dose to predict the relative effect of $O_3$ on leaf photosynthesis (Martin et al., 2001). Simulations with ECOPHYS found that $O_3$ decreased stem dry matter production, stem diameter and leaf dry matter production, induced earlier leaf abscission, and inhibited root growth (Martin et al., 2001). INTRASTAND is an hourly time step model for forest stand carbon and water budgets. LINKAGES is a monthly time step model simulating forest growth and community dynamics. Linking INTRASTAND with LINKAGES, Hanson et al. (2005) found that a simulated increase in $O_3$ concentration in 2100 (a mean 20-ppb increase over the current $O_3$ concentration) yields a 35% loss of carbon (C) in the net ecosystem exchange (NEE) with respect to the current conditions (174 g C/$m^2$·year).

### Regional and global scales

Since the publication of the 2006 $O_3$ AQCD, there is additional evidence suggesting that $O_3$ exposure alters ecosystem productivity and biogeochemical cycling at the regional scale, i.e., at scales ranging from watershed to subcontinental divisions, and at continental and global scales. Most of those studies were conducted by using process-based ecosystem models (Table 9-2) and are briefly reviewed in the following sections.

Ollinger et al. (1997a) simulated the effect of $O_3$ on hardwood forest productivity of 64 hardwood sites in the northeastern U.S. with PnET-II (Table 9-2). Their simulations indicated that $O_3$ caused a 3-16% reduction in NPP from 1987 to 1992 (Table 9-3) The interactive effects of $O_3$, N deposition, elevated $CO_2$ and land use history on C dynamics were estimated by PnET-CN (Table 9-2) (Ollinger et al., 2002). The results indicated that $O_3$ offset the increase in net C exchange caused by elevated $CO_2$ and N deposition by 13% (25.0 g C/$m^2$·year) under agriculture site history, and 23% (33.6 g C/$m^2$·year) under timber harvest site history. PnET-CN was

also used to assess changes in C sequestration of U.S. Mid-Atlantic temperate forest. Pan et al. (2009) designed a factorial modeling experiment to separate the effects of changes in atmospheric composition, historical climatic variability and land-disturbances on the C cycle. They found that $O_3$ acted as a negative factor, partially offsetting the growth stimulation caused by elevated $CO_2$ and N deposition in U.S. Mid-Atlantic temperate forest. Ozone decreased NPP of most forest types by 7-8%. Among all the forest types, spruce-fir forest was most resistant to $O_3$ damage, and NPP decreased by only 1% (Pan et al., 2009).

Felzer et al. (2004) developed TEM 4.3 (Table 9-2) to simulate the effects of $O_3$ on plant growth and estimated effects of $O_3$ on NPP and C sequestration of deciduous trees, conifers and crops in the conterminous United States. The results indicated that $O_3$ reduced NPP and C sequestration in the U.S. (Table 9-3) with the largest decreases (over 13% in some locations) in NPP occurring in the Midwest agricultural lands during the mid-summer. TEM was also used to evaluate the magnitude of $O_3$ damage at the global scale (Table 9-2) (Felzer et al., 2005). Simulations for the period 1860 to 1995 show that the largest reductions in NPP and net C exchange occurred in the mid western U.S., eastern Europe, and eastern China (Felzer et al., 2005). DLEM (Table 9-2) was developed to simulate the detrimental effect of $O_3$ on ecosystems, and has been used to examine the $O_3$ damage on NPP and C sequestration in Great Smoky Mountains National Park (Zhang et al., 2007a), grassland ecosystems and terrestrial ecosystems in China (Ren et al., 2007b; Ren et al., 2007a). Results of those simulations are listed in Table 9-3.

Instead of using AOT40 as their $O_3$ exposure metric as PnET, TEM and DLEM did, Sitch et al. (2007) incorporated a different $O_3$ metric named CUOt (cumulative stomatal uptake of $O_3$), derived from Pleijel et al. (2004a), into the MOSES-TRIFFID coupled model (Table 9-2). In the CUOt metric, the fractional reduction of plant production is dependent on $O_3$ uptake by stomata over a critical threshold for damage with this threshold level varying by plant functional type. Consistent with previous studies, their model simulation indicated that $O_3$ reduced global gross primary production (GPP), C-exchange rate and C sequestration (Table 9-3). The largest reductions in GPP and land-C storage were projected over North America, Europe, China and India. In the model, reduced ecosystem C uptake due to $O_3$ damage results in additional $CO_2$ accumulation in the atmosphere and an indirect radiative forcing of climate change. Their simulations indicated that the indirect radiative forcing caused by $O_3$ (0.62-1.09 W/m$^2$) could have even greater impact on global warming than the direct radiative forcing of $O_3$ (0.89 W/m$^2$) (Sitch et al., 2007).

Results from the various model studies presented in Table 9-3 are difficult to compare because of the various spatial and temporal scales used. However, all the studies showed that $O_3$ exposure decreased ecosystem productivity and C sequestration. These results are consistent and coherent with experimental results obtained from studies at the leaf, plant and ecosystem scales (Sitch et al., 2007; Felzer et al., 2005). Many of the models use the same underlying function to simulate the effect of $O_3$ exposure to C uptake. For example the functions of $O_3$ exposure

(AOT40) versus photosynthesis reduction for PnET-II, PnET-CN, TEM, DLEM were all from Reich et al. (1987) and Tjoelker et al. (1995). Therefore, it is not surprising that the results are similar. While these models can be improved and more evaluation with experimental data can be done, these models represent the state of the science for estimating the effect of $O_3$ exposure on productivity and C sequestration.

### 9.4.3.5 Summary

During the previous NAAQS reviews, there were very few studies that investigated the effect of $O_3$ exposure on ecosystem productivity and C sequestration. Recent studies from long-term FACE experiments, such as Aspen FACE, SoyFACE and the Kranzberg Forest (Germany), provide evidence of the association of $O_3$ exposure and reduced productivity at the ecosystem level of organization. Studies at the leaf and plant scales show that $O_3$ decreased photosynthesis and plant growth, which provides coherence and biological plausibility for the decrease in ecosystem productivity. Results across different ecosystem models, such as TREGRO, PnET, TEM and DLEM, are consistent with the FACE experimental evidence, which show that $O_3$ reduced productivity of various ecosystems. Productivity is measured by various metrics such as GPP, NPP, NEP, NCE, NEE and individual tree biomass gain. All these metrics indicate a decrease in $CO_2$ fixation by the systems that were studied.

Although $O_3$ generally causes negative effects on plant growth, the magnitude of the response varies among plant communities. For example, $O_3$ had little impact on white fir, but greatly reduced growth of ponderosa pine in southern California (Weinstein et al., 2005). Ozone decreased net primary production (NPP) of most forest types in the Mid-Atlantic region, but had small impacts on spruce-fir forest (Pan et al., 2009).

In addition to plant growth, other indicators that are typically estimated by model studies include net ecosystem $CO_2$ exchange (NEE), C sequestration, and crop yield. Model simulations consistently found that $O_3$ exposure caused negative impacts on these indicators, but the severity of these impacts was influenced by multiple interactions of biological and environmental factors. The suppression of ecosystem C sinks results in more $CO_2$ accumulation in the atmosphere. Globally, the indirect radiative forcing caused by $O_3$ exposure through lowering the ecosystem C sink could have an even greater impact on global warming than the direct radiative forcing of $O_3$ (Sitch et al., 2007). Ozone could also affect regional C budgets through interacting with multiple factors, such as N deposition, elevated $CO_2$ and land use history. Model simulations suggested that $O_3$ partially offset the growth stimulation caused by elevated $CO_2$ and N deposition in both Northeast- and Mid-Atlantic-region forest ecosystems of the U.S. (Pan et al., 2009; Ollinger et al., 2002).

The evidence is sufficient to infer that there **is a causal relationship between $O_3$ exposure and reduced productivity,** and **a likely causal relationship between $O_3$ exposure and reduced carbon sequestration in terrestrial ecosystems.**

## Table 9-3 Modeled effects of $O_3$ on primary production, C exchange, and C sequestration.

| | Scale | Model | Index | $O_3$ Impacts | Reference |
|---|---|---|---|---|---|
| **GPP** | Global | MOSES-TRIFFID | CUOt[a] | Decreased by 14-23% over the period 1901-2100 | Sitch et al. (2007) |
| **NPP** | Global | TEM | AOT40 | Decreased by 0.8% without agricultural management and a decrease of 2.9% with optimal agricultural management | Felzer et al. (2005) |
| | U.S. | TEM | AOT40 | Reduced by 2.3% without optimal N fertilization and 7.2% with optimal N fertilization from 1983-1993 | Felzer et al. (2005) |
| | U.S. | TEM | AOT40 | Reduced by 2.6–6.8% during the late 1980s to early 1990s. | Felzer et al. (2004) |
| | Northeastern U.S. | PnET | AOT40 | A reduction of 3-16% from 1987-1992 | Ollinger et al. (1997a) |
| | U.S. Mid-Atlantic | PnET | AOT40 | Decreased NPP of most forest types by 7-8% | Pan et al. (2009) |
| | China | DLEM | AOT40 | Reduced NPP of grassland in China by 8.5 Tg[b] C from 1960s to 1990s | Ren et al. (2007a) |
| **C exchange** | Global | TEM | AOT40 | Reduced net C exchange (1950–1995) by 0.1 Pg C/yr without agricultural management and 0.3 Pg C/yr with optimal agricultural management | Felzer et al. (2005) |
| | Global | MOSES-TRIFFID | CUOt | Decreased global mean land–atmosphere C fluxes by 1.3 Pg C/yr and 1.7 Pg C/yr for the 'high' and 'low' plant $O_3$ sensitivity models, respectively | Sitch et al. (2007) |
| **C sequestration** | Global | MOSES-TRIFFID | CUOt | Reduced land-C storage accumulation by between 143 Pg C/yr and 263 Pg C/yr from 1900–2100 | Sitch et al. (2007) |
| | U.S. | TEM | AOT40 | Reduced C sequestration by 18–38 Tg C/yr from 1950 to 1995 | Felzer et al. (2004) |
| | GSM National Park | DLEM | AOT40 | Decreased the ecosystem C storage of deciduous forests by 2.5% and pine forest by 1.4% from 1971 to 2001 | Zhang et al. (2007a) |
| | China | DLEM | AOT40 | Reduced total C storage by 0.06% in 1960s and 1.6% in 1990s in China's terrestrial ecosystems | Ren et al. (2007b) |
| | China | DLEM | AOT40 | $O_3$ exposure reduced the net C sink of China's terrestrial ecosystem by 7% from 1961 to 2005 | Tian et al. (2011) |
| | China | DLEM | AOT40 | Ozone induced net carbon exchange reduction ranged from 0.4-43.1%, depending on different forest type | Ren et al. (2011) |

[a]CUOt is defined as the cumulative stomatal uptake of $O_3$, using a constant $O_3$-uptake rate threshold of t nmol/$m^2$/sec.

[b]Pg equals $1 \times 10^{15}$ grams.

### 9.4.4 Crop Yield and Quality in Agricultural Systems

The detrimental effect of $O_3$ on crop production has been recognized since the 1960s and a large body of research has stemmed from that recognition. Previous $O_3$ AQCDs have extensively reviewed this body of literature. Table 9-4 summarizes recent experimental studies of $O_3$ effects on agricultural crops, exclusive of growth and yield. Growth and yield results are summarized in Table 9-17.

The actual concentration and duration threshold for $O_3$ damage varies from species to species and sometimes even among genotypes of the same species (Guidi et al., 2009; Sawada and Kohno, 2009; Biswas et al., 2008; Ariyaphanphitak et al., 2005; Dalstein and Vas, 2005; Keutgen et al., 2005). A number of comprehensive reviews and meta-analyses have recently been published discussing both the current understanding of the quantitative effects of $O_3$ concentration on a variety of crop species and the potential focus areas for biotechnological improvement to a future growing environment that will include higher $O_3$ concentrations (Bender and Weigel, 2011; Booker et al., 2009; Van Dingenen et al., 2009; Ainsworth, 2008; Feng et al., 2008b; Hayes et al., 2007; Mills et al., 2007a; Grantz et al., 2006; Morgan et al., 2003). Since the 2006 $O_3$ AQCD (U.S. EPA, 2006b), exposure-response indices for a variety of crops have been suggested (Mills et al., 2007a) and many reports have investigated the effects of $O_3$ concentration on seed or fruit quality to extend the knowledge base beyond yield quantity. This section will outline the key findings from these papers as well as highlight some of the recent research addressing the endpoints such as yields and crop quality.

This section will also highlight recent literature that focuses on $O_3$ damage to crops as influenced by other environmental factors. Genetic variability is not the only factor that determines crop response to $O_3$ damage. Ozone concentration throughout a growing-season is not homogeneous and other environmental conditions such as elevated $CO_2$ concentrations, drought, cold or nutrient availability may alleviate or exacerbate the oxidative stress response to a given $O_3$ concentration.

#### 9.4.4.1 Yield

It is well known that yield is negatively impacted in many crop species in response to high $O_3$ concentration. However, the concentrations at which damage is observed vary from species to species. Numerous analyses of experiments conducted in OTCs and with naturally occurring gradients demonstrate that the effects of $O_3$ exposure also vary depending on the growth stage of the plant; plants grown for seed or grain are often most sensitive to exposure during the seed or grain-filling period (Soja et al., 2000; Pleijel et al., 1998; Younglove et al., 1994; Lee et al., 1988a). AX9.5.4.1 of the 2006 $O_3$ AQCD summarized many previous studies on crop yield.

### Field studies and meta-analyses

The effect of $O_3$ exposure on U.S. crops remains an important area of research and several studies have been published on this topic since the 2006 $O_3$ AQCD (U.S. EPA, 2006b) (Table 9-4 and Table 9-17). For example, one study with cotton in a crop-weed interaction study (Grantz and Shrestha, 2006) utilizing OTCs suggests that 12-hour average $O_3$ concentrations of 79.9 ppb decreased cotton biomass by 25% and 12-hour average $O_3$ concentration of 122.7 ppb decreased cotton biomass by 75% compared to charcoal filtered control (12-h avg: 12.8 ppb). Further, this study suggests that the weed, yellow nutsedge, was less sensitive to increasing $O_3$ concentration, which would increase weed competition (Grantz and Shrestha, 2006). In a study of peanuts in North Carolina, near ambient and elevated exposures of $O_3$ reduced photosynthesis and yield compared to very low $O_3$ conditions (Booker et al., 2007; Burkey et al., 2007). In another study, Grantz and Vu (2009) reported that sugarcane biomass growth significantly declined under $O_3$ exposure.

The average yield loss reported across a number of meta-analytic studies have been published recently for soybean (Morgan et al., 2003), wheat (Feng et al., 2008b), rice (Ainsworth, 2008), semi-natural vegetation (Hayes et al., 2007), potato, bean and barley (Feng and Kobayashi, 2009). Meta-analysis allows for the objective development of a quantitative consensus of the effects of a treatment across a wide body of literature. Further, this technique allows for a compilation of data across a range of $O_3$ fumigation techniques, durations and concentrations in order to assemble the existing literature in a meaningful manner.

Morgan et al. (2003) reported an average seed yield loss for soybean of 24% compared to charcoal filtered air across all $O_3$ concentrations used in the 53 compiled studies. The decrease in seed yield appeared to be the product of nearly equal decreases (7-12%) in seed weight, seed number and pod number. As would be expected, the lowest $O_3$ concentration (30-59 ppb) resulted in the smallest yield losses, approximately 8%, while the highest $O_3$ concentration (80-120 ppb) resulted in the largest yield losses, approximately 35% (Morgan et al., 2003). Further, the oil/protein ratio within the soybean seed was altered due to growth at elevated $O_3$ concentrations, with a decrease in oil content. The studies included in this meta-analysis all used enclosed fumigation systems or growth chambers which may have altered the coupling of the atmosphere to the lower plant canopy (McLeod and Long, 1999), although the results of Morgan et al. (2006), Betzelberger (2010), and the comparisons presented in Section 9.6.3 strongly suggest that decreases in yield between ambient and elevated exposures are not affected by exposure method. Utilizing the Soybean Free Air gas Concentration Enrichment Facility (SoyFACE; www.soyface.illinois.edu). Morgan et al. (2006) reported a 20% seed yield loss due to a 23% increase in average daytime $O_3$ concentration (56-69 ppb) within a single soybean cultivar across two growing seasons in Illinois, which lies within the range predicted by the meta-analysis. A further breakdown of the effects of current $O_3$ concentrations (AOT40 of 4.7 ppm-h) on bean seed quality (*Phaseolus vulgaris*) has identified that growth at current $O_3$ concentrations compared to charcoal-filtered air raised total lipids, total crude protein and dietary fiber content (Iriti et al., 2009).

An increase in total phenolics was also observed, however the individual phenolic compounds responded differently, with significant decreases in anthocyanin content. The seeds from ambient $O_3$ exposed plants also displayed increased total antioxidant capacity compared to charcoal-filtered air controls (Iriti et al., 2009). Betzelberger et al. (2010) has recently utilized the SoyFACE facility to compare the impact of elevated $O_3$ concentrations across 10 soybean cultivars to investigate intraspecific variability of the $O_3$ response to find physiological or biochemical markers for eventual $O_3$ tolerance breeding efforts (Betzelberger et al., 2010). They report an average 17% decrease in yield across all 10 cultivars across two growing seasons due to a doubling of ambient $O_3$ concentrations, with the individual cultivar responses ranging from -7% to -36%. The exposure-response functions derived for these 10 current cultivars were similar to the response functions derived from the NCLAN studies conducted in the 1980s (Heagle, 1989), suggesting there has not been any selection for increased tolerance to $O_3$ in more recent cultivars. More complete comparisons between yield predictions based on data from cultivars used in NCLAN studies, and yield data for modern cultivars from SoyFACE are reported in Section 9.6.3 of this document. They confirm that the response of soybean yield to $O_3$ exposure has not changed in current cultivars.

A meta-analysis has also been performed on studies investigating the effects of $O_3$ concentrations on wheat (Feng et al., 2008b). Across 23 studies included, elevated $O_3$ concentrations (ranging from a 7-h daily average of 31-200 ppb) decreased grain yield by 29%. Winter wheat and spring wheat did not differ in their responses; however the response in both varieties to increasing $O_3$ concentrations resulted in successively larger decreases in yield, from a 20% decrease in 42 ppb to 60% in 153 ppb $O_3$. These yield losses were mainly caused by a combination of decreases in individual grain weight (-18%), ear number per plant (-16%), and grain number per ear (-11%). Further, the grain starch concentration decreased by 8% and the grain protein yield decreased by 18% due to growth at elevated $O_3$ concentrations as well. However, increases in grain calcium and potassium levels were reported (Feng et al., 2008b).

A recent meta-analysis found that growth at elevated $O_3$ concentrations negatively impacts nearly every aspect of rice performance as well (Ainsworth, 2008). While rice is not a major crop in the U.S., it provides a staple food for over half of the global population (IRRI, 2002) and the effects of rising $O_3$ concentrations on rice yields merit consideration. On average, rice yields decreased 14% in 62 ppb $O_3$ compared to charcoal-filtered air. This yield loss was largely driven by a 20% decrease in grain number (Ainsworth, 2008).

Feng and Kobayashi (2009) have recently compiled yield data for six major crop species, potato, barley, wheat, rice, bean and soybean and grouped the $O_3$ treatments used in those studies into three categories: baseline $O_3$ concentrations (<26 ppb), current ambient 7- or 12-h daily $O_3$ concentrations (31-50 ppb), and future ambient 7- or 12-h daily $O_3$ concentrations (51-75 ppb). Using these categories, they have effectively characterized the effects of current $O_3$ concentrations and the effects of future $O_3$ concentrations compared to baseline $O_3$ concentrations. At current $O_3$

concentrations, which ranged from 41-49 ppb in the studies included, soybean (-7.7%), bean (-19.0%), barley (-8.9%), wheat (-9.7%), rice (-17.5%) and potato (-5.3%) all had yield losses compared to the baseline $O_3$ concentrations (<26 ppb). At future $O_3$ concentrations, averaging 63 ppb, soybean (-21.6%), bean (-41.4%), barley (-14%), wheat (-28%), rice (-17.5%) and potato (-11.9%) all had significantly larger yield losses compared to the losses at current $O_3$ concentrations (<26 ppb) (Feng and Kobayashi, 2009).

A review of OTC studies has determined the AOT40 critical level that causes a 5% yield reduction across a variety of agricultural and horticultural species (Mills et al., 2007a). The authors classify the species studied into three groups: sensitive, moderate and tolerant. The sensitive crops, including watermelon, beans, cotton, wheat, turnip, onion, soybean, lettuce, and tomato, respond with a 5% reduction in yield under a 3-month AOT40 of 6 ppm-h. Watermelon was the most sensitive with a critical level of 1.6 ppm-h. The moderately sensitive crops, including sugar beet, oilseed rape, potato, tobacco, rice, maize, grape and broccoli, responded with a 5% reduction in yield between 8.6 and 20 ppm-h. The crops classified as tolerant, including strawberry, plum and barley, responded with a 5% yield reduction between 62-83.3 ppm-h (Mills et al., 2007a).

Feng and Kobayashi (2009) compared their exposure-response results to those published by Mills et al. (2007a) and found the ranges of yield loss to be similar for soybean, rice and bean. However, Feng and Kobayashi (2009) reported smaller yield losses for potato and wheat and larger yield losses for barley compared to the dose-response functions published by Mills et al. (2007a), which they attributed to their more lenient criteria for literature inclusion.

While the studies investigating the impact of various $O_3$ concentrations on yield are important and aid in determining the vulnerability of various crops to a variety of $O_3$ concentrations, there is still uncertainty as to how these crops respond under field conditions with interacting environmental factors such as temperature, soil moisture, $CO_2$ concentration, and soil fertility (Booker et al., 2009). Further, there appears to be a distinct developmental and genotype dependent influence on plant sensitivity to $O_3$ that has yet to be fully investigated across $O_3$ concentrations in a field setting. The potentially mitigating effect of breeding selection for $O_3$ resistance has received very little attention in the published scientific literature. Anecdotal reports suggest that such selection may have occurred in recent decades for some crops in areas of the country with high ambient exposures. However, the only published literature available is on soybean and these studies indicate that sensitivity has not changed in cultivars of soybean between the 1980s and the 2000s (Betzelberger et al., 2010). This conclusion for soybeans is confirmed by comparisons presented in Section 9.6.3 of this document.

### Yield loss at regional and global scales

Because $O_3$ is heterogeneous in both time and space and $O_3$ monitoring stations are predominantly near urban areas, the impacts of $O_3$ on current crop yields at large

geographical scales are difficult to estimate. Fishman et al. (2010) have used satellite observations to estimate $O_3$ concentrations in the contiguous tri-state area of Iowa, Illinois and Indiana and have combined that information with other measured environmental variables to model the historical impact of $O_3$ concentrations on soybean yield across the 2002-2006 growing seasons. When soybean yield across Iowa, Indiana and Illinois was modeled as a function of seasonal temperature, soil moisture and $O_3$ concentrations, $O_3$ had the largest contribution to the variability in yield for the southern-most latitudes included in the dataset. Fishman et al. (2010) determined that $O_3$ concentrations significantly reduced soybean yield by 0.38 to 1.63% for every additional ppb of exposure across the 5 years. This value is consistent with previous chamber studies (Heagle, 1989) and results from SoyFACE (Morgan et al., 2006). Satellite estimates of tropospheric $O_3$ concentrations exist globally (Fishman et al., 2008), therefore utilizing this historical modeling approach is feasible across a wider geographical area, longer time-span and perhaps for more crop species.

The detrimental effects of $O_3$ on crop production at regional or global scales were also assessed by several model studies. Two large scale field studies were conducted in the U.S. (NCLAN) and in Europe (European Open Top Chamber Programme, EOTCP) to assess the impact of $O_3$ on crop production. Ozone exposure-response regression models derived from the two programs have been widely used to estimate crop yield loss (Avnery et al., 2011a, b; Van Dingenen et al., 2009; Tong and Mauzerall, 2008; Wang and Mauzerall, 2004). Those studies found that $O_3$ generally reduced crop yield and that different crops showed different sensitivity to $O_3$ pollution (Table 9-5). Ozone was calculated to induce a possible 45-82 million metric tons loss for wheat globally. Production losses for rice, maize and soybean were on the order of 17-23 million metric tons globally (Van Dingenen et al., 2009). The largest yield losses occur in high-production areas exposed to high $O_3$ concentrations, such the Midwest and the Mississippi Valley regions in the U.S., Europe, China and India (Van Dingenen et al., 2009; Tong et al., 2007).

#### 9.4.4.2 Crop Quality

In general, it appears that increasing $O_3$ concentrations above current ambient concentrations can cause species-dependent biomass losses, decreases in root biomass and nutritive quality, accelerated senescence and shifts in biodiversity. A study conducted with highbush blackberry has demonstrated decreased nutritive quality with increasing $O_3$ concentration despite no change in biomass between charcoal-filtered control, ambient $O_3$ and 2 × ambient $O_3$ exposures (Ditchkoff et al., 2009). A study conducted with sedge using control (30 ppb), low (55 ppb), medium (80 ppb) and high (105 ppb) $O_3$ treatments has demonstrated decreased root biomass and accelerated senescence in the medium and high $O_3$ treatments (Jones et al., 2010). Alfalfa showed no biomass changes across two years of double ambient $O_3$ concentrations (AOT40 of 13.9 ppm-h) using FACE fumigation (Maggio et al., 2009). However a modeling study has demonstrated that 84% of the variability in the

relative feed value in high-yielding alfalfa was due to the variability in mean $O_3$ concentration from 1998-2002 (Lin et al., 2007). Further, in a managed grassland FACE system, the reduction in total biomass harvest over five years decreased twice as fast in the elevated treatment (AOT40 of 13-59 ppm-h) compared to ambient (AOT40 of 1-20.7 ppm-h). Compared with the ambient control, loss in annual dry matter yield was 23% after 5 year. Further, functional groups were differentially affected, with legumes showing the strongest negative response (Volk et al., 2006). However, a later study by Stampfli and Fuhrer (2010) at the same site suggested that Volk et al. (2006) likely overestimated the effects of $O_3$ on yield reduction because the overlapping effects of species dynamics caused by heterogeneous initial conditions and a change in management were not considered by these authors. An OTC study conducted with *Trifolium subterraneum* exposed to filtered (<15 ppb), ambient, and 40 ppb above ambient $O_3$ demonstrated decreases in biomass in the highest $O_3$ treatment as well as 10-20% decreased nutritive quality which was mainly attributed to accelerated senescence (Sanz et al., 2005). A study conducted with Eastern gamagrass and big bluestem in OTCs suggested that big bluestem was not sensitive to $O_3$, but gamagrass displayed decreased nutritive quality in the 2 × ambient $O_3$ treatment, due to higher lignin content and decreased N (Lewis et al., 2006).

#### 9.4.4.3 Summary

The detrimental effect of $O_3$ on crop production has been recognized since the 1960s and a large body of research has subsequently stemmed from those initial findings. Previous $O_3$ AQCDs have extensively reviewed this body of literature (U.S. EPA, 2006b). Current $O_3$ concentrations across the U.S. are high enough to cause yield loss for a variety of agricultural crops including, but not limited to, soybean, wheat, potato, watermelon, beans, turnip, onion, lettuce, and tomato. Continued increases in $O_3$ concentration may further decrease yield in these sensitive crops. Despite the well-documented yield losses due to increasing $O_3$ concentration, there is still a knowledge gap pertaining to the exact mechanisms of $O_3$-induced yield loss. Research has linked increasing $O_3$ concentration to decreased photosynthetic rates and accelerated senescence, which are related to yield.

Recent research has highlighted the effects of $O_3$ on crop quality. Increasing $O_3$ concentration decreases nutritive quality of grasses, decreases macro- and micro-nutrient concentrations in fruits and vegetable crops, and decreases cotton fiber quality. It is important to note that these effects, as well as those mentioned above can occur without the expression of visible injury on the leaves. These areas of research require further investigation to determine mechanisms and exposure-response relationships.

During the previous NAAQS reviews, there were very few studies that estimated $O_3$ impacts on crop yields at large geographical scales. Recent modeling studies found that $O_3$ generally reduced crop yield, but the impacts varied across regions and crop

species. For example, the largest $O_3$-induced crop yield losses occurred in high-production areas exposed to high $O_3$ concentrations, such the Midwest and the Mississippi Valley regions of the U.S. (Van Dingenen et al., 2009). Among crop species, the estimated yield loss for wheat and soybean were higher than for rice and maize (Van Dingenen et al., 2009). Using satellite air-column observations with direct air-sampling $O_3$ data, Fishman et al. (2010) modeled the yield-loss due to $O_3$ over the continuous tri-state area of Illinois, Iowa and Wisconsin. They determined that $O_3$ concentrations significantly reduced soybean yield, which further reinforces previous results from FACE-type experiments and OTC experiments. Evidence is sufficient to conclude that **there is a causal relationship between $O_3$ exposure and reduced yield and quality of agricultural crops.**

## Table 9-4 Summary of recent studies of $O_3$ effects on crops (exclusive of growth and yield).

| Species<br>Facility<br>Location | Exposure Duration | Ozone Exposure[a]<br>(Additional treatment) | Variable(s) measured | Percent (%) change from CF[b]<br>(% change from ambient) | Reference |
|---|---|---|---|---|---|
| Alfalfa (*Medicago sativa* cv. Beaver)<br>Growth chambers | 1, 2 or 4 days | 3 or 5 hours/day 85 ppb<br>(Exposure duration) | Relative feed value | n.s.<br>*high variability among treatment groups (N/A) | Muntifering et al. (2006b) |
| Bean (*Phaseolus vulgaris* I. cv Borlotto)<br>OTC, ground-planted<br>Curno, Italy | 4 months | Seasonal AOT40:<br>CF = 0.5 ppm-h;<br>Ambient = 4.6 ppm-h<br>(N/A) | Seed lipid,<br>Protein content<br>Fiber content | +28.5 (N/A)<br>+7.88 (N/A)<br>+14.54 (N/A) | Iriti et al. (2009) |
| Big Blue Stem (*Andropogon gerardii*)<br>OTC<br>Alabama, U.S. | 4 months | 12-h avg:<br>CF = 14 ppb;<br>Ambient = 29 ppb;<br>Elevated = 71 ppb<br>(N/A) | Relative feed value | n.s. (n.s.) | Lewis et al. (2006) |
| *Brassica napus*<br>Growth chambers<br>Belgium | 4 days | CF & 176 ppb for 4 hours/day<br>(N/A) | Glucosinolates | −41 (N/A) | Gielen et al. (2006) |
| Brassica napus cv. Westar<br>Growth chambers<br>Finland | 17-26 days | 8-h avg:<br>CF & 100 ppb<br>(Bt/non-Bt; herbivory) | VOC emissions | −30.7 (N/A);<br>−34 (N/A) | Himanen et al. (2009b) |
| Eastern Gamagrass (*Tripsacum dactyloides*)<br>OTC<br>Alabama, U.S. | 4 months | 12-h avg:<br>CF = 14 ppb;<br>Ambient = 29 ppb;<br>Elevated = 71 ppb<br>(N/A) | Relative feed value | −17 (−12) | Lewis et al. (2006) |
| Lettuce (*Lactuca sativa*)<br>OTC<br>Carcaixent Experimental Station, Spain | 30 days | 12-h mean:<br>CF = 10.2 ppb;<br>NF = 30.1 ppb;<br>NF+$O_3$ = 62.7 ppb<br>(4 cultivars) | Lipid peroxidation;<br>Root length | +77 (+38)<br>−22 (−14) | Calatayud et al. (2002) |
| Peanut (*Arachis hypogaea*)<br>OTC<br>Raleigh, NC; U.S. | 3 yr | 12-h avg:<br>CF = 22 ppb;<br>Ambient = 46 ppb;<br>Elevated = 75 ppb<br>($CO_2$: 375 ppm; 548 ppm; 730 ppm) | Harvest biomass | −40 (−10) | Booker et al. (2007) |

| Species<br>Facility<br>Location | Exposure Duration | Ozone Exposure[a]<br>(Additional treatment) | Variable(s) measured | Percent (%) change from CF[b]<br>(% change from ambient) | Reference |
|---|---|---|---|---|---|
| *Poa pratensis*<br>OTC<br>Braunschweig, Germany | 3 yr;<br>4-5 weeks in the spring | 8-h avg:<br>CF+25 = 21.7 ppb;<br>NF+50 = 73.1 ppb<br>(Competition) | Relative feed value | N/A (n.s.; −8) | Bender et al. (2006) |
| Potato (*Solanum tuberosum* cv. Bintje)<br>OTC<br>Sweden & Finland | 2 yr | CF = 10 ppb;<br>Ambient = 25 ppb);<br>Ambient(+) = (36 ppb);<br>Ambient(++) = (47 ppb)<br>(N/A) | [K], [Ca], [Mg], [P], [N] per dry weight of tubers *dose-response regression, report significant positive or negative slope with increasing [$O_3$] | [N] [P] [Ca] n.s.;<br>[K] & [Mg] sig +<br>(N/A) | Piikki et al. (2007) |
| Potato (Solanum tuberosum cv. Indira)<br>Climate chambers<br>Germany | 8 weeks | CF = 10 ppb;<br>Ambient = 50 ppb;<br>2× Ambient = 100 ppb<br>($CO_2$: 400 ppm & 700 ppm) | Pathogen infestation using percent necrosis | +52 (n.s.) | Plessl et al. (2007) |
| Soybean<br>OTC<br>Italy | 3 yr | AOT40:<br>CF = 0 ppm-h;<br>Ambient = 3.4 ppm-h;<br>Elevated = 9.0 ppm-h<br>(Well-watered & water-stressed) | Daily evapotranspiration | −28 (−14) | Bou Jaoudé et al. (2008a) |
| Soybean (*Glycine max* cv. 93B15)<br>SoyFACE<br>Urbana, IL; U.S. | 3 yr<br>May-Oct | AOT40:<br>Ambient = 5-22 ppm-h;<br>Elevated = 20-43 ppm-h<br>($CO_2$: 550 ppm; environmental variability) | Photosynthesis in new leaves, | N/A (n.s.) | Bernacchi et al. (2006) |
| Soybean (*Glycine max* cv. 93B15)<br>SoyFACE<br>Urbana, IL; U.S. | 4 months | 8-h avg:<br>Ambient = 38.5 ppb;<br>Elevated = 52 ppb<br>(Herbivory) | Herbivory defense-related genes | N/A (N/A) | Casteel et al. (2008) |
| Soybean (*Glycine max* cv. Essex)<br>OTC, ground-planted<br>Raleigh, NC; U.S. | 2 yr | 12-h avg:<br>CF = 21 ppb;<br>1.5× Ambient = 74 ppb<br>($CO_2$: 370 ppm & 714 ppm) | Post-harvest residue | N/A (−15.46) | Booker et al. (2005) |
| Soybean (*Glycine max* cv. Essex)<br>OTCs, 21 L pots<br>Raleigh, NC; U.S. | 3 months | 12-h avg:<br>CF = 18 ppb);<br>Elevated = 72 ppb)<br>($CO_2$: 367 & 718) | Water-use efficiency | n.s. (N/A) | Booker et al. (2004b) |

| Species Facility Location | Exposure Duration | Ozone Exposure[a] (Additional treatment) | Variable(s) measured | Percent (%) change from CF[b] (% change from ambient) | Reference |
|---|---|---|---|---|---|
| Soybean (*Glycine max*) 10 cultivars)<br>SoyFACE<br>Urbana, IL; U.S. | 2 yr | 8-h avg (ppb):<br>Ambient = 46.3 & 37.9; Elevated = 82.5 & 61.3<br>(Cultivar comparisons) | Total antioxidant capacity | N/A (+19) | Betzelberger et al. (2010) |
| Spring Wheat (*Triticum aestivum* cv. Minaret; Satu; Drabant; Dragon)<br>OTCs<br>Belgium, Finland, & Sweden | 7 yr | Seasonal AOT40s ranged from:<br>0 to16 ppm-h<br>(N/A) | Seed protein content; | N/A (Significant negative correlation) | Piikki et al. (2008b) |
| | | | 1,000-seed weight regressed across all experiments | N/A (Significant negative correlation) | |
| Strawberry (*Fragaria x ananassa* Duch. Cv. Korona & Elsanta)<br>Growth chambers<br>Bonn, Germany | 2 months | 8-h avg:<br>CF = 0 ppb;<br>Elevated = 78 ppb<br>(N/A) | Total leaf area | −16 (N/A) | Keutgen et al. (2005) |
| Sweet Potato<br>Growth Chambers<br>Bonn, Germany | 4 weeks | 8-h avg:<br>CF = 0 ppb;<br>Ambient <40 ppb; Elevated = 255 ppb<br>(N/A) | Tuber weight | −14 (−11.5) | Keutgen et al. (2008) |
| Tomato (*Lycopersicon esculentum*)<br>OTC<br>Valencia, Spain | 133 days | 8- mean:<br>CF = 16.3 ppb;<br>NF = 30.1 ppb;<br>NF(+) = 83.2 ppb<br>(Various cultivars; early & late harvest) | Brix degree | −7.2 (−3.6) | Dalstein et al. (2005) |
| *Trifolium repens* & *Trifolium pretense*<br>Aspen FACE<br>Rhinelander, WI; U.S. | 3 months | 3-mo daylight avg:<br>Ambient = 34.8 ppb;<br>1.2× Ambient = 42.23 ppb<br>($CO_2$; 560 ppm) | Lignin;<br>Dry-matter digestibility | N/A (+19.3)<br>N/A (−4.2) | Muntifering et al. (2006a) |

[a]Ozone exposure in ppb unless otherwise noted.

[b]CF = Carbon-filtered air; NF = Non-filtered air.

**Table 9-5 Modeled effects of $O_3$ on crop yield loss at regional and global scales.**

| Scale | Index | $O_3$ Impacts | Reference |
|---|---|---|---|
| Global | M7a; M12b; AOT40 | Reduced by 7.3% to 12.3% for wheat, 5.4% to 15.6% for soybean, 2.8% to 3.7% for rice, and 2.4% to 4.1% for maize in year 2000. | Van Dingenen et al. (2009) |
| Global | M12b; AOT40 | $O_3$-induced global yield reductions ranged from 8.5-14% for soybean, 3.9-15% for wheat, and 2.2-5.5% for maize in year 2000. Global crop production losses totaled 79-121 million metric tons, worth $11-18 billion annually (in U.S. Dollars; 2000). | Avnery et al. (2011a) |
| U.S. | M7; M12; AOT40 | Reduced by 4.1% to 4.4% for wheat, 7.1% to 17.7% for soybean, 2.6% to 3.2% for rice, and 2.2% to 3.6% for maize in year 2000. | Van Dingenen et al. (2009) |
| U.S. | SUM06 | Caused a loss of 53.8 million to 438 million bushels in soybean production, which account for 1.7–14.2% of total U.S. soybean production in 2005 | Tong et al. (2007) |
| East Asia | M7; M12 | Reduced the yield of wheat, rice and corn by 1–9% and soybean by 23–27% in China, Japan and South Korea in 1990 | Wang et al. (2004) |

[a]M7 is defined as 7-hour mean $O_3$ concentration (ppb).

[b]M12 is defined as 12-hour mean $O_3$ concentration (ppb).

### 9.4.5 Water Cycling

Ozone can affect water use in plants and ecosystems through several mechanisms including damage to stomatal functioning and loss of leaf area. Figure 9-7 provides a simple illustration of potential effects of $O_3$ exposure on water cycling. Section 9.3.2 reviewed possible mechanisms for effects of $O_3$ exposure on stomatal functioning. This section on water cycling discusses how this alteration of stomatal functioning may affect water use in leaves, whole plants, a planted forest and watersheds. .

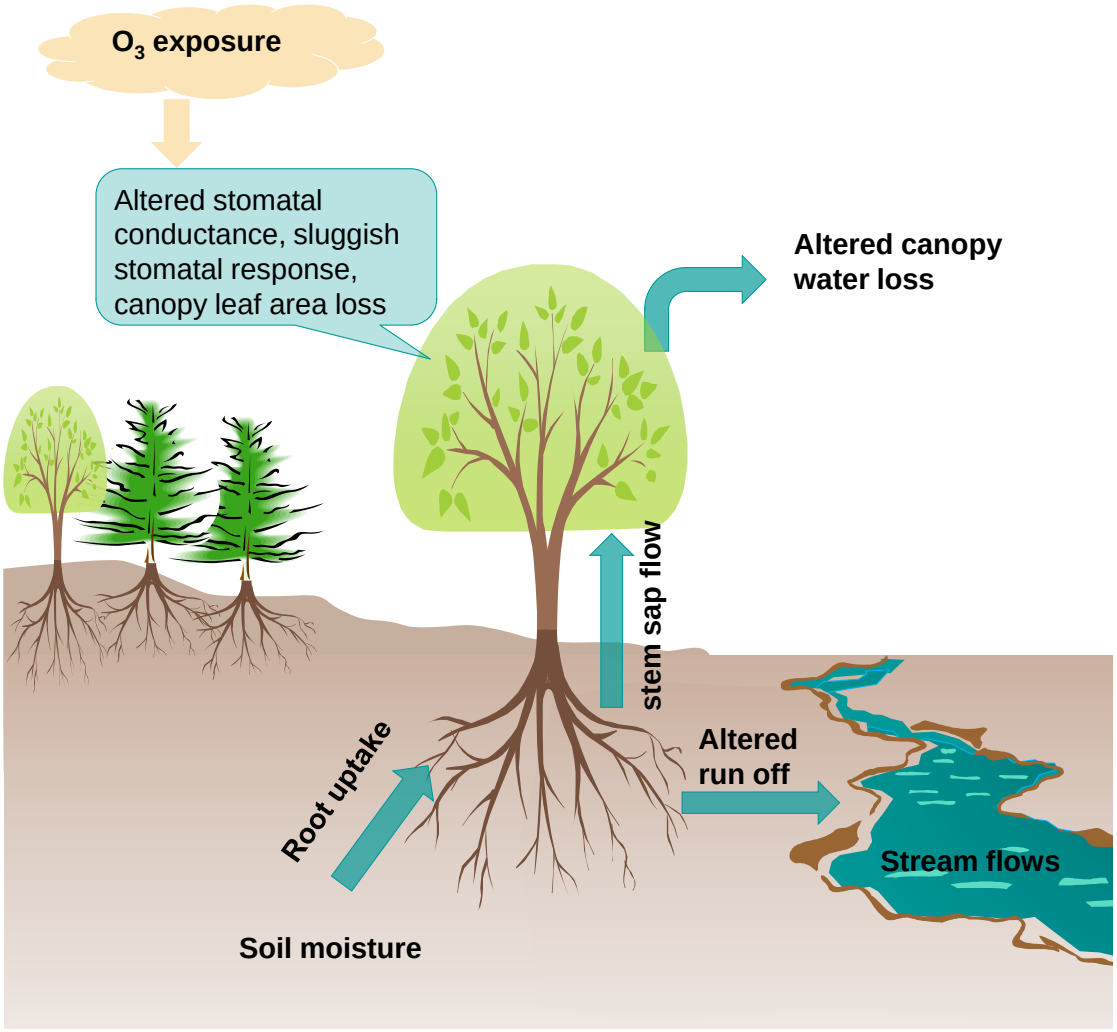



**Figure 9-7 The potential effects of $O_3$ exposure on water cycling.**

In the literature, there is not a clear consensus on the nature of leaf-level stomatal conductance response to $O_3$ exposure. At the leaf level, $O_3$ exposure is known to result in stomatal patchiness (Paoletti and Grulke, 2005; Omasa et al., 1987; Ellenson and Amundson, 1982), i.e., the heterogeneous aperture widths of stomata on the leaf surface, and, as a result, the collective response of groups of stomata on leaves and canopies determines larger-scale responses to $O_3$. When measured at steady-state high light conditions, leaf-level stomatal conductance is often found to be reduced when exposed to $O_3$. For example, a meta-analysis of 55 studies found that $O_3$ reduced stomatal conductance by 11% (Wittig et al., 2007). However, these steady-state measurements were generally taken at saturating light conditions and steady-state vapor pressure deficit (VPD). Saturating light and steady-state VPD conditions are not common in the field since many parts of the plant canopy are shaded throughout the day. When studied under varying environmental conditions, many

studies have reported incomplete stomatal closure with elevated $O_3$ exposure during the day (Mills et al., 2009; Grulke et al., 2007b; Matyssek et al., 1995; Wieser and Havranek, 1995) or at night (Grulke et al., 2004). This may be due to sluggish stomatal response. Sluggish stomatal response, defined as a delay in stomatal response to changing environmental factors relative to controls (Paoletti and Grulke, 2010) has also been documented by several researchers (Grulke et al., 2007c; Matyssek et al., 1995; Pearson and Mansfield, 1993; Wallin and Skärby, 1992; Lee et al., 1990; Skarby et al., 1987; Keller and Häsler, 1984; Reich and Lassoie, 1984). Sluggish stomatal response associated with $O_3$ exposure suggests an uncoupling of the normally tight relationship between carbon assimilation and stomatal conductance as measured under steady-state conditions (Gregg et al., 2006; Paoletti and Grulke, 2005). Several tree and ecosystem models, such as TREGRO, PnET and DLEM, rely on this tight relationship to simulate water and carbon dynamics. The $O_3$-induced impairment of stomatal control may be more pronounced for plants growing under water stress (Wilkinson and Davies, 2010; Grulke et al., 2007a; Paoletti and Grulke, 2005; Bonn et al., 2004; Kellomaki and Wang, 1997; Tjoelker et al., 1995; Reich and Lassoie, 1984). Since leaf-level stomatal regulation is usually assessed in a steady state rather than as a dynamic response to changing environmental conditions, steady state measurements cannot detect sluggish stomatal response. Because of sluggish stomatal responses, water loss from plants could be greater or reduced under dynamic environmental conditions over days and months. In situations where stomata fail to close under low light or water stressed conditions, water loss may be greater over time. In other situations, it is possible that slugglish stomata may fail to completely open in response to environmental stimuli and result in decreased water loss.

In addition to the impacts on stomatal performance, $O_3$-induced physiological changes, such as reduced leaf area index and accelerated leaf senescence could alter water use efficiency. It is well established from chamber and field studies that $O_3$ exposure is correlated with lower foliar retention (Karnosky et al., 2003; Topa et al., 2001; Pell et al., 1999; Grulke and Lee, 1997; Karnosky et al., 1996; Miller et al., 1972; Miller et al., 1963). However, Lee et al. (2009a) did not find changes in needle area of ponderosa pine and reported that greater canopy conductance followed by water stress under elevated $O_3$ may have been caused by stomatal dysfunction. At the Aspen FACE experiment, stand-level water use, as indicated by sap flux per unit ground area, was not significantly affected by elevated $O_3$ despite a 22% decrease in leaf area index and 20% decrease in basal area (Uddling et al., 2008). The same study reported a substantial increase in maximum sap flow per unit leaf area under elevated $O_3$, indicating higher canopy conductance compared to controls. A subsequent study at Aspen FACE (Uddling et al., 2009) reported that leaf-level conductance was not reduced by elevated $O_3$ as observed in most short-term experiments on tree seedlings (Wittig et al., 2007). The mean values of leaf-level conductance were always higher in elevated $O_3$ compared to controls, although this increase was not always statistically significant (Uddling et al., 2009). The authors also reported a less sensitive stomatal closure response to increasing vapor pressure deficit in pure aspen stands exposed to elevated $O_3$. This indicated that there was some evidence of impaired stomatal control. These studies at Aspen FACE also suggested that long-

term cumulative effects of elevated $O_3$ on tree and stand structure may be more important than the primary stomatal responses for understanding the effect of $O_3$ on stand-level water use (Uddling et al., 2009; 2008). Elevated $O_3$ could also affect evapotranspiration by altering tree crown interception of precipitation. Ozone was shown to change branch architectural parameters, and the effects were species-dependent at the Aspen FACE experiment (Rhea et al., 2010). The authors found that there was a significant correlation between canopy architecture parameters and stemflow (the flow of intercepted water down the stem of a tree) for birch but not aspen.

It is difficult to scale up physiology measurements from leaves to ecosystems. Thus, the current understanding of how stomatal response at the leaf scale is integrated at the scale of whole forest canopies, and therefore how it influences tree and forest stand water use is limited. Field studies by (McLaughlin et al., 2007a; 2007b) provided valuable insight into the possible consequences of stomatal sluggishness for ecosystem water cycling. McLaughlin et al. (2007a; 2007b) indicated that $O_3$ increased water use in a mixed deciduous forest in eastern Tennessee. McLaughlin et al. (2007a; 2007b) found that $O_3$, with daily maximum levels ranging from 69 to 83 ppb, reduced stem growth by 30-50% in the high-$O_3$ year 2002. The decrease in growth rate was caused in part by amplification of diurnal cycles of water loss and recovery. Peak hourly $O_3$ exposure increased the rate of water loss through transpiration as indicated by the increased stem sap flow. The authors suggested that a potential mechanism for the increased sap flow could be altered stomatal regulation from $O_3$ exposure, but this was inferred through sap flow measurements and was not directly measured. Alternatively, stomatal conductance may have increased under higher $O_3$ conditions (Paoletti and Grulke, 2010). The increased canopy water loss resulted in higher water uptake by the trees as reflected in the reduced soil moisture in the rooting zone. The change in tree water use led to further impacts on the hydrological cycle at the landscape level. Increased water use under high $O_3$ exposure was reported to reduce late-season modeled streamflow in three forested watersheds in eastern Tennessee (McLaughlin et al., 2007b).

Felzer et al. (2009) used TEM-Hydro to assess the interactions of $O_3$, climate, elevated $CO_2$ and N limitation on the hydrological cycle in the eastern United States. They found that elevated $CO_2$ decreased evapotranspiration by 2-4% and increased runoff by 3-7%, as compared to the effects of climate alone. When $O_3$ damage and N limitation were included, evapotranspiration was reduced by an additional 4-7% and runoff was increased by an additional 6-11% (Felzer et al., 2009). Based upon simulation with INTRAST and LINKAGES, Hanson et al. (2005) found that increasing $O_3$ concentration by 20 ppb above the current ambient level yields a modest 3% reduction in water use. Those ecological models were generally built on the assumption that $O_3$ induces stomatal closure and have not incorporated possible stomatal sluggishness due to $O_3$ exposure. Because of this assumption, results of those models normally found that $O_3$ reduced water use.

#### 9.4.5.1 Summary

Although the evidence was from a limited number of field and modeling studies, findings showed an association between $O_3$ exposure and alteration of water use and cycling in vegetation, and at the watershed level. There is not a clear consensus on the nature of leaf-level stomatal conductance response to $O_3$ exposure. When measured under steady-state high light conditions, leaf-level stomatal conductance is often found to be reduced when plants are exposed to $O_3$. However, measurements of stomatal conductance under dynamic light and VPD conditions indicate sluggish responses under elevated $O_3$ exposure, which could potentially lead to increased water loss from vegetation in some situations. Field studies conducted by McLaughlin et al. (2007a; 2007b) suggested that peak hourly $O_3$ exposure increased the rate of water loss from several tree species, and led to a reduction in the late-season modeled stream flow in three forested watersheds in eastern Tennessee. Sluggish stomatal responses during $O_3$ exposure was suggested as a possible mechanism for increased water loss during peak $O_3$ exposure. Currently, the $O_3$-induced reduction in stomatal aperture is the biological assumption for most process-based models. Because of this assumption, results of those models normally found that $O_3$ reduced water loss. For example, Felzer et al. (2009) found that $O_3$ damage and N limitation together reduced evapotranspiration and increased runoff.

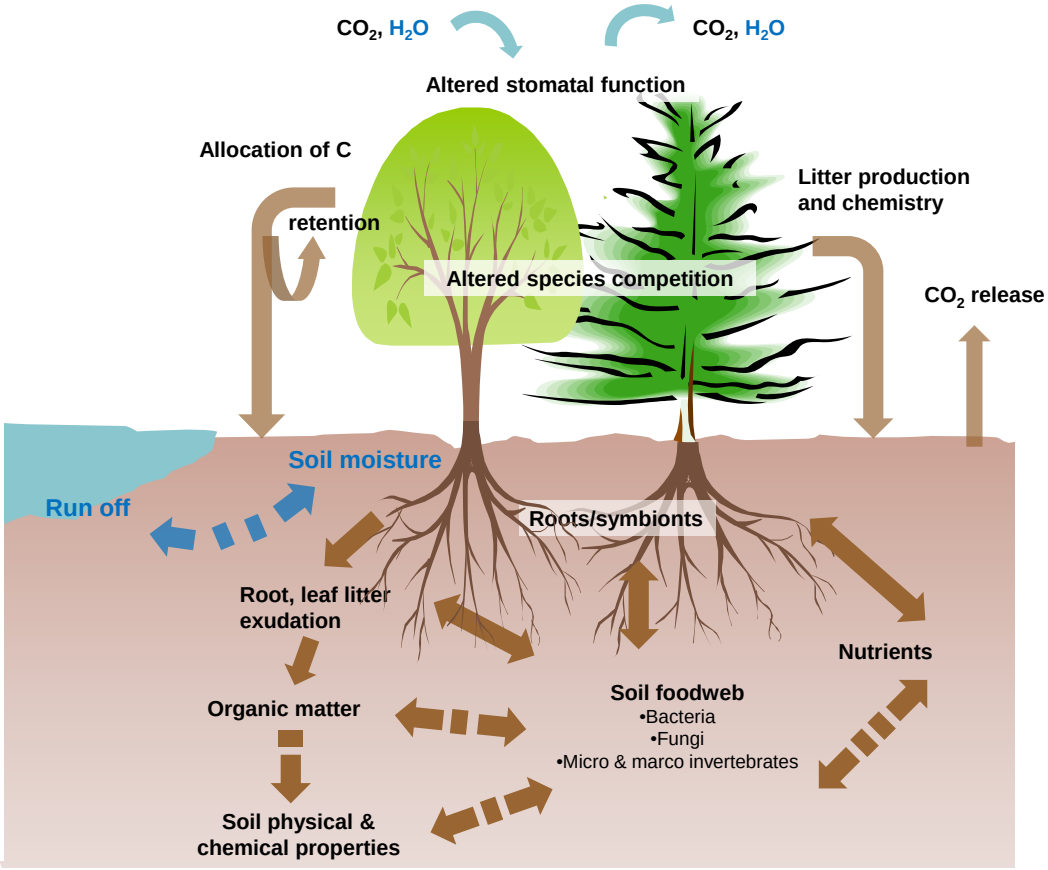
Although the direction of the response differed among studies, the evidence is sufficient to conclude that there **is likely to be a causal relationship between $O_3$ exposure and the alteration of ecosystem water cycling.**

### 9.4.6 Below-Ground Processes

Above-ground and below-ground processes are tightly interconnected. Because roots and soil organisms are not exposed directly to $O_3$, below-ground processes are affected by $O_3$ through alterations in the quality and quantity of C supply from photosynthates and litterfall (Andersen, 2003). Ozone can decrease leaf C uptake by reducing photosynthesis (Section 9.3). Ozone can also increase metabolic costs by stimulating the production of chemical compounds for defense and repair processes, and by increasing the synthesis of antioxidants to neutralize free radicals (see Section 9.3), both of which increase the allocation of carbon for above-ground processes. Therefore, $O_3$ could significantly reduce the amount of C available for allocation to below-ground by decreasing C uptake while increasing C consumption of above-ground processes (Andersen, 2003).

Since the 2006 $O_3$ AQCD, there is additional evidence for $O_3$ effects on below-ground processes. Ozone has been found to alter root growth, soil food web structure, decomposer activities, C turnover, water cycling and nutrient flow (Figure 9-8). Ozone effects on root development and root biomass production and soil food web structure are reviewed in Section 9.4.3.1 and Section 9.4.9.2, respectively. The focus

in this section is on the response of litter input, decomposer activities, soil respiration, soil C formation and nutrient cycling.




Note: Arrows denote C flux pathways that are affected by $O_3$. Dashed lines indicate where the impact of $O_3$ is suspected but unknown.

Source: Modified from Andersen et al. (2003).

**Figure 9-8 Conceptual diagram showing where $O_3$ alters C, water and nutrient flow in a tree-soil system, including transfer between biotic and abiotic components below ground that influence soil physical and chemical properties.**

### 9.4.6.1 Litter Carbon Chemistry, Litter Nutrient and Their Ecosystem Budgets

Consistent with previous findings, recent studies show that, although the responses are often species-dependent, $O_3$ tends to alter litter chemistry (U.S. EPA, 2006b). Alterations in chemical parameters, such as changes in C chemistry and nutrient concentrations, were observed in both leaf and root litter (Table 9-6).

At the Aspen FACE site, several studies investigated litter chemistry changes (Parsons et al., 2008; Johnson and Pregitzer, 2007; Chapman et al., 2005; Liu et al., 2005). In both aspen and birch leaf litter, elevated $O_3$ increased the concentrations of soluble sugars, soluble phenolics and condensed tannins (Parsons et al., 2008; Liu et al., 2005). Compared to other treatments, aspen litter under elevated $O_3$ had the highest fiber concentration, with the lowest concentration associated with the birch litter under the same conditions (Parsons et al., 2008). Chapman et al. (2005) measured chemical changes in fine root litter and found that elevated $O_3$ decreased lignin concentration. $O_3$-induced chemistry changes were also reported from other experimental sites. Results from an OTC study in Finland suggested that elevated $O_3$ increased the concentration of acid-soluble lignin, but had no significant impact on other chemicals such as total sugars, hemicelluloses, cellulose or total lignin in the litter of silver birch (Kasurinen et al., 2006). Results from the free air canopy $O_3$ exposure experiment at Kranzberg Forest showed that $O_3$ increased starch concentrations but had no impact on cellulose and lignin in beech and spruce leaf litter (Aneja et al., 2007). The effect of $O_3$ on three antioxidants (ascorbate, glutathione and $\alpha$-tocopherol) in fine roots of beech was also assessed at Kranzberg Forest. The results indicated that $O_3$ had no significant effect on $\alpha$-tocopherol and ascorbate concentrations, but decreased glutathione concentrations in fine roots (Haberer et al., 2008). In addition to changing C chemistry, $O_3$ also altered nutrient concentrations in green leaves and litter (Table 9-6).

The combined effects of $O_3$ on biomass productivity and chemistry changes may alter C chemicals and nutrient contents at the canopy or stand level. For example, although $O_3$ had different impacts on their concentrations, annual fluxes of C chemicals (soluble sugar, soluble phenolics, condensed tannins, lipid and hemicelluloses), macro nutrients (N, P, K and S) and micro nutrients (Mg, B, Cu and Zn) to soil were all reduced due to lower litter biomass productivity at Aspen FACE (Liu et al., 2007a; Liu et al., 2005). In a 2-year growth chamber experiment in Germany, N content of a spruce canopy in a mixed culture and Ca content of a beech canopy in a monoculture was increased due to elevated $O_3$, although leaf production was not significantly altered by $O_3$ (Rodenkirchen et al., 2009).

**Table 9-6 The effect of elevated $O_3$ on leaf/litter nutrient concentrations.**

| Study Site | Species | $O_3$ Concentration | Response | Reference |
|---|---|---|---|---|
| Suonenjoki Research Station, Finland | Silver birch | Ambient: 10-60 ppb<br>Elevated: 2× ambient | Decreased the concentration of P, Mn, Zn and B in leaf litter | Kasurinen et al. (2006) |
| Aspen FACE | Aspen and birch | Ambient: 50-60 ppb<br>Elevated: 1.5× ambient | Decreased the concentrations of P, S, Ca and Zn, but had no impact on the concentrations of N, K, Mg, Mn, B and Cu in leaf litter. | Liu et al. (2007a) |
| Aspen FACE | Birch | Ambient: 50-60 ppb<br>Elevated: 1.5× ambient | Increase N concentration in birch litter | Parsons et al. (2008) |
| Kranzberg Forest, Germany | Beech and spruce | Ambient: 9-41 ppb<br>Elevated: 2× ambient | Increased N concentration in beach leaf, but not in spruce needle | Kozovits et al. (2005) |
| Kranzberg Forest, Germany | Beech and spruce | Ambient: 9-41 ppb<br>Elevated: 2× ambient | (1) Had no significant effects on spruce needle chemistry;<br>(2) increased Ca concentration in beech leaves in monoculture, but had no impacts on other nutrients | Rodenkirchen et al. (2009) |
| Salerno, Italy | Holm oak | Non-filtered OTC: 29 ppb<br>Filtered OTC: 17ppb | $O_3$ had no significant impacts on litter C, N, lignin and cellulose concentrations | Baldantoni et al. (2011) |
| Kuopio University Research Garden, Finland | Red Clover | Ambient: 25.7 ppb<br>Elevated: 1.5× ambient | Increased the total phenolic content of leaves and had minor effects on the concentrations of individual phenolic compounds | Saviranta et al. (2010) |

### 9.4.6.2 Decomposer Metabolism and Litter Decomposition

The above- and below-ground physiological changes caused by $O_3$ exposure cascade through the ecosystem and affect soil food webs. In the 2006 $O_3$ AQCD, there were very few studies on the effect of $O_3$ on the structure and function of soil food webs, except two studies conducted by Larson et al. (2002) and Phillips et al. (2002). Since the last $O_3$ AQCD, new studies have provided more information on how $O_3$ affects the metabolism of soil microbes and soil fauna.

Chung et al. (2006) found that the activity of the cellulose-degrading enzyme 1,4-β-glucosidase was reduced by 25% under elevated $O_3$ at Aspen FACE. The decrease in cellulose-degrading enzymatic activity was associated with the lower cellulose availability under elevated $O_3$ (Chung et al., 2006). However, a later study at the same site, which was conducted in the 10th year of the experiment, found that $O_3$ had no impact on cellulolytic activity in soil (Edwards and Zak, 2011). In a lysimeter study of beech trees (*Fagus sylvatica*) in Germany, soil enzyme activity was found to be suppressed by $O_3$ exposure (Esperschutz et al., 2009; Pritsch et al., 2009). Except for xylosidase, enzyme activities involved in plant cell wall

degradation (cellobiohydrolase, beta-glucosidase and glucuronidase) were decreased in rhizosphere soil samples under elevated $O_3$ (2 × ambient level) (Pritsch et al., 2009). Similarly, Chen et al. (2009) found $O_3$ exposure, with a 3-month AOT40 of 21-44 ppm-h, decreased the microbial metabolic capability in the rhizosphere and bulk soil of wheat, although the observed reduction in bulk soil was not significant.

Ozone-induced change in soil organisms' activities could affect litter decomposition rates. Results of recent studies indicated that $O_3$ slightly reduced or had no impacts on litter decomposition (Liu et al., 2009b; Parsons et al., 2008; Kasurinen et al., 2006) (Baldantoni et al., 2011). The responses varied among species, sites and exposure length. Parsons et al. (2008) collected litter from aspen and birch seedlings at Aspen FACE site, and conducted a 23-month field litter incubation starting in 1999. They found that elevated $O_3$ had different impacts on the decomposition of aspen and birch litter. Elevated $O_3$ was found to reduce aspen litter decomposition. However, $O_3$ accelerated birch litter decomposition under ambient $CO_2$, but reduced it under elevated $CO_2$ (Parsons et al., 2008). Liu et al. (2009b) conducted another litter decomposition study at Aspen FACE from 2003 to 2006, when stand leaf area index (LAI) reached its maximum. During the 935-day field incubation, elevated $O_3$ was shown to reduce litter mass loss in the first year, but not in the second year. They suggested that higher initial tannin and phenolic concentrations under elevated $O_3$ reduced microbial activity in the first year (Liu et al., 2009b). In an OTC experiment, Kasurinen et al. (2006) collected silver birch leaf litter from three consecutive growing seasons and conducted three separate litter-bag incubation experiments. Litter decomposition was not affected by $O_3$ exposure in the first two incubations, but a slower decomposition rate was found in the third incubation. Their principle component analysis indicated that the litter chemistry changes caused by $O_3$ (decreased Mn, P, B and increased C:N) might be partially responsible for the decreased mass loss of their third incubation. In another OTC experiment, Baldantoni et al. (2011) found that $O_3$ significantly reduced leaf litter decomposition of *Quercus ilex* L, although litter C, N, lignin and cellulose concentrations were not altered by $O_3$ exposure.

### 9.4.6.3 Soil Respiration and Carbon Formation

Ozone could reduce the availability of photosynthates for export to roots, and thus, indirectly increase root mortality and turnover rates. Ozone has also been shown to reduce above-ground litter productivity and alter litter chemistry, which would affect the quality and quantity of the C supply to soil organisms (Section 9.4.6.1). The complex interactions among those changes make it difficult to predict the response of soil C cycling under elevated $O_3$. The 2006 $O_3$ AQCD concluded that $O_3$ had no consistent impact on soil respiration (U.S. EPA, 2006b). Ozone could increase or decrease soil respiration, depending on the approach and timing of the measurements. Ozone may also alter soil C formation. However, very few experiments directly measured changes in soil organic matter content under $O_3$ fumigation (U.S. EPA, 2006b). Recent studies on soil respiration and soil C content

also found mixed responses. Most importantly, recent results from long-term fumigation experiments, such as the Aspen FACE experiment, suggest that ecosystem response to $O_3$ exposure can change over time. Observations made during the late exposure years can be inconsistent with those during the early years, highlighting the need for caution when assessing $O_3$ effects based on short-term studies (Table 9-7).

### Soil Respiration

Ozone has shown inconsistent impacts on soil respiration. A sun-lit controlled-environment chamber study found that $O_3$ had no significant effects on soil respiration, fine root biomass or any of the soil organisms in a reconstructed ponderosa pine/soil-litter system (Tingey et al., 2006). In an adult European beech/Norway spruce forest at Kranzberg Forest, the free air $O_3$ fumigation (AOT40 of 10.2-117 ppm-h) increased soil respiration under both beech and spruce during a humid year (Nikolova et al., 2010). The increased soil respiration under beech has been accompanied by the increase in fine root biomass and ectomycorrhizal fungi diversity and turnover (Grebenc and Kraigher, 2007). The stimulating effect on soil respiration disappeared under spruce in a dry year, which was associated with a decrease in fine root production in spruce under drought. This finding suggested that drought was a more dominant stress than $O_3$ for spruce (Nikolova et al., 2010). Andersen et al. (2010) labeled the canopies of European beech and Norway spruce with $CO_2$ depleted in $^{13}C$ at the same site. They did not observe any significant changes in soil respiration for either species.

The nearly 10 year long studies at Aspen FACE indicated that the response of soil respiration to $O_3$ interacted with $CO_2$ exposure and varied temporally (Table 9-7) (Pregitzer et al., 2008; Pregitzer et al., 2006; King et al., 2001). Ozone treatment alone generally had the lowest mean soil respiration rates, although those differences between control and elevated $O_3$ were usually not significant. However, soil respiration rates were different with $O_3$ alone and when acting in combination with elevated $CO_2$. In the first five years (1998-2002), soil respiration under $+CO_2+O_3$ treatment was similar to that under control and lower than that under $+CO_2$ treatment (Pregitzer et al., 2006; King et al., 2001). Since 2003, $+CO_2+O_3$ treatment started to show the greatest impact on soil respiration. Compared to elevated $CO_2$, soil respiration rate under $+CO_2+O_3$ treatment was 15-25% higher from 2003-2004, and 5-10% higher from 2005-2007 (Pregitzer et al., 2008; Pregitzer et al., 2006). Soil respiration was highly correlated with the biomass of roots with diameters of <2 mm and <1 mm, across plant community and atmospheric treatments. The authors suggested that the increase in soil respiration rate may be due to $+CO_2+O_3$ increased fine root (<1.0 mm) biomass production (Pregitzer et al., 2008).

**Table 9-7 The temporal variation of ecosystem responses to $O_3$ exposure at Aspen FACE site**

| Endpoint | Period of Measurement | Response | Reference |
|---|---|---|---|
| Litter decomposition | 1999-2001 | $O_3$ reduced aspen litter decomposition. However, $O_3$ accelerated birch litter decomposition under ambient $CO_2$, but reduced it under elevated $CO_2$ | Parsons et al. (2008) |
| | 2003-2006 | $O_3$ reduced litter mass loss in the first year, but not in the second year. | Liu et al. (2009b) |
| Fine root production | 1999 | $O_3$ had no significant impact on fine root biomass | King et al. (2001) |
| | 2002, 2005 | $O_3$ increased fine root biomass | Pregitzer et al. (2008) |
| Soil respiration | 1998-1999 | Soil respiration under $+CO_2+O_3$ treatment was lower than that under $+CO_2$ treatment | King et al. (2001) |
| | 2003-2007 | Soil respiration under $+CO_2+O_3$ treatment was 5-25% higher than under elevated $CO_2$ treatment. | Pregitzer et al. (2008; 2006) |
| Soil C formation | 1998-2001 | $O_3$ reduced the formation rates of total soil C by 51% and acid-insoluble soil C by 48% | Loya et al. (2003) |
| | 2004-2008 | No significant effect of $O_3$ on the new C formed under elevated $CO_2$ | Talhelm et al. (2009) |

Changes in leaf chemistry and productivity due to $O_3$ exposure have been shown to affect herbivore growth and abundance (see Section 9.4.9.1). Canopy insects could affect soil carbon and nutrient cycling through frass deposition, or altering chemistry and quantity of litter input to the forest floor. A study at the Aspen FACE found that although elevated $O_3$ affected the chemistry of frass and greenfall, these changes had small impact on microbial respiration and no effect on nitrogen leaching (Hillstrom et al., 2010a). However, respiratory carbon loss and nitrate immobilization were nearly double in microcosms receiving herbivore inputs than those receiving no herbivore inputs (Hillstrom et al., 2010a).

### Soil Carbon Formation

Ozone-induced reductions in plant growth can result in reduced C input to soil and therefore soil C content (Andersen, 2003). The simulations of most ecosystem models support this prediction (Ren et al., 2007b; Zhang et al., 2007a; Felzer et al., 2004). However, very few studies have directly measured soil C dynamics under elevated $O_3$. After the first four years of fumigation (from 1998 to 2001) at the Aspen FACE site, Loya et al. (2003) found that forest stands exposed to both elevated $O_3$ and $CO_2$ accumulated 51% less total soil C, and 48% less acid-insoluble soil C compared to stands exposed only to elevated $CO_2$. Soil organic carbon (SOC) was continuously monitored at the Aspen FACE site, and the later data showed that the initial reduction in new C formation (soil C derived from plant litter since the start of the experiment) by $O_3$ under elevated $CO_2$ is only a temporary effect (Table 9-7) (Talhelm et al., 2009). The amount of new soil C in the elevated $CO_2$ and

the combined elevated $CO_2$ and $O_3$ treatments has converged since 2002. There was no significant effect of $O_3$ on the new C formed under elevated $CO_2$ over the last four years of the study (2004-2008). Talhelm et al. (2009) suggested the observed reduction in the early years of the experiment might be driven by a suppression of C allocated to fine root biomass. During the early exposure years, $O_3$ had no significant impact on fine root production (King et al., 2001). However, the effect of $O_3$ on fine root biomass was observed later in the experiment. Ozone increased fine root production and the highest fine root biomass was observed under the combined elevated $CO_2$ and $O_3$ treatment in the late exposure years (Table 9-7) (Pregitzer et al., 2006). This increase in fine root production was due to changes in community composition, such as better survival of an $O_3$-tolerant aspen genotype, birch and maple, rather than changes in C allocation at the individual tree level (Pregitzer et al., 2008; Zak et al., 2007).

#### 9.4.6.4 Nutrient Cycling

Ozone can affect nutrient cycling by changing nutrient release from litter, nutrient uptake by plants, and soil microbial activity. Nitrogen is the limiting nutrient for most temperate ecosystems, and several studies examined N dynamics under elevated $O_3$. Nutrient mineralization from decomposing organic matter is important for sustaining ecosystem production. Holmes et al. (2006) found that elevated $O_3$ decreased gross N mineralization at the Aspen FACE site, indicating that $O_3$ may reduce N availability. Other N cycling processes, such as $NH_4^+$ immobilization, gross nitrification, microbial biomass N and soil organic N, were not affected by elevated $O_3$ (Holmes et al., 2006). Similarly, Kanerva et al. (2006) found total N, $NO_3$-, microbial biomass N, potential nitrification and denitrification in their meadow mesocosms were not affected by elevated $O_3$ (40-50 ppb). Ozone was found to decrease soil mineral N content at SoyFACE, which was likely caused by a reduction in plant material input and increased denitrification (Pujol Pereira et al., 2011). Ozone also showed small impact on other micro and macro nutrients. Liu et al. (2007a) assessed N, P, K, S, Ca, Mg, Mn, B, Zn and Cu release dynamics at Aspen FACE, and they found that $O_3$ had no effects on most nutrients, except to decrease N and Ca release from litter. These studies reviewed above suggest that soil N cycling processes are not affected or slightly reduced by $O_3$ exposure. However, in a lysimeter study with young beech trees, Stoelken et al. (2010) found that elevated $O_3$ stimulated N release from litter which was largely attributed to an enhanced mobilization of inert nitrogen fraction.

Using the SImple NItrogen Cycle model (SINIC), Hong et al. (2006) evaluated the impacts of $O_3$ exposure on soil N dynamics and streamflow nitrate flux.
The detrimental effect of $O_3$ on plant growth was found to reduce plant uptake of N and therefore increase nitrate leaching. Their model simulation indicated that ambient $O_3$ exposure increased the mean annual stream flow nitrate export by 12% (0.042 g $N/m^2 \cdot$year) at the Hubbard Brook Experimental Watershed from 1964-1994 (Hong et al., 2006).

### 9.4.6.5 Dissolved Organic Carbon and Biogenic Trace Gases Emission

The $O_3$-induced changes in plant growth, C and N fluxes to soil and microbial metabolism can alter other biogeochemical cycling processes, such as soil dissolved organic carbon (DOC) turnover and trace gases emission.

Jones et al. (2009) collected fen cores from two peatlands in North Wales, UK and exposed them to one of four levels of $O_3$ (AOT40 of 0, 3.69, 5.87 and 13.80 ppm-h for 41 days). They found the concentration of porewater DOC in fen cores was significantly decreased by increased $O_3$ exposure. A reduction of the low molecular weight fraction of DOC was concurrent with the observed decrease in DOC concentration. Their results suggested that $O_3$ damage to overlying vegetation may decrease utilizable C flux to soil. Microbes, therefore, have to use labile C in the soil to maintain their metabolism, which, the authors hypothesized, leads to a decreased DOC concentration with a shift of the DOC composition to more aromatic, higher molecular weight organic compounds.

Several studies since the 2006 $O_3$ AQCD have examined the impacts of $O_3$ on nitrous oxide ($N_2O$) and methane ($CH_4$) emission. Kanerva et al. (2007) measured the fluxes of $N_2O$ and $CH_4$ in meadow mesocosms, which were exposed to elevated $CO_2$ and $O_3$ in OTCs in south-western Finland. They found that the daily $N_2O$ fluxes were decreased in the NF+$O_3$ (non-filtered air + elevated $O_3$, 40-50 ppb) after three seasons of exposure. Elevated $O_3$ alone or combined with $CO_2$ did not have any significant effect on the daily fluxes of $CH_4$ (Kanerva et al., 2007). In another study conducted in central Finland, the 4 year open air $O_3$ fumigation (AOT40 of 20.8-35.5 ppm-h for growing season) slightly increased potential $CH_4$ oxidation by 15% in the peatland microcosms, but did not affect the rate of potential $CH_4$ production or net $CH_4$ emissions, which is the net result of the potential $CH_4$ production and oxidation (Morsky et al., 2008). However, several studies found that $O_3$ could significantly reduce $CH_4$ emission. Toet et al. (2011) exposed peatland mesocosms to $O_3$ in OTCs for two years, and found that $CH_4$ emissions were significantly reduced by about 25% during midsummer periods of both years. In an OTC study of rice paddy, Zheng et al. (2011) found that the daily mean $CH_4$ emissions were significantly lower under elevated $O_3$ treatments than those in charcoal-filtered air and nonfiltered air treatments. They found that the seasonal mean $CH_4$ emissions were negatively related with AOT40, but positively related to the relative rice yield, aboveground biomass and underground biomass.

### 9.4.6.6 Summary

Since the 2006 $O_3$ AQCD, more evidence has shown that although the responses are often site specific, $O_3$ altered the quality and quantity of litter input to soil, microbial community composition, and C and nutrient cycling. Biogeochemical cycling of below-ground processes is fueled by C input from plants. Studies at the leaf and plant

level have provided biologically plausible mechanisms, such as reduced photosynthetic rates, increased metabolic cost, and reduced root C allocation for the association of $O_3$ exposure and the alteration of below-ground processes.

Results from Aspen FACE and other experimental studies consistently found that $O_3$ reduced litter production and altered C chemistry, such as soluble sugars, soluble phenolics, condensed tannins, lignin, and macro/micro nutrient concentration in litter (Parsons et al., 2008; Kasurinen et al., 2006; Liu et al., 2005). Under elevated $O_3$, the changes in substrate quality and quantity could alter microbial metabolism and therefore soil C and nutrient cycling. Several studies indicated that $O_3$ suppressed soil enzyme activities (Pritsch et al., 2009; Chung et al., 2006). However, the impact of $O_3$ on litter decomposition was inconsistent and varied among species, sites and exposure length. Similarly, $O_3$ had inconsistent impacts on dynamics of micro and macro nutrients.

Studies from the Aspen FACE experiment suggested that the response of below-ground C cycle to $O_3$ exposure, such as litter decomposition, soil respiration and soil C content, changed over time. For example, in the early part of the experiment (1998-2003), $O_3$ had no impact on soil respiration but reduced the formation rates of total soil C under elevated $CO_2$. However, after 10-11 years of exposure, $O_3$ was found to increase soil respiration but have no significant impact on soil C formation under elevated $CO_2$.

The evidence is sufficient to infer that there **is a causal relationship between $O_3$ exposure and the alteration of below-ground biogeochemical cycles.**

### 9.4.7 Community Composition

The effects of $O_3$ on species competition (AX9.3.3.4) and community composition (AX9.6.4) were summarized in the 2006 $O_3$ AQCD. Plant species differ in their sensitivity to $O_3$. Further, different genotypes of a given species also vary in their sensitivity. This differential sensitivity could change the competitive interactions that lead to loss in $O_3$ sensitive species or genotypes. In addition, $O_3$ exposure has been found to alter reproductive processes in plants (see Section 9.4.3.3). Changes in reproductive success could lead to changes in species composition. However, since ecosystem-level responses result from the interaction of organisms with one another and with their physical environment, it takes longer for a change to develop to a level of prominence at which it can be identified and measured. A shift in community composition in forest and grassland ecosystems noted in the 2006 $O_3$ AQCD has continued to be observed from experimental and gradient studies. Additionally, research since the last review has shown that $O_3$ can alter community composition and diversity of soil microbial communities.

### 9.4.7.1 Forest

In the San Bernardino Mountains in southern California, $O_3$ pollution caused a significant decline in ponderosa pine (*Pinus ponderosa*) and Jeffrey pine (*Pinus jeffreyi*) (U.S. EPA, 2006b). Pine trees in the young mature age class group exhibited higher mortality rates compared with mature trees at a site with severe $O_3$ visible foliar injury. The vulnerability of young mature pines was most likely caused by the fact that trees in this age class were emerging into the canopy, where higher $O_3$ concentrations were encountered (McBride and Laven, 1999). Because of the loss of $O_3$-sensitive pines, mixed forests of ponderosa pine, Jeffery Pine and white fir (*Abies concolor*) shifted to predominantly white fir (Miller, 1973). Ozone may have indirectly caused the decline in understory diversity in coniferous forests in the San Bernardino Mountains through an increase in pine litterfall. This increase in litterfall from $O_3$ exposure results in an understory layer that may prohibit the establishment of native herbs, but not the exotic annual *Galium aparine* (Allen et al., 2007).

Ozone damage to conifer forests has also been observed in several other regions. In the Valley of Mexico, a widespread mortality of sacred fir (*Abies religiosa*) was observed in the heavily polluted area of the Desierto de los Leones National Park in the early 1980s (de Lourdes de Bauer and Hernandez-Tejeda, 2007; Fenn et al., 2002). Ozone damage was widely believed to be an important causal factor in the dramatic decline of sacred fir. In alpine regions of southern France and the Carpathians Mountains, $O_3$ was also considered as the major cause of the observed decline in cembran pine (*Pinus cembra*) (Wieser et al., 2006). However, many environmental factors such as light, temperature, nutrient and soil moisture, and climate extremes such as unusual dry and wet periods could interact with $O_3$ and alter the response of forest to $O_3$ exposure. For those pollution gradient studies, several confounding factors, such as drought, insect outbreak and forest management, may also contribute to or even be the dominant factors causing the mortality of trees (de Lourdes de Bauer and Hernandez-Tejeda, 2007; Wieser et al., 2006).

Recent evidence from long-term free $O_3$ fumigation experiments provided additional support for the potential impacts of $O_3$ on species competition and community composition changes in forest ecosystems. At the Aspen FACE site, community composition at both the genetic and species levels was altered after seven years of fumigation with $O_3$ (Kubiske et al., 2007). In the pure aspen community, $O_3$ fumigation reduced growth and increased mortality of sensitive clone 259, while the $O_3$ tolerant clone 8L emerged as the dominant clone. Growth of clone 8L was even greater under elevated $O_3$ compared to controls, probably due to $O_3$ alleviated competitive pressure on clone 8L by reducing growth of other clones. In the mixed aspen-birch and aspen-maple communities, $O_3$ reduced the competitive capacity of aspen compared to birch and maple (Kubiske et al., 2007). In a phytotron study, $O_3$ fumigation reduced growth of beech but not spruce in mixed culture, suggesting a higher susceptibility of beech to $O_3$ under interspecific competition (Kozovits et al., 2005).

### 9.4.7.2 Grassland and Agricultural Land

The response of managed pasture, often cultivated as a mixture of grasses and clover, to $O_3$ pollution has been studied for many years. The tendency for $O_3$-exposure to shift the biomass of grass-legume mixtures in favor of grass species, reported in the previous $O_3$ AQCD has been generally confirmed by recent studies. In a mesocosm study, *Trifolium repens* and *Lolium perenne* mixtures were exposed to an episodic rural $O_3$ regime within solardomes for 12 weeks. *T. repens* showed significant changes in biomass but not *L. perenne*, and the proportion of *T. repens* decreased in $O_3$-exposed mixtures compared to the control (Hayes et al., 2009). The changes in community composition of grass-legume-forb mixtures were also observed at the Le Mouret FACE experiment, Switzerland. During the 5-year $O_3$ fumigation (AOT40 of 13.3-59.5 ppm-h), the dominance of legumes in fumigated plots declined more quickly than those in the control plots (Volk et al., 2006). However, Stampfli and Fuhrer (2010) reanalyzed the species and soil data and suggested that Volk et al. (2006) overestimated the $O_3$ effect. Stampfli and Fuhrer (2010) found that the difference in the species dynamics between control and $O_3$ treatment was more caused by heterogeneous initial conditions than $O_3$ exposure. Several studies also suggested that mature/species-rich ecosystems were more resilient to $O_3$ exposure. At another FACE experiment, located at Alp Flix, Switzerland, $O_3$ fumigation (AOT40 of 15.2-64.9 ppm-h) showed no significant impact on community composition of this species-rich pasture (Bassin et al., 2007b). Although most studies demonstrated an increase in grass:forb ratio with $O_3$ exposure (Hayes et al., 2009; U.S. EPA, 2006b), a study on a simulated upland grassland community showed that $O_3$ reduced the grass:forb ratio (Hayes et al., 2010) which may be due to the grass species in this community. The grass species studied by Hayes et al. (2010), *Anthoxanthum odoratum*, was more sensitive to $O_3$ than other grass species such as *L. perenne* (Hayes et al., 2009). Pfleeger et al. (2010) collected seed bank soil from an agricultural field and examined how the plant community responded over several generations to elevated $O_3$ exposures. Sixty plant species from 22 families emerged in the chambers over their four year study. Overall, they found that $O_3$ appeared to have small impacts on seed germination and only a minor effect on species richness of pioneer plant communities.

Several review papers have discussed the physiological and ecological characteristics of $O_3$-sensitive herbaceous plants. Hayes et al. (2007) assessed species traits associated with $O_3$ sensitivity by the changes in biomass caused by $O_3$ exposure. Plants of the therophyte (e.g., annual) life form were particularly sensitive to $O_3$. Species with higher mature leaf N concentration tended to be more sensitive than those with lower leaf N concentration. Plants growing under high oxidative stress environments, such as high light or high saline, were more sensitive to $O_3$. Using the same dataset from Hayes et al. (2007), Mills et al. (2007b) identified the $O_3$ sensitive communities. They found that the largest number of these $O_3$ sensitive communities were associated with grassland ecosystems. Among grassland ecosystems, alpine grassland, sub-alpine grassland, woodland fringe, and dry grassland were identified as the most sensitive communities.

### 9.4.7.3 Microbes

Several methods have been used to study microbial composition changes associated with elevated $O_3$. Phospholipid fatty acid (PLFA) analysis is widely used to determine whether $O_3$ elicits an overall effect on microbial community composition. However, since PLFA markers cover a broad range of different fungi, resolution of this method may be not fine enough to detect small changes in the composition of fungal communities. Methods, such as microscopic analyses and polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE), have better resolution to specifically analyze the fungal community composition. The resolution differences among those methods needs to be considered when assessing the $O_3$ impact on microbial community composition.

Kanerva et al. (2008) found that elevated $O_3$ (40-50 ppb) decreased total, bacterial, actinobacterial and fungal PLFA biomass values as well as fungal:bacterial PLFA biomass ratio in their meadow mesocosms in south-western Finland. The relative proportions of individual PLFAs between the control and elevated $O_3$ treatments were significantly different, suggesting that $O_3$ modified the structure of the microbial community. Morsky et al. (2008) exposed boreal peatland microcosms to elevated $O_3$, with growing season AOT40 of 20.8-35.3 ppm-h, in an open-air $O_3$ exposure field in Central Finland. They also found that microbial composition was altered after three growing seasons with $O_3$ fumigation, as measured by PLFA. Ozone tended to increase the presence of Gram-positive bacteria and the biomass of fungi in the peatland microcosms. Ozone also resulted in higher microbial biomass, which co-occurred with the increases in concentrations of organic acids and leaf density of sedges (Morsky et al., 2008). In a lysimeter experiment in Germany, $O_3$ was found to alter the PLFA profiles in the upper 0-20 cm rhizosphere soil of European beech. Elevated $O_3$ reduced bacterial abundance but had no detectable effect on fungal abundance (Pritsch et al., 2009). Using microscopic analyses, Kasurinen et al. (2005) found that elevated $O_3$, with 5 or 6 months of AOT40 of 20.6-30.9 ppm-h, decreased the proportions of black and liver-brown mycorrhizas and increased that of light brown/orange mycorrhizas. In an herbaceous plant study, SSCP (single-strand conformation polymorphism) profiles indicated that $O_3$ stress (about 75 ppb) had a very small effect on the structural diversity of the bacterial community in rhizospheres (Dohrmann and Tebbe, 2005). At the Aspen FACE site, $O_3$ had no significant effect on fungal relative abundance, as indicated by PLFA profile. However, elevated $O_3$ altered fungal community composition, according to the identification of 39 fungal taxonomic units from soil using polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE) (Chung et al., 2006). In another study at Aspen FACE, phylogenetic analysis suggested that $O_3$ exposure altered the agaricomycete community. The ectomycorrhizal communities developing under elevated $O_3$ had higher proportions of *Cortinarius* and *Inocybe* species, and lower proportions of *Laccaria* and *Tomentella* (Edwards and Zak, 2011). Ozone was found to change microbial community composition in an agricultural system. Chen et al. (2010b) found elevated $O_3$ (100-150 ppb) had significant effects on soil microbial composition expressed as PLFA percentage in a rice paddy in China.

#### 9.4.7.4 Summary

In the 2006 $O_3$ AQCD, the impact of $O_3$ exposure on species competition and community composition was assessed. Ozone was found to cause a significant decline in ponderosa and Jeffrey pine in the San Bernardino Mountains in southern California. Ozone exposure also tended to shift the grass-legume mixtures in favor of grass species (U.S. EPA, 2006b). Since the 2006 $O_3$ AQCD, more evidence has shown that $O_3$ exposure changed the competitive interactions and could lead to loss of $O_3$ sensitive species or genotypes. Studies at plant level found that the severity of $O_3$ damage on growth, reproduction, and foliar injury varied among species, which provided the biological plausibility for the alteration of community composition. Additionally, research since the last review has shown that $O_3$ can alter community composition and diversity of soil microbial communities.

The decline of conifer forests under $O_3$ exposure was continually observed in several regions. Ozone damage was believed to be an important causal factor in the dramatic decline of sacred fir in the valley of Mexico (de Lourdes de Bauer and Hernandez-Tejeda, 2007), as well as cembran pine in southern France and the Carpathian Mountains (Wieser et al., 2006). Results from the Aspen FACE site indicated that $O_3$ could alter community composition of broadleaf forests as well. At the Aspen FACE site, $O_3$ reduced growth and increased mortality of a sensitive aspen clone, while the $O_3$ tolerant clone emerged as the dominant clone in the pure aspen community. In the mixed aspen-birch and aspen-maple communities, $O_3$ reduced the competitive capacity of aspen compared to birch and maple (Kubiske et al., 2007).

The tendency for $O_3$-exposure to shift the biomass of grass-legume mixtures in favor of grass species, was reported in the 2006 $O_3$ AQCD and has been generally confirmed by recent studies. However, in a high elevation mature/species-rich grass-legume pasture, $O_3$ fumigation showed no significant impact on community composition (Bassin et al., 2007b).

Ozone exposure not only altered community composition of plant species, but also microorganisms. The shift in community composition of bacteria and fungi has been observed in both natural and agricultural ecosystems, although no general patterns could be identified (Kanerva et al., 2008; Morsky et al., 2008; Kasurinen et al., 2005).

The evidence is sufficient to conclude that there **is likely to be a causal relationship between $O_3$ exposure and the alteration of community composition of some ecosystems.**

### 9.4.8 Factors that Modify Functional and Growth Response

Many biotic and abiotic factors, including insects, pathogens, root microbes and fungi, temperature, water and nutrient availability, and other air pollutants, as well as elevated $CO_2$, influence or alter plant response to $O_3$. These modifying factors were

comprehensively reviewed in AX9.3 of the 2006 $O_3$ AQCD and thus, this section serves mainly as a brief summary of the previous findings. A limited number of new studies published since the 2006 $O_3$ AQCD add to the understanding of the role of these interactions in modifying $O_3$-induced plant responses. Many of these modifying factors and interactions are integrated into discussions elsewhere in this chapter and the reader is directed to those sections.

#### 9.4.8.1 Genetics

It is well known that species vary greatly in their responsiveness to $O_3$. Even within a given species, individual genotypes or populations can also vary significantly with respect to $O_3$ sensitivity (U.S. EPA, 2006b). Therefore, caution should be taken when considering a species' degree of sensitivity to $O_3$. Plant response to $O_3$ is determined by genes that are directly related to oxidant stress and to an unknown number of genes that are not specifically related to oxidants, but instead control leaf and cell wall thickness, stomatal conductance, and the internal architecture of the air spaces. It is rarely the case that single genes are responsible for $O_3$ tolerance. Studies using molecular biological tools and transgenic plants have positively verified the role of various genes and gene products in $O_3$ tolerance and are continuing to increase the understanding of $O_3$ toxicity and differences in $O_3$ sensitivity. See Section 9.3.3.2 of this document for a discussion of recent studies related to gene expression changes in response to $O_3$.

#### 9.4.8.2 Environmental Biological Factors

As stated in the 2006 $O_3$ AQCD, the biological factors within the plant's environment that may influence its response to $O_3$ encompass insects and other animal pests, diseases, weeds, and other competing plant species. Ozone may influence the severity of a disease or infestation by a pest or weed, either by direct effects on the causal species, or indirectly by affecting the host, or both. In addition, the interaction between $O_3$, a plant, and a pest, pathogen, or weed may influence the response of the target host species to $O_3$ (U.S. EPA, 2006b). Several recent studies on the effects of $O_3$ on insects via their interactions with plants are discussed in Section 9.4.9.1 In addition, $O_3$ has also been shown to alter soil fauna communities (Section 9.4.9.2).

In contrast to detrimental biological interactions, there are mutually beneficial relationships or symbioses involving higher plants and bacteria or fungi. These include (1) the nitrogen-fixing species *Rhizobium* and *Frankia* that nodulate the roots of legumes and alder and (2) the mycorrhizae that infect the roots of many crop and tree species, all of which may be affected by exposure of the host plants to $O_3$. Some discussion of mycorrhizae can be found in Section 9.4.6.

In addition to the interactions involving animal pests, $O_3$ also has indirect effects on higher herbivorous animals, e.g., livestock, due to $O_3$-induced changes in feed quality. Recent studies on the effects of $O_3$ on nutritive quality of plants are discussed in Section 9.4.4.2.

Intra- and interspecific competition are also important factors in determining vegetation response to $O_3$. Plant competition involves the ability of individual plants to acquire the environmental resources needed for growth and development: light, water, nutrients, and space. Intraspecific competition involves individuals of the same species, typically in monoculture crop situations, while interspecific competition refers to the interference exerted by individuals of different species on each other when they are in a mixed culture. This topic was previously reviewed in AX9.3.3.4 of the 2006 $O_3$ AQCD. Recent studies on competition and its implications for community composition are discussed in Section 9.4.7.

### 9.4.8.3 Physical Factors

Physical or abiotic factors play a large role in modifying plant response to $O_3$, and have been extensively discussed in previous $O_3$ AQCDs. This section summarizes those findings as well as recent studies published since the last review.

Although some studies have indicated that $O_3$ impact significantly increases with increased ambient temperature (Ball et al., 2000; Mills et al., 2000), other studies have indicated that temperature has little effect (Balls et al., 1996; Fredericksen et al., 1996). A recent study by Riikonen et al. (2009) at the Ruohoniemi open air exposure field in Kuopio, Finland found that the effects of temperature and $O_3$ on total leaf area and photosynthesis of *Betula pendula* were counteractive. Elevated $O_3$ reduced the saplings' ability to utilize the warmer growth environment by increasing the stomatal limitation for photosynthesis and by reducing the redox state of ascorbate in the apoplast in the combination treatment as compared to temperature alone (Riikonen et al., 2009).

Temperature affects the rates of all physiological processes based on enzyme catalysis and diffusion; each process and overall growth (the integral of all processes) has a distinct optimal temperature range. It is important to note that a plant's response to changes in temperature will depend on whether it is growing near its optimum temperature for growth or near its maximum temperature (Rowland-Bamford, 2000). However, temperature is very likely an important variable affecting plant $O_3$ response in the presence of the elevated $CO_2$ levels contributing to global climate change. In contrast, some evidence suggests that $O_3$ exposure sensitizes plants to low temperature stress (Colls and Unsworth, 1992) and, also, that $O_3$ decreases below-ground carbohydrate reserves, which may lead to responses in perennial species ranging from rapid demise to impaired growth in subsequent seasons (i.e., carry-over effects) (Andersen et al., 1997).

Light, a component of the plant's physical environment, is an essential "resource" of energy content that drives photosynthesis and C assimilation. It has been suggested that increased light intensity may increase the $O_3$ sensitivity of light-tolerant species while decreasing that of shade-tolerant species, but this appears to be an oversimplification with many exceptions. Several studies suggest that the interaction between $O_3$ sensitivity and light environment is complicated by the developmental stage as well as the light environment of individual leaves in the canopy (Kitao et al., 2009; Topa et al., 2001; Chappelka and Samuelson, 1998).

Although the relative humidity of the ambient air has generally been found to increase the effects of $O_3$ by increasing stomatal conductance (thereby increasing $O_3$ flux into the leaves), abundant evidence also indicates that the ready availability of soil moisture results in greater $O_3$ sensitivity (Mills, 2002). The partial "protection" against the effects of $O_3$ afforded by drought has been observed in field experiments (Low et al., 2006) and modeled in computer simulations (Broadmeadow and Jackson, 2000). Conversely, drought may exacerbate the effects of $O_3$ on plants (Pollastrini et al., 2010; Grulke et al., 2003b). There is also some evidence that $O_3$ can predispose plants to drought stress (Maier-Maercker, 1998). Hence, the nature of the response is largely species-specific and will depend to some extent upon the sequence in which the stressors occur.

### 9.4.8.4 Interactions with Other Pollutants

#### Ozone-nitrogen interactions

Elevated $O_3$ exposure and N deposition often co-occur. However, the interactions of $O_3$ exposure and N deposition on vegetation are complex and less well understood compared to their independent effects. Consistent with the conclusion of the 2006 $O_3$ AQCD, the limited number of studies published since the last review indicated that the interactive effects of N and $O_3$ varied among species and ecosystems (Table 9-8). Nitrogen deposition could stimulate relative growth rate (RGR), and lead to increased stomatal conductance. Therefore, plants might become more susceptible to $O_3$ exposure. Alternatively, N deposition may increase the availability of photosynthates for use in detoxification and plants could become more tolerant to $O_3$ (Bassin et al., 2007a). Elevated $O_3$ exposure and N deposition could also act in concert to increase plant susceptibility to disease (von Tiedemann, 1996). To better understand these interactions in ecosystems across the U.S., more information is needed considering combined $O_3$ exposure and N deposition related effects.

Only a few recent studies have investigated the interactive effects of $O_3$ and N in the United States. Grulke et al. (2005) measured stomatal conductance of California black oak (*Quercus kelloggii*) at a long-term N-enrichment site located in the San Bernardino Mountains, which is accompanied by high $O_3$ exposure (80 ppb, 24-h avg. over a six month growing season). The authors found that N amendment led to poor stomatal control in full sun in midsummer of the average precipitation

years, but enhanced stomatal control in shade leaves of California black oak. In an OTC study, Handley and Grulke (2008) found that $O_3$ lowered photosynthetic ability and water-use efficiency, and increased leaf chlorosis and necrosis of California black oak. Nitrogen fertilization tended to reduce plant sensitivity to $O_3$ exposure; however, the interaction was not statistically significant. In another study, Grulke et al. (2008) reported that various lines of phenomenological and experimental evidence indicate that N deposition and $O_3$ pollution contribute to the susceptibility of forests to wildfire in the San Bernadino Mountains by increasing stress due to drought, weakening trees, and predisposing them to bark beetle infestation (U.S. EPA, 2008b).

Studies conducted outside the U.S. are also summarized in Table 9-8. Generally, the responses were species specific. The $O_3$-induced reduction in photosynthetic rate and biomass loss were greater in the relatively high N treatment for watermelon (*Citrillus lanants*) (Calatayud et al., 2006) and Japanese beech (*Fagus crenata*) seedlings (Yamaguchi et al., 2007). However, there was no significant interactive effect of $O_3$ and N on biomass production for *Quercus serrata* seedlings (Watanabe et al., 2007), young Norway spruce (*Picea abies*) trees (Thomas et al., 2005), and young European beech (*Fagus sylvatica*) trees Thomas et al. (2006).

## Table 9-8 Response of plants to the interactive effects of elevated $O_3$ exposure and nitrogen enrichment.

| Site | Species | Ozone exposure | N addition | Responses | References |
|---|---|---|---|---|---|
| San Bernardino Mountains, U.S. | California black oak (*Quercus kelloggii*) | 80 ppb | 0, and 50 kg N/ha·yr | N-amended trees had lower late summer C gain and greater foliar chlorosis in the drought year, and poor stomatal control and lower leaf water use efficiency and in midsummer of the average precipitation year. | Grulke et al. (2005) |
| San Bernardino Mountains, U.S. | California black oak (*Quercus kelloggii*) | 0, 75, and 150 ppb | 0, and 50 kg N/ha·yr | N fertilization tended to reduce plant sensitivity to $O_3$ exposure; however the interaction was not statistically significant. | Handley and Grulke (2008) |
| Switzerland | Spruce trees (*Picea abies*) | Filtered (19.4-28.1 ppb); Ambient (37.6-47.4 ppb) | 0, 20, 40 and 80 kg N/ha·yr | Higher N levels alleviated the negative impact of $O_3$ on root starch concentrations | Thomas et al. (2005) |
| Switzerland | Beech trees (*Fagus sylvatica*) | Filtered (19.4-28.1 ppb); Ambient (37.6-47.4 ppb) | 0, 20, 40 and 80 kg N/ha·yr | N addition amplified the negative effects of $O_3$ on leaf area and shoot elongation. | Thomas et al. (2006) |
| Switzerland | Alpine pasture | Ambient (AOT40 of 11.1-12.6 ppm-h); 1.2 ambient (AOT40 of 15.2-29.5 ppm-h); 1.6 ambient (28.4-64.9 ppm-h) | 0, 5, 10, 25, 50 kg N/ha·yr | The positive effects of N addition on canopy greenness were counteracted by accelerated leaf senescence in the highest $O_3$ treatment. | Bassin et al. (2007b) |
| Switzerland | Alpine pasture | Ambient (AOT40 of 11.1-12.6 ppm-h); 1.2 ambient (AOT40 of 15.2-29.5 ppm-h); 1.6 ambient (28.4-64.9 ppm-h) | 0, 5, 10, 25, 50 kg N/ha·yr | Only a small number of species showed significant $O_3$ and N interactive effects on leaf chlorophyll concentration, leaf weight and change in $^{18}O$, and the patterns were not consistent. | Bassin et al. (2009) |
| Switzerland | Alpine pasture | Ambient (AOT40 of 11.1-12.6 ppm-h); 1.2 ambient (AOT40 of 15.2-29.5 ppm-h); 1.6 ambient (28.4-64.9 ppm-h) | 0, 5, 10’ 25, 50 kg N/ha·yr | Highest N addition resulted in carbon loss, but there was no interaction between $O_3$ and N treatments. | Volk et al. (2011) |

| Site | Species | Ozone exposure | N addition | Responses | References |
|---|---|---|---|---|---|
| Spain | Watermelon (*Citrillus lanants*) | $O_3$ free (AOT40 of 0 ppm-h), Ambient (AOT40 of 5.1-6.3 ppm-h); Elevated $O_3$ (AOT40 of 32.5-35.6 ppm-h) | 140, 280, and 436 kg N/ha·yr | High N concentration enhanced the detrimental effects of $O_3$ on Chlorophyll a fluorescence parameters, lipid peroxidation, and the total yield. | Calatayud et al. (2006) |
| Spain | Clover *Trifolium striatum* | Filtered (24-h avg. of 8-22 ppb); Ambient (29-34 ppb), Elevated $O_3$ (35-56 ppb) | 10, 30, and 60 kg N/ha·yr | $O_3$ reduced total aerial biomass. N fertilization counterbalanced $O_3$-induced effects only when plants were exposed to moderate $O_3$ levels (ambient) but not under elevated $O_3$ concentrations. | Sanz et al. (2007) |
| Japan | Japanese beech seedlings (*Fagus crenata*) | Filtered (24-h avg. of 10.3-13.2 ppb); Ambient (42.0-43.3 ppb), 1.5 Ambient (62.6-63.9 ppb); 2.0 ambient (82.7-84.7 ppb) | 0, 20 and 50 kg N/ha·yr | The $O_3$-induced reduction in net photosynthesis and whole-plant dry mass were greater in the relatively high N treatment than that in the low N treatment. | Yamaguchi et al. (2007) |
| Japan | Japanese tree *(Quercus serrata)* seedlings | Filtered (24-h avg. of 10.3-13.2 ppb); Ambient (42.0-43.3 ppb), 1.5 ambient (62.6-63.9 ppb); 2.0 ambient (82.7-84.7 ppb) | 0, 20 and 50 kg N/ha·yr | No significant interactive effects of $O_3$ and N load on the growth and net photosynthetic rate were detected. | Watanabe et.al. (2007) |

### Ozone-carbon dioxide interactions

Several decades of research has shown that exposure to elevated $CO_2$ increases photosynthetic rates (Bernacchi et al., 2006; Bernacchi et al., 2005; Tissue et al., 1999; Tissue et al., 1997; Will and Ceulemans, 1997), decreases stomatal conductance (Ainsworth and Rogers, 2007; Paoletti et al., 2007; Bernacchi et al., 2006; Leakey et al., 2006; Medlyn et al., 2001) and generally increases the growth of plants (McCarthy et al., 2009; Norby et al., 2005). This is in contrast to the decrease in photosynthesis and growth in many plants that are exposed to elevated $O_3$. The interactive effects on vegetation have been the subject of research in the past two decades due to the implications on productivity and water use of ecosystems. This area of research was discussed in detail in AX9.3.8.1 of the 2006 $O_3$ AQCD and the conclusions made then are still relevant (U.S. EPA, 2006b).

The bulk of the available evidence shows that, under the various experimental conditions used (which almost exclusively employed abrupt or "step" increases in $CO_2$ concentration, as discussed below), increased $CO_2$ levels (ambient + 200 to 400 ppm) may protect plants from the negative effects of $O_3$ on growth. This protection may be afforded in part by $CO_2$ acting together with $O_3$ in inducing stomatal closure, thereby reducing $O_3$ uptake, and in part by $CO_2$ reducing the negative effects of $O_3$ on Rubisco and its activity in $CO_2$-fixation. Although both $CO_2$-induced and $O_3$-induced decreases in stomatal conductance have been observed primarily in short-term studies, recent data show a long-term and sustained reduction in stomatal conductance under elevated $CO_2$ for a number of species (Ainsworth and Long, 2005; Ellsworth et al., 2004; Gunderson et al., 2002). Instances of increased stomatal conductance have also been observed in response to $O_3$ exposure, suggesting partial stomatal dysfunction after extended periods of exposure (Paoletti and Grulke, 2010; Grulke et al., 2007a; Maier-Maercker, 1998).

Important caveats must be raised with regard to the findings presented in published research. The first caveat concerns the distinctly different natures of the exposures to $O_3$ and $CO_2$ experienced by plants in the field. Changes in the ambient concentrations of these gases have very different dynamics. In the context of climate change, $CO_2$ levels increase relatively slowly (globally 2 ppm/year) and may change little over several seasons of growth. On the other hand, $O_3$ presents a fluctuating stressor with considerable hour-to-hour, day-to-day and regional variability (Polle and Pell, 1999). Almost all of the evidence presented comes from experimentation involving plants subjected to an abrupt step increase to a higher, steady $CO_2$ concentration. In contrast, the $O_3$ exposure concentrations usually varied from day to day. Luo and Reynolds (1999), Hui et al. (2002), and Luo (2001) noted the difficulties in predicting the likely effects of a gradual $CO_2$ increase from experiments involving a step increase or those using a range of $CO_2$ concentrations. It is also important to note that the levels of elevated $CO_2$ in many of the studies will not be experienced in the field for 30 or 40 years, but elevated levels of $O_3$ can occur presently in several areas of the United States. Therefore, the $CO_2 \times O_3$ interaction studies may be less relevant for current ambient conditions.

Another caveat concerns the interactions of $O_3$ and $CO_2$ with other climatic variables, such as temperature and precipitation. In light of the key role played by temperature in regulating physiological processes and modifying plant response to increased $CO_2$ levels (Morison and Lawlor, 1999; Long, 1991) and the knowledge that relatively modest increases in temperature may lead to dramatic consequences in terms of plant development (Lawlor, 1998), it is important to consider that studying $CO_2$ and $O_3$ interactions alone may not create a complete understanding of effects on plants under future climate change.

## 9.4.9 Insects and Other Wildlife

### 9.4.9.1 Insects

Insects may respond indirectly to changes in plants (i.e., increased reactive oxygen species, altered phytochemistry, altered nutrient content) that occur under elevated $O_3$ conditions, or $O_3$ can have a direct effect on insect performance (Menendez et al., 2009). Effects of $O_3$ on insects occur at the species level (i.e., growth, survival, reproduction, development, feeding behavior) and at the population and community-level (i.e., population growth rate, community composition). In general, effects of $O_3$ on insects are highly context- and species-specific (Lindroth, 2010; Bidart-Bouzat and Imeh-Nathaniel, 2008). Furthermore, plant responses to $O_3$ exposure and herbivore attack have been demonstrated to share signaling pathways, complicating characterization of these stressors (Lindroth, 2010; Menendez et al., 2010, 2009). Although both species-level and population and community-level responses to elevated $O_3$ are observed in field and laboratory studies discussed below, there is no consensus on how insects respond to feeding on $O_3$–exposed plants.

#### Species-level responses

In considering insect growth, survival and reproduction in elevated $O_3$ conditions, several studies have indicated an effect while others have found no correlation. The performance of five herbivore species (three moths and two weevils) was assessed in an OTC experiment at 2 × ambient concentration (Peltonen et al., 2010). Growth of larvae of the Autumnal moth, *Epirrita autumna*, was significantly decreased in the $O_3$ treatment while no effects were observed in the other species. In an aphid oviposition preference study using birch buds grown in a three year OTC experiment, $O_3$ had neither a stimulatory or deterring effect on egg-laying (Peltonen et al., 2006). Furthermore, changes in birch bud phenolic compounds associated with the doubled ambient concentrations of $O_3$ did not correlate with changes in aphid oviposition (Peltonen et al., 2006). Reproduction in *Popillia japonica*, that were fed soybeans and grown under elevated $O_3$ appeared to be unaffected (O'Neill et al., 2008). In a meta-analysis of effects of elevated $O_3$ on 22 species of trees and 10 species of insects, the rates of survival, reproduction and food consumption were typically unaffected while development times were reduced and pupal masses were increased (Valkama et al., 2007).

At the Aspen FACE site insect performance under elevated (50-60 ppb) $O_3$ conditions (approximately 1.5 × background ambient levels of 30-40 ppb $O_3$) have been considered for several species. Cumulative fecundity of aphids (*Cepegillettea betulaefoliae),* that were reared on $O_3$-exposed paper birch (*Betula papyrifera*) trees, was lower than aphids from control plots (Awmack et al., 2004). No effects on growth, development, adult weight, embryo number and birth weight of newborn nymphs were observed. In a study conducted using three aspen genotypes,

performance of the aspen beetle (*Chrysomela crochi*) decreased across all parameters measured (development time, adult mass and survivorship) under elevated $O_3$ (Vigue and Lindroth, 2010). There was an increase in the development time of male and female aspen beetle larvae although the percentages varied across genotypes. Decreased beetle adult mass and survivorship was observed across all genotypes under elevated $O_3$ conditions. Another study from the Aspen FACE site did not find any significant effects of elevated $O_3$ on performance (longevity, fecundity, abundance) of the invasive weevil (*Polydrusus sericeus*) (Hillstrom et al., 2010b).

Since the 2006 $O_3$ AQCD, several studies have considered the effect of elevated $O_3$ on feeding behavior of insects. In a feeding preference study, the common leaf weevil (*Phyllobius pyri*) consumed significantly more leaf discs from one aspen clone when compared to a second clone under ambient air conditions (Freiwald et al., 2008). In a moderately elevated $O_3$ environment (1.5 × ambient), this preference for a certain aspen clone was less evident, however, leaves from $O_3$-exposed trees were significantly preferred to leaves grown under ambient conditions. Soybeans grown under enriched $O_3$ had significantly less loss of leaf tissue to herbivory in August compared to earlier in the growing season (July) when herbivory was not affected (Hamilton et al., 2005). Other plant-herbivore interactions have shown no effects of elevated $O_3$ on feeding. Feeding behavior of Japanese beetles (*P. japonica*) appeared to be unchanged when beetles were fed soybean leaves grown under elevated $O_3$ conditions (O'Neill et al., 2008). At the Aspen FACE site, feeding by the invasive weevil (*Polydrusus sericeus*), as measured by leaf area consumption, was not significantly different between foliage that was grown under elevated $O_3$ versus ambient conditions (Hillstrom et al., 2010b).

### Population-level and community-level responses

Recent data on insects provide evidence of population-level and community-level responses to $O_3$. Elevated levels of $O_3$ can affect plant phytochemistry and nutrient content which in turn can alter population density and structure of the associated herbivorous insect communities and impact ecosystem processes (Cornelissen, 2011; Lindroth, 2010). In 72-hour exposures to elevated $O_3$, mean relative growth rate of the aphid *Diuraphis noxia* increased with $O_3$ concentration suggesting that more rapid population growth may occur when atmospheric $O_3$ is elevated (Summers et al., 1994). In a long-term study of elevated $O_3$ on herbivore performance at the Aspen FACE site, individual performance and population-level effects of the aphid *C. betulaefoliae* were assessed. Elevated $O_3$ levels had a strong positive effect on the population growth rates of the aphids; although effects were not detected by measuring growth, development, adult weight, embryo number or birth weight of newborn nymphs (Awmack et al., 2004). Conversely, a lower rate of population growth was observed in aphids previously exposed to $O_3$ in an OTC (Menendez et al., 2010). No direct effects of $O_3$ were observed; however, nymphs born from adults exposed to and feeding on $O_3$ exposed plants were less capable of infesting new plants when compared to nymphs in the control plots (Menendez et al., 2010). Elevated $O_3$ reduced total arthropod abundance by 17% at Aspen FACE, largely as a

result of the negative effects on parasitoids, although phloem-feeding insects may benefit (Hillstrom and Lindroth, 2008). Herbivore communities affected by $O_3$ and N were sampled along an air pollution gradient in the Los Angeles basin (Jones and Paine, 2006). Abundance, diversity, and richness of herbivores were not affected. However, a shift in community structure, from phloem-feeding to chewing dominated communities, was observed along the gradient. No consistent effect of elevated $O_3$ on herbivory or insect population size was detected at SoyFACE (O'Neill et al., 2010; Dermody et al., 2008).

Evidence of modification of insect populations and communities in response to elevated $O_3$ includes genotypic and phenotypic changes. In a study conducted at the Aspen FACE site, elevated $O_3$ altered the genotype frequencies of the pea aphid (*Acyrthosiphon pisum*) grown on red clover (*Trifolium pratense*) over multiple generations (Mondor et al., 2005). Aphid color was used to distinguish between the two genotypes. Ozone increased the genotypic frequencies of pink-morph:green-morph aphids from 2:1 to 9:1, and depressed wing-induction responses more strongly in the pink than the green genotype (Mondor et al., 2005). Growth and development of individual green and pink aphids reared as a single genotype or mixed genotypes were unaffected by elevated $O_3$ (Mondor et al., 2010). However, growth of pea aphid populations is not readily predictable using individual growth rates.

### 9.4.9.2 Wildlife

#### Herpetofauna

Since the 2006 $O_3$ AQCD, direct effects of $O_3$ exposure including physiological changes and alterations of ecologically important behaviors such as feeding and thermoregulation have been observed in wildlife. These studies have been conducted in limited laboratory exposures, and the levels of $O_3$ treatment (e.g., 0.2-0.8 ppm) were often unrealistically higher than the ambient levels. Amphibians may be especially vulnerable to airborne oxidants due to the significant gas exchange that occurs across the skin (Andrews et al., 2008; Dohm et al., 2008). Exposure to 0.2 ppm to 0.8 ppm $O_3$ for 4 hours resulted in a decrease of oxygen consumption and depressed lung ventilation in the California tree frog *Pseudacris cadaverina* (Mautz and Dohm, 2004). Following a single 4-h inhalation exposure to 0.8 ppm $O_3$, reduced pulmonary macrophage phagocytosis was observed at 1 and 24 hours postexposure in the marine toad (*Bufo marinus*) indicating an effect on immune system function (Dohm et al., 2005). There was no difference in macrophage function at 48 hours postexposure in exposed and control individuals.

Behavioral effects of $O_3$ observed in amphibians include responses to minimize the surface area of the body exposed to the air and a decrease in feeding rates (Dohm et al., 2008; Mautz and Dohm, 2004). The adoption of a low-profile “water conservation posture” during $O_3$ exposure was observed in experiments with the

California tree frog (Mautz and Dohm, 2004). Marine toads, *Bufo marinus*, exposed to 0.06 µL/L (ppm) $O_3$ for 4 hours ate significantly fewer mealworms at 1 hour and 48 hours postexposure than control toads (Dohm et al., 2008). In the same study, escape/exploratory behavior as measured by total distance moved was not negatively affected in the $O_3$-exposed individuals as compared to the controls (Dohm et al., 2008).

Water balance and thermal preference in herpetofauna are altered with elevated $O_3$. Marine toads exposed to 0.8 ppm $O_3$ for 4 hours exhibited behavioral hypothermia when temperature selection in the toads was assessed at 1, 24 and 48 hours postexposure (Dohm et al., 2001). Ozone-exposed individuals lost almost 5g more body mass on average than controls due to evaporative water loss. At 24 hours after exposure, the individuals that had lost significant body mass selected lower body temperatures (Dohm et al., 2001). Behavioral hypothermia was also observed in reptiles following 4-h exposures to 0.6 ppm $O_3$. Exposure of the Western Fence Lizard (*Sceloporus occidentalis*) at 25°C induced behavioral hypothermia that recovered to control temperatures by 24 hours (Mautz and Dohm, 2004).
The behavioral hypothermic response persisted in lizards exposed to $O_3$ at 35°C at 24 hours postexposure resulting in a mean body temperature of 3.3°C over controls.

#### Soil fauna communities

Ozone has also been shown to alter soil fauna communities (Meehan et al., 2010; Kasurinen et al., 2007; Loranger et al., 2004). Abundance of Acari (mites and ticks) decreased by 47% under elevated $O_3$ at Aspen FACE site, probably due to the higher secondary metabolites and lower N concentrations in litter and foliage under elevated $O_3$ (Loranger et al., 2004). In another study from the Aspen FACE site, leaf litter collected from aspen grown under elevated $O_3$ conditions was higher in fiber and lignin concentrations than litter from trees grown under ambient conditions. These chemical characteristics of the leaves were associated with increased springtail population growth following 10 weeks in a laboratory microcosm (Meehan et al., 2010). Consumption rates of earthworms fed on leaf litter for 6 weeks from trees grown under elevated $O_3$ conditions and ambient air did not vary significantly between treatments (Meehan et al., 2010). In another study on juvenile earthworms *Lumbricus terrestris*, individual growth was reduced when worms were fed high-$O_3$ birch litter from trees exposed for three years to elevated $O_3$ in an OTC system (Kasurinen et al., 2007). In the same study no significant growth or mortality effects were observed in isopods.

### 9.4.9.3 Indirect Effects on Wildlife

In addition to the direct effects of $O_3$ exposure on physiological and behavioral endpoints observed in the laboratory, there are indirect effects to wildlife. These effects include changes in biomass and nutritive quality of $O_3$-exposed plants (reviewed in Section 9.4.4) that are consumed by wildlife. Reduced digestibility of

$O_3$-exposed plants may alter dietary intake and foraging strategies in herbivores. In a study using native highbush blackberry (*Rubus argutus*) relative feed value of the plants decreased in bushes exposed to double ambient concentrations of $O_3$ (Ditchkoff et al., 2009). Indirect effects of elevated $O_3$ on wildlife include changes in chemical signaling important in ecological interactions reviewed below.

### Chemical signaling in ecological interactions

Ozone has been shown to degrade or alter biogenic VOC signals important to ecological interactions including; (1) attraction of pollinators and seed dispersers; (2) defense against herbivory; and (3) predator-prey interactions (Pinto et al., 2010; McFrederick et al., 2009; Yuan et al., 2009; Pinto et al., 2007a; Pinto et al., 2007b). Each signal released by emitters has an atmospheric lifetime and a unique chemical signature comprised of different ratios of individual hydrocarbons that are susceptible to atmospheric oxidants such as $O_3$ (Yuan et al., 2009; Wright et al., 2005). Under elevated $O_3$ conditions, these olfactory cues may travel shorter distances before losing their specificity (McFrederick et al., 2009; McFrederick et al., 2008). Additional non-phytogenic VOC-mediated interrelationships with the potential to be modified by $O_3$ include territorial marking, pheromones for attraction of mates and various social interactions including scent trails, nestmate recognition and signals involved in aggregation behaviors (McFrederick et al., 2009). For example, the alcohols, ketones and aldehydes comprising sex pheromones in moths could be especially vulnerable to degradation by $O_3$, since some males travel >100 meters to find mates (Carde and Haynes, 2004). In general, effects of $O_3$ on scent-mediated ecological interactions are highly context- and species-specific (Lindroth, 2010; Bidart-Bouzat and Imeh-Nathaniel, 2008).

### Pollination and seed dispersal

Phytogenic VOC's attract pollinators and seed dispersers to flowers and fruits (Dudareva et al., 2006; Theis and Raguso, 2005). These floral scent trails in plant-insect interactions may be destroyed or transformed by $O_3$ (McFrederick et al., 2008). Using a Lagrangian model, the rate of destruction of phytogenic VOC's was estimated in air parcels at increasing distance from a source in response to increased regional levels of $O_3$, hydroxyl and nitrate radicals (McFrederick et al., 2008). Based on the model, the ability of pollinators to locate highly reactive VOCs from emitting flowers may have decreased from kilometers during pre-industrial times to <200 meters at current ambient conditions (McFrederick et al., 2008). Scents that travel shorter distances (0-10 meters) are less susceptible to air pollutants, while highly reactive scents that travel longer distances (10 to 100s of meters), are at a higher risk for degradation (McFrederick et al., 2009). For example, male euglossine bees can detect bait stations from a distance of at least one kilometer (Dobson, 1994).

### Defense against herbivory

Ozone can alter the chemical signature of VOCs emitted by plants and these VOCs are subsequently detected by herbivores (Blande et al., 2010; Iriti and Faoro, 2009; Pinto et al., 2007a; Vuorinen et al., 2004; Jackson et al., 1999; Cannon, 1990). These modifications can make the plant either more attractive or repellant to phytophagous insects (Pinto et al., 2010). For example, under elevated $O_3$, the host plant preference by forest tent caterpillars increased for birch compared to aspen (Agrell et al., 2005). Ozone-induced emissions from red spruce needles were found to repel spruce budworm larvae (Cannon, 1990). Transcriptional profiles of field grown soybean (*Glycine max*) grown in elevated $O_3$ conditions were altered due to herbivory by Japanese beetles. The herbivory resulted in a higher number of transcripts in the leaves of $O_3$-exposed plants and upregulation of antioxidant metabolism associated with plant defense (Casteel et al., 2008).

Ozone may modify signals involved in plant-to-plant interactions and plant defense against pathogens (Blande et al., 2010; Pinto et al., 2010; McFrederick et al., 2009; Yuan et al., 2009). In a recent study with lima beans, 80 ppb $O_3$ degraded several herbivore-induced VOCs, reducing the distance over which plant-to-plant signaling occurred (Blande et al., 2010).

### Predator-prey interactions

Elevated $O_3$ conditions are associated with disruption of pheromone-mediated interactions at higher trophic levels (e.g., predators and parasitoids of herbivores). In a study from the Aspen FACE site, predator escape behaviors of the aphid (*Chatophorus stevensis*) were enhanced on $O_3$-fumigated aspen trees although the mechanism of this response remains unknown (Mondor et al., 2004). The predatory mite *Phytoseiulus persimilis* can distinguish between the VOC signature of ozonated lima bean plants and ozonated lima bean plants simultaneously damaged by *T. urticae* (Vuorinen et al., 2004) however, other tritrophic interactions have shown no effect (Pinto et al., 2007b).

There are few studies that consider host location behaviors of parasites under elevated $O_3$. In closed chambers fumigated with $O_3$, the searching efficiency and proportion of the host larval fruit flies parasitized by *Asobara tabida* declined when compared to filtered air controls (Gate et al., 1995). The host location behavior and rate of parasitism of the wasp (*Coesia plutellae*) on *Plutella xylostella*-infested potted cabbage plants was tested under ambient and doubled $O_3$ conditions in an open-air fumigation system (Pinto et al., 2008). The number of wasps found in the field and the percentages of parasitized larvae were not significantly different from controls under elevated $O_3$.

Elevated $O_3$ has the potential to perturb specialized food-web communication in transgenic crops. In insect-resistant oilseed rape *Brassica napus* grown under 100 ppb $O_3$ in a growth chamber, reduced feeding damage by *Putella xylostella* led to deceased attraction of the endoparasitoid (*Costesia vestalis*), however this

tritrophic interaction was influenced by the degree of herbivore feeding (Himanen et al., 2009a; Himanen et al., 2009b). Under chronic $O_3$-exposure, the insect resistance trait BT cry1Ac in transgenic *B. napus* was higher than the control (Himanen et al., 2009c). There was a negative relative growth rate of the Bt target herbivore, *P. xylostella*, in all $O_3$ treatments.

#### 9.4.9.4 Summary

Recent information on $O_3$ effects on insects and other wildlife is limited to a few species and there is no consensus on how these organisms respond to elevated $O_3$. Studies published since the last review show impacts of elevated $O_3$ on both species-level responses (reproduction, growth, feeding behavior) and community and ecosystem-level responses (population growth, abundance, shift in community structure) in some insects and soil fauna. Changes in ecologically important behaviors such as feeding and thermoregulation have recently been observed with $O_3$ exposure in amphibians and reptiles, however, these responses occur at concentrations of $O_3$ much higher than ambient levels.

Recent information available since the last review considers the effects of $O_3$ on chemical signaling in insect and wildlife interactions. Specifically, studies on $O_3$ effects on pollination and seed dispersal, defenses against herbivory and predator-prey interactions all consider the ability of $O_3$ to alter the chemical signature of VOCs emitted during these pheromone-mediated events. The effects of $O_3$ on chemical signaling between plants, herbivores and pollinators as well as interactions between multiple trophic levels is an emerging area of study that may result in further elucidation of $O_3$ effects at the species, community and ecosystem-level.

## 9.5 Effects-based Air Quality Exposure Indices and Dose Modeling

### 9.5.1 Introduction

Exposure indices are metrics that quantify exposure as it relates to measured plant response (e.g., reduced growth). They are summary measures of monitored ambient $O_3$ concentrations over time, intended to provide a consistent metric for reviewing and comparing exposure-response effects obtained from various studies. Such indices may also provide a basis for developing a biologically-relevant air quality standard for protecting vegetation and ecosystems. Effects on plant growth and/or yield have been a major focus of the characterization of $O_3$ impacts on plants for purposes of the air quality standard setting process (U.S. EPA, 2007b, 1996e, 1986). The relationship of $O_3$ and plant responses can be characterized quantitatively as “dose-response” or “exposure-response.” The distinction is in how the pollutant concentration is

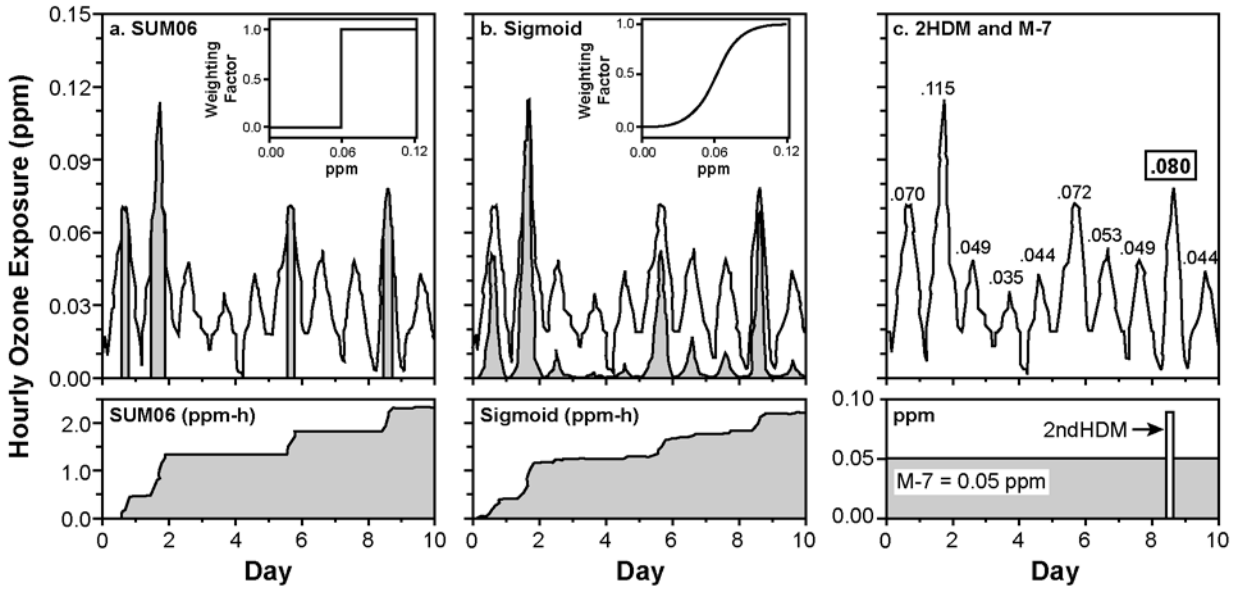
expressed: “dose” is the pollutant concentration absorbed by the leaf over some time period, and is very difficult to measure directly, whereas “exposure” is the ambient air concentration measured near the plant over some time period, and summarized for that period using an index. Exposure indices have been most useful in considering the form of the secondary $O_3$ NAAQS, in large part because they only require ambient air quality data rather than more complex indirect calculations of dose to the plant. The attributes of exposure indices that are most relevant to plant response are the weighting of $O_3$ concentrations and the daily and seasonal time-periods. Several different types of exposure indices are discussed in Section 9.5.2.

From a theoretical perspective, a measure of plant $O_3$ uptake or dose from ambient air (either rate of uptake or cumulative seasonal uptake) might be a better predictor of plant response to $O_3$ than an exposure index and may be useful in improving risk assessment. An uptake estimate would have to integrate all those environmental factors that influence stomatal conductance, including but not limited to temperature, humidity, and soil water status (Section 9.5.4). Therefore, uptake values are generally obtained with simulation models that require knowledge of species- and site-specific values for the variables mentioned. However, a limitation of modeling dose is that environmental variables are poorly characterized. In addition, it has also been recognized that $O_3$ detoxification processes and the temporal dynamics of detoxification must be taken into account in dose modeling (Heath et al., 2009) (Section 9.5.4). Because of this, research has focused historically on predictors of $O_3$ damage to plants based only on exposure as a summary measure of monitored ambient pollutant concentration over some integral of time, rather than dose (U.S. EPA, 1996c; Costa et al., 1992; Lee et al., 1988b; U.S. EPA, 1986; Lefohn and Benedict, 1982; O'Gara, 1922).

## 9.5.2 Description of Exposure Indices Available in the Literature

Mathematical approaches for summarizing ambient air quality information in biologically meaningful forms for $O_3$ vegetation effects assessment purposes have been explored for more than 80 years (U.S. EPA, 1996b; O'Gara, 1922). In the context of national standards that protect for “known or anticipated” effects on many plant species in a variety of habitats, exposure indices provide a numerical summary of very large numbers of ambient observations of concentration over extended periods. Like any summary statistic, exposure indices retain information on some, but not all, characteristics of the original observations. Several indices have been developed to attempt to incorporate some of the biological, environmental, and exposure factors that influence the magnitude of the biological response and contribute to observed variability (Hogsett et al., 1988). In the 1996 $O_3$ AQCD (U.S. EPA, 1996a), the exposure indices were arranged into five categories; (1) One event, (2) Mean, (3) Cumulative, (4) Concentration weighted, and (5) Multicomponent, and were discussed in detail (Lee et al., 1989). Figure 9-9 illustrates how several of the indices weight concentration and accumulate exposure. For example, the SUM06 index (panel a) is a threshold-based approach wherein concentrations below

0.06 ppm are given a weight of zero and concentrations at or above 0.06 ppm are given a weight of 1.0 that is summed, usually over 3 to 6 months. The Sigmoid approach (panel b), which is similar to the W126 index (Lefohn et al., 1988; Lefohn and Runeckles, 1987), is a non-threshold approach wherein all concentrations are given a weight that increases from zero to 1.0 with increasing concentration and summed.




(a) SUM06: the upper graphic (within panel a) illustrates an episodic exposure profile; the shaded area under some of the peaks illustrates the concentrations greater than or equal to 0.06 ppm that are accumulated in the index. The insert shows the concentration weighting (0 or 1) function. The lower portion of panel a graphically illustrates how concentration is accumulated over the exposure period. (b) SIGMOID: the upper graphic illustrates an episodic exposure profile; the variable shaded area under the peaks illustrates the concentration-dependent weights that are accumulated in the index. The insert shows the sigmoid concentration weighting function. This is similar to the W126 function. The lower portion of the graphic illustrates how concentration is accumulated over the exposure period. (c) second HDM and M-7: the upper graphic illustrates an episodic exposure profile. The lower portion of the graphic illustrates that the second HDM considers only a single exposure peak, while the M-7 (average of 7-h daily means) applies a constant exposure value over the exposure period.

Source: Reprinted with permission of Air and Waste Management Association (Tingey et al., 1991).

**Figure 9-9 Diagrammatic representation of several exposure indices illustrating how they weight concentration and accumulate exposure.**

This section will primarily discuss SUM06, W126 and AOTx exposure metrics. Below are the definitions of the three cumulative index forms:

- **SUM06:** Sum of all hourly $O_3$ concentrations greater than or equal to 0.06 ppm observed during a specified daily and seasonal time window (Figure 9-9, Panel A).
- **AOTx:** Sum of the differences between hourly $O_3$ concentrations greater than a specified threshold during a specified daily and seasonal time window. For example, AOT40 is sum of the differences between hourly concentarions above 0.04 ppm.
- **W126:** Sigmoidally weighted sum of all hourly $O_3$ concentrations observed during a specified daily and seasonal time window (Lefohn et al., 1988; Lefohn and Runeckles, 1987), similar to Figure 9-9, Panel B. The sigmoidal weighting of hourly $O_3$ concentration is given in the equation below, where *C* is the hourly $O_3$ concentration in ppm:

$$W_c = \frac{1}{1 + 4403e^{-126C}}$$

**Equation 9-1**

These indices have a variety of relevant time windows that may be applied and are discussed in Section 9.5.3.

Various factors with known or suspected bearing on the exposure-response relationship, including concentration, time of day, respite time, frequency of peak occurrence, plant phenology, predisposition, etc., have been weighted with various functions in a large set of indices. The resulting indices were evaluated by ranking them according to the goodness-of-fit of a regression model of growth or yield response (Lee et al., 1989). The statistical evaluations for each of these indices were completed using growth or yield response data from many earlier exposure studies (e.g., NCLAN). This retrospective approach was necessary because there were no studies specifically designed to test the goodness-of-fit of the various indices. The goodness-of-fit of a set of linear and nonlinear models for exposure-response was ranked as various proposed indices were used in turn to quantify exposure. This approach provided evidence for the best indices. The results of retrospective analyses are described below.

Most of the early retrospective studies reporting regression approaches used data from the NCLAN program or data from Corvallis, Oregon or California (Costa et al., 1992; Lee et al., 1988b; Lefohn et al., 1988; Musselman et al., 1988; Lee et al., 1987; U.S. EPA, 1986). These studies were previously reviewed by the EPA (U.S. EPA, 1996c; Costa et al., 1992) and were in general agreement that the best fit to the data resulted from using cumulative concentration-weighted exposure indices (e.g., W126, SUM06). Lee et al. (1987) suggested that exposure indices that included all the 24-h data performed better than those that used only 7 hours of data; this was

consistent with the conclusions of Heagle et al. (1987) that plants receiving exposures for an additional 5 hours/day showed 10% greater yield loss than those exposed for 7 hours/day. In an analysis using the National Crop Loss Assessment Network (NCLAN) data, Lee et al.(1988b) found several indices which only cumulated and weighted higher concentrations (e.g., W126, SUM06, SUM08, and AOT40) performed very well. Amongst this group no index had consistently better fits than the other indices across all studies and species (Heagle et al., 1994b; Lefohn et al., 1988; Musselman et al., 1988). Lee et al. (1988b) found that adding phenology weighting to the index somewhat improved the performance of the indices. The "best" exposure index was a phenologically weighted cumulative index, with sigmoid weighting on concentration and a gamma weighting function as a surrogate for plant growth stage. This index provided the best statistical fit when used in the models under consideration, but it required data on species and site conditions, making specification of weighting functions difficult for general use.

Other factors, including predisposition time (Hogsett et al., 1988; McCool et al., 1988) and crop development stage (Tingey et al., 2002; Heagle et al., 1991) contributed to variation in the biological response and suggested the need for weighting $O_3$ concentrations to account for predisposition time and phenology. However, the roles of predisposition and phenology in plant response vary considerably with species and environmental conditions; therefore, specification of a weighting function for general use in characterizing plant exposure has not been possible.

European scientists took a similar approach in developing indices describing growth and yield loss in crops and tree seedlings, using OTCs with modified ambient exposures, but many fewer species and study locations were employed in the European studies. There is evidence from some European studies that a lower (Pleijel et al., 1997) or higher (Finnan et al., 1997; Finnan et al., 1996) cutoff value in indices with a threshold may provide a better statistical fit to the experimental data. Finnan et al. (1997) used seven exposure studies of spring wheat to confirm that cumulative exposure indices emphasizing higher $O_3$ concentrations were best related to plant response and that cumulative exposure indices using weighting functions, including cutoff concentrations, allometric and sigmoidal, provided a better fit and that the ranking of these indices differed depending on the exposure-response model used. Weighting those concentrations associated with sunshine hours in an attempt to incorporate an element of plant uptake did not improve the index performance (Finnan et al., 1997). A more recent study using data from several European studies of Norway spruce, analyzed the relationship between relative biomass accumulation and several cumulative, weighted indices, including the AOT40 (area over a threshold of 40ppb) and the SUM06 (Skarby et al., 2004). All the indices performed relatively well in regressing biomass and exposure index, with the AOT20 and AOT30 doing slightly better than others ($r^2$ = 0.46-0.47). In another comparative study of four independent data sets of potato yield and different cumulative uptake indices with different cutoff values, a similarly narrow range of $r^2$ was observed ($r^2$ = 0.3-0.4) (Pleijel et al., 2004b).

In Europe, the cutoff concentration-weighted index AOT40 was selected in developing exposure-response relationships based on OTC studies of a limited number of crops and trees (Grunhage and Jager, 2003). The United Nations Economic Commission for Europe (UNECE, 1988) adopted the critical levels approach for assessment of $O_3$ risk to vegetation across Europe. As used by the UNECE, the critical levels are not like the air quality regulatory standards used in the U.S., but rather function as planning targets for reductions in pollutant emissions to protect ecological resources. Critical levels for $O_3$ are intended to prevent long-term deleterious effects on the most sensitive plant species under the most sensitive environmental conditions, but not intended to quantify $O_3$ effects. A critical level was defined as "the concentration of pollutant in the atmosphere above which direct adverse effects on receptors, such as plants, ecosystems, or materials may occur according to present knowledge" (UNECE, 1988). The nature of the "adverse effects" was not specified in the original definition, which provided for different levels for different types of harmful effect (e.g., visible injury or loss of crop yield). There are also different critical levels for crops, forests, and semi-natural vegetation. The caveat, "according to present knowledge" is important because critical levels are not rigid; they are revised periodically as new scientific information becomes available. For example, the original critical level for $O_3$ specified concentrations for three averaging times, but further research and debate led to the current critical level being stated as the cumulative exposure (concentration × hours) over a cutoff concentration of 40 ppb (AOT40) (Fuhrer et al., 1997).

More recently in Europe, a decision was made to work toward a flux-based approach (see Section 9.5.4) for the critical levels ("Level II"), with the goal of modeling $O_3$ flux-effect relationships for three vegetation types: crops, forests, and semi-natural vegetation (Grunhage and Jager, 2003). Progress has been made in modeling flux (U.S. EPA, 2006b) and the Mapping Manual is being revised (Ashmore et al., 2004a, b; Grennfelt, 2004; Karlsson et al., 2003). The revisions may include a flux-based approach for three crops: wheat, potatoes, and cotton. However, because of a lack of flux-response data, a cumulative, cutoff concentration-based (AOTx) exposure index will remain in use for the near future for most crops and for forests and semi-natural herbaceous vegetation (Ashmore et al., 2004b).

In both the U.S. and Europe, the adequacy of these numerical summaries of exposure in relating biomass and yield changes have, for the most part, all been evaluated using data from studies not necessarily designed to compare one index to another (Skarby et al., 2004; Lee et al., 1989; Lefohn et al., 1988). Very few studies in the U.S. have addressed this issue since the 2006 $O_3$ AQCD. McLaughlin et al. (2007a) reported that the cumulative exposure index of AOT60 related well to reductions in growth rates at forest sites in the southern Appalachian Mountains. However, the authors did not report an analysis to compare multiple indices. Overall, given the available data from previous $O_3$ AQCDs and the few recent studies, the cumulative, concentration-weighted indices perform better than the peak or mean indices. It is still not possible, however, to distinguish the differences in performance among the cumulative, concentration-weighted indices.

The main conclusions from the 1996 and 2006 $O_3$ AQCDs regarding an index based on ambient exposure are still valid. No information has come forth since the 2006 $O_3$ AQCD to alter those conclusions. These key conclusions can be restated as follows:

- ozone effects in plants are cumulative;
- higher $O_3$ concentrations appear to be more important than lower concentrations in eliciting a response;
- plant sensitivity to $O_3$ varies with time of day and plant development stage;
- quantifying exposure with indices that accumulate the $O_3$ hourly concentrations and preferentially weight the higher concentrations improves the explanatory power of exposure/response models for growth and yield, over using indices based on mean and peak exposure values.

Following the 2006 criteria review process (U.S. EPA, 2006b), the EPA proposed an alternative form of the secondary NAAQS for $O_3$ using a cumulative, concentration-weighted exposure index to protect vegetation from damage (72 FR 37818). The EPA considered two specific concentration-weighted indices: the cutoff concentration weighted SUM06 and the sigmoid-weighted W126 exposure index (U.S. EPA, 2007b). These two indices performed equally well in predicting the exposure-response relationships observed in the crop and tree seedlings studies (Lee et al., 1989). At a workshop convened to consider the science supporting these indices (Heck and Cowling, 1997) there was a consensus that these cumulative concentration-weighted indices being considered were equally capable of predicting plant response. It should be noted that there are some important differences between the SUM06 and W126. When considering the response of vegetation to $O_3$ exposures represented by the threshold (e.g., SUM06) and non-threshold (e.g., W126) indices, the W126 metric does not have a cut-off in the weighting scheme as does SUM06 and thus it includes consideration of potentially damaging exposures below 60 ppb. The W126 metric also adds increasing weight to hourly concentrations from about 40 ppb to about 100 ppb (Lefohn et al., 1988; Lefohn and Runeckles, 1987). This is unlike cut-off metrics such as the SUM06 where all concentrations above 60 ppb are treated equally. This is an important feature of the W126 since as hourly concentrations become higher, they become increasingly likely to overwhelm plant defenses and are known to be more detrimental to vegetation (see Section 9.5.3.1).

## 9.5.3 Important Components of Exposure Indices

In the previous $O_3$ AQCDs it was established that higher hourly concentrations have greater effects on vegetation than lower concentrations (U.S. EPA, 2006b, 1996c). Further, it was determined that the diurnal and seasonal duration of exposure is important for plant response. Weighting of hourly concentrations and the diurnal and seasonal time window of exposure are the most important variables in a cumulative exposure index and will be discussed below. However, these variables should be looked at in the context of plant phenology, diurnal conductance rates, plant canopy structure, and detoxification mechanisms of vegetation as well as the climate and

meteorology, all of which are determinants of plant response. These more specific factors will be discussed in the uptake and dose modeling Section 9.5.4.

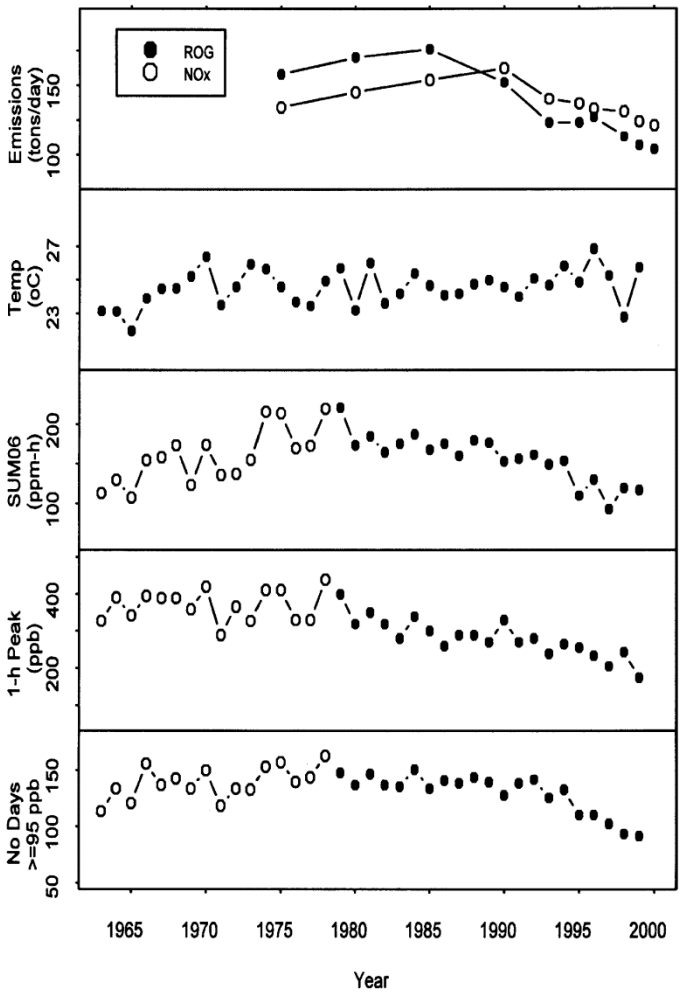
### 9.5.3.1 Role of Concentration

The significant role of peak $O_3$ concentrations was established based on several experimental studies (U.S. EPA, 1996c). Several studies (Oksanen and Holopainen, 2001; Yun and Laurence, 1999; Nussbaum et al., 1995) have added support for the important role that peak concentrations, as well as the pattern of occurrence, plays in plant response to $O_3$. Oksanen and Holopainen (2001) found that the peak concentrations and the shape of the $O_3$ exposure (i.e., duration of the event) were important determinants of foliar injury in European white birch saplings, but growth reductions were found to be more related to total cumulative exposure. Based on air quality data from 10 U.S. cities, three 4-week exposure treatments having the same SUM06 value were constructed by Yun and Laurence (1999). The authors used different exposure regimes to explore effects of treatments with variable versus uniform peak occurrence during the exposure period. The authors reported that the variable peak exposures were important in causing injury, and that the different exposure treatments, although having the same SUM06, resulted in very different patterns of foliar injury. Nussbaum et al. (1995) also found peak concentrations and the pattern of occurrence to be critical in determining the measured response. The authors recommended that to describe the effect on total forage yield, peak concentrations >0.11 ppm must be emphasized by using an AOT with higher threshold concentrations.

A greater role for peak concentrations in effects on plant growth might be inferred based on air quality analyses for the southern California area (Tingey et al., 2004; Lee et al., 2003a). In the late 1960s and 1970s, extremely high $O_3$ concentrations had impacted the San Bernardino National Forest. However, over the past 20+ years, significant reductions in $O_3$ exposure have occurred (Bytnerowicz et al., 2008; Lee et al., 2003a; Lefohn and Shadwick, 2000; Davidson, 1993). An illustration of this improvement in air quality is shown by the 37-year history of $O_3$ air quality at the Crestline site in the San Bernardino Mountains (Figure 9-10) (Lee et al., 2003a). Ozone exposure increased from 1963 to 1979 concurrent with increased population and vehicular miles, followed by a decline to the present mirroring decreases in precursor emissions. The pattern in exposure was evident in various exposure indices including the cumulative concentration weighted (SUM06), as well as maximum peak event (1-h peak), and the number of days having hourly averaged $O_3$ concentrations greater than or equal to 95 ppb. The number of days having hourly averaged $O_3$ concentrations greater than or equal to 95 ppb declined significantly from 163 days in 1978 to 103 days in 1997. The changes in ambient $O_3$ air quality for the Crestline site were reflected in the changes in frequency and magnitude of the peak hourly concentration and the duration of exposure (Figure 9-10). Considering the role of exposure patterns in determining response, the seasonal and diurnal

patterns in hourly $O_3$ concentration did not vary appreciably from year to year over the 37-year period (Lee et al., 2003a).

The potential importance of exposure to peak concentrations comes both from results of measures of tree conditions on established plots and from results of model simulations. Across a broad area of the San Bernardino National Forest, the Forest Pest Management (FPM) method of injury assessment indicated an improvement in crown condition from 1974 to 1988; and the area of improvement in injury assessment is coincident with an improvement in $O_3$ air quality (Miller and Rechel, 1999). A more recent analysis of forest changes in the San Bernardino National Forest, using an expanded network of monitoring sites, has verified significant changes in growth, mortality rates, basal area, and species composition throughout the area since 1974 (Arbaugh et al., 2003). A model simulation of ponderosa pine growth over the 40-year period in the San Bernardino National Forest showed a significant impact of $O_3$ exposure on tree growth and indicates improved growth with reduced $O_3$ concentrations. This area has also experienced elevated N deposition and based on a number of environmental indicators, it appears that this area is experiencing N saturation (Fenn et al., 1996). To account for this potential interaction, the model simulations were conducted under conditions of unlimited soil N. The actual interactions are not known. The improvement in growth over the years was attributed to improved air quality, but no distinction was made regarding the relative role of "mid-range" and higher hourly concentrations, only that improved growth tracked decreasing SUM06, maximum peak concentration, and number of days of hourly $O_3$ >95 ppb (Tingey et al., 2004). A summary of air quality data from 1980 to 2000 for the San Bernardino National Forest area of the number of "mid-range" hourly concentrations indicated no dramatic changes over this 20-year period, ranging from about 1,500 to 2,000 hours per year (Figure 9-11). There was a slow increase in the number of "mid-range" concentrations from 1980 to 1986, which corresponds to the period after implementation of the air quality standard. Another sharper increase was observed in the late 1990s. This pattern of occurrence of mid-range hourly concentrations suggests a lesser role for these concentration ranges compared to the higher values in either of the ground-level tree injury observations of the model simulation of growth over the 40-year period.




Note: Annual ROG and $NO_X$ emissions data for San Bernardino County were obtained from Alexis et al. (2001a) and the California Air Resource Board's emission inventory available at http://www.arb.ca.gov/html/ds.htm (Cal/EPA, 2010).

Source: Reprinted with permission of Elsevier Science Ltd. (Lee et al., 2003a).

**Figure 9-10 Trends in May to September: 12-hour SUM06, Peak 1-hour $O_3$ concentration and number of daily exceedances of 95 ppb for the Crestline site in 1963 to 1999; in relation to trends in mean daily maximum temperature for Crestline and daily reactive organic gases (ROG) and oxides of nitrogen ($NO_X$) for San Bernardino County.**

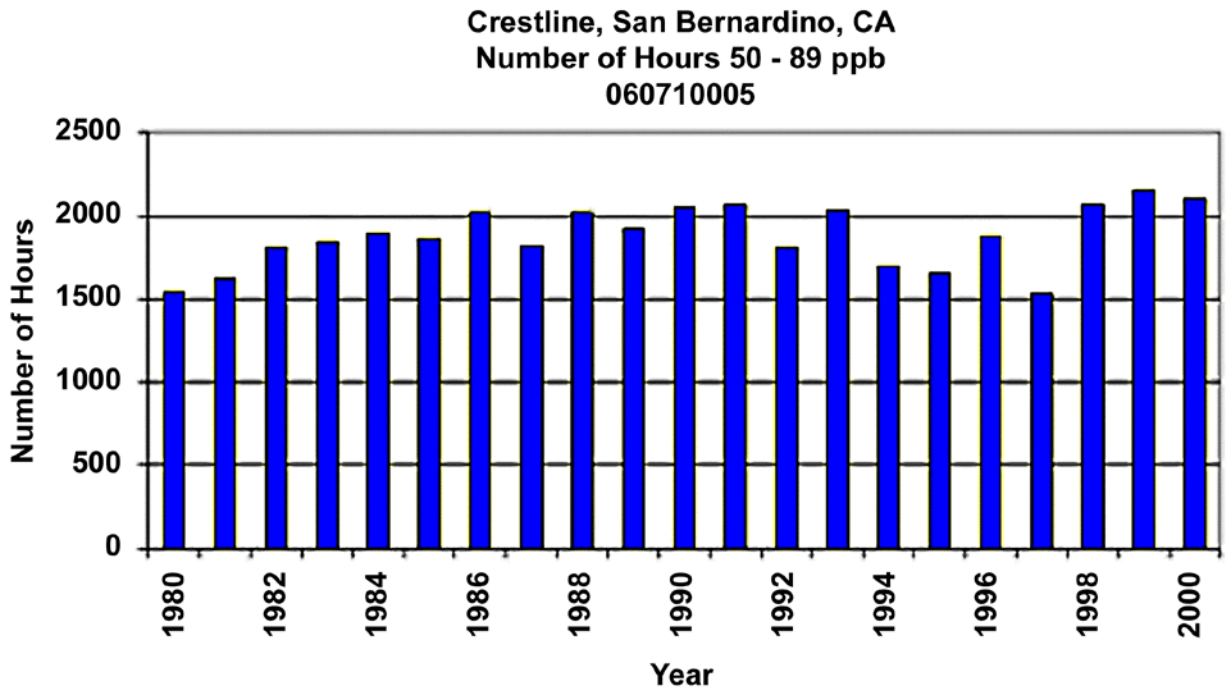



**Figure 9-11 The number of hourly average concentrations between 50 and 89 ppb for the period 1980-2000 for the Crestline, San Bernardino County, CA, monitoring site.**

### 9.5.3.2 Diurnal and Seasonal Exposure

#### Diurnal Exposure

The diurnal patterns of maximal leaf/needle conductance and occurrence of higher ambient concentrations can help determine which hours during the day over a season should be included in an exposure index. Stomatal conductance is species and phenology dependent and is linked to both diurnal and seasonal meteorological activity as well as to soil/site conditions (e.g., VPD, soil moisture). Daily patterns of leaf/needle conductance are often highest in midmorning, whereas higher ambient $O_3$ concentrations generally occur in early to late afternoon when stomata are often partially closed and conductances are lower. Total $O_3$ flux depends on atmospheric and boundary layer resistances, both of which exhibit variability throughout the day. Experimental studies with tree species demonstrated the decoupling of ambient $O_3$ exposure, peak occurrence, and gas exchange, particularly in areas of drought (Panek, 2004). Several studies have suggested that ponderosa pine trees in the southern and northern Sierra Nevada Mountains may not be as susceptible to high $O_3$ concentrations as to lower concentrations, due to reduced needle conductance and $O_3$ uptake during the period when the highest concentrations occur (Panek et al., 2002; Panek and Goldstein, 2001; Bauer et al., 2000; Arbaugh et al., 1998). Panek et al.

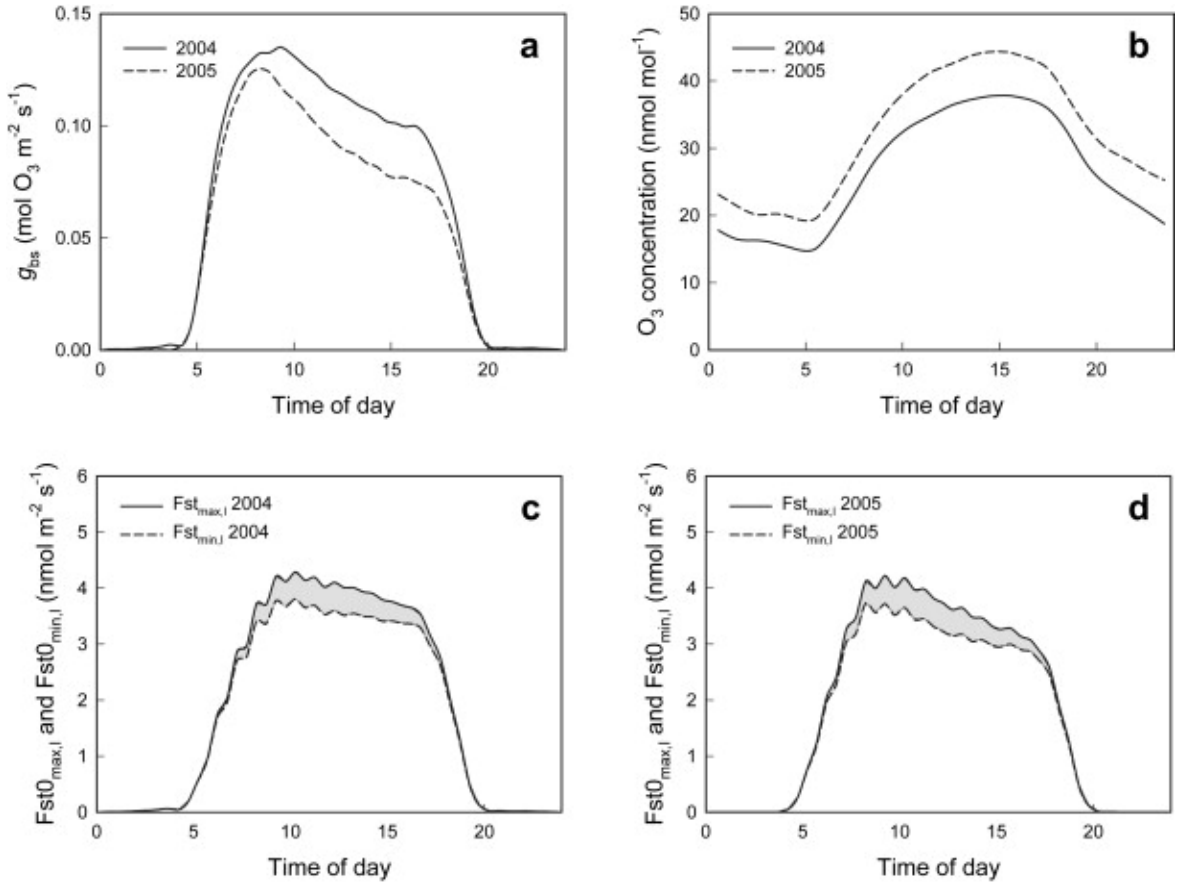
(2002) compared direct $O_3$ flux measurements into a canopy of ponderosa pine and demonstrated a lack of correlation of daily patterns of conductance and $O_3$ occurrence, especially in the late season drought period; the authors concluded that a consideration of climate or season was essential, especially considering the role of soil moisture and conductance/uptake. In contrast, Grulke et al. (2002) reported high conductance when $O_3$ concentrations were high in the same species, but under different growing site conditions. The longer-term biological responses reported by Miller and Rechel (1999) for ponderosa pine in the same region, and the general reduction in recent years in ambient $O_3$ concentrations, suggest that stomatal conductance alone may not be a sufficient indicator of potential vegetation injury or damage. Another consideration for the effect of $O_3$ uptake is the diurnal pattern of detoxification capacity of the plant. The detoxification capacity may not follow the same pattern as stomatal conductance (Heath et al., 2009).

The use of a 12-h (8:00 a.m. to 8:00 p.m.) daylight period for a W126 cumulating exposure was based primarily on evidence that the conditions for uptake of $O_3$ into the plant occur mainly during the daytime hours. In general, plants have the highest stomatal conductance during the daytime and in many areas atmospheric turbulent mixing is greatest during the day as well (Uddling et al., 2010; U.S. EPA, 2006b). However, notable exceptions to maximum daytime conductance are cacti and other plants with crassulacean acid metabolism (CAM photosynthesis) which only open their stomata at night. This section will focus on plants with C3 and C4 photosynthesis, which generally have maximum stomatal conductance during the daytime.

Recent reviews of the literature reported that a large number of species had varying degrees of nocturnal stomatal conductance (Caird et al., 2007; Dawson et al., 2007; Musselman and Minnick, 2000). The reason for night-time water loss through stomata is not well understood and is an area of active research (e.g., Christman et al., 2009; Howard et al., 2009). Night-time stomatal opening may be enhanced by $O_3$ damage that could result in loss of stomatal control, and less complete closure of stomata, than under low $O_3$ conditions (Caird et al., 2007; Grulke et al., 2007b). In general, the rate of stomatal conductance at night is much lower than during the day (Caird et al., 2007). Atmospheric turbulence at night is also often low, which results in stable boundary layers and unfavorable conditions for $O_3$ uptake into vegetation (Finkelstein et al., 2000). Nevertheless, nocturnal turbulence does intermittently occur and may result in non-negligible $O_3$ flux into the plants. In addition, plants might be more susceptible to $O_3$ exposure at night than during the daytime, because of potentially lower plant defenses (Heath et al., 2009; Loreto and Fares, 2007; Musselman et al., 2006; Musselman and Minnick, 2000). For significant nocturnal stomatal flux and $O_3$ effects to occur, specific conditions must exist. A susceptible plant with nocturnal stomatal conductance and low defenses must be growing in an area with relatively high night-time $O_3$ concentrations and appreciable nocturnal atmospheric turbulence. It is unclear how many areas there are in the U.S. where these conditions occur. It may be possible that these conditions exist in mountainous areas of southern California, front-range of Colorado (Turnipseed et al., 2009) and the Great Smoky Mountains of North Carolina and Tennessee. Tobiessen

(1982) found that shade intolerant tree species showed opening of stomata in the dark and did not find this in shade tolerant species. This may indicate shade intolerant trees may be more likely to be susceptible to $O_3$ exposure at night. More information is needed in locations with high night-time $O_3$ to assess the local $O_3$ patterns, micrometeorology and responses of potentially vulnerable plant species.

Several field studies have attempted to quantify night-time $O_3$ uptake with a variety of methods. However, many of these studies have not linked the night-time flux to measured effects on plants. Grulke et al. (2004) showed that the stomatal conductance at night for ponderosa pine in the San Bernardino National Forest (CA) ranged from one tenth to one fourth that of maximum daytime stomatal conductance. In June, at a high-elevation site, it was calculated that 11% of the total daily $O_3$ uptake of pole-sized trees occurred at night. In late summer, however, $O_3$ uptake at night was negligible. However, this study did not consider the turbulent conditions at night. Finkelstein et al. (2000) investigated $O_3$ deposition velocity to forest canopies at three different sites. The authors found the total flux (stomatal and non-stomatal) to the canopy to be very low during night-time hours as compared to day-time hours. However, the authors did note that higher nocturnal deposition velocities at conifer sites may be due to some degree of stomatal opening at night (Finkelstein et al., 2000). Work by Mereu et al. (2009) in Italy on Mediterranean species indicated that nocturnal uptake was from 10 to 18% of total daily uptake during a weak drought and up to 24% as the drought became more pronounced. The proportion of night-time uptake was greater during the drought due to decreases in daytime stomatal conductance (Mereu et al., 2009). In a study conducted in California, (Fares et al., 2011) reported that calculated mean percentages of nocturnal uptake were 5%, 12.5%, 6.9% of total $O_3$ uptake for lemon, mandarin, and orange, respectively. In another recent study at the Aspen FACE site in Wisconsin, calculated leaf-level stomatal $O_3$ flux was near zero from the night-time hours of 8:00 p.m. to 5:00 a.m. (Uddling et al., 2010). This was likely due to low horizontal wind speed (>1 meter/sec) and low $O_3$ concentrations (<25 ppb) during those same night-time hours (Figure 9-12).




Note: Subscripts "max" and "min" refer to stomatal fluxes calculated neglecting and accounting for potential non-stomatal $O_3$ flux, respectively.

Source: Reprinted with permission of Elsevier Ltd. (Uddling et al., 2010).

**Figure 9-12 Diurnal (a) conductance through boundary layer and stomata (gbs), (b) ozone concentration, and leaf-level stomatal $O_3$ flux (Fst0l) in control plots from mid-June through August, in (c) 2004 and (d) 2005 in the Aspen FACE experiment.**

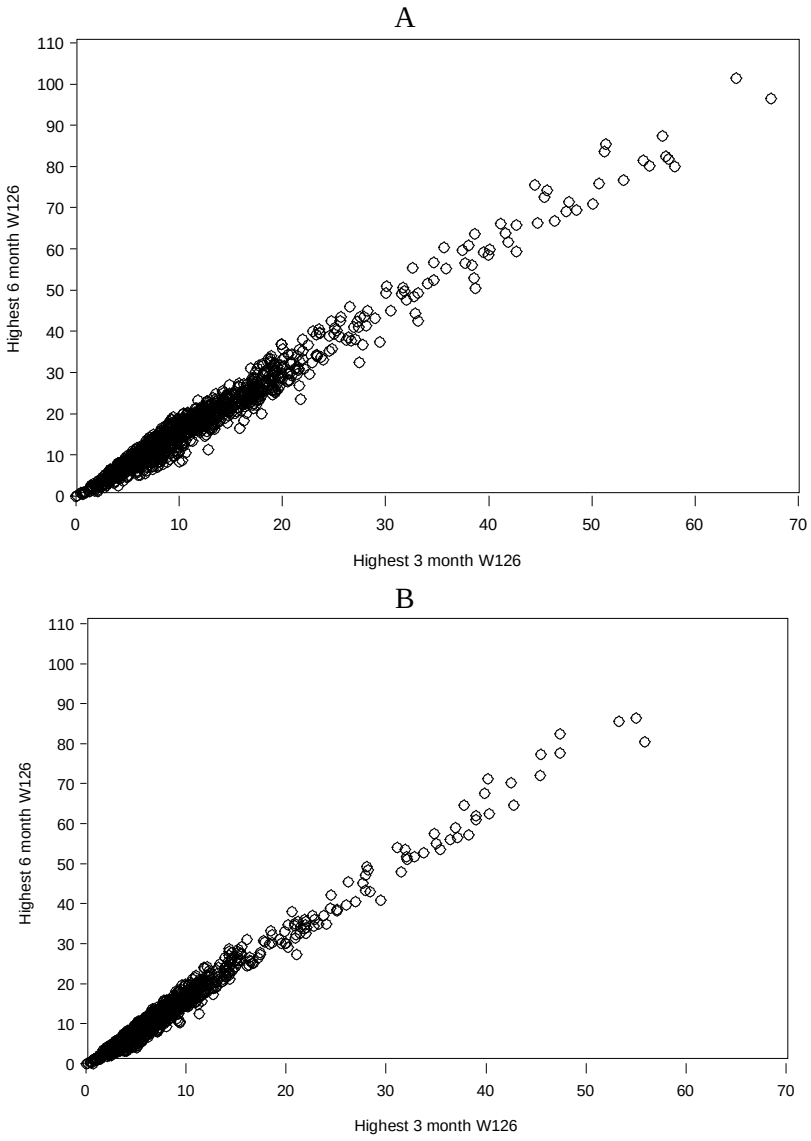
A few studies have tested the biological effects of night-time $O_3$ exposure on vegetation in controlled chambers. Biomass of ponderosa pine seedlings was significantly reduced when seedlings were exposed to either daytime or nighttime episodic profiles (Lee and Hogsett, 1999). However, the biomass reductions were much greater with daytime peak concentrations than with nighttime peak concentrations. Similarly, birch cuttings grown in field chambers that were exposed to $O_3$ at night only, daytime only, and 24 hours showed similar reductions in biomass in night only and day only treatments. Birch seedling showed greater reductions in growth in 24-h exposures than those exposed to $O_3$ at night or day only (Matyssek et al., 1995). Field mustard (*Brassica rapa*) plants exposed to $O_3$ during the day or night showed little significant difference in the amounts of injury or reduced growth response to $O_3$ treatment, although the stomatal conductance was 70-80% lower at

night (Winner et al., 1989). These studies show that effects can be seen with night-time exposures to $O_3$ but when atmospheric conditions are stable at night, it is uncertain how these exposures may affect plants and trees with complex canopies in the field.

### Seasonal exposure

Vegetation across the U.S. has widely varying periods of physiological activity during the year due to variability in climate and phenology. In order for a particular plant to be vulnerable to $O_3$ pollution, it must have foliage and be physiologically active. Annual crops are typically grown for periods of two to three months. In contrast, perennial species may be photosynthetically active longer (up to 12 months each year for some species) depending on the species and where it is grown. In general, the period of maximum physiological activity and thus, potential $O_3$ uptake for vegetation coincides with some or all of the intra-annual period defined as the $O_3$ season, which varies on a state-by-state basis (Figure 3-24). This is because the high temperature and high light conditions that typically promote the formation of tropospheric $O_3$ also promote physiological activity in vegetation. There are very limited exceptions to this pattern where $O_3$ can form in the winter in areas in the western U.S. with intense natural gas exploration (Pinto, 2009), but this is typically when plants are dormant and there is little chance of $O_3$ uptake. Given the significant variability in growth patterns and lengths of growing season among the wide range of vegetation species that may experience adverse effects associated with $O_3$ exposure, no single time window of exposure can work perfectly for all types of vegetation.

Various intra-annual averaging and accumulation time periods have been considered for the protection of vegetation. The 2007 proposal for the secondary $O_3$ standard (75 FR 37818) proposed to use the maximum consecutive 3-month period within the $O_3$ season. The U.S. Forest Service and federal land managers have used a 24-h W126 accumulated for 6 months from April through September (U.S. Forest Service, 2000). However, some monitors in the U.S. are operational for as little as four months and would not have enough data for a 6-month seasonal window. The exposure period in the vast majority of $O_3$ exposure studies conducted in the U.S. has been much shorter than 6 months. Most of the crop studies done through NCLAN had exposures less than three months with an average of 77 days. Open-top chamber studies of tree seedlings, compiled by the EPA, had an average exposure of just over three months or 99 days. In more recent FACE experiments, SoyFACE exposed soybeans for an average of approximately 120 days per year and the Aspen FACE experiment exposed trees to an average of approximately 145 days per year of elevated $O_3$, which included the entire growing season at those particular sites. Despite the possibility that plants may be exposed to ambient $O_3$ longer than 3 months in some locations, there is generally a lack of exposure experiments conducted for longer than 3 months.




Note: Data are from the AQS and CASTNET monitors for the years 2008 and 2009. (A) W126, 3 month versus 6 month, 2008 (Pearson correlation = 0.99); (B) W126, 3 month versus 6 month, 2009 (Pearson correlation = 0.99).

**Figure 9-13 Maximum 3-month, 12-h W126 plotted against maximum 6-month, 12-h W126.**

In an analysis of the 3- and 6-month maximum W126 values calculated for over 1,200 AQS (Air Quality System) and CASTNET (Clean Air Status and Trend Network) EPA monitoring sites for the years 2008-2009, it was found that these 2 accumulation periods resulted in highly correlated metrics (Figure 9-13). The two accumulation periods were centered on the yearly maximum for each monitoring site, and it is possible that this correlation would be weaker if the two periods were not temporally aligned. In the U.S., W126 cumulated over 3 months, and W126

cumulated over 6 months are proxies of one another, as long as the period in which daily W126 is accumulated corresponds to the seasonal maximum. Therefore, it is expected that either statistic will predict vegetation response equally well. In other words, the strength of the correlation between maximum 3-month W126 and maximum 6-month W126 is such that there is no material difference in their predictive value for vegetation response.

### 9.5.4 Ozone Uptake/Dose Modeling for Vegetation

Another approach for improving risk assessment of vegetation response to ambient $O_3$ is based on estimating the $O_3$ concentration from the atmosphere that enters the leaf (i.e., flux or deposition). Interest has been increasing in recent years, particularly in Europe, in using mathematically tractable flux models for $O_3$ assessments at the regional, national, and European scale (Matyssek et al., 2008; Paoletti and Manning, 2007; ICP M&M, 2004; Emberson et al., 2000b; Emberson et al., 2000a). Some researchers have claimed that using flux models can be used to better predict vegetation responses to $O_3$ than exposure-based approaches (Matyssek et al., 2008). However, other research has suggested that flux models do not predict vegetation responses to $O_3$ better than exposure-based models, such as AOT40 (Gonzalez-Fernandez et al., 2010). While some efforts have been made in the U.S. to calculate $O_3$ flux into leaves and canopies (Fares et al., 2010a; Turnipseed et al., 2009; Uddling et al., 2009; Bergweiler et al., 2008; Hogg et al., 2007; Grulke et al., 2004; Grantz et al., 1997; Grantz et al., 1995), little information has been published relating these fluxes to effects on vegetation. The lack of flux data in the U.S. and the lack of understanding of detoxification processes have made this technique less viable for vulnerability and risk assessments in the U.S.

Flux calculations are data intensive and must be carefully implemented. Reducing uncertainties in flux estimates for areas with diverse surface or terrain conditions to within ± 50% requires "very careful application of dry deposition models, some model development, and support by experimental observations" (Wesely and Hicks, 2000). As an example, the annual average deposition velocity of $O_3$ among three nearby sites in similar vegetation was found to vary by ± 10%, presumably due to terrain (Brook et al., 1997). Moreover, the authors stated that the actual variation was even greater, because stomatal uptake was unrealistically assumed to be the same among all sites, and flux is strongly influenced by stomatal conductance (Brook et al., 1997; Massman and Grantz, 1995; Fuentes et al., 1992; Reich, 1987; Leuning et al., 1979). This uptake-based approach to quantify the vegetation impact of $O_3$ requires inclusion of those factors that control the diurnal and seasonal $O_3$ flux to vegetation (e.g., climate patterns, species and/or vegetation-type factors and site-specific factors). The models have to distinguish between stomatal and non-stomatal components of $O_3$ deposition to adequately estimate actual concentration reaching the target tissue of a plant to elicit a response (Uddling et al., 2009). Determining this $O_3$ uptake via canopy and stomatal conductance relies on models to predict flux and ultimately the "effective" flux (Grunhage et al., 2004; Massman, 2004; Massman et

al., 2000). "Effective flux" has been defined as the balance between $O_3$ flux and detoxification processes (Heath et al., 2009; Musselman and Massman, 1999; Grunhage and Haenel, 1997; Dammgen et al., 1993). The time-integrated "effective flux" is termed "effective dose." The uptake mechanisms and the resistances in this process, including stomatal conductance and biochemical defense mechanisms, are discussed below. The flux-based index is the goal for the "Level II" critical level for assessment of $O_3$ risk to vegetation and ecosystems across Europe (Ashmore et al., 2004a).

An important consideration in both $O_3$ exposure and uptake is how the $O_3$ concentration at the top of low vegetation such as, crops and tree seedlings may be lower than the height at which the measurement is taken. Ambient monitor inlets in the U.S. are typically at heights of 3 to 5 meters. During daytime hours, the vertical $O_3$ gradient can be relatively small because turbulent mixing maintains the downward flux of $O_3$. For example, Horvath et al. (1995) calculated a 7% decrease in $O_3$ going from a height of 4 meters down to 0.5 meters above the surface during unstable (or turbulent) conditions in a study over low vegetation in Hungary [see Section AX3.3.2. of the 2006 $O_3$ AQCD (U.S. EPA, 2006b)]. There have been several studies indicating decreased $O_3$ concentrations under tree canopies (Kolb et al., 1997; Samuelson and Kelly, 1997; Joss and Graber, 1996; Fredericksen et al., 1995; Lorenzini and Nali, 1995; Enders, 1992; Fontan et al., 1992; Neufeld et al., 1992). In contrast, for forests, measured data may underestimate $O_3$ concentration at the top of the canopy. The difference between measurement height and canopy height is a function of several factors, the intensity of turbulent mixing in the surface layer and other meteorological factors, canopy height and total deposition to the canopy. Some researchers have used deposition models to estimate $O_3$ concentration at canopy-top height based on concentrations at measurement height (Emberson et al., 2000a). However, deposition models usually require meteorological data inputs that are not always available or well characterized across large geographical scales.

Soil moisture is a critical factor in controlling $O_3$ uptake through its effect on plant water status and stomatal conductance. In an attempt to relate uptake, soil moisture, and ambient air quality to identify areas of potential risk, available $O_3$ monitoring data for 1983 to 1990 were used along with literature-based seedling exposure-response data from regions within the southern Appalachian Mountains that might have experienced $O_3$ exposures sufficient to inhibit growth (Lefohn et al., 1997). In a small number of areas within the region, $O_3$ exposures and soil moisture availability were sufficient to possibly cause growth reductions in some $O_3$ sensitive species (e.g., black cherry). The conclusions were limited, however, because of the uncertainty in interpolating $O_3$ exposures in many of the areas and because the hydrologic index used might not reflect actual water stress.

The non-stomatal component of plant defenses are the most difficult to quantify, but some studies are available (Heath et al., 2009; Barnes et al., 2002; Plochl et al., 2000; Chen et al., 1998; Massman and Grantz, 1995). Massman et al. (2000) developed a conceptual model of a dose-based index to determine how plant injury response to $O_3$ relates to the traditional exposure-based parameters. The index used time-

varying-weighted fluxes to account for the fact that flux was not necessarily correlated with plant injury or damage. The model applied only to plant foliar injury and suggested that application of flux-based models for determining plant damage (yield or biomass) would require a better understanding and quantification of the relationship between injury and damage.

### 9.5.5 Summary

Exposure indices are metrics that quantify exposure as it relates to measured plant damage (i.e., reduced growth). They are summary measures of monitored ambient $O_3$ concentrations over time intended to provide a consistent metric for reviewing and comparing exposure-response effects obtained from various studies. No recent information is available since 2006 that alters the basic conclusions put forth in the 2006 and 1996 $O_3$ AQCDs. These AQCDs focused on the research used to develop various exposure indices to help quantify effects on growth and yield in crops, perennials, and trees (primarily seedlings). The performance of indices was compared through regression analyses of earlier studies designed to support the estimation of predictive $O_3$ exposure-response models for growth and/or yield of crops and tree (seedling) species.

Another approach for improving risk assessment of vegetation response to ambient $O_3$ is based on determining the $O_3$ concentration from the atmosphere that enters the leaf (i.e., flux or deposition). Interest has been increasing in recent years, particularly in Europe, in using mathematically tractable flux models for $O_3$ assessments at the regional, national, and European scale (Matyssek et al., 2008; Paoletti and Manning, 2007; ICP M&M, 2004; Emberson et al., 2000b; Emberson et al., 2000a). While some efforts have been made in the U.S. to calculate $O_3$ flux into leaves and canopies (Turnipseed et al., 2009; Uddling et al., 2009; Bergweiler et al., 2008; Hogg et al., 2007; Grulke et al., 2004; Grantz et al., 1997; Grantz et al., 1995), little information has been published relating these fluxes to effects on vegetation. There is also concern that not all $O_3$ stomatal uptake results in a yield reduction, which depends to some degree on the amount of internal detoxification occurring with each particular species. Those species having high amounts of detoxification potential may, in fact, show little relationship between $O_3$ stomatal uptake and plant response (Musselman and Massman, 1999). The lack of data in the U.S. and the lack of understanding of detoxification processes have made this technique less viable for vulnerability and risk assessments in the U.S.

The main conclusions from the 1996 and 2006 $O_3$ AQCDs regarding indices based on ambient exposure are still valid. These key conclusions can be restated as follows:

- Ozone effects in plants are cumulative;
- higher $O_3$ concentrations appear to be more important than lower concentrations in eliciting a response;
- plant sensitivity to $O_3$ varies with time of day and plant development stage;
- quantifying exposure with indices that accumulate the $O_3$ hourly concentrations and preferentially weight the higher concentrations improves the explanatory power of exposure/response models for growth and yield, over using indices based on mean and peak exposure values.

Various weighting functions have been used, including threshold-weighted (e.g., SUM06) and continuous sigmoid-weighted (e.g., W126) functions. Based on statistical goodness-of-fit tests, these cumulative, concentration-weighted indices could not be differentiated from one another using data from previous exposure studies. Additional statistical forms for $O_3$ exposure indices have been discussed in Lee et al. (1988b). The majority of studies published since the 2006 $O_3$ AQCD do not change earlier conclusions, including the importance of peak concentrations, and the duration and occurrence of $O_3$ exposures in altering plant growth and yield.

Given the current state of knowledge and the best available data, exposure indices that cumulate and differentially weight the higher hourly average concentrations and also include the "mid-level" values continue to offer the most defensible approach for use in developing response functions and comparing studies, as well as for defining future indices for vegetation protection.

## 9.6 Ozone Exposure-Plant Response Relationships

### 9.6.1 Introduction

The adequate characterization of the effects of $O_3$ on plants for the purpose of setting air quality standards is contingent not only on the choice of the index used (i.e., SUM06, W126) to summarize $O_3$ concentrations (Section 9.5), but also on quantifying the response of the plant variables of interest at specific values of the selected index. The many factors that determine the response of plants to $O_3$ exposure have been discussed in previous sections. They include species, genotype and other genetic characteristics (Section 9.3), biochemical and physiological status (Section 9.3), previous and current exposure to other stressors (Section 9.4.8), and characteristics of the exposure itself (Section 9.5). Establishing a secondary air quality standard entails the capability to generalize those observations, in order to obtain predictions that are reliable enough under a broad variety of conditions, taking into account these factors. This section reviews results that have related specific quantitative observations of $O_3$ exposure with quantitative observations of plant

responses, and the predictions of responses that have been derived from those observations through empirical models.

For four decades, exposure to $O_3$ at ambient concentrations found in many areas of the U.S. has been known to cause detrimental effects in plants (U.S. EPA, 2006b, 1996b, 1984, 1978a). Results published after the 2006 $O_3$ AQCD continue to support this finding, and the following sections deal with the quantitative characterizations of the relationship, and what new insights may have appeared since 2006. Detrimental effects on plants include visible injury, decreases in the rate of photosynthesis, reduced growth, and reduced yield of marketable plant parts. Most published exposure-response data have reported $O_3$ effects on the yield of crops and the growth of tree seedlings, and those two variables have been the focus of the characterization of ecological impacts of $O_3$ for the purpose of setting secondary air quality standards. In order to support quantitative modeling of exposure-response relationships, data should preferably include more than three levels of exposure, and some control of potential confounding or interacting factors should be present in order to model the relationship with sufficient accuracy. Letting potential confounders, such as other stressors, vary freely when generating $O_3$ exposure-response data might improve the 'realism' of the data, but it also greatly increases the amount of data necessary to extract a clear quantitative description of the relationship. Conversely however, experimental settings should not be so exhaustively restrictive as to make generalization outside of them problematic. During the last four decades, many of the studies of the effects of $O_3$ on growth and yield of plants have not included enough levels of $O_3$ to parameterize more than the simplest linear model. The majority of these studies have only contrasted two levels, ambient and elevated, or sometimes three by adding carbon filtration in OTC studies, with little or no consideration of quantitatively relating specific values of exposure to specific values of growth or yield. This is not to say that studies that did not include more than two or three levels of $O_3$ exposure, or studies that were conducted in uncontrolled environments, do not provide exposure-response information that is highly relevant to reviewing air quality standards. In fact, they can be essential in verifying the agreement between predictions obtained through the empirical models derived from experiments such as NCLAN, and observations. The consensus of model predictions and observations from a variety of studies conducted in other locations, at other times, and using different exposure methods, greatly increases confidence in the reliability of both. Furthermore, if they are considered in the aggregate, studies with few levels of exposure or high unaccounted variability can provide additional independent estimates of decrements in plant growth and yield, at least within a few broad categories of exposure.

Extensive exposure-response information on a wide variety of plant species has been produced by two long-term projects that were designed with the explicit aim of obtaining quantitative characterizations of the response of such an assortment of crop plants and tree seedlings to $O_3$ under North American conditions: the NCLAN project for crops, and the EPA National Health and Environmental Effects Research Laboratory, Western Ecology Division tree seedling project (NHEERL/WED). The NCLAN project was initiated by the EPA in 1980 primarily to improve

estimates of yield loss under field conditions and to estimate the magnitude of crop losses caused by $O_3$ throughout the U.S. (Heck et al., 1991; Heck et al., 1982). The cultural conditions used in the NCLAN studies approximated typical agronomic practices, and the primary objectives were: (1) to define relationships between yields of major agricultural crops and $O_3$ exposure as required to provide data necessary for economic assessments and development of $O_3$ NAAQS; (2) to assess the national economic consequences resulting from $O_3$ exposure of major agricultural crops; and (3) to advance understanding of cause-and-effect relationships that determine crop responses to pollutant exposures.

NCLAN experiments yielded 54 exposure-response curves for 12 crop species, some of which were represented by multiple cultivars at several of 6 locations throughout the United States. The NHEERL/WED project was initiated by EPA in 1988 with similar objectives for tree species, and yielded 49 exposure-responses curves for multiple genotypes of 11 tree species grown for up to three years in Oregon, Michigan, and the Great Smoky Mountains National Park. Both projects used OTCs to expose plants to three to five levels of $O_3$. Eight of the 54 crop datasets were from plants grown under a combination of $O_3$ exposure and experimental drought conditions. Figure 9-14 through Figure 9-17 summarizes some of the NCLAN and NHEERL/WED results.

It should be noted that data from FACE experiments might also be used for modeling exposure-response. They only use two levels of $O_3$ (ambient concentration at the site and a multiple of it), but given that the value of both levels of exposure changes every year, and that they are typically run for many consecutive years, aggregating data over time produces twice as many levels of $O_3$ as there are years. As described in Section 9.2.4, FACE experiments seek to impose fewer constraints on the growth environment than OTCs. As a consequence, FACE studies have to contend with larger variability, especially year-to-year variability, but the difference in experimental conditions between the two methodologies makes comparisons between their results especially useful.

Growth and yield of at least one crop (soybean) has been investigated in yearly experiments since 2001 at a FACE facility in Illinois (UIUC, 2010; Morgan et al., 2006). However, almost all analyses of SoyFACE published so far have been based on subsets of one or two years, and have only contrasted ambient versus elevated $O_3$ as categorical variables. They have not modeled the response of growth and yield to $O_3$ exposure continuously over the range of exposure values that have occurred over time. The only exception is a study by Betzelberger et al. (2010), who used a linear regression model on data pooled over 2 years. Likewise, trees of three species (trembling aspen, paper birch, and sugar maple) were grown between 1998 and 2009 in a FACE experiment located in Rhinelander, Wisconsin (Pregitzer et al., 2008; Dickson et al., 2000). The Aspen FACE experiment has provided extensive data on responses of trees beyond the seedling stage under long-term exposure, and also on ecosystem-level responses (Section 9.4), but the only attempt to use those data in a continuous model of the response of tree growth to $O_3$ exposure (Percy et al., 2007) suffered from severe methodological problems, some of which are discussed in

Section 9.6.3. Finally, one experiment was able to exploit a naturally occurring gradient of $O_3$ concentrations to fit a linear regression model to the growth of cottonwood (Gregg et al., 2006, 2003). Factors such as genotype, soil type and soil moisture were under experimental control, and the authors were able to partition out the effects of potential confounders such as temperature, atmospheric N deposition, and ambient $CO_2$.

A serious difficulty in assessing results of exposure-response research is the multiplicity of $O_3$ metrics that have been used in reporting. As described in Section 9.5, metrics that entail either weighting or thresholding of hourly values cannot be algebraically converted into one another, or into unweighted metrics such as hourly average. When computing $O_3$ exposure using weighted or thresholded metrics, each metric has to be computed separately from the original hourly data. Comparisons of exposure-response models can only be made between studies that used the same metric, and the value of exposure at which a given plant response is expected using one metric of exposure cannot be exactly converted to another metric. Determining the exposure value at which an effect would be observed in a different metric can only be accomplished by first computing the experimental exposures in this metric from the hourly data, then estimating (fitting) model coefficients again. This problem is irremediable, although useful comparisons might be made using categorical exposures such as 'current ambient exposure' or '2050 projected exposure', which can serve as a common reference for quantitative values expressed in various metrics. Studies that contained growth or yield exposure-response data at few levels of exposure, and/or using metrics other than W126 are summarized in Table 9-17 and Table 9-18.

## 9.6.2 Estimates of Crop Yield Loss and Tree Seedling Biomass Loss in the 1996 and 2006 Ozone AQCDs

The 1996 and 2006 $O_3$ AQCDs relied extensively on analyses of NCLAN and NHEERL/WED by Lee et al. (1994; 1989, 1988b, 1987), Hogsett et al. (1997), Lee and Hogsett (1999), Heck et al. (1984), Rawlings and Cure (1985), Lesser et al. (1990), and Gumpertz and Rawlings (1992). Those analyses concluded that a three-parameter Weibull model –

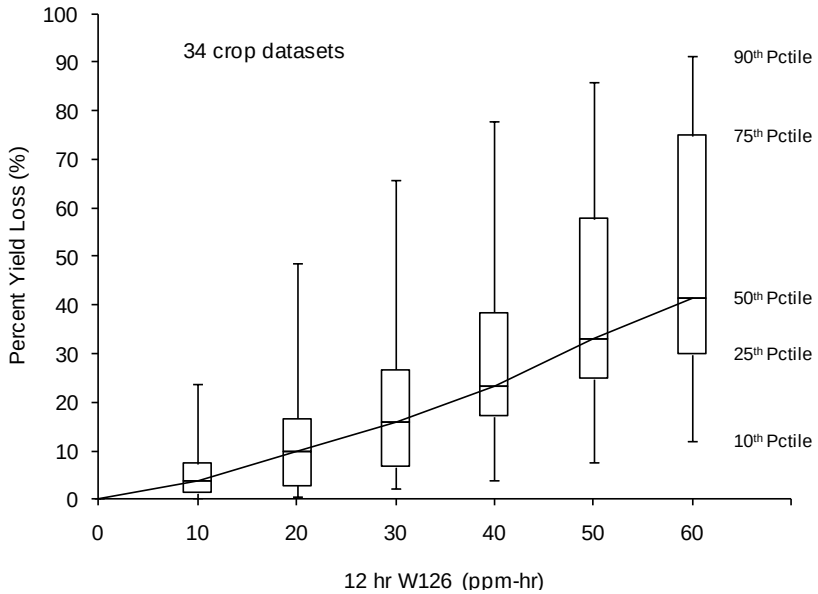
$$Y = \alpha e^{-\left(\frac{W126}{\eta}\right)^{\beta}}$$

**Equation 9-2**

is the most appropriate model for the response of absolute yield and growth to $O_3$ exposure, because of the interpretability of its parameters, its flexibility (given the small number of parameters), and its tractability for estimation. In addition, removing the intercept $\alpha$ results in a model of relative yield (yield relative to [yield at exposure=0]) without any further reparameterization. Formulating the model in terms

of relative yield or relative yield loss (yield loss=[1 − relative yield]) is essential in comparing exposure-response across species, genotypes, or experiments for which absolute values of the response may vary greatly. In the 1996 and 2006 $O_3$ AQCDs, the two-parameter model of relative yield was used in deriving common models for multiple species, multiple genotypes within species, and multiple locations.

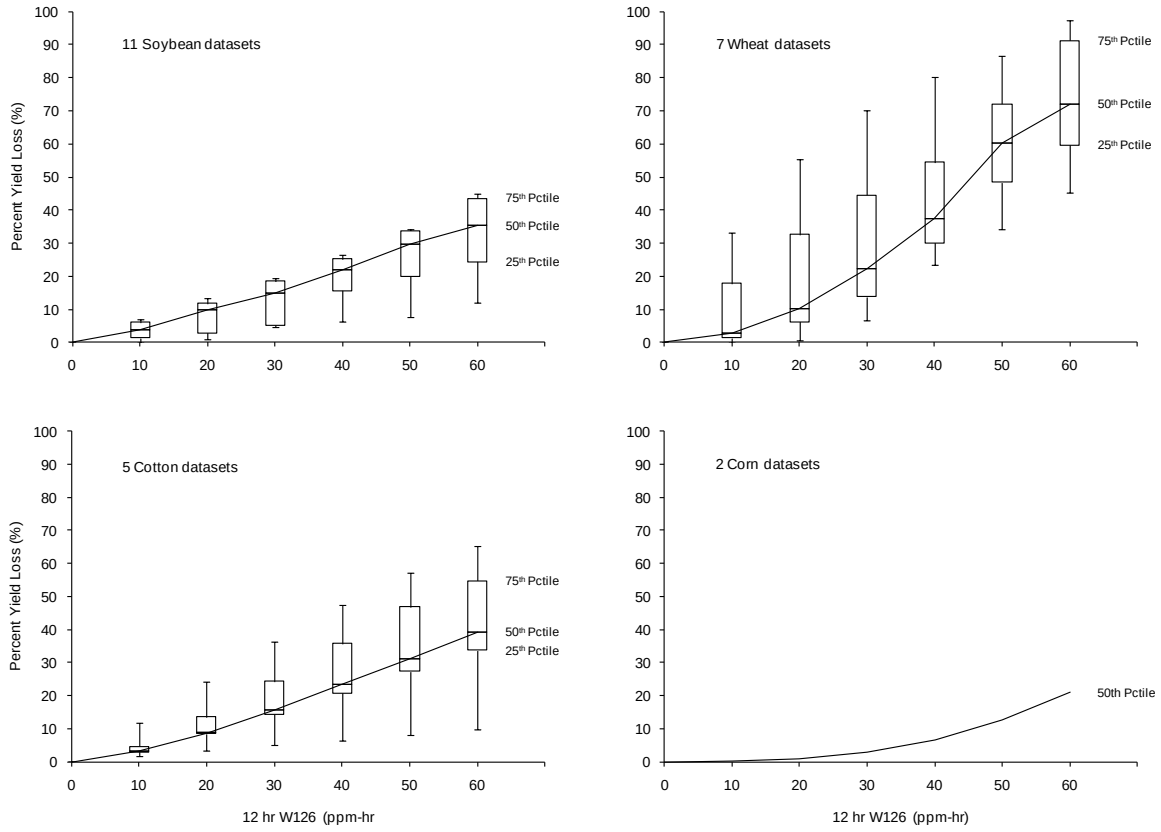
Given the disparate species, genotypes, and locations that were included in the NCLAN and NHEERL/WED projects, and in the absence of plausible distributional assumptions with respect to those variables, a three step process using robust methods was used to obtain parameter estimates that could be generalized.
The models that were derived for each species or group of species were referred to as median composite functions. In the first step, the three parameters of the Weibull model were computed for absolute yield or biomass data from each NCLAN and NHEERL/WED experiment (54 crop datasets and 49 tree seedling datasets), using nonlinear regression. When data were only available for three levels of exposure because of experimental problems, the shape parameter $\beta$ was constrained to 1, reducing the model to an exponential decay model. In the second step, $\alpha$ was dropped, and predicted values of relative yield or biomass were then computed for 12-hour W126 exposures between 0 and 60 ppm-h. At each of these W126 exposure values, the 25th, 50th, and 75th percentiles of the response were identified among the predicted curves of relative response. For example, for the 34 NCLAN studies of 12 crop species grown under non-droughted conditions for a complete cropping cycle (Figure 9-14), the 3 quartiles of the response were identified at every integer value of W126 between 0 and 60. The third step fitted a two-parameter Weibull model to those percentiles, yielding the median composite function for the relative yield or biomass response to $O_3$ exposure for each grouping of interest (e.g., all crops, all trees, all datasets for one species), as well as composite functions for the other quartiles. In the 1996 and 2006 $O_3$ AQCDs this modeling of crop yield loss and tree seedling biomass loss was conducted using the SUM06 metric for exposure. This section updates those results by using the 12-hour W126 as proposed in 2007 (72 FR 37818) and 2010 (75 FR 2938, page 3,003). Figure 9-14 through Figure 9-17 present quantiles of predicted relative yield or biomass loss at seven values of the 12-h W126 for some representative groupings of NCLAN and NHEERL/WED results. Table 9-9 through Table 9-11 give the 90-day 12-h W126 $O_3$ exposure values at which 10 and 20% yield or biomass losses are predicted in 50 and 75% of crop or tree species using the composite functions.




Note: Quantiles of the predicted relative yield loss at 7 values of 12-hour W126 for 34 Weibull curves estimated using nonlinear regression on data from 34 studies of 12 crop species grown under well-watered conditions for the full duration of 1 cropping cycle.

Source of Weibull parameters: Lee and Hogsett (1996).

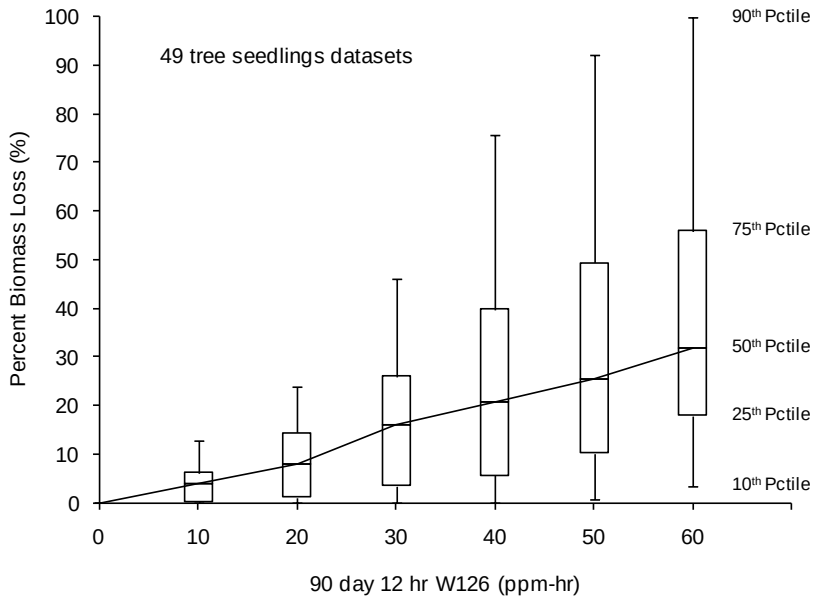
**Figure 9-14 Quantiles of predicted relative yield loss for 34 NCLAN crop experiments.**




Notes: Quantiles of the predicted relative yield loss at 7 values of 12-h W126 for Weibull curves estimated using nonlinear regression for 4 species grown under well-watered conditions for the full duration of 1 cropping cycle. The number of studies available for each species is indicated on each plot.

Source of Weibull parameters: Lee and Hogsett (1996).

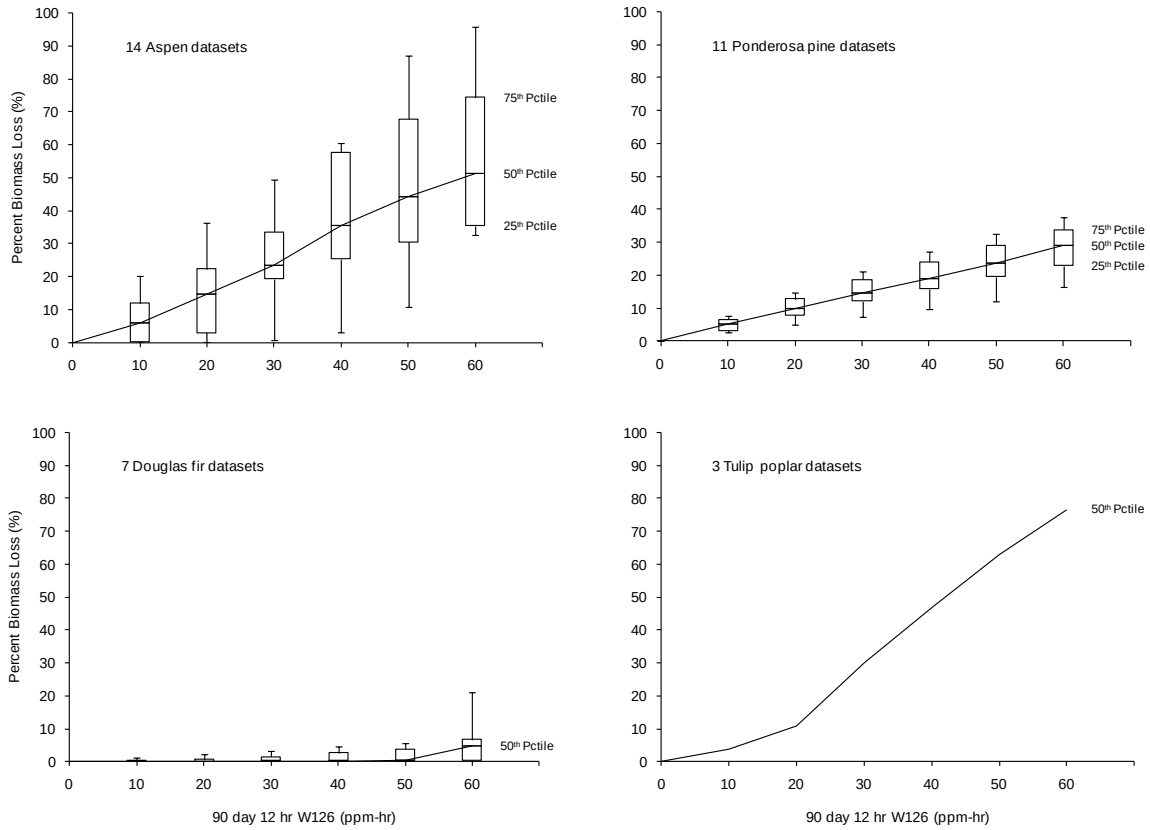
## Figure 9-15 Quantiles of predicted relative yield loss for 4 crop species in NCLAN experiments.




Note: Quantiles of the predicted relative above-ground biomass loss at 7 values of 12-h W126 for 49 Weibull curves estimated using nonlinear regression on data from 49 studies of 11 tree species grown under well-watered conditions for 1 or 2 years. Curves were standardized to 90-day W126.

Source of Weibull parameters: Lee and Hogsett (1996).

**Figure 9-16 Quantiles of predicted relative biomass loss for 49 studies of 11 tree species in NHEERL/WED experiments.**




Note: Quantiles of the predicted relative above-ground biomass loss at 7 exposure values of 12-h W126 for Weibull curves estimated using nonlinear regression on data for 4 tree species grown under well-watered conditions for 1 or 2 year. Curves were standardized to 90-day W126. The number of studies available for each species is indicated on each plot.

Source of Weibull parameters: Lee and Hogsett (1996).

## Figure 9-17 Quantiles of predicted relative biomass loss for 4 tree species in NHEERL/WED experiments.

## Table 9-9 Ozone exposures at which 10 and 20% yield loss is predicted for 50 and 75% of crop species.

| Predited Yield Loss for Crop Species[a] | 90-day 12-h W126 for 10% yield loss (ppm-h) | 90-day 12-h W126 for 20% yield loss (ppm-h) |
|---|---|---|
| Model for the 50th Percentile of 34 curves | | |
| Relative yield=exp(-(W126/104.82)**1.424) | 22 | 37 |
| Model for the 75th Percentile of 34 curves | | |
| Relative yield=exp(-(W126/78.12)**1.415) | 16 | 27 |

[a]Based on composite functions for the 50th and 75th percentiles of 34 Weibull curves for relative yield loss data from 34 non-droughted NCLAN studies of 12 crop species; curves were standardized to 90-day W126.

Source of parameters for the 34 curves: Lee and Hogsett (1996).

## Table 9-10 Ozone exposures at which 10 and 20% yield loss is predicted for 50 and 75% of crop species (Droughted versus Watered conditions).

| Predicted Yield Loss for Crop Species[a] | | 90 day 12-h W126 for 10% yield loss (ppm-h) | 90 day 12-h W126 for 20% yield loss (ppm-h) |
|---|---|---|---|
| **Model for the 50th Percentile of 2×8 curves** | | | |
| Watered | Relative yield=exp(-(W126/132.86)**1.170) | 19 | 37 |
| Droughted | Relative yield=exp(-(W126/179.84)**1.713) | 48 | 75 |
| **Model for the 75th Percentile of 2×8 curves** | | | |
| Watered | Relative yield=exp(-(W126/90.43)**1.310) | 16 | 29 |
| Droughted | Relative yield=exp(-(W126/105.16)**1.833) | 31 | 46 |

[a]Under drought conditions and adequate moisture based on composite functions for the 50th and 75th percentiles of 16 Weibull curves for relative yield loss data from 8 NCLAN studies that paired droughted and watered conditions for the same genotype; curves were standardized to 90-day W126.

Source of parameters for the 16 curves: Lee and Hogsett (1996).

**Table 9-11 Ozone exposures at which 10 and 20% biomass loss is predicted for 50 and 75% of tree species.**

| Predicted Biomass Loss for Tree Species[a] | 90 day 12 h W126 for 10% yield loss (ppm-h) | 90 day 12 h W126 for 20% yield loss (ppm-h) |
|---|---|---|
| **Model for the 50th Percentile of 49 curves** | | |
| Relative yield=exp(-(W126/131.57)**1.242) | 21 | 39 |
| **Model for the 75th Percentile of 49 curves** | | |
| Relative yield=exp(-(W126/65.49)**1.500) | 15 | 24 |

[a]Based on composite functions for the 50th and 75th percentiles of 49 Weibull curves for relative above-ground biomass loss data from 49 studies of 11 tree species grown under well-watered conditions for 1 or 2 year; curves were standardized to 90-day W126.

Source of parameters for the 49 curves: Lee and Hogsett (1996).

### 9.6.3 Validation of 1996 and 2006 Ozone AQCD Models and Methodology Using the 90-day 12-h W126 and Current FACE Data

Since the completion of the NCLAN and NHEERL/WED projects, almost no studies have been published that could provide a basis for estimates of exposure-response that can be compared to those of the 1996 and 2006 $O_3$ AQCDs. Most experiments, regardless of exposure methodology, include only two levels of exposure. In addition, very few studies have included measurements of exposure using the W126 metric, or the hourly $O_3$ concentration data that would allow computing exposure using the W126. Two FACE projects, however, were conducted over multiple years, and by adding to the number of exposure levels over time, can support independent model estimation and prediction using the same model and the same robust process as summarized in Section 9.6.2. Hourly $O_3$ data were available from both FACE projects.

The SoyFACE project is situated near Champaign, IL, and comprises 32 octagonal rings (20m-diameter), 4 of which in a given year are exposed to ambient conditions, and 4 of which are exposed to elevated $O_3$ as a fixed proportion of the instantaneous ambient concentration (Betzelberger et al., 2010; UIUC, 2010; Morgan et al., 2006; Morgan et al., 2004). Since 2002, yield data have been collected for up to 8 genotypes of soybean grown in subplots within each ring. The Aspen FACE project is situated in Rhinelander, WI, and comprises 12 rings (30m-diameter), 3 of which are exposed to ambient conditions, and 3 of which are exposed to $O_3$ as a fixed proportion of the instantaneous ambient concentration (Pregitzer et al., 2008; Karnosky et al., 2005; Dickson et al., 2000). In the summer of 1997, half the area of each ring was planted with small (five to seven leaf sized) clonally propagated plants of five genotypes of trembling aspen, which were left to grow in those environments until 2009. Biomass data are currently available for the years 1997-2005 (King et al., 2005). Ozone exposure in these two FACE projects can be viewed as a categorical

variable with two levels: ambient, and elevated. However, this overlooks the facts that not only do both ambient and elevated exposure vary from year to year, but the proportionality between them also changes yearly. This change has two sources: first, the dispensing of $O_3$ into the elevated exposure rings varies from the set point for the ambient/elevated proportionality to some extent, and for SoyFACE, the set point changed between years. Second, when using threshold or concentration-weighted cumulative metrics (such as AOT40, SUM06 or W126), the proportionality does not propagate regularly from the hourly data to the yearly value. For example, hourly average elevated exposures that are a constant 1.5 times greater than ambient do not result in AOT40, SUM06 or W126 values that are some constant multiple of the ambient values of those indices. Depending on the fraction of hourly values that are above the threshold or heavily weighted, the same average yearly exposure will result in different exposure values when using thresholded or weighted metrics. In some years, elevated exposures in FACE experiments experience many more values above the threshold, or more heavily weighted than the ambient exposures; thus in those years, the distance between ambient and elevated exposure values increases relative to other years. As a consequence, the number of exposure levels in multi-year experiments is twice the number of years. In the case of SoyFACE for the period between 2002 and 2008, ambient exposure in the highest year was approximately equal to elevated exposure in the lowest year, with 14 levels of $O_3$ exposure evenly distributed from lowest to highest. The particular conditions of the Aspen FACE experiment resulted in 12 exposure levels between 1998 and 2003, but they were not as evenly distributed between minimum and maximum over the 6-year period.

There are necessary differences in the modeling of exposure-response in annual plants such as soybean, and in perennial plants such as aspen trees, when exposure takes place over multiple years. In annual plants, responses recorded at the end of the life cycle, i.e., yearly, are analyzed in relationship to that year's exposure. Yield of soybeans is affected by exposure during the year the crop was growing, and a new crop is planted every year. Thus an exposure-response relationship can be modeled from yearly responses matched to yearly exposures, with those exposure-response data points having been generated in separate years. For perennial organisms, which are not harvested yearly and continue to grow from year to year, such pairing of exposure and response cannot be done without accounting for time. Not only does the size of the organism at the beginning of each year of exposure increase, but size is also dependent on the exposure from previous years. Therefore the relationship of response and exposure must be analyzed either one year at a time, or by standardizing the response as a yearly increment relative to size at the beginning of each year. Furthermore, the relevant measurement of exposure is cumulative, or cumulative yearly average exposure, starting in the year exposure was initiated, up to the end of the year of interest. When analyzing the growth of trees over several years, it would be evidently incorrect to pair the exposure level in every discrete year with absolute size of the trees that year, and posit a direct relationship between them, without taking increasing age into consideration. In the Aspen FACE experiment, for example, one could not establish an exposure-response relationship by matching 12 yearly exposures and 12 yearly tree sizes, while disregarding age as if size did not

also depend on it. This is the basis of the 2007 study of Aspen FACE data by Percy et al. (2007), which compares the size of trees of various ages as if they were all the same age, and was therefore not informative.

#### 9.6.3.1 Comparison of NCLAN-Based Prediction and SoyFACE Data

For this ISA, EPA conducted a comparison between yield of soybean as predicted by the composite function three-step process (Section 9.6.2) using NCLAN data, and observations of yield in SoyFACE. The median composite function for relative yield was derived for the 11 NCLAN soybean Weibull functions for non-droughted studies, and comparisons between the predictions of the median composite and SoyFACE observations were conducted as follows.

For the years 2007 and 2008, SoyFACE yield data were available for 7 and 6 genotypes, respectively. The EPA used those data to compare the relative change in yield observed in SoyFACE in a given year between ambient $O_3$ and elevated $O_3$, versus the relative change in yield predicted by the NCLAN-based median composite function between those same two values of $O_3$ exposure. The two parameter median composite function for relative yield of soybean based on NCLAN data was used to predict yield response at the two values of exposure observed in SoyFACE in each year, and the change between yield under ambient and elevated was compared to the change observed in SoyFACE for the relevant year (Table 9-12). This approach results in a direct comparison of predicted versus observed change in yield. Because the value of relative response between any two values of $O_3$ exposure is independent of the intercept $\alpha$, this comparison does not require prediction of the absolute values of the responses.

Since comparisons of absolute values might be of interest, the predictive functions were also scaled to the observed data: SoyFACE data were used to compute an intercept $\alpha$ while the shape and scale parameters ($\beta$ and $\eta$) were held at their value in the NCLAN predictive model. This method gives a comparison of prediction and observation that takes all the observed information into account to provide the best possible estimate of the intercept, and thus the best possible scaling (Table 9-13 and Figure 9-18). For the comparison of NCLAN and SoyFACE, this validation was possible for 2007 and 2008, where data for 7 and 6 soybean genotypes, respectively, were available. The median composite function for relative yield was derived for the 11 NCLAN soybean Weibull functions for nondroughted studies, and the values of median yield under ambient exposure at SoyFACE in 2007 and 2008 were used to obtain an estimate of the intercept $\alpha$ for the NCLAN median function in each of the two years. Table 9-12 presents the results of ambient/elevated relative yield comparisons between the NCLAN-derived predictions and SoyFACE observations. Table 9-13 and Figure 9-18 present the results of comparisons between NCLAN-derived predictions and SoyFACE observations of yield, with the predictive function scaled to provide absolute yield values.

## Table 9-12 Comparison between change in yield observed in the SoyFACE experiment between elevated and ambient $O_3$, and change predicted at the same values of $O_3$ by the median composite function for NCLAN.

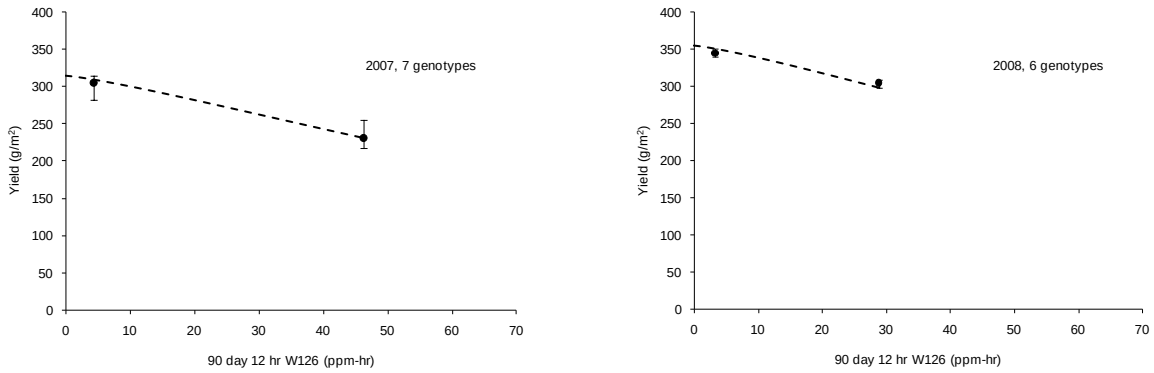
| | 90-day 12-h W126 (ppm-h) observed at SoyFACE | | Yield in Elevated $O_3$ Relative to Ambient $O_3$ (%) | |
|---|---|---|---|---|
| Year | Ambient | Elevated | Predicted by NCLAN[a] | Observed at SoyFACE |
| 2007 | 4.39 | 46.23 | 75 | 76 |
| 2008 | 3.23 | 28.79 | 85 | 88 |

[a]Two-parameter relative yield model.

## Table 9-13 Comparison between yield observed in the SoyFACE experiment and yield predicted at the same values of $O_3$ by the median composite function for NCLAN.

| | 90-day 12-h W126 (ppm-h) observed at SoyFACE | | Yield predicted by NCLAN[a] ($g/m^2$) | | Yield observed at SoyFACE ($g/m^2$) | |
|---|---|---|---|---|---|---|
| Year | Ambient | Elevated | Ambient | Elevated | Ambient | Elevated |
| 2007 | 4.39 | 46.23 | 309.2 | 230.6 | 305.2 | 230.6 |
| 2008 | 3.23 | 28.79 | 350.3 | 298.2 | 344.8 | 304.4 |

[a]Three-parameter absolute yield model with intercept scaled to SoyFACE data.

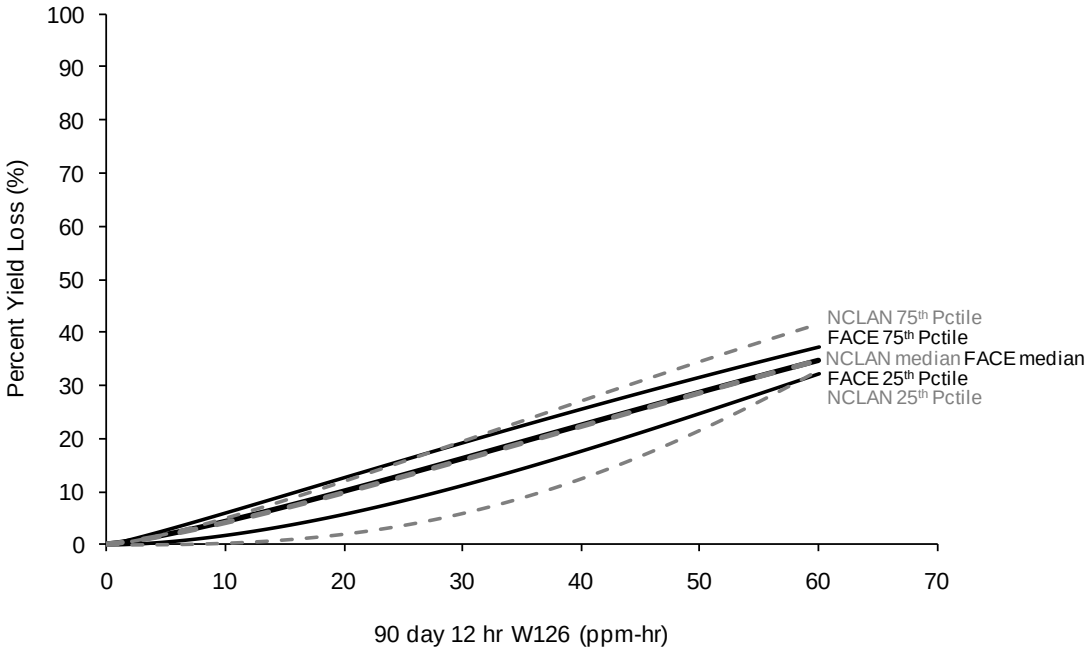



Note: Black dots are the median of 7 or 6 soybean genotypes in SoyFACE (2007, 2008); bars are Inter-Quartile Range for genotypes; dashed line is median composite model for 11 studies in NCLAN.

Source of data: Betzelberger et al. (2010), Morgan et al. (2006), Lee and Hogsett (1996).

**Figure 9-18 Comparison of yield observed in SoyFACE experiment in a given year with yield predicted by the median composite function based on NCLAN.**

Finally, a composite function for the 25th, 50th, and 75th percentiles was developed from SoyFACE annual yield data, and compared to the NCLAN-based function. The process described in Section 9.6.2 was applied to SoyFACE data for individual genotypes, aggregated over the years during which each was grown; one genotype from 2003 to 2007, and six genotypes in 2007 and 2008. First, the three parameter Weibull model described in Section 9.6.2 was estimated using nonlinear regression on exposure-yield data for each genotype separately, over the years for which data were available, totaling seven curves. The 25th, 50th, and 75th percentiles of the predicted values for the two parameter relative yield curves were then identified at every integer of W126 between 0 and 60, and a two-parameter Weibull model estimated by regression for the three quartiles. The comparison between these composite functions for the quartiles of relative yield loss in SoyFACE and the corresponding composite functions for NCLAN is presented in Figure 9-19.




Source of data: Betzelberger et al. (2010), Morgan et al. (2006), Lee and Hogsett (1996).

**Figure 9-19 Comparison of composite functions for the quartiles of 7 curves for 7 genotypes of soybean grown in the SoyFACE experiment, and for the quartiles of 11 curves for 5 genotypes of soybean grown in the NCLAN project.**

As seen in Table 9-13 and Table 9-14 and in Table 9-18, the agreement between predictions based on NCLAN data and SoyFACE observations was notably close in single-year comparisons. Together with the very high agreement between median composite models for NCLAN and SoyFACE (Figure 9-19), it provides very strong mutual confirmation of those two projects' results with respect to the response of yield of soybeans to $O_3$ exposure. It is readily apparent from these results that the methodology described in Section 9.6.2 for obtaining predictions of yield or yield loss from NCLAN data is strongly validated by SoyFACE results. As described in Section 9.2, the exposure technologies used in the two projects were in sharp contrast, specifically with respect to the balance each achieved between control of potential interacting factors or confounders, and fidelity to natural conditions. The comparisons that EPA conducted therefore demonstrate that the methodology used in developing the composite functions is resistant to the influence of nuisance variables and that predictions are reliable. They may also suggest that the aspects in which the two exposure technologies differ have less influence on exposure-response than initially supposed. These results are also in agreement with comparative studies reviewed in Section 9.2.6.

### 9.6.3.2 Comparison of NHEERL/WED-Based Prediction of Tree Biomass Response and Aspen FACE Data

EPA also conducted two comparisons between prediction of above-ground biomass loss based on NHEERL/WED results and observations from Aspen FACE. The median composite function was developed from NHEERL/WED data for 11 studies that used wild-type seedlings of aspen as well as four clonally propagated genotypes. All plants were grown in OTCs for one growing season before being destructively harvested. Aspen FACE data were from clonally propagated trees of five genotypes grown from 1998 to 2003, with above-ground biomass calculated using allometric equations derived from data for trees harvested destructively in 2000 and 2002 (King et al., 2005).

The two parameter median composite function for relative biomass was used to predict biomass response under the observed elevated exposure, relative to its value under observed ambient exposure, for each separate year of Aspen FACE. EPA first compared Aspen FACE observations of the change in biomass between ambient and elevated exposure with the corresponding prediction at the same values of exposure. Comparisons between observed and predicted absolute biomass values were then conducted for each year by scaling the predictive function to yearly Aspen FACE data as described for soybean data in Section 9.6.3.1. In all cases, yearly 90 day 12-hour W126 values for Aspen FACE were computed as the cumulative average from the year of planting up to the year of interest. A comparison of composite functions between NHEERL/WED and Aspen FACE, similar to the one performed for NCLAN and SoyFACE, was not possible: as discussed in the introduction to Section 9.6, the pairing of 12 exposure values from separate years and 12 values of biomass cannot be the basis for a model of exposure-response, because the trees continued growing for the six-year period of exposure. Because the same trees were used for the entire duration, and continued to grow, data could not be aggregated over years. Table 9-14 presents the results of ambient/elevated relative biomass comparisons between the NHEERL/WED-derived predictions and Aspen FACE observations. Table 9-15 and Figure 9-20 present the results of the comparison between NHEERL/WED-derived predictions and Aspen FACE observations for absolute biomass, using Aspen FACE data to scale the NHEERL/WED-derived composite function.

## Table 9-14 Comparison between change in above-ground biomass elevated and ambient $O_3$ in Aspen FACE experiment in 6 year, and change predicted at the same values of $O_3$ by the median composite function for NHEERL/WED.

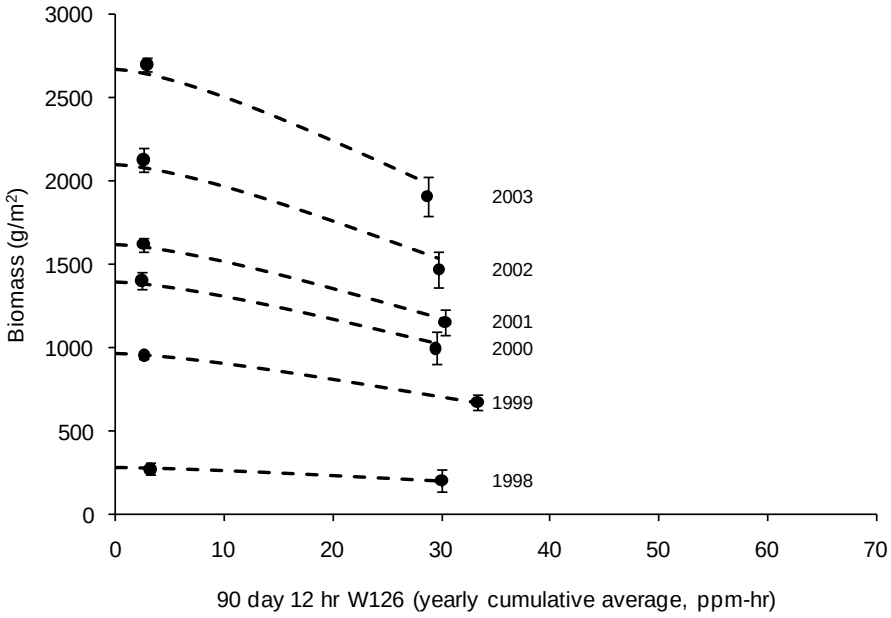
| | 90-day 12-h W126 (ppm-h) Cumulative Average observed at Aspen FACE | | Above-Ground Biomass in Elevated $O_3$, Relative to Ambient $O_3$ (%) | |
|---|---|---|---|---|
| Year | Ambient | Elevated | Predicted by NHEERL/WED[a] | Observed at Aspen FACE |
| 1998 | 3.19 | 30.08 | 74 | 75 |
| 1999 | 2.61 | 33.85 | 70 | 70 |
| 2000 | 2.43 | 30.16 | 74 | 71 |
| 2001 | 2.55 | 31.00 | 73 | 71 |
| 2002 | 2.51 | 30.27 | 74 | 69 |
| 2003 | 2.86 | 29.12 | 75 | 71 |

[a]Two-parameter relative biomass model

## Table 9-15 Comparison between above-ground biomass observed in Aspen FACE experiment in 6 year and biomass predicted by the median composite function based on NHEERL/WED.

| | 90 day 12-h W126 (ppm-h) Cumulative Average observed at Aspen FACE | | Biomass Predicted by NHEERL/WED[a] ($g/m^2$) | | Biomass Observed at Aspen FACE ($g/m^2$) | |
|---|---|---|---|---|---|---|
| Year | Ambient | Elevated | Ambient | Elevated | Ambient | Elevated |
| 1998 | 3.19 | 30.08 | 276.0 | 203.2 | 274.7 | 204.9 |
| 1999 | 2.61 | 33.85 | 958.7 | 668.3 | 955.3 | 673.3 |
| 2000 | 2.43 | 30.16 | 1,382.4 | 1,022.8 | 1,400.3 | 998.6 |
| 2001 | 2.55 | 31.00 | 1,607.0 | 1,173.7 | 1,620.7 | 1,154.9 |
| 2002 | 2.51 | 30.27 | 2,079.0 | 1,532.1 | 2,125.9 | 1,468.4 |
| 2003 | 2.86 | 29.12 | 2,640.1 | 1,981.2 | 2,695.2 | 1,907.8 |

[a]Three-parameter absolute biomass model with intercept scaled to Aspen FACE data.




Note: Black dots are aspen biomass/$m^2$ for 3 FACE rings filled with an assemblage of 5 clonal genotypes of aspen at Aspen FACE; bars are SE for 3 rings; dashed line is median composite model for 4 clonal genotypes and wild-type seedlings in 11 NHEERL/WED 1-year OTC studies.

Source of data: King et al. (2005), Lee and Hogsett (1996).

**Figure 9-20 Comparison between above-ground biomass observed in Aspen FACE experiment in 6 year and biomass predicted by the median composite function based on NHEERL/WED.**

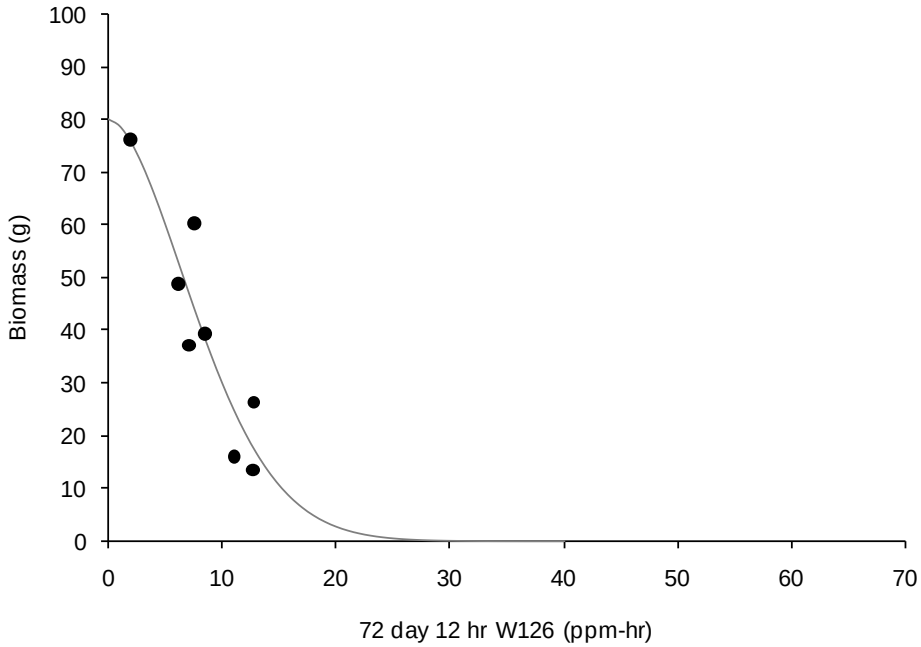
As in the comparisons between NCLAN and SoyFACE, the agreement between predictions based on NHEERL/WED data and Aspen FACE observations was very close. The results of the two projects strongly reinforce each other with respect to the response of aspen biomass to $O_3$ exposure. The methodology used for obtaining the median composite function is shown to be capable of deriving a predictive model despite potential confounders, and despite the added measurement error that is expected from calculating biomass using allometric equations. In addition, the function based on one year of growth was shown to be applicable to subsequent years.

The results of experiments that used different exposure methodologies, different genotypes, locations, and durations converged to the same values of response to $O_3$ exposure for each of two very dissimilar plant species, and predictions based on the earlier experiments were validated by the data from current ones. However, in these comparisons, the process used in establishing predictive functions involved aggregating data over variables such as time, locations, and genotypes, and the use of a robust statistic (quartiles) for that aggregation. The validating data, from SoyFACE and Aspen FACE, were in turn aggregated over the same variables. The accuracy of

predictions is not expected to be conserved for individual values of those variables over which aggregation occurred. For example, the predicted values for soybean, based on data for five genotypes, are not expected to be valid for each genotype separately. As shown in the validation, however, aggregation that occurred over different values of the same variable did not affect accuracy: composite functions based on one set of genotypes were predictive for another set, as long as medians were used for both sets. A study of cottonwood (*Populus deltoides*) conducted using a naturally occurring gradient of $O_3$ exposure (Gregg et al., 2006, 2003) may provide an illustration of the response of an individual species whose response is far from the median response for an aggregation of species.

#### 9.6.3.3 Exposure-Response in a Gradient Study

Gregg et al. (2003) grew saplings of one clonally propagated genotype of cottonwood (*Populus deltoides*) in seven locations within New York City and in the surrounding region between July and September in 1992, 1993 and 1994, and harvested them 72 days after planting. Owing to regional gradients of atmospheric $O_3$ concentration, the experiment yielded eight levels of exposure (Figure 9-21), and the authors were able to rule out environmental variables other than $O_3$ to account for the large differences in biomass observed after one season of growth. The deficit in growth increased substantially faster with increasing $O_3$ exposure than has been observed in aspen, another species of the same genus (*Populus tremuloides*, Section 9.6.3.2). Using a three parameter Weibull model (Figure 9-21), the biomass of cottonwood at a W126 exposure of 15 ppm-h, relative to biomass at 5 ppm-h, is estimated to be 0.18 (18% of growth at 5 ppm-h). The relative biomass of trembling aspen within the same 5-15 ppm-h range of exposure is estimated to be 0.92, using the median composite model for aspen whose very close agreement with Aspen FACE data was shown in Section 9.6.3.2. Using a median composite function for all deciduous trees in the NHEERL/WED project (6 species in 21 studies) also gives predictions that are very distant from the cottonwood response observed in this experiment. For all deciduous tree species in NHEERL/WED, biomass at a W126 exposure of 15 ppm-h, relative to biomass at 5 ppm-h, was estimated to be 0.87.




Note: Line represents the three-parameter Weibull model.
Source: Modified with permission of Nature Publishing Group (Gregg et al., 2003).

**Figure 9-21 Above-ground biomass for one genotype of cottonwood grown in seven locations for one season in 3 years.**

As shown in Section 9.6.2, the median models available for trembling aspen and soybean have verifiable predictive ability for those particular species. This suggests that the corresponding NCLAN- and NHEERL/WED-based models for multiple crop and tree species can provide reliable estimates of losses for similar assortments of species. However, their predictive ability would likely be poor for individual species not tested.

The cottonwood data of Gregg et al. (2003) show an extremely severe response to $O_3$. They are consistent with the expectation that among species and genotypes, some are likely to be substantially more sensitive than a median measure, such as the estimate produced by NHEERL/WED (Figure 9-16), but the sensitivity of this particular species has not been studied elsewhere.

An alternative hypothesis for the difference between the response of cottonwood in this experiment and deciduous tree species in NHEERL/WED, or the difference between the response of cottonwood and aspen in NHEERL/WED and Aspen FACE, could be the presence of confounding factors in the environments where the experiment was conducted. However, variability in temperature, moisture, soil fertility, and atmospheric deposition of N were all ruled out by Gregg et al. (2003) as contributing to the observed response to $O_3$. In addition, this hypothesis would imply

that the unrecognized confounder(s) were either absent from both OTC and FACE studies, or had the same value in both. This is not impossible, but the hypothesis that cottonwood is very sensitive to $O_3$ exposure is more parsimonious, and sufficient.

### 9.6.3.4 Meta-analyses of Growth and Yield Studies

Since the 2006 $O_3$ AQCD, five studies have used meta-analytic methods to integrate results from experimental studies of crops or tree species relevant to the United States. It is possible to obtain exposure-response data for growth and yield from those meta-analyses, but because all of them provided summary measurements of $O_3$ exposure as hourly averages of various lengths of exposures, comparisons with exposure-response results where exposure is expressed as W126 are problematic. Table 9-16 summarizes the characteristics of the five meta-analyses. They all included studies conducted in the U.S. and other locations worldwide, and all of them expressed responses as comparative change between levels of exposure to $O_3$, with carbon filtered air (CF) among those levels. Using hourly average concentration to summarize exposure, CF rarely equates with absence of $O_3$, although it almost always near zero when exposure is summarized as W126, SUM06, or AOT40.

**Table 9-16 Meta-analyses of growth or yield studies published since 2005.**

| Study | Number of articles included | Years of publication surveyed | Crop, species or genera | Response | Number of $O_3$ levels | Duration of exposure |
|---|---|---|---|---|---|---|
| Ainsworth (2008) | 12 | 1980-2007 | Rice | Yield | 2 | unreported |
| Feng et al. (2008b) | 53 | 1980-2007 | Wheat | Yield | 5 | >10 days |
| Feng and Kobayashi (2009) | All crops together: 81 | 1980-2007 | Potato, barley, wheat, rice, bean, soybean | Yield | 3 | >10 days |
| Grantz et al. (2006) | 16 | 1992-2004 | 34 Herbaceous dicots<br>21 Herbaceous monocots<br>5 Tree species | Relative Growth Rate | 2 | 2-24 weeks |
| Wittig et al. (2009) | All responses:263<br>Articles that included biomass:unreported | 1970-2006 | 4 Gymnosperm tree genera<br>11 Angiosperm tree genera | Total biomass | 4 | >7 days |

The only effect of $O_3$ exposure on yield of rice reported in Ainsworth (2008) was a decrease of 14% with exposure increasing from CF to 62 ppb average concentration. Feng et al. (2008b) were able to separate exposure of wheat into four classes with average concentrations of 42, 69, 97, and 153 ppb, in data where $O_3$ was the only treatment. Mean responses relative to CF were yield decreases of 17, 25, 49, and 61% respectively. Feng et al. (2008b) observed that wheat yield losses were smaller under conditions of drought, and that Spring wheat and Winter wheat appeared similarly affected. However, mean exposure in studies of Winter wheat was substantially higher than in studies of Spring wheat (86 versus 64 ppb), which suggests that the yield of Spring wheat was in fact more severely affected, since yield was approximately the same, even though Spring wheat was exposed to lower concentrations. Exposures of the six crops considered in Feng and Kobayahi (2009) were classified into two ranges, each compared to CF air. In the lower range of exposure (41-49 ppb), potato studies had the highest average exposure (45 ppb) and wheat and rice the lowest (41 ppb). In the higher range (51-75 ppb), wheat studies had the highest average exposure (65 ppb), and potato, barley and rice the lowest (63 ppb). In other words, across the studies included, all crops were exposed to very similar levels of $O_3$. At approximately 42 ppb, the yield of potato, barley, wheat, rice, bean, and soybean declined by 5.3, 8.9, 9.7, 17.5, 19, and 7.7% respectively, relative to CF air. At approximately 64 ppb $O_3$, declines were 11.9, 12.5, 21.1, 37.5, 41.4, and 21.6%. Grantz et al. (2006) reported Relative Growth Rate (RGR) rather than growth, and did not report $O_3$ exposure values in a way that would allow calculation of mean exposure for each of the three categories of plants for which RGR changes are reported. All studies used only two levels of exposure, with CF air as the lower one, and most used elevated exposure in the range of 40 to 70 ppb. Decline in RGR was 8.2% for the 34 herbaceous dicots, 4.5% for the 21 herbaceous monocots, and 17.9% for the 5 tree species. Finally, Wittig et al. (2009) divided the studies analyzed into three classes of comparisons: CF versus ambient, CF versus elevated, and ambient versus elevated, but reported comparisons between three average levels of exposure besides CF: 40 ppb, 64 ppb, and 97 ppb. Corresponding decreases in total biomass relative to CF were 7, 17, and 17%.

These meta-analyses provide very strong confirmation of EPA's conclusions from previous $O_3$ AQCDs: compared to lower levels of ambient $O_3$, current levels in many locations are having a substantial detrimental effect on the growth and yield of a wide variety of crops and natural vegetation. They also confirm strongly that decreases in growth and yield continue at exposure levels higher than current ambient levels. However, direct comparisons with the predictions of exposure-response models that use concentration-weighted cumulative metrics are difficult.

#### 9.6.3.5 Additional Exposure-Response Data

The studies summarized in Table 9-17 and Table 9-18 contain growth or yield exposure-response data at too few levels of exposure for exposure-response models, and/or used metrics other than W126. These tables update Tables AX9-16 through AX9-19 of the 2006 $O_3$ AQCD.

### 9.6.4 Summary

None of the information on effects of $O_3$ on vegetation published since the 2006 $O_3$ AQCD has modified the assessment of quantitative exposure-response relationships that was presented in that document. This assessment updates the 2006 exposure-response models by computing them using the W126 metric, cumulated over 90 days. Almost all of the experimental research on the effects of $O_3$ on growth or yield of plants published since 2006 used only two levels of exposure. In addition, hourly $O_3$ concentration data that would allow calculations of exposure using the W126 metric are generally unavailable. However, two long-term experiments, one with a crop species (soybean), one with a tree species (aspen), have produced data that can be used to validate the exposure-response models presented in the 2006 $O_3$ AQCD, and methodology used to derive them.

Quantitative characterization of exposure-response in the 2006 $O_3$ AQCD was based on experimental data generated for that purpose by the National Crop Loss Assessment Network (NCLAN) and EPA National Health and Environmental Effects Research Laboratory, Western Ecology Division (NHEERL-WED) projects, using OTCs to expose crops and trees seedling to $O_3$. In recent years, yield and growth results for two of the species that had provided extensive exposure-response information in those projects have become available from studies that used FACE technology, which is intended to provide conditions much closer to natural environments (Pregitzer et al., 2008; Morgan et al., 2006; Morgan et al., 2004; Dickson et al., 2000). The robust methods that were used previously with exposure measured as SUM06 were applied to the NCLAN and NHEERL-WED data with exposure measured as W126, in order to derive single-species median models for soybean and aspen from studies involving different genotypes, years, and locations. The resulting models were used to predict the change in yield of soybean and biomass of aspen between the two levels of exposure reported in recent FACE experiments. Results from these new experiments were exceptionally close to predictions from the models. The accuracy of model predictions for two widely different plant species provides support for the validity of the corresponding multiple-species models for crops and trees in the NCLAN and NHEERL-WED projects. However, variability among species in those projects indicates that the range of sensitivity is likely quite wide. This was confirmed by a recent experiment with cottonwood in a naturally occurring gradient of exposure (Gregg et al., 2006), which established the occurrence of species with responses substantially more severe

under currently existing conditions than are predicted by the median model for multiple species.

Results from several meta-analyses have provided approximate values for responses of yield of soybean, wheat, rice and other crops under broad categories of exposure, relative to charcoal-filtered air (Ainsworth, 2008; Feng et al., 2008b; Morgan et al., 2003). Likewise, Feng and Kobayashi (2009) have summarized yield data for six crop species under various broad comparative exposure categories, while Wittig et al. (2009) reviewed 263 studies that reported effects on tree biomass. However, these analyses have proved difficult to compare with exposure-response models, especially given that exposure was not expressed in the same W126 metric.

**Table 9-17 Summary of studies of effects of $O_3$ exposure on growth and yield of agricultural crops.**

| Species<br>Facility<br>Location | Exposure Duration | $O_3$ Exposure<br>(Additional Treatment) | Response Measured | Percent Change from CF<br>(Percent Change from Ambient) | Reference |
|---|---|---|---|---|---|
| Alfalfa (*Medicago sativa*)<br>OTC; 0.27$m^3$ pots<br>Federico, Italy | 2 yr, 2005, 2006 | AOT40: CF 0 ppm-h<br>13.9 ppm-h (2005), 10.1 ppm-h (2006)<br>(NaCl: 0.29, 0.65, 0.83, 1.06 deciSiemens/meter) | Total shoot yield | n.s. (N/A) | Maggio et al. (2009) |
| Bean (*Phaseolus vulgaris* l. cv Borlotto)<br>OTC; ground-planted<br>Curno, Italy | 3 months, 2006 | Seasonal AOT40:<br>CF (0.5 ppm-h);<br>ambient (4.6 ppm-h)<br>(N/A) | # Seeds per plant;<br>100-seed weight | -33 (N/A)<br>n.s. (N/A) | Gerosa et al. (2009) |
| Big Blue Stem (*Andropogon gerardii*)<br>OTC<br>Alabama | 4 months, 2003 | 12-h avg:<br>CF (14 ppb),<br>Ambient (29 ppb),<br>Elevated (71 ppb)<br>(N/A) | Final harvest biomass;<br>RVF | n.s. (n.s.)<br>-7 (-7) | Lewis et al. (2006) |
| *Brassica napus* cv. Westar<br>Growth chambers<br>Finland | 17-26 days | 8-h avg:<br>CF (0 ppb), 100 ppb<br>(Bt/non-Bt; herbivory) | Shoot biomass | -30.70 (N/A) | Himanen et al. (2009b) |
| Corn (*Zea mays* cv. Chambord)<br>OTC<br>France | 33 days | AOT40 ppm-h: 1.1; 1.3; 4.9; 7.2; 9.3; 12.8<br>(N/A) | Total above-ground biomass | N/A (Highest treatment caused -26% change) | Leitao et al. (2007a) |
| Cotton cv. Pima<br>OTC; 9-L pots<br>San Joaquin Valley, CA | 8 weeks | 12-h avg: 12.8 ± 0.6; 79.9 ± 6.3; 122.7 ± 9.7<br>(N/A) | Above-ground biomass | -76 (n.s.) | Grantz and Shrestha (2006) |

| Species<br>Facility<br>Location | Exposure Duration | $O_3$ Exposure (Additional Treatment) | Response Measured | Percent Change from CF (Percent Change from Ambient) | Reference |
|---|---|---|---|---|---|
| Eastern Gamagrass (*Tripsacum dactyloides*)<br>OTC<br>Alabama | 4 months, 2003 | 12-h avg:<br>CF (14ppb),<br>Ambient (29 ppb),<br>Elevated (71 ppb)<br>(N/A) | Final harvest biomass;<br>RVF | +68 (+42);<br>-17 (-12) | Lewis et al. (2006) |
| Grapevine (*Vitis vinivera*)<br>OTC<br>Austria | 3 yr, May-Oct | AOT40 ppm-h:<br>CF (0),<br>Ambient (7-20),<br>Elevated. 1 (20-30),<br>Elevated. 2 (38-48) | Total fruit yield/<br>Sugar yield | -20 to -80 in different yr<br>(-20 to -90 in different yr) | Soja et al. (2004) |
| Mustard (*Brassica campestris*)<br>Chambers;<br>7.5-cm pots | 10 days | CF &<br>67.8 ppb for 7 h<br>(N/A) | Seeds/plant | n.s. (N/A) | Black et al. (2007) |
| Oilseed Rape (*Brassica napus*)<br>OTC<br>Yangtze Delta, China | 39 days | Daily avg: 100 ppb, one with diurnal variation and one with constant concentration<br>(N/A) | Biomass and pods per plant | Diurnal variability reduced both biomass and pod number more than constant fumigation (N/A) | Wang et al. (2008) |
| Peanut (*Arachis hypogaea*)<br>OTC<br>Raleigh, NC | 3 yr | 12-h avg:<br>CF (22 ppb),<br>Ambient (46 ppb),<br>Elevated (75ppb)<br>($CO_2$: 375 ppm; 548 ppm; 730 ppm) | Yield (seed weight, g/m) | -33 (-8) | Burkey et al. (2007) |
| *Poa pratensis*<br>OTC<br>Braunschweig, Germany | 2000-2002: 4-5 weeks in the Spring | 8-h avg:<br>CF+25 (21.7),<br>NF+50 (73.1)<br>(Competition) | Total biomass (g DW/pot) | N/A (n.s.) | Bender et al. (2006) |
| Potato (*Solanum tuberosum*)<br>OTC; CHIP<br>6 northern European locations | 1988,1999. Emergence to harvest | AOT40:CF (0);<br>Ambient (0.27-5.19); NF (0.002-2.93)<br>NF+ (3.10-24.78<br>(N/A) | Tuber yield averaged across 5 field-sites;<br>Tuber starch content regressed against [$O_3$] report sig. ± slope with increasing [$O_3$] | N/A (-27% -+27%, most comparisons n.s.) Linear regression slope = -0.0098) | Vandermeiren et al. (2005) |
| Rice (*Oryza sativa*)<br>OTC<br>Raleigh, NC | 1997-1998, June-September | 12-h mean ppb:<br>CF (27.5),<br>Elevated (74.8)<br>($CO_2$) | Total biomass;<br>Seed yield | -25(N/A)<br>-13 to 20 (N/A) | Reid and Fiscus (2008) |

| Species<br>Facility<br>Location | Exposure Duration | $O_3$ Exposure (Additional Treatment) | Response Measured | Percent Change from CF (Percent Change from Ambient) | Reference |
|---|---|---|---|---|---|
| Rice (*Oryza sativa*) 20 Asian cultivars<br>OTC<br>Gunma Prefecture, Japan | 2008 growing season | Daily avg (ppb):<br>CF (2),<br>0.8×ambient (23);<br>1 ×ambient (28);<br>1.5×ambient (42);<br>2×ambient (57)<br>(Cultivar comparisons) | Yield | From n.s. to -30 across all cultivars | Sawada and Kohno (2009) |
| Seminatural grass<br>FACE<br>Le Mouret, Switzerland | 5 yr | Seasonal AOT40: Ambient (0.1-7.2 ppm-h);<br>Elevated. (1.8-24.1 ppm-h)<br>(N/A) | Relative annual yield | N/A (2×faster decrease in yield/yr) | Volk et al. (2006) |
| Soybean<br>OTC; CRA<br>Bari, Italy | 2003-2005 growing seasons | Seasonal AOT40 ppm-h: CF (0),<br>Ambient (3.4), High (9.0)<br>(Drought) | Yield | -46 (-9) | Bou Jaoudé et al. (2008b) |
| Soybean (*Glycine max* cv. 93B15)<br>SoyFACE<br>Urbana, IL | 2002, 2003 growing seasons | 8-h avg:<br>Ambient (62 & 50 ppb), Elevated (75 & 63 ppb)<br>(N/A) | Yield | N/A<br>(-15 in 2002;<br>-25 in 2003) | Morgan et al. (2006) |
| Soybean (*Glycine max* cv. Essex)<br>Chambers; 21 L<br>Raleigh, NC | 2×3 months | 12-h avg: CF (28),<br>Elevated (79),<br>Elevated flux (112)<br>($CO_2$: 365 & 700) | Seed mass per plant | -30 (N/A) | Booker and Fiscus (2005) |
| Soybean (*Glycine max* cv. Essex)<br>OTCs; 21-L pots<br>Raleigh, NC | 2×3 months | 12-h avg: CF (18);<br>Elevated (72)<br>($CO_2$: 367 & 718) | Seed mass per plant | -34 (N/A) | Booker et al. (2004b) |
| Soybean (*Glycine max* cv. Tracaja)<br>Chambers; pots<br>Brazil | 20 days | 12-h avg: CF & 30 ppb<br>(N/A) | Biomass | -18 (N/A) | Bulbovas et al. (2007) |
| Soybean (*Glycine max*) 10 cultivars<br>SoyFACE<br>Urbana, IL | 2007 & 2008 | 8-h avg: Ambient (46.3 & 37.9), Elevated (82.5 & 61.3)<br>(Cultivar comparisons) | Yield | N/A (-17.20) | Betzelberger et al. (2010) |
| Spring Wheat (*Triticum aestivum* cv. Minaret; Satu; Drabant; Dragon)<br>OTCs<br>Belgium, Finland, & Sweden | 1990-2006 | Seasonal AOT40s ranged from 0 to16 ppm-h<br>(N/A) | Seed protein content;<br>1,000-seed weight regressed across all experiments | N/A (significant negative correlation)<br>N/A (sig negative correlation) | Piikki et al. (2008b) |
| Strawberry (*Fragaria x ananassa* Duch. Cv Korona & Elsanta)<br>Growth chambers<br>Bonn, Germany | 2 months | 8-h avg: CF (0 ppb) &<br>Elevated (78 ppb)<br>(N/A) | Fruit yield (weight/plant) | -16 (N/A) | Keutgen et al. (2005) |

| Species<br>Facility<br>Location | Exposure Duration | $O_3$ Exposure (Additional Treatment) | Response Measured | Percent Change from CF (Percent Change from Ambient) | Reference |
|---|---|---|---|---|---|
| Sugarbeet (*Beta vulgaris* cv. Patriot)<br>OTC<br>Belgium | 2003, 2004; 5 months | 8-h avg: Ambient (36 ppb); Elevated (62 ppb)<br>(N/A) | Sugar yield | N/A (-9) | De Temmerman et al. (2007) |
| Sugarcane (*Saccharum spp*)<br>CSTR<br>San Joaquin Valley, CA | 2007; 11-13 weeks. | 12-h avg: CF (4 ppb); Ambient (58); Elevated (147)<br>(N/A) | Total biomass (g/plant) | -40 (-30) | Grantz and Vu (2009) |
| Sweet Potato<br>Growth chambers<br>Bonn, Germany | 4 weeks | 8-h avg: CF (0 ppb), Ambient (<40 ppb) Elevated (255 ppb)<br>(N/A) | Tuber weight | -14 (-11.5) | Keutgen et al. (2008) |
| Tomato (*Lycopersicon esculentum*)<br>OTC<br>Valencia, Spain | 133 days in 1998 | 8-h mean ppb: CF 16.3, NF 30.1, NF+ 83.2<br>(Various cultivars; early & late harvest) | Yield | n.s (n.s.) | Dalstein and Vas (2005) |
| *Trifolium Subterraneum*<br>OTC; 2.5-L pots<br>Madrid, Spain | 29 days | 12-h avg: CF (<7.9 ± 6.3); Ambient (34.4 ± 10.8); Elevated (56.4 ± 22.3)<br>(N: 5, 15 & 30 kg/ha) | Above-ground biomass | -45 (-35) | Sanz et al. (2005) |
| Watermelon (*Citrullus lanatus)*<br>OTC<br>Valencia, Spain | 2000, 2001. 90 days | AOT40: CF (0 ppm-h) Ambient (5.7 ppm-h), Elevated (34.1 ppm-h)<br>(N:0, 14.0 & 29.6 g/pot) | total fruit yield (kg) | n.s. (54) | Calatayud et al. (2006) |
| Yellow Nutsedge<br>OTC; 9-L pots<br>San Joaquin Valley, CA | 8 weeks | 12-h avg: 12.8 ± 0.6; 79.9 ± 6.3; 122.7 ± 9.7<br>(N/A) | above-ground biomass | n.s. (n.s.) | Grantz and Shrestha (2006) |

In studies where variables other than $O_3$ were included in the experimental design, response to $O_3$ is only provided for the control level of those variables.

## Table 9-18 Summary of studies of effects of $O_3$ exposure on growth of natural vegetation.

| Species<br>Facility<br>Location | Exposure Duration | $O_3$ Exposure (Additional Treatment) | Response Measured | Response | Reference |
|---|---|---|---|---|---|
| Yellow nutsedge (*Cyperus esculentus*)<br>CSTR<br>San Joaquin Valley, CA | 53 days in 2008 | 12-h mean ppb:<br>CF (4); CF+ (60);<br>CF2+ (115) | Above-ground biomass; tubers (g/plant) | ns; CF(4.1) CF+(3.9) CF2+(2.7) | Grantz et al. (2010a) |
| 35 herbaceous species<br>OTC<br>Corvallis, OR | 1999-2002, May-August | 4-yr avg; yearly W126 ppm-h:<br>CF (0),<br>CF+ (21),<br>CF 2+ (49.5) | Total community above-ground biomass (35 species) after 4 years | CF (459 g/m$^2$), CF+ (457 g/m$^2$), CF2+ (398 g/m$^2$) | Pfleeger et al. (2010) |
| Highbush blackberry (*Rubus argutus*)<br>OTC<br>Auburn, AL | 2004, May-August | 12-h mean ppb:<br>CF (21.7),<br>Ambient (32.3),<br>Elevated (73.3) | Vegetative regrowth after pruning | CF (75.1 g/plant),<br>Ambient (76.4 g/plant),<br>Elevated (73.1 g/plant) | Ditchkoff et al. (2009) |
| Horseweed (*Conyza canadensis*)<br>CSTR<br>San Joaquin Valley, CA | 2005, 2 runs, 28 days each (July-Aug, Sept) | W126 ppm-h:<br>CF(0),<br>CF+ (11),<br>CF 2+ (30)<br>(Glyphosate resistance) | Total biomass (g/plant) | Glyphosate sensitive:<br>CF (0.354)<br>CF+ (0.197)<br>CF2+ (0.106)<br>Glyphosate resistant: CF(0.510)<br>CF+ (0.313)<br>CF2+ (0.143) | Grantz et al. (2008) |
| Red Oak (*Quercus rubrum*)<br>Forest sites<br>Look Rock & Twin Creeks Forests, TN | 2001-2003, April-October | AOT60:<br>2001 (11.5),<br>2002 (24.0),<br>2003 (11.7)<br>(Observational study with multiple environmental variables) | Annual circumference increment (change relative to 2001 in year 2002;2003) | -42.8%; +1% | McLaughlin et al. (2007a) |
| Pine species<br>Forest sites<br>Look Rock Forest, TN | 2001-2003, April-October | AOT60:<br>2001 (11.5),<br>2002 (24.0),<br>2003 (11.7)<br>(Observational study with multiple environmental variables) | Annual circumference increment (change relative to 2001 in year 2002;2003) | -62.5%; -2.9% | McLaughlin et al. (2007a) |

| Species<br>Facility<br>Location | Exposure Duration | $O_3$ Exposure (Additional Treatment) | Response Measured | Response | Reference |
|---|---|---|---|---|---|
| Hickory species<br>Forest sites<br>Look Rock Forest, TN | 2001-2003, April-October | AOT60:<br>2001 (11.5),<br>2002 (24.0),<br>2003 (11.7)<br>(Observational study with multiple environmental variables) | Annual circumference increment (change relative to 2001 in year 2002;2003) | -14%; +30% | McLaughlin et al. (2007a) |
| Chestnut Oak (*Quercus prinus*)<br>Forest sites<br>Look Rock Forest, TN | 2001-2003, April-October | AOT60:<br>2001 (11.5),<br>2002 (24.0),<br>2003 (11.7)<br>(Observational study with multiple environmental variables) | Annual circumference increment (change relative to 2001 in year 2002;2003) | +44%; +55% | McLaughlin et al. (2007a) |
| Black Cherry (*Prunus rigida*)<br>Forest sites<br>Twin Creeks Forest, TN | 2002-2003, April-October | AOT60:<br>2002 (24.0),<br>2003 (11.7)<br>(Observational study with multiple environmental variables) | Annual circumference increment (change relative to 2003 in year 2002) | -75% | McLaughlin et al. (2007a) |
| Shortleaf pine (*Pinus echinata*)<br>Forest sites<br>Twin Creeks Forest, TN | 2002-2003, April-October | AOT60:<br>2002 (24.0),<br>2003 (11.7)<br>(Observational study with multiple environmental variables) | Annual circumference increment (change relative to 2003 in year 2002) | -16.8% | McLaughlin et al. (2007a) |
| Hemlock (*Tsuga canadensis*)<br>Forest sites<br>Twin Creeks Forest, TN | 2002-2003, April-October | AOT60:<br>2002 (24.0),<br>2003 (11.7)<br>(Observational study with multiple environmental variables) | Annual circumference increment (change relative to 2003 in year 2002) | -21.9% | McLaughlin et al. (2007a) |
| Red Maple (*Acer rubrum*)<br>Forest sites<br>Twin Creeks Forest, TN | 2002-2003, April-October | AOT60:<br>2002 (24.0),<br>2003 (11.7)<br>(Observational study with multiple environmental variables) | Annual circumference increment (change relative to 2003 in year 2002) | -59.6% | McLaughlin et al. (2007a) |

| Species<br>Facility<br>Location | Exposure Duration | $O_3$ Exposure (Additional Treatment) | Response Measured | Response | Reference |
|---|---|---|---|---|---|
| Yellow Poplar (*Liriodendron tulipifera*)<br>Forest sites<br>Look Rock, Oak Ridge, & Twin Creeks Forest, TN | 2002-2003, April-October | AOT60:<br>2002 (24.0),<br>2003 (11.7)<br>(Observational study with multiple environmental variables) | Annual circumference increment (change relative to 2001 in years 2002; 2003) | -45.9%; -15.25% | McLaughlin et al. (2007a) |
| Sugar Maple (*Acer saccharum*)<br>Forest sites<br>Twin Creeks Forest, TN | 2002-2003, April-October | AOT60:<br>2002 (24.0),<br>2003 (11.7)<br>(Observational study with multiple environmental variables) | Annual circumference increment (change relative to 2003 in year 2002) | -63.8% | McLaughlin et al. (2007a) |
| Trembling aspen (*Populus tremuloides*), 5 genotypes<br>Aspen FACE<br>Rhinelander, WI | 1998-2004, May-October | Cumulative avg 90-day 12-h W126.<br>Ambient 3.1 ppm-h Elevated: 27.2 ppm-h<br>(Competition with birch, maple) | main stem volume after 7 years | Ambient: 6.22 $dm^3$;<br>Elevated: 4.73 $dm^3$ | Kubiske et al. (2006) |
| Hybrid Poplar (*Populus trichocarpa* x *Populus deltoides*)<br>OTC<br>Seattle, WA | 2003, 3 months | Daily mean (µg/g):<br>CF(<9),<br>Elevated (85-128) | Total biomass | CF to elevated:<br>-12.9% | Woo and Hinckley (2005) |

In studies where variables other than $O_3$ were included in the experimental design, response to $O_3$ is only provided for the control level of those variables.

## 9.7 Summary and Conclusions

Based on the evidence presented in Chapter 9 and summarized here, $O_3$ is causally related or likely to be causally related to effects observed on vegetation and ecosystems. The evidence for these effects spans the entire continuum of biological organization, from the cellular and subcellular level to the whole plant, and up to ecosystem-level processes, and includes evidence for effects at lower levels of organization, leading to effects at higher levels. Given the current state of knowledge, exposure indices that cumulate and differentially weight the higher hourly average concentrations and also include the mid-level values are the most appropriate for use in developing response functions and comparing studies. The framework for causal determinations (see Preamble) has been applied to the body of scientific evidence to examine effects attributed to $O_3$ exposure collectively and the determinations are presented in Table 9-19.

## Table 9-19 Summary of $O_3$ causal determinations for vegetation and ecosystem effects.

| Vegetation and Ecosystem Effects | Conclusions from 2006 $O_3$ AQCD | Conclusions from this ISA |
|---|---|---|
| Visible Foliar Injury Effects on Vegetation | Data published since the 1996 $O_3$ AQCD strengthen previous conclusions that there is strong evidence that current ambient $O_3$ concentrations cause impaired aesthetic quality of many native plants and trees by increasing foliar injury. | Causal Relationship |
| Reduced Vegetation Growth | Data published since the 1996 $O_3$ AQCD strengthen previous conclusions that there is strong evidence that current ambient $O_3$ concentrations cause decreased growth and biomass accumulation in annual, perennial and woody plants, including agronomic crops, annuals, shrubs, grasses, and trees. | Causal Relationship |
| Reduced Productivity in Terrestrial Ecosystems | There is evidence that $O_3$ is an important stressor of ecosystems and that the effects of $O_3$ on individual plants and processes are scaled up through the ecosystem, affecting net primary productivity. | Causal Relationship |
| Reduced Carbon (C) Sequestration in Terrestrial Ecosystems | Limited studies in the 2006 $O_3$ AQCD. | Likely to be a Causal Relationship |
| Reduced Yield and Quality of Agricultural Crops | Data published since the 1996 $O_3$ AQCD strengthen previous conclusions that there is strong evidence that current ambient $O_3$ concentrations cause decreased yield and/or nutritive quality in a large number of agronomic and forage crops. | Causal Relationship |
| Alteration of Terrestrial Ecosystem Water Cycling | Ecosystem water quantity may be affected by $O_3$ exposure at the landscape level. | Likely to be a Causal Relationship |
| Alteration of Below-ground Biogeochemical Cycles | Ozone-sensitive species have well known responses to $O_3$ exposure, including altered C allocation to below-ground tissues; and also altered rates of leaf and root production, turnover, and decomposition. These shifts can affect overall C and N loss from the ecosystem in terms of respired C, and leached aqueous dissolved organic and inorganic C and N. | Causal Relationship |
| Alteration of Terrestrial Community Composition | Ozone may be affecting above- and below -ground community composition through impacts on both growth and reproduction. Significant changes in plant community composition resulting directly from $O_3$ exposure have been demonstrated. | Likely to be a Causal Relationship |

## References


Agrell, J; Kopper, BJ; McDonald, EP; Lindroth, RL. (2005). CO2 and O3 effects on host plant preferences of the forest tent caterpillar (Malacosoma disstria). Global Change Biol 11: 588-599. http://dx.doi.org/10.1111/j.1365-2486.2005.00924.x

Ahlfors, R; Brosche, M; Kollist, H; Kangasjarvi, J. (2009). Nitric oxide modulates ozone-induced cell death, hormone biosynthesis and gene expression in Arabidopsis thaliana. Plant J 58: 1-12. http://dx.doi.org/10.1111/j.1365-313X.2008.03756.x

Ahsan, N; Nanjo, Y; Sawada, H; Kohno, Y; Komatsu, S. (2010). Ozone stress-induced proteomic changes in leaf total soluble and chloroplast proteins of soybean reveal that carbon allocation is involved in adaptation in the early developmental stage. Proteomics 10: 2605-2619. http://dx.doi.org/10.1002/pmic.201000180

Ainsworth, EA. (2008). Rice production in a changing climate: a meta-analysis of responses to elevated carbon dioxide and elevated ozone concentration. Global Change Biol 14: 1642-1650. http://dx.doi.org/10.1111/j.1365-2486.2008.01594.x

Ainsworth, EA; Long, SP. (2005). What have we learned from 15 years of free-air CO2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO2 [Review]. New Phytol 165: 351-371. http://dx.doi.org/10.1111/j.1469-8137.2004.01224.x

Ainsworth, EA; Rogers, A. (2007). The response of photosynthesis and stomatal conductance to rising [CO2]: Mechanisms and environmental interactions [Review]. Plant Cell Environ 30: 258-270. http://dx.doi.org/10.1111/j.1365-3040.2007.01641.x

Alexis, A; Garcia, A; Nystrom, M; Rosenkranz, K. (2001a). The 2001 California almanac of emissions and air quality. Sacremento, CA: California Air Resources Board. http://www.arb.ca.gov/aqd/almanac/almanac01/almanac01.htm

Allen, EB; Temple, PJ; Bytnerowicz, A; Arbaugh, MJ; Sirulnik, AG; Rao, LE. (2007). Patterns of understory diversity in mixed coniferous forests of southern California impacted by air pollution. ScientificWorldJournal 7: 247-263. http://dx.doi.org/10.1100/tsw.2007.72

Alonso, R; Bermejo, V; Sanz, J; Valls, B; Elvira, S; Gimeno, BS. (2007). Stomatal conductance of semi-natural Mediterranean grasslands: Implications for the development of ozone critical levels. Environ Pollut 146: 692-698. http://dx.doi.org/10.1016/j.envpol.2006.06.009

Amthor, JS. (1988). Growth and maintenance respiration in leaves of bean (Phaseolus vulgaris L) exposed to ozone in open-top chambers in the field. New Phytol 110: 319-325. http://dx.doi.org/10.1111/j.1469-8137.1988.tb00268.x

Andersen, CP. (2003). Source-sink balance and carbon allocation below ground in plants exposed to ozone. New Phytol 157: 213-228.

Andersen, CP; Ritter, W; Gregg, J; Matyssek, R; Grams, TEE. (2010). Below-ground carbon allocation in mature beech and spruce trees following long-term, experimentally enhanced O3 exposure in southern Germany. Environ Pollut 158: 2604-2609. http://dx.doi.org/10.1016/j.envpol.2010.05.008

Andersen, CP; Wilson, R; Plocher, M; Hogsett, WE. (1997). Carry-over effects of ozone on root growth and carbohydrate concentrations of ponderosa pine seedlings. Tree Physiol 17: 805-811.

Andrews, KM; Gibbons, JW; Jochimsen, DM. (2008). Ecological effects of roads on amphibians and reptiles: A literature review. In JC Mitchell; REJ Brown; B Bartholomew (Eds.), Urban Herpetology (pp. 121-143). Salt Lake City: Society for the Study of Amphibians and Reptiles. http://www.uga.edu/srel/Reprint/3091.htm

Aneja, MK; Sharma, S; Fleischmann, F; Stich, S; Heller, W; Bahnweg, G; Munch, JC; Schloter, M. (2007). Influence of ozone on litter quality and its subsequent effects on the initial structure of colonizing microbial communities. Microb Ecol 54: 151-160. http://dx.doi.org/10.1007/s00248-006-9183-0

Arbaugh, M; Bytnerowicz, A; Grulke, N; Fenn, M; Poth, M; Temple, P; Miller, P. (2003). Photochemical smog effects in mixed conifer forests along a natural gradient of ozone and nitrogen deposition in the San Bernardino Mountains. Environ Int 29: 401-406. http://dx.doi.org/10.1016/S0160-4120(02)00176-9

Arbaugh, MJ; Miller, PR; Carroll, JJ; Takemoto, BL; Proctor, T. (1998). Relationships of ozone exposure to pine injury in the Sierra Nevada and San Bernardino Mountains of California, USA. Environ Pollut 101: 291-301. http://dx.doi.org/10.1016/S0269-7491(98)00027-X

Ariyaphanphitak, W; Chidthaisong, A; Sarobol, E; Bashkin, VN; Towprayoon, S. (2005). Effects of elevated ozone concentrations on Thai Jasmine rice cultivars (Oryza sativa L.). Water Air Soil Pollut 167: 179-200. http://dx.doi.org/10.1007/s11270-005-8650-4

Ashmore, M; Emberson, L; Karlsson, PE; Pleijel, H. (2004a). Introduction for ozone deposition special issue. Atmos Environ 38: 2211-2212.

Ashmore, M; Emberson, L; Karlsson, PE; Pleijel, H. (2004b). New directions: A new generation of ozone critical levels for the protection of vegetation in Europe (correspondence). Atmos Environ 38: 2213-2214.

Ashmore, MR. (2002). Effects of oxidants at the whole plant and community level. In JNB Bell; M Treshow (Eds.), Air pollution and plant life (pp. 89-118). London: Wiley.

Ashmore, MR; Bell, JNB; Mimmack, A. (1988). Crop growth along a gradient of ambient air pollution. Environ Pollut 53: 99-121. http://dx.doi.org/10.1016/0269-7491(88)90028-0

Avnery, S; Mauzerall, DL; Liu, J; Horowitz, LW. (2011a). Global crop yield reductions due to surface ozone exposure: 1. Year 2000 crop production losses and economic damage. Atmos Environ 45: 2284-2296. http://dx.doi.org/10.1016/j.atmosenv.2010.11.045

Avnery, S; Mauzerall, DL; Liu, J; Horowitz, LW. (2011b). Global crop yield reductions due to surface ozone exposure: 2. Year 2030 potential crop production losses and economic damage under two scenarios of O3 pollution. Atmos Environ 45: 2297-2309. http://dx.doi.org/10.1016/j.atmosenv.2011.01.002

Awmack, CS; Harrington, R; Lindroth, RL. (2004). Aphid individual performance may not predict population responses to elevated CO2 or O3. Global Change Biol Biol.10: 1414-1423.

Awmack, CS; Mondor, EB; Lindroth, RL. (2007). Forest understory clover populations in enriched CO2 and O-3 atmospheres: Interspecific, intraspecific, and indirect effects. Environ Exp Bot 59: 340-346. http://dx.doi.org/10.1016/j.envexpbot.2006.04.003

Bagard, M; Le Thiec, D; Delacote, E; Hasenfratz-Sauder, MP; Banvoy, J; Gerard, J; Dizengremel, P; Jolivet, Y. (2008). Ozone-induced changes in photosynthesis and photorespiration of hybrid poplar in relation to the developmental stage of the leaves. Physiol Plant 134: 559-574. http://dx.doi.org/10.1111/j.1399-3054.2008.01160.x

Baier, M; Kandlbinder, A; Golldack, D; Dietz, K. (2005). Oxidative stress and ozone: Perception; signalling and response. Plant Cell Environ 28: 1012-1020. http://dx.doi.org/10.1111/j.1365-3040.2005.01326.x

Baldantoni, D; Fagnano, M; Alfani, A. (2011). Tropospheric ozone effects on chemical composition and decomposition rate of Quercus ilex L. leaves. Sci Total Environ 409: 979-984. http://dx.doi.org/10.1016/j.scitotenv.2010.11.022

Ball, GR; Palmer-Brown, D; Fuhrer, J; Skarby, L; Gimeno, BS; Mills, G. (2000). Identification of non-linear influences on the seasonal ozone dose-response of sensitive and resistant clover clones using artificial neural networks. Ecol Modell 129: 153-168.

Balls, GR; Palmer-Brown, D; Sanders, GE. (1996). Investigating microclimatic influences on ozone injury in clover (Trifolium subterraneum) using artificial neural networks. New Phytol 132: 271-280. http://dx.doi.org/10.1111/j.1469-8137.1996.tb01846.x

Bandeff, JM; Pregitzer, KS; Loya, WM; Holmes, WE; Zak, DR. (2006). Overstory community composition and elevated atmospheric CO2 and O3 modify understory biomass production and nitrogen acquisition. Plant Soil 282: 251-259. http://dx.doi.org/10.1007/s11104-005-5930-0

Barnes, J; Zheng, Y; Lyons, T. (2002). Plant resistance to ozone: The role of ascorbate. In K Omasa; H Saji; S Youssefian; N Kondo (Eds.), Air pollution and plant biotechnology - Prospects for phytomonitoring and phytoremediation (pp. 235-252). Tokyo: Springer-Verlag.

Bassin, S; Volk, M; Fuhrer, J. (2007a). Factors affecting the ozone sensitivity of temperate European grasslands: An overview [Review]. Environ Pollut 146: 678-691. http://dx.doi.org/10.1016/j.envpol.2006.06.010

Bassin, S; Volk, M; Suter, M; Buchmann, N; Fuhrer, J. (2007b). Nitrogen deposition but not ozone affects productivity and community composition of subalpine grassland after 3 yr of treatment. New Phytol 175: 523-534. http://dx.doi.org/10.1111/j.1469-8137.2007.02140.x

Bassin, S; Werner, RA; Sorgel, K; Volk, M; Buchmann, N; Fuhrer, J. (2009). Effects of combined ozone and nitrogen deposition on the in situ properties of eleven key plant species of a subalpine pasture. Oecologia 158: 747-756. http://dx.doi.org/10.1007/s00442-008-1191-y

Bauer, MR; Hultman, NE; Panek, JA; Goldstein, AH. (2000). Ozone deposition to a ponderosa pine plantation in the Sierra Nevada Mountains (CA): A comparison of two different climatic years. J Geophys Res 105: 22,123-122,136. http://dx.doi.org/10.1029/2000JD900168

Bender, J; Muntifering, RB; Lin, JC; Weigel, HJ. (2006). Growth and nutritive quality of Poa pratensis as influenced by ozone and competition. Environ Pollut 142: 109-115. http://dx.doi.org/10.1016/j.envpol.2005.09.012

Bender, J; Weigel, HJ. (2011). Changes in atmospheric chemistry and crop health: A review [Review]. Agron Sustain Dev 31: 81-89. http://dx.doi.org/10.1051/agro/2010013

Bennett, JP; Jepsen, EA; Roth, JA. (2006). Field responses of Prunus serotina and Asclepias syriaca to ozone around southern Lake Michigan. Environ Pollut 142: 354-366. http://dx.doi.org/10.1016/j.envpol.2005.09.024

Benoit, LF; Skelly, JM; Moore, LD; Dochinger, LS. (1982). Radial growth reductions of Pinus strobus L correlated with foliar ozone sensitivity as an indicator of ozone-induced losses in eastern forests. Can J For Res 12: 673-678. http://dx.doi.org/10.1139/x82-101

Bergweiler, CJ; Manning, WJ. (1999). Inhibition of flowering and reproductive success in spreading dogbane (Apocynum androsaemifolium) by exposure to ambient ozone. Environ Pollut 105: 333-339. http://dx.doi.org/10.1016/S0269-7491(99)00044-5

Bergweiler, CJ; Manning, WJ; Chevone, BI. (2008). Seasonal and diurnal gas exchange differences in ozone-sensitive common milkweed (Asclepias syriaca L.) in relation to ozone uptake. Environ Pollut 152: 403-415. http://dx.doi.org/10.1016/j.envpol.2007.06.019

Bernacchi, CJ; Leaky, ADB; Heady, LE; Morgan, PB; Dohleman, FG; McGrath, JM; Gillespie, KM; Wittig, VE; Rogers, A; Long, SP; Ort, DR. (2006). Hourly and seasonal variation in photosynthesis and stomatal conductance of soybean grown at future CO2 and ozone concentrations for 3 years under fully open-air field conditions. Plant Cell Environ 29: 2077-2090. http://dx.doi.org/10.1111/j.1365-3040.2006.01581.x

Bernacchi, CJ; Morgan, PB; Ort, DR; Long, SP. (2005). The growth of soybean under free air CO2 enrichment (FACE) stimulates photosynthesis while decreasing in vivo Rubisco capacity. Planta 220: 434-446. http://dx.doi.org/10.1007/s00425-004-1320-8

Betzelberger, AM; Gillespie, KM; McGrath, JM; Koester, RP; Nelson, RL; Ainsworth, EA. (2010). Effects of chronic elevated ozone concentration on antioxidant capacity, photosynthesis and seed yield of 10 soybean cultivars. Plant Cell Environ 33: 1569-1581. http://dx.doi.org/10.1111/j.1365-3040.2010.02165.x

Bidart-Bouzat, MG; Imeh-Nathaniel, A. (2008). Global change effects on plant chemical defenses against insect herbivores. J Integr Plant Biol 50: 1339-1354. http://dx.doi.org/10.1111/j.1744-7909.2008.00751.x

Billings, WD. (1978). Plants and the ecosystem. Belmont, CA: Wadsworth Publishing Company, Inc.

Biswas, DK; Xu, H; Li, YG; Sun, JZ; Wang, XZ; Han, XG; Jiang, GM. (2008). Genotypic differences in leaf biochemical, physiological and growth responses to ozone in 20 winter wheat cultivars released over the past 60 years. Global Change Biol 14: 46-59. http://dx.doi.org/10.1111/j.1365-2486.2007.01477.x

Black, VJ; Black, CR; Roberts, JA; Stewart, CA. (2000). Impact of ozone on the reproductive development of plants. New Phytol 147: 421-447.

Black, VJ; Stewart, CA; Roberts, JA; Black, CR. (2007). Ozone affects gas exchange, growth and reproductive development in Brassica campestris (Wisconsin Fast Plants). New Phytol 176: 150-163. http://dx.doi.org/10.1111/j.1469-8137.2007.02163.x

Black, VJ; Stewart, CA; Roberts, JA; Black, CR. (2010). Direct effects of ozone on reproductive development in Plantago major L. populations differing in sensitivity. Environ Exp Bot 69: 121-128. http://dx.doi.org/10.1016/j.envexpbot.2010.04.006

Blande, JD; Holopainen, JK; Li, T. (2010). Air pollution impedes plant-to-plant communication by volatiles. Ecol Lett 13: 1172-1181. http://dx.doi.org/10.1111/j.1461-0248.2010.01510.x

Bohler, S; Bagard, M; Oufir, M; Planchon, S; Hoffmann, L; Jolivet, Y; Hausman, JF; Dizengremel, P; Renaut, J. (2007). A DIGE analysis of developing poplar leaves subjected to ozone reveals major changes in carbon metabolism. Proteomics 7: 1584-1599. http://dx.doi.org/10.1002/pmic.200600822

Bohler, S; Sergeant, K; Lefèvre, I; Jolivet, Y; Hoffmann, L; Renaut, J; Dizengremel, P; Hausman, JF. (2010). Differential impact of chronic ozone exposure on expanding and fully expanded poplar leaves. Tree Physiol 30: 1415-1432. http://dx.doi.org/10.1093/treephys/tpq082

Bonn, B; Von Kuhlmann, R; Lawrence, MG. (2004). High contribution of biogenic hydroperoxides to secondary organic aerosol formation. Geophys Res Lett 31: L10108. http://dx.doi.org/10.1029/2003GL019172

Booker, FL; Burkey, KO; Jones, AM. (2012). Re-evaluating the role of ascorbic acid and phenolic glycosides in ozone scavenging in the leaf apoplast of Arabidopsis thaliana L. Plant Cell Environ 35: 1456-1466. http://dx.doi.org/10.1111/j.1365-3040.2012.02502.x

Booker, FL; Burkey, KO; Overmyer, K; Jones, AM. (2004a). Differential responses of G-protein Arabidopsis thaliana mutants to ozone. New Phytol 162: 633-641.

Booker, FL; Burkey, KO; Pursley, WA; Heagle, AS. (2007). Elevated carbon dioxide and ozone effects on peanut: I. Gas-exchange, biomass, and leaf chemistry. Crop Sci 47: 1475-1487. http://dx.doi.org/10.2135/cropsci2006.08.0537

Booker, FL; Fiscus, EL. (2005). The role of ozone flux and antioxidants in the suppression of ozone injury by elevated CO2 in soybean. J Exp Bot 56: 2139-2151. http://dx.doi.org/10.1093/jxb/eri214

Booker, FL; Fiscus, EL; Miller, JE. (2004b). Combined effects of elevated atmospheric carbon dioxide and ozone on soybean whole-plant water use. Environ Manage 33: S355-S362. http://dx.doi.org/10.1007/s00267-003-9144-z

Booker, FL; Muntifering, R; McGrath, M; Burkey, K; Decoteau, D; Fiscus, E; Manning, W; Krupa, S; Chappelka, A; Grantz, D. (2009). The ozone component of global change: Potential effects on agricultural and horticultural plant yield, product quality and interactions with invasive species. J Integr Plant Biol 51: 337-351. http://dx.doi.org/10.1111/j.1744-7909.2008.00805.x

Booker, FL; Prior, SA; Torbert, HA; Fiscus, EL; Pursley, WA; Hu, S. (2005). Decomposition of soybean grown under elevated concentrations of CO2 and O3. Global Change Biol 11: 685-698. http://dx.doi.org/10.1111/j.1365-2486.2005.00939.x

Booker, FL; Reid, CD; Brunschon-Harti, S; Fiscus, EL; Miller, JE. (1997). Photosynthesis and photorespiration in soybean [Glycine max (L) Merr] chronically exposed to elevated carbon dioxide and ozone. J Exp Bot 48: 1843-1852.

Borowiak, K; Rucinska-Sobkowiak, R; Rymer, K; Gwozdz, EA; Zbierska, J. (2009). Biochemical markers of tropospheric ozone: Experimentation with test-plants. Polish Journal of Ecology 57: 3-14.

Bou Jaoudé, M; Katerji, N; Mastrorilli, M; Rana, G. (2008a). Analysis of the effect of ozone on soybean in the Mediterranean region I: The consequences on crop-water status. Eur J Agron 28: 508-518. http://dx.doi.org/10.1016/j.eja.2007.09.002

Bou Jaoudé, M; Katerji, N; Mastrorilli, M; Rana, G. (2008b). Analysis of the ozone effect on soybean in the Mediterranean region II. The consequences on growth, yield and water use efficiency. Eur J Agron 28: 519-525. http://dx.doi.org/10.1016/j.eja.2007.09.001

Broadmeadow, MSJ; Jackson, SB. (2000). Growth responses of Quercus petraea, Fraxinus excelsior and Pinus sylvestris to elevated carbon dioxide, ozone and water supply. New Phytol 146: 437-451. http://dx.doi.org/10.1046/j.1469-8137.2000.00665.x

Brook, JR; DiGiovanni, F; Cakmak, S; Meyers, TP. (1997). Estimation of dry deposition velocity using inferential models and site-specific meteorology--uncertainty due to siting of meteorological towers. Atmos Environ 31: 3911-3919.

Bulbovas, P; de Souza, SR; de Moraes, RM; Luizao, F; Artaxo, P. (2007). Soybean 'Tracaja' seedlings exposed to ozone under controlled conditions. Pesqui Agropecu Bras 42: 641-646. http://dx.doi.org/10.1590/S0100-204X2007000500005

Burkey, KO; Booker, FL; Pursley, WA; Heagle, AS. (2007). Elevated carbon dioxide and ozone effects on peanut: II. Seed yield and quality. Crop Sci 47: 1488-1497. http://dx.doi.org/10.2135/cropsci2006.08.0538

Burkey, KO; Eason, G; Fiscus, EL. (2003). Factors that affect leaf extracellular ascorbic acid content and redox status. Physiol Plant 117: 51-57. http://dx.doi.org/10.1034/j.1399-3054.2003.1170106.x

Burkey, KO; Neufeld, HS; Souza, L; Chappelka, AH; Davison, AW. (2006). Seasonal profiles of leaf ascorbic acid content and redox state in ozone-sensitive wildflowers. Environ Pollut 143: 427-434. http://dx.doi.org/10.1016/j.envpol.2005.12.009

Bytnerowicz, A; Arbaugh, M; Schilling, S; Fraczek, W; Alexander, D. (2008). Ozone distribution and phytotoxic potential in mixed conifer forests of the San Bernardino Mountains, Southern California. Environ Pollut 155: 398-408. http://dx.doi.org/10.1016/j.envpol.2008.01.046

Caird, MA; Richards, JH; Donovan, LA. (2007). Nighttime stomatal conductance and transpiration in C-3 and C-4 plants. Plant Physiol 143: 4-10. http://dx.doi.org/10.1104/pp.106.092940

Cal/EPA (California Environmental Protection Agency). (2010). Air quality data branch main page. Available online at http://www.arb.ca.gov/aqd/aqdpage.htm (accessed January 28, 2011).

Calatayud, A; Alvarado, JW; Barreno, E. (2002). Similar effects of ozone on four cultivars of lettuce in open top chambers during winter. Photosynthetica 40: 195-200. http://dx.doi.org/10.1023/A:1021333305592

Calatayud, A; Pomares, F; Barreno, E. (2006). Interactions between nitrogen fertilization and ozone in watermelon cultivar Reina de Corazones in open-top chambers. Effects on chlorophyll alpha fluorescence, lipid peroxidation, and yield. Photosynthetica 44: 93-101. http://dx.doi.org/10.1007/s11099-005-0163-2

Calatayud, V; Cervero, J; Sanz, MJ. (2007a). Foliar, physiologial and growth responses of four maple species exposed to ozone. Water Air Soil Pollut 185: 239-254. http://dx.doi.org/10.1007/s11270-007-9446-5

Calatayud, V; Marco, F; Cerveró, J; Sánchez-Peña, G; Sanz, MJ. (2010). Contrasting ozone sensitivity in related evergreen and deciduous shrubs. Environ Pollut 158: 3580-3587. http://dx.doi.org/10.1016/j.envpol.2010.08.013

Calatayud, V; Sanz, MJ; Calvo, E; Cervero, J; Ansel, W; Klumpp, A. (2007b). Ozone biomonitoring with Bel-W3 tobacco plants in the city of Valencia (Spain). Water Air Soil Pollut 183: 283-291. http://dx.doi.org/10.1007/s11270-007-9376-2

Campbell, SJ; Wanek, R; Coulston, JW. (2007). Ozone injury in west coast forests: 6 years of monitoring - Introduction. Portland, OR: U.S. Department of Agriculture.

Cannon, WN. (1990). Olfactory response of eastern spruce budworm larvae to red spruce needles exposed to acid rain and elevated levels of ozone. J Chem Ecol 16: 3255-3261. http://dx.doi.org/10.1007/BF00982096

Carde, RT; Haynes, KF. (2004). Stucture of the pheromone communication channel in moths. In Advances in insect chemical ecology. Cambridge: Cambridge University Press. http://dx.doi.org/10.1017/CBO9780511542664.009

Casteel, CL; O'Neill, BF; Zavala, JA; Bilgin, DD; Berenbaum, MR; DeLucia, EH. (2008). Transcriptional profiling reveals elevated CO2 and elevated O3 alter resistance of soybean (Glycine max) to Japanese beetles (Popillia japonica). Plant Cell Environ 31: 419-434. http://dx.doi.org/10.1111/j.1365-3040.2008.01782.x

Chapman, JA; King, JS; Pregitzer, KS; Zak, DR. (2005). Effects of elevated concentrations of atmospheric CO2 and tropospheric O-3 on decomposition of fine roots. Tree Physiol 25: 1501-1510.

Chappelka, A; Skelly, J; Somers, G; Renfro, J; Hildebrand, E. (1999a). Mature black cherry used as a bioindicator of ozone injury. Water Air Soil Pollut 116: 261-266.

Chappelka, A; Somers, G; Renfro, J. (1999b). Visible ozone injury on forest trees in Great Smoky Mountains National Park, USA. Water Air Soil Pollut 116: 255-260.

Chappelka, AH. (2002). Reproductive development of blackberry (Rubus cuneifolius) as influenced by ozone. New Phytol 155: 249-255. http://dx.doi.org/10.1046/j.1469-8137.2002.00464.x

Chappelka, AH; Neufeld, HS; Davison, AW; Somers, GL; Renfro, JR. (2003). Ozone injury on cutleaf coneflower (Rudbeckia laciniata) and crown-beard (Verbesina occidentalis) in Great Smoky Mountains National Park. Environ Pollut 125: 53-60.

Chappelka, AH; Samuelson, LJ. (1998). Ambient ozone effects on forest trees of the eastern United States: A review [Review]. New Phytol 139: 91-108. http://dx.doi.org/10.1046/j.1469-8137.1998.00166.x

Chappelka, AH; Somers, GL; Renfro, JR. (2007). Temporal patterns of foliar ozone symptoms on tall milkweed (Asclepias exaltata L) in Great Smoky Mountains National Park. Environ Pollut 149: 358-365. http://dx.doi.org/10.1016/j.envpol.2007.05.015

Chen, CW; Tsai, WT; Lucier, AA. (1998). A model of air-tree-soil system for ozone impact analysis. Ecol Modell 111: 207-222.

Chen, Z; Gallie, DR. (2005). Increasing tolerance to ozone by elevating foliar ascorbic acid confers greater protection against ozone than increasing avoidance. Plant Physiol 138: 1673-1689. http://dx.doi.org/10.1104/pp.105.062000

Chen, Z; Wang, XK; Feng, ZZ; Xiao, Q; Duan, XN. (2009). Impact of elevated O3 on soil microbial community function under wheat crop. Water Air Soil Pollut 198: 189-198. http://dx.doi.org/10.1007/s11270-008-9838-1

Chen, Z; Wang, XK; Yao, FF; Zheng, FX; Feng, ZZ. (2010b). Elevated ozone changed soil microbial community in a rice paddy. Soil Sci Soc Am J 74: 829-837. http://dx.doi.org/10.2136/sssaj2009.0258

Cheng, FY; Burkey, KO; Robinson, JM; Booker, FL. (2007). Leaf extracellular ascorbate in relation to O3 tolerance of two soybean cultivars. Environ Pollut 150: 355-362. http://dx.doi.org/10.1016/j.envpol.2007.01.022

Cho, K; Shibato, J; Agrawal, GK; Jung, YH; Kubo, A; Jwa, NS; Tamogami, S; Satoh, K; Kikuchi, S; Higashi, T; Kimura, S; Saji, H; Tanaka, Y; Iwahashi, H; Masuo, Y; Rakwal, R. (2008). Integrated transcriptomics, proteomics, and metabolomics analyses to survey ozone responses in the leaves of rice seedling. J Proteome Res 7: 2980-2998. http://dx.doi.org/10.1021/pr800128q

Christman, MA; Donovan, LA; Richards, JH. (2009). Magnitude of nighttime transpiration does not affect plant growth or nutrition in well-watered Arabidopsis. Physiol Plant 136: 264-273. http://dx.doi.org/10.1111/j.1399-3054.2009.01216.x

Chung, HG; Zak, DR; Lilleskov, EA. (2006). Fungal community composition and metabolism under elevated CO2 and O3. Oecologia 147: 143-154. http://dx.doi.org/10.1007/s00442-005-0249-3

Colls, JJ; Unsworth, MH. (1992). Air pollution interactions with natural stressors. In JR Barker; DT Tingey (Eds.), Air pollution effects on biodiversity. New York, NY: Van Nostrand Reinhold.

Cornelissen, T. (2011). Climate change and its effects on terrestrial insects and herbivory patterns [Review]. Neotrop Entomol 40: 155-163. http://dx.doi.org/10.1590/S1519-566X2011000200001

Costa, DL; Folinsbee, LJ; Raub, JA; Tilton, B; Tingey, DT. (1992). Summary of selected new information on effects of ozone on health and vegetation: Supplement to 1986 air quality criteria for ozone and other photochemical oxidants. (EPA/600/8-88/105F). Research Triangle Park, NC: U.S. Environmental Protection Agency. http://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=31093

Coulston, JW; Smith, GC; Smith, WD. (2003). Regional assessment of ozone sensitive tree species using bioindicator plants. Environ Monit Assess 83: 113-127.

Crous, KY; Vandermeiren, K; Ceulemans, R. (2006). Physiological responses to cumulative ozone uptake in two white clover (Trifolium repens L. cv. Regal) clones with different ozone sensitivity. Environ Exp Bot 58: 169-179. http://dx.doi.org/10.1016/j.envexpbot.2005.07.007

D'Haese, D; Vandermeiren, K; Asard, H; Horemans, N. (2005). Other factors than apoplastic ascorbate contribute to the differential ozone tolerance of two clones of Trifolium repens L. Plant Cell Environ 28: 623-632. http://dx.doi.org/10.1111/j.1365-3040.2005.01308.x

Dalstein, L; Vas, N. (2005). Ozone concentrations and ozone-induced symptoms on coastal and alpine mediterranean pines in southern France. Water Air Soil Pollut 160: 181-195.

Dammgen, U; Grunhage, L; Haenel, HD; Jager, HJ. (1993). Climate and stress in ecotoxicology: A coherent system of definitions and terms. J Appl Bot Food Qual 67: 157-162.

Darbah, JNT; Kubiske, ME; Neilson, N; Oksanen, E; Vaapavuori, E; Karnosky, DF. (2007). Impacts of elevated atmospheric CO2 and O3 on paper birch (Betula papyrifera): Reproductive fitness. ScientificWorldJournal 7: 240-246. http://dx.doi.org/10.1100/tsw.2007.42

Darbah, JNT; Kubiske, ME; Nelson, N; Oksanen, E; Vapaavuori, E; Kamosky, DF. (2008). Effects of decadal exposure to interacting elevated CO2 and/or O-3 on paper birch (Betula papyrifera) reproduction. Environ Pollut 155: 446-452. http://dx.doi.org/10.1016/j.envpol.2008.01.033

Davidson, A. (1993). Update of ozone trends in California's South Coast Air Basin. Air Waste 43: 226-227. http://dx.doi.org/10.1080/1073161X.1993.10467130

Davis, DD. (2007a). Ozone-induced symptoms on vegetation within the Moosehorn National Wildlife Refuge in Maine. Northeast Nat 14: 403-414. http://dx.doi.org/10.1656/1092-6194(2007)14[403:OSOVWT]2.0.CO;2

Davis, DD. (2007b). Ozone injury to plants within the Seney National Wildlife Refuge in northern Michigan. Northeast Nat 14: 415-424.

Davis, DD. (2009). Ozone-induced stipple on plants in the Cape Romain National Wildlife Refuge, South Carolina. Southeastern Naturalist 8: 471-478.

Davis, DD; Orendovici, T. (2006). Incidence of ozone symptoms on vegetation within a National Wildlife Refuge in New Jersey, USA. Environ Pollut 143: 555-564. http://dx.doi.org/10.1016/j.envpol.2005.10.051

Dawson, TE; Burgess, SS; Tu, KP; Oliveira, RS; Santiago, LS; Fisher, JB; Simonin, KA; Ambrose, AR. (2007). Nighttime transpiration in woody plants from contrasting ecosystems. Tree Physiol 27: 561-575. http://dx.doi.org/10.1093/treephys/27.4.561

de Lourdes de Bauer, M; Hernandez-Tejeda, T. (2007). A review of ozone-induced effects on the forests of central Mexico [Review]. Environ Pollut 147: 446-453. http://dx.doi.org/10.1016/j.envpol.2006.12.020

De Temmerman, L; Legrand, G; Vandermeiren, K. (2007). Effects of ozone on sugar beet grown in open-top chambers. Eur J Agron 26: 1-9. http://dx.doi.org/10.1016/j.eja.2006.08.001

Degl'Innocenti, E; Guidi, L; Soldatini, GF. (2007). Effects of elevated ozone on chlorophyll a fluorescence in symptomatic and asymptomatic leaves of two tomato genotypes. Biol Plantarum 51: 313-321. http://dx.doi.org/10.1007/s10535-007-0061-5

Dermody, O; O'Neill, BF; Zangerl, AR; Berenbaum, MR; DeLucia, EH. (2008). Effects of elevated CO2 and O3 on leaf damage and insect abundance in a soybean agroecosystem. Arthropod-Plant Inte 2: 125-135.

Di Baccio, D; Castagna, A; Paoletti, E; Sebastiani, L; Ranieri, A. (2008). Could the differences in O3 sensitivity between two poplar clones be related to a difference in antioxidant defense and secondary metabolic response to O3 influx? Tree Physiol 28: 1761-1772.

Dickson, RE; Lewin, KF; Isebrands, JG; Coleman, MD; Heilman, WE; Riemenschneider, DE; Sober, J; Host, GE; Zak, DR; Hendrey, GR; Pregitzer, KS; Karnosky, DF. (2000). Forest Atmosphere Carbon Transfer and Storage (FACTS-II) the Aspen Free-Air CO2 and O3 Enrichment (FACE) project: An overview. (General Technical Report NC-214). St. Paul, MN: U.S. Dept. of Agriculture, Forest Service. http://nrs.fs.fed.us/pubs/278

Ditchkoff, SS; Lewis, JS; Lin, JC; Muntifering, RB; Chappelka, AH. (2009). Nutritive quality of highbush blackberry (Rubus argutus) exposed to tropospheric ozone. Rangeland Ecol Manag 62: 364-370.

Dizengremel, P; Le Thiec, D; Bagard, M; Jolivet, Y. (2008). Ozone risk assessment for plants: Central role of metabolism-dependent changes in reducing power. Environ Pollut 156: 11-15. http://dx.doi.org/10.1016/j.envpol.2007.12.024

Dizengremel, P; Le Thiec, D; Hasenfratz-Sauder, MP; Vaultier, MN; Bagard, M; Jolivet, Y. (2009). Metabolic-dependent changes in plant cell redox power after ozone exposure. Plant Biol (Stuttg) 11: 35-42. http://dx.doi.org/10.1111/j.1438-8677.2009.00261.x

Dizengremel, P; Sasek, T; Brown, K; Richardson, C. (1994). Ozone-induced changes in primary carbon metabolism enzymes of loblolly pine needles. J Plant Physiol 144: 300-306.

Dobson, HEM. (1994). Floral volatiles in insect biology. In EA Bernays (Ed.), Insect-plant interactions: Vol 5 (pp. 47-82). Boca Raton, FL: CRC Press.

Dohm, MR; Mautz, WJ; Andrade, JA; Gellert, KS; Salas-Ferguson, LJ; Nicolaisen, N; Fujie, N. (2005). Effects of ozone exposure on nonspecific phagocytic capacity of pulmonary macrophages from an amphibian, Bufo marinus. Environ Toxicol Chem 24: 205-210.

Dohm, MR; Mautz, WJ; Doratt, RE; Stevens, JR. (2008). Ozone exposure affects feeding and locomotor behavior of adult Bufo marinus. Environ Toxicol Chem 27: 1209-1216. http://dx.doi.org/10.1897/07-388.1

Dohm, MR; Mautz, WJ; Looby, PG; Gellert, KS; Andrade, JA. (2001). Effects of ozone on evaporative water loss and thermoregulatory behavior of marine toads (Bufo marinus). Environ Res 86: 274-286.

Dohrmann, AB; Tebbe, CC. (2005). Effect of elevated tropospheric ozone on the structure of bacterial communities inhabiting the rhizosphere of herbaceous plants native to Germany. Appl Environ Microbiol 71: 7750-7758. http://dx.doi.org/10.1128/AEM.71.12.7750-7758.2005

Drogoudi, PD; Ashmore, M. (2001). 14C-allocation of flowering and deblossomed strawberry in response to elevated ozone. New Phytol 152: 455-461. http://dx.doi.org/10.1046/j.0028-646X.2001.00270.x

Drogoudi, PD; Ashmore, MR. (2000). Does elevated ozone have differing effects in flowering and deblossomed strawberry? New Phytol 147: 561-569. http://dx.doi.org/10.1046/j.1469-8137.2000.00718.x

Dudareva, N; Negre, F; Nagegowda, DA; Orlova, I. (2006). Plant volatiles: Recent advances and future perspectives [Review]. Crit Rev Plant Sci 25: 417-440.

Ederli, L; Morettini, R; Borgogni, A; Wasternack, C; Miersch, O; Reale, L; Ferranti, F; Tosti, N; Pasqualini, S. (2006). Interaction between nitric oxide and ethylene in the induction of alternative oxidase in ozone-treated tobacco plants. Plant Physiol 142: 595-608. http://dx.doi.org/10.1104/pp.106.085472

Edwards, IP; Zak, DR. (2011). Fungal community composition and function after long-term exposure of northern forests to elevated atmospheric CO2 and tropospheric O3. Global Change Biol 17: 2184-2195. http://dx.doi.org/10.1111/j.1365-2486.2010.02376.x

Ellenson, JL; Amundson, RG. (1982). Delayed light imaging for the early detection of plant stress. Science 215: 1104-1106. http://dx.doi.org/10.1126/science.215.4536.1104

Ellsworth, DS; Reich, PB; Naumburg, ES; Koch, GW; Kubiske, ME; Smith, SD. (2004). Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO2 across four free-air CO2 enrichment experiments in forest, grassland and desert. Global Change Biol 10: 2121-2138. http://dx.doi.org/10.1111/j.1365-2486.2004.00867.x

Eltayeb, AE; Kawano, N; Badawi, GH; Kaminaka, H; Sanekata, T; Morishima, I; Shibahara, T; Inanaga, S; Tanaka, K. (2006). Enhanced tolerance to ozone and drought stresses in transgenic tobacco overexpressing dehydroascorbate reductase in cytosol. Physiol Plant 127: 57-65. http://dx.doi.org/10.1111/j.1399-3054.2005.00624.x

Eltayeb, AE; Kawano, N; Badawi, GH; Kaminaka, H; Sanekata, T; Shibahara, T; Inanaga, S; Tanaka, K. (2007). Overexpression of monodehydroascorbate reductase in transgenic tobacco confers enhanced tolerance to ozone, salt and polyethylene glycol stresses. Planta 225: 1255-1264. http://dx.doi.org/10.1007/s00425-006-0417-7

Emberson, L; Ashmore, MR; Cambridge, HM; Simpson, D; Tuovinen, JP. (2000a). Modelling stomatal ozone flux across Europe. Environ Pollut 109: 403-413. http://dx.doi.org/10.1016/S0269-7491(00)00043-9

Emberson, LD; Wieser, G; Ashmore, MR. (2000b). Modelling of stomatal conductance and ozone flux of Norway spruce: Comparison with field data. Environ Pollut 109: 393-402. http://dx.doi.org/10.1016/S0269-7491(00)00042-7

Enders, G. (1992). Deposition of ozone to a mature spruce forest: Measurements and comparison to models. Environ Pollut 75: 61-67. http://dx.doi.org/10.1016/0269-7491(92)90057-H

Esperschutz, J; Pritsch, K; Gattinger, A; Welzl, G; Haesler, F; Buegger, F; Winkler, JB; Munch, JC; Schloter, M. (2009). Influence of chronic ozone stress on carbon translocation pattern into rhizosphere microbial communities of beech trees (Fagus sylvatica L.) during a growing season. Plant Soil 323: 85-95. http://dx.doi.org/10.1007/s11104-009-0090-2

Fares, S; Barta, C; Brilli, F; Centritto, M; Ederli, L; Ferranti, F; Pasqualini, S; Reale, L; Tricoli, D; Loreto, F. (2006). Impact of high ozone on isoprene emission, photosynthesis and histology of developing Populus alba leaves directly or indirectly exposed to the pollutant. Physiol Plant 128: 456-465. http://dx.doi.org/10.1111/j.1399-3054.2006.00750.x

Fares, S; Gentner, DR; Park, JH; Ormeno, E; Karlik, J; Goldstein, AH. (2011). Biogenic emissions from Citrus species in California. Atmos Environ 45: 4557-4568. http://dx.doi.org/10.1016/j.atmosenv.2011.05.066

Fares, S; Loreto, F; Kleist, E; Wildt, J. (2008). Stomatal uptake and stomatal deposition of ozone in isoprene and monoterpene emitting plants. Plant Biol (Stuttg) 10: 44-54. http://dx.doi.org/10.1055/s-2007-965257

Fares, S; McKay, M; Holzinger, R; Goldstein, AH. (2010a). Ozone fluxes in a Pinus ponderosa ecosystem are dominated by non-stomatal processes: Evidence from long-term continuous measurements. Agr Forest Meteorol 150: 420-431. http://dx.doi.org/10.1016/j.agrformet.2010.01.007

Fares, S; Oksanen, E; Lannenpaa, M; Julkunen-Tiitto, R; Loreto, F. (2010b). Volatile emissions and phenolic compound concentrations along a vertical profile of Populus nigra leaves exposed to realistic ozone concentrations. Photosynth Res 104: 61-74. http://dx.doi.org/10.1007/s11120-010-9549-5

Felzer, B; Kicklighter, D; Melillo, J; Wang, C; Xhuang, Q; Prinn, R. (2004). Effects of ozone on net primary production and carbon sequestration in the conterminous United States using a biogeochemistry model. Tellus B Chem Phys Meteorol 56: 230-248. http://dx.doi.org/10.1111/j.1600-0889.2004.00097.x

Felzer, B; Reilly, J; Melillo, J; Kicklighter, D; Sarofim, M; Wang, C; Prinn, R; Zhuang, Q. (2005). Future effects of ozone on carbon sequestration and climate change policy using a global biogeochemical model. Clim Change 73: 345-373. http://dx.doi.org/10.1007/s10584-005-6776-4

Felzer, BS; Cronin, TW; Melillo, JM; Kicklighter, DW; Schlosser, CA. (2009). Importance of carbon-nitrogen interactions and ozone on ecosystem hydrology during the 21st century. J Geophys Res 114: G01020.

Feng, YW; Komatsu, S; Furukawa, T; Koshiba, T; Kohno, Y. (2008a). Proteome analysis of proteins responsive to ambient and elevated ozone in rice seedlings. Agric Ecosyst Environ 125: 255-265. http://dx.doi.org/10.1016/j.agee.2008.01.018

Feng, Z; Pang, J; Nouchi, I; Kobayashi, K; Yamakawa, T; Zhu, J. (2010). Apoplastic ascorbate contributes to the differential ozone sensitivity in two varieties of winter wheat under fully open-air field conditions. Environ Pollut 158: 3539-3545. http://dx.doi.org/10.1016/j.envpol.2010.08.019

Feng, ZZ; Kobayashi, K. (2009). Assessing the impacts of current and future concentrations of surface ozone on crop yield with meta-analysis. Atmos Environ 43: 1510-1519. http://dx.doi.org/10.1016/j.atmosenv.2008.11.033

Feng, ZZ; Kobayashi, K; Ainsworth, EA. (2008b). Impact of elevated ozone concentration on growth, physiology, and yield of wheat (Triticum aestivum L.): A meta-analysis. Global Change Biol 14: 2696-2708. http://dx.doi.org/10.1111/j.1365-2486.2008.01673.x

Fenn, ME; de Bauer, LI; Hernández-Tejeda, T. (2002). Summary of air pollution impacts on forests in the Mexico City air basin. In Urban air pollution and forests. New York, NY: Springer-Verlag.

Fenn, ME; Poth, MA; Johnson, DW. (1996). Evidence for nitrogen saturation in the San Bernardino Mountains in southern California. For Ecol Manage 82: 211-230. http://dx.doi.org/10.1016/0378-1127(95)03668-7

Findley, DA; Keever, GJ; Chappelka, AH; Eakes, DJ; Gillian, DJ. (1997). Differential responses of buddleia (Buddleia davidii Franch) to ozone. Environ Pollut 98: 105-111.

Finkelstein, PL; Ellestad, TG; Clarke, JF; Meyers, TP; Schwede, DB; Hebert, EO; Neal, JA. (2000). Ozone and sulfur dioxide dry deposition to forests: Observations and model evaluation. J Geophys Res 105: 15365-15377.

Finnan, JM; Burke, JL; Jones, MB. (1997). An evaluation of indices that describe the impact of ozone on the yield of spring wheat (Triticum aestivum L). Atmos Environ 31: 2685-2693. http://dx.doi.org/10.1016/S1352-2310(97)00105-2

Finnan, JM; Jones, MB; Burke, JI. (1996). A time-concentration study on the effects of ozone on spring wheat (Triticum aestivum L): 2. A comparison of indices. Agric Ecosyst Environ 57: 169-177. http://dx.doi.org/10.1016/0167-8809(95)01004-1

Fiscus, EL; Booker, FL; Burkey, KO. (2005). Crop responses to ozone: Uptake, modes of action, carbon assimilation and partitioning. Plant Cell Environ 28: 997-1011.

Fiscus, EL; Philbeck, R; Britt, AM; Booker, FL. (1999). Growth of Arabidopsis flavonoid mutants under solar radiation and UV filters. Environ Exp Bot 41: 231-245. http://dx.doi.org/10.1016/S0098-8472(99)00011-8

Fishman, J; Bowman, KW; Burrows, JP; Richter, A; Chance, KV; Edwards, DP; Martin, RV; Morris, GA; Pierce, RB; Ziemke, JR; Al-Saadi, JA; Creilson, JK; Schaack, TK; Thompson, AM. (2008). Remote sensing of tropospheric pollution from space. Bull Am Meteorol Soc 89: 805-821. http://dx.doi.org/10.1175/2008BAMS2526.1

Fishman, J; Creilson, JK; Parker, PA; Ainsworth, EA; Vining, GG; Szarka, J; Booker, FL; Xu, XJ. (2010). An investigation of widespread ozone damage to the soybean crop in the upper Midwest determined from ground-based and satellite measurements. Atmos Environ 44: 2248-2256. http://dx.doi.org/10.1016/j.atmosenv.2010.01.015

Flagler, RB. (1998). Recognition of air pollution injury to vegetation: A pictorial atlas (2nd ed.). Pittsburgh, PA: Air & Waste Management Association.

Flowers, MD; Fiscus, EL; Burkey, KO; Booker, FL; Dubois, JJB. (2007). Photosynthesis, chlorophyll fluorescence, and yield of snap bean (Phaseolus vulgaris L.) genotypes differing in sensitivity to ozone. Environ Exp Bot 61: 190-198. http://dx.doi.org/10.1016/j.envexpbot.2007.05.009

Fontan, JA; Minga, A; Lopez, A; Druilhet, A. (1992). Vertical ozone profiles in a pine forest. Atmos Environ 26: 863-869. http://dx.doi.org/10.1016/0960-1686(92)90245-G

Foyer, CH; Noctor, G. (2005a). Oxidant and antioxidant signalling in plants: A re-evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ 28: 1056-1071.

Foyer, CH; Noctor, G. (2005b). Redox homeostasis and antioxidant signaling: A metabolic interface between stress perception and physiological responses [Review]. Plant Cell 17: 1866-1875. http://dx.doi.org/10.1105/tpc.105.033589

Fredericksen, TS; Joyce, BJ; Skelly, JM; Steiner, KC; Kolb, TE; Kouterick, KB; Savage, JE; Snyder, KR. (1995). Physiology, morphology, and ozone uptake of leaves of black cherry seedlings, saplings, and canopy trees. Environ Pollut 89: 273-283. http://dx.doi.org/10.1016/0269-7491(94)00077-Q

Fredericksen, TS; Kolb, TE; Skelly, JM; Steiner, KC; Joyce, BJ; Savage, JE. (1996). Light environment alters ozone uptake per net photosynthetic rate in black cherry trees. Tree Physiol 16: 485-490.

Freiwald, V; Haikio, E; Julkunen-Tiitto, R; Holopainen, JK; Oksanen, E. (2008). Elevated ozone modifies the feeding behaviour of the common leaf weevil on hybrid aspen through shifts in developmental, chemical, and structural properties of leaves. Entomol Exp Appl 128: 66-72. http://dx.doi.org/10.1111/j.1570-7458.2008.00677.x

Fuentes, JD; Gillespie, TJ; den Hartog, G; Neumann, HH. (1992). Ozone deposition onto a deciduous forest during dry and wet conditions. Agr Forest Meteorol 62: 1-18. http://dx.doi.org/10.1016/0168-1923(92)90002-L

Fuhrer, J. (1994). Effects of ozone on managed pasture: 1. Effects of open-top chambers on microclimate, ozone flux, and plant growth. Environ Pollut 86: 297-305.

Fuhrer, J; Skarby, L; Ashmore, MR. (1997). Critical levels for ozone effects on vegetation in Europe. Environ Pollut 97: 91-106. http://dx.doi.org/10.1016/S0269-7491(97)00067-5

Gate, IM; McNeill, S; Ashmore, MR. (1995). Effects of air pollution on the searching behaviour of an insect parasitoid. Water Air Soil Pollut 85: 1425-1430. http://dx.doi.org/10.1007/BF00477181

Geiser, LH; Neitlich, PN. (2007). Air pollution and climate gradients in western Oregon and Washington indicated by epiphytic macrolichens. Environ Pollut 145: 203-218. http://dx.doi.org/10.1016/j.envpol.2006.03.024

Gerosa, G; Marzuoli, R; Rossini, M; Panigada, C; Meroni, M; Colombo, R; Faoro, F; Iriti, M. (2009). A flux-based assessment of the effects of ozone on foliar injury, photosynthesis, and yield of bean (Phaseolus vulgaris L. cv. Borlotto Nano Lingua di Fuoco) in open-top chambers. Environ Pollut 157: 1727-1736. http://dx.doi.org/10.1016/j.envpol.2008.06.028

Gielen, B; Vandermeiren, K; Horemans, N; D'Haese, D; Serneels, R; Valcke, R. (2006). Chlorophyll a fluorescence imaging of ozone-stressed Brassica napus L. plants differing in glucosinolate concentrations. Plant Biol (Stuttg) 8: 698-705. http://dx.doi.org/10.1055/s-2006-924150

Gitay, H; Brown, S; Easterling, W; Jallow, B. (2001). Ecosystems and their goods and services. In Climate change 2001: Impacts, adaptation and vulnerability: Contribution of Working Group II to the third assessment report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom: Cambridge University Press.

Gombert, S; Asta, J; Seaward, MRD. (2006). Lichens and tobacco plants as complementary biomonitors of air pollution in the Grenoble area (Isere, southeast France). Ecol Indicat 6: 429-443. http://dx.doi.org/10.1016/j.ecolind.2005.06.001

Gonzalez-Fernandez, I; Kaminska, A; Dodmani, M; Goumenaki, E; Quarrie, S; Barnes, JD. (2010). Establishing ozone flux-response relationships for winter wheat: Analysis of uncertainties based on data for UK and Polish genotypes. Atmos Environ 44: 621-630. http://dx.doi.org/10.1016/j.atmosenv.2009.11.021

Goumenaki, E; Taybi, T; Borland, A; Barnes, J. (2010). Mechanisms underlying the impacts of ozone on photosynthetic performance. Environ Exp Bot 69: 259-266. http://dx.doi.org/10.1016/j.envexpbot.2010.04.011

Grantz, DA; Gunn, S; Vu, HB. (2006). O3 impacts on plant development: A meta-analysis of root/shoot allocation and growth. Plant Cell Environ 29: 1193-1209. http://dx.doi.org/10.1111/j.1365-3040.2006.01521.x

Grantz, DA; Shrestha, A. (2006). Tropospheric ozone and interspecific competition between yellow nutsedge and pima cotton. Crop Sci 46: 1879-1889. http://dx.doi.org/10.2135/cropsci2005.06.0167

Grantz, DA; Shrestha, A; Vu, HB. (2008). Early vigor and ozone response in horseweed (Conyza canadensis) biotypes differing in glyphosate resistance. Weed Sci 56: 224-230. http://dx.doi.org/10.1614/ws-07-130.1

Grantz, DA; Shrestha, A; Vu, HB. (2010a). Ozone impacts on assimilation and allocation to reproductive sinks in the vegetatively propagated C-4 weed, yellow nutsedge. Crop Sci 50: 246-252. http://dx.doi.org/10.2135/cropsci2009.03.0127

Grantz, DA; Vu, HB. (2009). O3 sensitivity in a potential C4 bioenergy crop: Sugarcane in California. Crop Sci 49: 643-650.

Grantz, DA; Vu, HB; Aguilar, C; Rea, MA. (2010b). No interaction between methyl jasmonate and ozone in Pima cotton: Growth and allocation respond independently to both. Plant Cell Environ 33: 717-728. http://dx.doi.org/10.1111/j.1365-3040.2009.02096.x

Grantz, DA; Zhang, XJ; Massman, W; Delany, A; Pederson, R. (1997). Ozone deposition to a cotton (Gossypium hirsutum L) field: Stomatal and surface wetness effects during the California Ozone Deposition experiment. Agr Forest Meteorol 85: 19-31. http://dx.doi.org/10.1016/S0168-1923(96)02396-9

Grantz, DA; Zhang, XJ; Massman, WJ; Den Hartog, G; Neumann, HH; Pederson, JR. (1995). Effects of stomatal conductance and surface wetness on ozone deposition in field-grown grape. Atmos Environ 29: 3189-3198. http://dx.doi.org/10.1016/1352-2310(95)00129-M

Grebenc, T; Kraigher, H. (2007). Changes in the community of ectomycorrhizal fungi and increased fine root number under adult beech trees chronically fumigated with double ambient ozone concentration. Plant Biol (Stuttg) 9: 279-287. http://dx.doi.org/10.1055/s-2006-924489

Gregg, JW; Jones, CG; Dawson, TE. (2003). Urbanization effects on tree growth in the vicinity of New York City [Letter]. Nature 424: 183-187. http://dx.doi.org/10.1038/nature01728

Gregg, JW; Jones, CG; Dawson, TE. (2006). Physiological and developmental effects of O3 on cottonwood growth in urban and rural sites. Ecol Appl 16: 2368-2381. http://dx.doi.org/10.1890/1051-0761(2006)016[2368:PADEOO]2.0.CO;2

Grennfelt, P. (2004). New directions: Recent research findings may change ozone control policies. Atmos Environ 38: 2215-2216.

Groppa, MD; Benavides, MP. (2008). Polyamines and abiotic stress: Recent advances. Amino Acids 34: 35-45. http://dx.doi.org/10.1007/s00726-007-0501-8

Grulke, N; Neufeld, H; Davison, A; Roberts, M; Chappelka, A. (2007a). Stomatal behavior of ozone-sensitive and -insensitive coneflowers (Rudbeckia laciniata var. digitata) in Great Smoky Mountains National Park. New Phytol 173: 100-109. http://dx.doi.org/10.1111/j.1469-8137.2006.01872.x

Grulke, NE. (1999). Physiological responses of ponderosa pine to gradients of environmental stressors. In PR Miller; JR McBride (Eds.), Oxidant air pollution impacts in the montane forests of Southern California (pp. 126-163). New York, NY: Springer-Verlag.

Grulke, NE; Alonso, R; Nguyen, T; Cascio, C; Dobrowolski, W. (2004). Stomata open at night in pole-sized and mature ponderosa pine: Implications for O3 exposure metrics. Tree Physiol 24: 1001-1010.

Grulke, NE; Dobrowolski, W; Mingus, P; Fenn, ME. (2005). California black oak response to nitrogen amendment at a high O3, nitrogen-saturated site. Environ Pollut 137: 536-545. http://dx.doi.org/10.1016/j.envpol.2005.01.039

Grulke, NE; Johnson, R; Esperanza, A; Jones, D; Nguyen, T; Posch, S; Tausz, M. (2003a). Canopy transpiration of Jeffrey pine in mesic and xeric microsites: O3 uptake and injury response. Trees Struct Funct 17: 292-298.

Grulke, NE; Johnson, R; Monschein, S; Nikolova, P; Tausz, M. (2003b). Variation in morphological and biochemical O3 injury attributes of mature Jeffrey pine within canopies and between microsites. Tree Physiol 23: 923-929.

Grulke, NE; Lee, EH. (1997). Assessing visible ozone-induced injury in ponderosa pine. Can J For Res 27: 1658-1668.

Grulke, NE; Minnich, RA; Paine, TD; Seybold, SJ; Chavez, DJ; Fenn, ME; Riggan, PJ; Dunn, A. (2008). Air pollution increases forest susceptibility to wildfires: A case study in the San Bernardino Mountains in southern California. In A Bytnerowicz; MJ Arbaugh; AR Riebau; C Anderson (Eds.), Wildland fires and air pollution; Section III: Ecological Impacts of Forest Fires and Air Pollution (pp. 365-403). Amsterdam, The Netherlands: Elsevier Ltd. http://dx.doi.org/10.1016/S1474-8177(08)00017-X

Grulke, NE; Paoletti, E; Heath, RL. (2007b). Chronic vs. short-term acute O3 exposure effects on nocturnal transpiration in two Californian oaks. ScientificWorldJournal 7: 134-140. http://dx.doi.org/10.1100/tsw.2007.33

Grulke, NE; Paoletti, E; Heath, RL. (2007c). Comparison of calculated and measured foliar O3 flux in crop and forest species. Environ Pollut 146: 640-647. http://dx.doi.org/10.1016/j.envpol.2006.04.014

Grulke, NE; Preisler, HK; Rose, C; Kirsch, J; Balduman, L. (2002). O3 uptake and drought stress effects on carbon acquisition of ponderosa pine in natural stands. New Phytol 154: 621-631. http://dx.doi.org/10.1046/j.1469-8137.2002.00403.x

Grunhage, L; Haenel, HD. (1997). PLATIN (PLant-ATmosphere-INteraction) I: A model of plant-atmosphere interaction for estimating absorbed doses of gaseous air pollutants. Environ Pollut 98: 37-50. http://dx.doi.org/10.1016/S0269-7491(97)00114-0

Grunhage, L; Jager, HJ. (2003). From critical levels to critical loads for ozone: a discussion of a new experimental and modelling approach for establishing flux-response relationships for agricultural crops and native plant species. Environ Pollut 125: 99-110.

Grunhage, L; Krupa, SV; Legge, AH; Jager, HJ. (2004). Ambient flux-based critical values of ozone for protecting vegetation: Differing spatial scales and uncertainties in risk assessment. Atmos Environ 38: 2433-2437. http://dx.doi.org/10.1016/j.atmosenv.2003.12.039

Guenther, A; Karl, T; Harley, P; Wiedinmyer, C; Palmer, PI; Geron, C. (2006). Estimates of global terrestrial isoprene emissions using MEGAN (Model of Emissions of Gases and Aerosols from Nature). Atmos Chem Phys 6: 3181-3210. http://dx.doi.org/10.5194/acp-6-3181-2006

Guidi, L; Degl'Innocenti, E; Martinelli, F; Piras, M. (2009). Ozone effects on carbon metabolism in sensitive and insensitive Phaseolus cultivars. Environ Exp Bot 66: 117-125. http://dx.doi.org/10.1016/j.envexpbot.2008.12.005

Guidi, L; Degl'lnnocenti, E. (2008). Ozone effects on high light-induced photoinhibition in Phaseolus vulgaris. Plant Sci 174: 590-596. http://dx.doi.org/10.1016/j.plantsci.2008.03.003

Gül, H; Gaga, EO; Döğeroğlu, T; Ozden, O; Ayvaz, O; Ozel, S; Güngör, G. (2011). Respiratory health symptoms among students exposed to different levels of air pollution in a Turkish city. Int J Environ Res Public Health 8: 1110-1125. http://dx.doi.org/10.3390/ijerph8041110

Gumpertz, ML; Rawlings, JO. (1992). Nonlinear regression with variance components: Modeling effects of ozone on crop yield. Crop Sci 32: 219-224. http://dx.doi.org/10.2135/cropsci1992.0011183X003200010045x

Gunderson, CA; Sholtis, JD; Wullschleger, SD; Tissue, DT; Hanson, PJ; Norby, RJ. (2002). Environmental and stomatal control of photosynthetic enhancement in the canopy of a sweetgum (Liquidambar styraciflua L) plantation during 3 years of CO2 enrichment. Plant Cell Environ 25: 379-393. http://dx.doi.org/10.1046/j.0016-8025.2001.00816.x

Haberer, K; Herbinger, K; Alexou, M; Rennenberg, H; Tausz, M. (2008). Effects of drought and canopy ozone exposure on antioxidants in fine roots of mature European beech (Fagus sylvatica). Tree Physiol 28: 713-719. http://dx.doi.org/10.1093/treephys/28.5.713

Hamel, LP; Miles, GP; Samuel, MA; Ellis, BE; Seguin, A; Beaudoin, N. (2005). Activation of stress-responsive mitogen-activated protein kinase pathways in hybrid poplar (Populus trichocarpa x Populus deltoides). Tree Physiol 25: 277-288. http://dx.doi.org/10.1093/treephys/25.3.277

Hamilton, JG; Dermody, O; Aldea, M; Zangerl, AR; Rogers, A; Berenbaum, MR; DeLucia, EH. (2005). Anthropogenic changes in tropospheric composition increase susceptibility of soybean to insect herbivory. Environ Entomol 34: 479-485.

Handley, T; Grulke, NE. (2008). Interactive effects of O3 exposure on California black oak (Quercus kelloggii Newb.) seedlings with and without N amendment. Environ Pollut 156: 53-60. http://dx.doi.org/10.1016/j.envpol.2008.01.002

Hanson, PJ; Wullschleger, SD; Norby, RJ; Tschaplinski, TJ; Gunderson, CA. (2005). Importance of changing CO2, temperature, precipitation, and ozone on carbon and water cycles of an upland-oak forest: incorporating experimental results into model simulations. Global Change Biol 11: 1402-1423. http://dx.doi.org/10.1111/j.1365-2486.2005.00991.x

Harward, M; Treshow, M. (1975). Impact of ozone on the growth and reproduction of understorey plants in the Aspen zone of western USA. Environ Conserv 2: 17-23. http://dx.doi.org/10.1017/S0376892900000564

Hassan, R; Scholes, R; Ash, N. (2005). Ecosystems and human well-being: Current state and trends. Washington, DC: Island Press.

Hayes, F; Jones, MLM; Mills, G; Ashmore, M. (2007). Meta-analysis of the relative sensitivity of semi-natural vegetation species to ozone. Environ Pollut 146: 754-762. http://dx.doi.org/10.1016/j.envpol.2006.06.011

Hayes, F; Mills, G; Ashmore, M. (2009). Effects of ozone on inter- and intra-species competition and photosynthesis in mesocosms of Lolium perenne and Trifolium repens. Environ Pollut 157: 208-214. http://dx.doi.org/10.1016/j.envpol.2008.07.002

Hayes, F; Mills, G; Ashmore, M. (2010). How Much Does the Presence of a Competitor Modify the Within-Canopy Distribution of Ozone-Induced Senescence and Visible Injury? Water Air Soil Pollut 210: 265-276. http://dx.doi.org/10.1007/s11270-009-0248-9

He, XY; Fu, SL; Chen, W; Zhao, TH; Xu, S; Tuba, Z. (2007). Changes in effects of ozone exposure on growth, photosynthesis, and respiration of Ginkgo biloba in Shenyang urban area. Photosynthetica 45: 555-561. http://dx.doi.org/10.1007/s11099-007-0095-0

He, XY; Ruan, YN; Chen, W; Lu, T. (2006). Responses of anti-oxidative system in leaves of Ginkgo biloba to elevated ozone concentration in urban area. Botanical Studies 47: 409-416.

Heagle, AS. (1979). Effects of growth media, fertiliser rate and hour and season of exposure on sensitivity of four soybean cultivars to ozone. Environ Pollut 18: 313-322.

Heagle, AS. (1989). Ozone and crop yield*. Annu Rev Phytopathol 27: 397-423. http://dx.doi.org/10.1146/annurev.py.27.090189.002145

Heagle, AS; Body, DE; Heck, WW. (1973). An open-top field chamber to assess the impact of air pollution on plants. J Environ Qual 2: 365-368.

Heagle, AS; Brandenburg, RL; Burns, JC; Miller, JE. (1994a). Ozone and carbon dioxide effects on spider mites in white clover and peanut. J Environ Qual 23: 1168-1176. http://dx.doi.org/10.2134/jeq1994.00472425002300060006x

Heagle, AS; Heck, WW; Lesser, VM; Rawlings, JO. (1987). Effects of daily ozone exposure duration and concentration fluctuation on yield of tobacco. Phytopathology 77: 856-862. http://dx.doi.org/10.1094/Phyto-77-856

Heagle, AS; Kress, LW; Temple, PJ; Kohut, RJ; Miller, JE; Heggestad, HE. (1988). Factors influencing ozone dose-yield response relationships in open-top field chamber studies. In WW Heck; OC Taylor; DT Tingey (Eds.), Assessment of crop loss from air pollutants: Proceedings of an international conference (pp. 141-179). New York, NY: Elsevier Applied Science.

Heagle, AS; Letchworth, MB; Mitchell, CA. (1983). Effects of growth medium and fertilizer rate on the yield response of soybeans exposed to chronic doses of ozone. Phytopathology 73: 134-139. http://dx.doi.org/10.1094/Phyto-73-134

Heagle, AS; Miller, JE; Rawlings, JO; Vozzo, SF. (1991). Effect of growth stage on soybean response to chronic ozone exposure. J Environ Qual 20: 562-570. http://dx.doi.org/10.2134/jeq1991.00472425002000030010x

Heagle, AS; Miller, JE; Sherrill, DE. (1994b). A white clover system to estimate effects of tropospheric ozone on plants. J Environ Qual 23: 613-621. http://dx.doi.org/10.2134/jeq1994.00472425002300030030x

Heagle, AS; Reinert, RA; Miller, JE. (1996). Response of white clover to ozone in different environments. J Environ Qual 25: 273-278. http://dx.doi.org/10.2134/jeq1996.00472425002500020010x

Heath, RL. (2008). Modification of the biochemical pathways of plants induced by ozone: What are the varied routes to change? Environ Pollut 155: 453-463. http://dx.doi.org/10.1016/j.envpol.2008.03.010

Heath, RL; Lefohn, AS; Musselman, RC. (2009). Temporal processes that contribute to nonlinearity in vegetation responses to ozone exposure and dose. Atmos Environ 43: 2919-2928. http://dx.doi.org/10.1016/j.atmosenv.2009.03.011

Heck, WW; Cowling, EB. (1997). The need for a long term cumulative secondary ozone standard - An ecological perspective. EM January: 23-33.

Heck, WW; Cure, WW; Rawlings, JO; Zaragoza, LJ; Heagle, AS; Heggestad, HE; Kohut, RJ; Kress, LW; Temple, PJ. (1984). Assessing impacts of ozone on agricultural crops: II. Crop yield functions and alternative exposure statistics. J Air Pollut Control Assoc 34: 810-817.

Heck, WW; Heagle, AS; Miller, JE; Rawlings, JO. (1991). A national program (NCLAN) to assess the impact of ozone on agricultural resources. In RL Berglund; DR Lawson; DJ McKee (Eds.), Tropospheric ozone and the environment: papers from an international conference; March 1990; Los Angeles, CA (pp. 225-254). Pittsburgh, PA: Air & Waste Management Association.

Heck, WW; Philbeck, RB; Dunning, JA. (1978). A continuous stirred tank reactor (CSTR) system for exposing plants to gaseous air contaminants: Principles, specifications, construction, and operation. Washington, DC: U.S. Government Printing Office.

Heck, WW; Taylor, OC; Adams, R; Bingham, G; Miller, J; Preston, E; Weinstein, L. (1982). Assessment of crop loss from ozone. J Air Pollut Control Assoc 32: 353-361.

Heggestad, HE. (1991). Origin of Bel-W3, Bel-C and Bel-B tobacco varieties and their use as indicators of ozone. Environ Pollut 74: 263-291. http://dx.doi.org/10.1016/0269-7491(91)90076-9

Heidenreich, B; Haberer, G; Mayer, K; Sandermann, H; Ernst, D. (2005). CDNA array-analysis of mercury- and ozone-induced genes in Arabidopsis thaliana. Acta Physiologiae Plantarum 27: 45-51. http://dx.doi.org/10.1007/s11738-005-0035-1

Hendrey, GR; Ellsworth, DS; Lewin, KF; Nagy, J. (1999). A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO2. Global Change Biol 5: 293-309. http://dx.doi.org/10.1046/j.1365-2486.1999.00228.x

Hendrey, GR; Kimball, BA. (1994). The FACE program. Agr Forest Meteorol 70: 3-14. http://dx.doi.org/10.1016/0168-1923(94)90044-2

Hildebrand, E; Skelly, JM; Fredericksen, TS. (1996). Foliar response of ozone-sensitive hardwood tree species from 1991 to 1993 in the Shenandoah National Park, Virginia. Can J For Res 26: 658-669.

Hillstrom, M; Meehan, TD; Kelly, K; Lindroth, RL. (2010a). Soil carbon and nitrogen mineralization following deposition of insect frass and greenfall from forests under elevated CO2 and O3. Plant Soil 336: 75-85. http://dx.doi.org/10.1007/s11104-010-0449-4

Hillstrom, ML; Lindroth, RL. (2008). Elevated atmospheric carbon dioxide and ozone alter forest insect abundance and community composition. Insect Conservation and Diversity 1: 233-241. http://dx.doi.org/10.1111/j.1752-4598.2008.00031.x

Hillstrom, ML; Vigue, LM; Coyle, DR; Raffa, KF; Lindroth, RL. (2010b). Performance of the invasive weevil Polydrusus sericeus is influenced by atmospheric CO2 and host species. Agr Forest Entomol 12: 285-292. http://dx.doi.org/10.1111/j.1461-9563.2010.00474.x

Himanen, SJ; Nerg, AM; Holopainen, JK. (2009a). Degree of herbivore feeding damage as an important contributor to multitrophic plant-parasitoid signaling under climate change [Comment]. Plant Signalling & Behavior 4: 249-251.

Himanen, SJ; Nerg, AM; Nissinen, A; Pinto, DM; Stewart, CN; Poppy, GM; Holopainen, JK. (2009b). Effects of elevated carbon dioxide and ozone on volatile terpenoid emissions and multitrophic communication of transgenic insecticidal oilseed rape (Brassica napus). New Phytol 181: 174-186. http://dx.doi.org/10.1111/j.1469-8137.2008.02646.x

Himanen, SJ; Nerg, AM; Nissinen, A; Stewart, CN; Poppy, GM; Holopainen, JK. (2009c). Elevated atmospheric ozone increases concentration of insecticidal Bacillus thuringiensis (Bt) Cry1Ac protein in Bt Brassica napus and reduces feeding of a Bt target herbivore on the non-transgenic parent. Environ Pollut 157: 181-185. http://dx.doi.org/10.1016/j.envpol.2008.07.006

Hogg, A; Uddling, J; Ellsworth, D; Carroll, MA; Pressley, S; Lamb, B; Vogel, C. (2007). Stomatal and non-stomatal fluxes of ozone to a northern mixed hardwood forest. Tellus B Chem Phys Meteorol 59: 514-525. http://dx.doi.org/10.1111/j.1600-0889.2007.00269.x

Hogsett, WE; Olszyk, D; Ormrod, DP; Taylor, GE, Jr; Tingey, DT. (1987a). Air pollution exposure systems and experimental protocols: Volume 2: Description of facilities. (EPA/600/3-87/037b). Corvallis, OR: U.S. Environmental Protection Agency, Environmental Research Laboratory. http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=30000KQH.txt

Hogsett, WE; Olszyk, D; Ormrod, DP; Taylor, GE, Jr; Tingey, DT. (1987b). Air pollution exposure systems and experimental protocols: Volume I: A review and evaluation of performance. (EPA/600/3-87/037a). Corvallis, OR: U.S. Environmental Protection Agency.

Hogsett, WE; Tingey, DT; Holman, SR. (1985). A programmable exposure control system for determination of the effects of pollutant exposure regimes on plant growth. Atmos Environ 19: 1135-1145. http://dx.doi.org/10.1016/0004-6981(85)90198-2

Hogsett, WE; Tingey, DT; Lee, EH. (1988). Ozone exposure indices: Concepts for development and evaluation of their use. In Assessment of crop loss from air pollutants: Proceedings of an international conference. New York: Elsevier Applied Science.

Hogsett, WE; Tingey, DT; Lee, EH; Beedlow, PA; Andersen, CP. (2008). An approach for evaluating the effectiveness of various ozone Air Quality Standards for protecting trees. Environ Manage 41: 937-948. http://dx.doi.org/10.1007/s00267-007-9057-3

Hogsett, WE; Weber, JE; Tingey, D; Herstrom, A; Lee, EH; Laurence, JA. (1997). Environmental auditing: An approach for characterizing tropospheric ozone risk to forests. J Environ Manage 21: 105-120. http://dx.doi.org/10.1007/s002679900010

Holmes, WE; Zak, DR; Pregitzer, KS; King, JS. (2006). Elevated CO2 and O3 alter soil nitrogen transformations beneath trembling aspen, paper birch, and sugar maple. Ecosystems 9: 1354-1363. http://dx.doi.org/10.1007/s10021-006-0163-5

Hong, B; Weinstein, D; Swaney, D. (2006). Assessment of ozone effects on nitrate export from Hubbard Brook Watershed 6. Environ Pollut 141: 8-21. http://dx.doi.org/10.1016/j.envpol.2005.08.030

Horvath, L; Nagy, Z; Weidinger, T; Artz, R; Luke, WT; Valigura, R; Pinto, JP; Womack, J. (1995). Measurement of fluxes of trace gases (O3, NOX, SO2, CO2, HNO3), particulate sulfate and nitrate, water vapour over short vegetation by gradient and eddy correlation techniques in Hungary [Abstract]. Ann Geophys 13: C490.

Howard, AR; van Iersel, MW; Richards, JH; Donovan, LA. (2009). Night-time transpiration can decrease hydraulic redistribution. Plant Cell Environ 32: 1060-1070. http://dx.doi.org/10.1111/j.1365-3040.2009.01988.x

Hui, D; Sims, DA; Johnson, DW; Chang, W; Luo, Y. (2002). Effects of gradual versus step increases in carbon dioxide on Plantago photosynthesis and growth in a microcosm study. Environ Exp Bot 47: 51-66.

ICP M&M (International Cooperative Programme on Modelling and Mapping). (2004). Mapping critical levels for vegetation. In Manual on methodologies and criteria for modelling and mapping critical loads and levels, and air pollution effects, risks and trends. http://www.rivm.nl/en/themasites/icpmm/manual-and-downloads/manual-english/index.html

Innes, JL; Skelly, JM; Schaub, M. (2001). Ozon, Laubholz- und Krautpflanzen: Ein Fuhrer zum Bestimmen von Ozonsymptomen. Bern, Switzerland: Paul Haupt Publishers.

IPCC (Intergovernmental Panel on Climate Change). (2007a). Climate change 2007: Impacts, adaptation and vulnerability. Cambridge, UK: Cambridge University Press.

Iriti, M; Di Maro, A; Bernasconi, S; Burlini, N; Simonetti, P; Picchi, V; Panigada, C; Gerosa, G; Parente, A; Faoro, F. (2009). Nutritional traits of bean (Phaseolus vulgaris) seeds from plants chronically exposed to ozone pollution. J Agric Food Chem 57: 201-208. http://dx.doi.org/10.1021/jf802819m

Iriti, M; Faoro, F. (2009). Chemical diversity and defence metabolism: How plants cope with pathogens and ozone pollution. International Journal of Molecular Sciences 10: 3371-3399. http://dx.doi.org/10.3390/ijms10083371

IRRI (International Rice Research Institute). (2002). IRRI annual report 2001-2002. Los Baños, Laguna in the Philippines. http://www.scribd.com/collections/2641758/Annual-Reports

Isebrands, JG; Dickson, RE; Rebbeck, J; Karnosky, DF. (2000). Interacting effects of multiple stresses on growth and physiological processes in northern forest trees. In RA Mickler; RA Birsdey; J Hom (Eds.), Responses of northern US forests to environmental change (pp. 149-180). New York, NY: Springer-Verlag.

Isebrands, JG; McDonald, EP; Kruger, E; Hendrey, G; Percy, K; Pregitzer, K; Sober, J; Karnosky, DF. (2001). Growth responses of Populus tremuloides clones to interacting carbon dioxide and tropospheric ozone. Environ Pollut 115: 359-371.

Jackson, DM; Heagle, AS; Eckel, RVW. (1999). Ovipositional response of tobacco hornworm moths (Lepidoptera: Sphyngidae) to tobacco plants grown under elevated levels of ozone. Environ Entomol 28: 566-571.

Janzik, I; Preiskowski, S; Kneifel, H. (2005). Ozone has dramatic effects on the regulation of the prechorismate pathway in tobacco (Nicotiana tabacum L. cv. Bel W3). Planta 223: 20-27. http://dx.doi.org/10.1007/s00425-005-0060-8

Johnson, RM; Pregitzer, KS. (2007). Concentration of sugars, phenolic acids, and amino acids in forest soils exposed to elevated atmospheric CO2 and O3. Soil Biol Biochem 39: 3159-3166. http://dx.doi.org/10.1016/j.soilbio.2007.07.010

Jones, ME; Paine, TD. (2006). Detecting changes in insect herbivore communities along a pollution gradient. Environ Pollut 143: 377-387. http://dx.doi.org/10.1016/j.envpol.2005.12.013

Jones, MLM; Hodges, G; Mills, G. (2010). Nitrogen mediates above-ground effects of ozone but not below-ground effects in a rhizomatous sedge. Environ Pollut 158: 559-565. http://dx.doi.org/10.1016/j.envpol.2009.08.002

Jones, TG; Freeman, C; Lloyd, A; Mills, G. (2009). Impacts of elevated atmospheric ozone on peatland below-ground doc characteristics. Ecol Eng 35: 971-977. http://dx.doi.org/10.1016/j.ecoleng.2008.08.009

Joo, JH; Wang, SY; Chen, JG; Jones, AM; Fedoroff, NV. (2005). Different signaling and cell death roles of heterotrimeric G protein alpha and beta subunits in the arabidopsis oxidative stress response to ozone. Plant Cell 17: 957-970. http://dx.doi.org/10.1105/tpc.104.029603

Joss, U; Graber, WK. (1996). Profiles and simulated exchange of H2O, O3, NO2 between the atmosphere and the HartX Scots pine plantation. Theor Appl Climatol 53: 157-172.

Jovan, S; McCune, B. (2006). Using epiphytic macrolichen communities for biomonitoring ammonia in forests of the greater Sierra Nevada, California. Water Air Soil Pollut 170: 69-93.

Kanerva, T; Palojarvi, A; Ramo, K; Manninen, S. (2008). Changes in soil microbial community structure under elevated tropospheric O3 and CO2. Soil Biol Biochem 40: 2502-2510. http://dx.doi.org/10.1016/j.soilbio.2008.06.007

Kanerva, T; Palojarvi, A; Ramo, K; Ojanpera, K; Esala, M; Manninen, S. (2006). A 3-year exposure to CO2 and O3 induced minor changes in soil N cycling in a meadow ecosystem. Plant Soil 286: 61-73. http://dx.doi.org/10.1007/s11104-006-9026-2

Kanerva, T; Regina, K; Ramo, K; Ojanpera, K; Manninen, S. (2007). Fluxes of N2O, CH4 and CO2 in a meadow ecosystem exposed to elevated ozone and carbon dioxide for three years. Environ Pollut 145: 818-828. http://dx.doi.org/10.1016/j.envpol.2006.03.055

Kangasjarvi, J; Jaspers, P; Kollist, H. (2005). Signalling and cell death in ozone-exposed plants. Plant Cell Environ 28: 1021-1036.

Karlsson, PE; Sellden, G; Plaijel, H. (2003). Establishing ozone critical levels II: UNECE workshop report. Gothenburg, Sweden: IVL Swedish Environmental Institute.

Karlsson, PE; Uddling, J; Braun, S; Broadmeadow, M; Elvira, S; Gimeno, BS; Le Thiec, D; Okansen, E; Vandermeiren, K; Wilkinson, M; Emberson, L. (2004). New critical levels for ozone effects on young trees based on AOT40 and simulated cumulative leaf uptake of ozone. Atmos Environ 38: 2283-2294.

Karnosky, DF; Gagnon, ZE; Dickson, RE; Coleman, MD; Lee, EH; Isebrands, JG. (1996). Changes in growth, leaf abscission, biomass associated with seasonal tropospheric ozone exposures of Populus tremuloides clones and seedlings. Can J For Res 26: 23-37.

Karnosky, DF; Mankovska, B; Percy, K; Dickson, RE; Podila, GK; Sober, J; Noormets, A; Hendrey, G; Coleman, MD; Kubiske, M; Pregitzer, KS; Isebrands, JG. (1999). Effects of tropospheric ozone on trembling aspen and interaction with CO2: Results from an O3-gradient and a FACE experiment. Water Air Soil Pollut 116: 311-322.

Karnosky, DF; Pregitzer, KS; Zak, DR; Kubiske, ME; Hendrey, GR; Weinstein, D; Nosal, M; Percy, KE. (2005). Scaling ozone responses of forest trees to the ecosystem level in a changing climate. Plant Cell Environ 28: 965-981. http://dx.doi.org/10.1111/j.1365-3040.2005.01362.x

Karnosky, DF; Zak, DR; Pregitzer, KS; Awmack, CS; Bockheim, JG; Dickson, RE; Hendrey, GR; Host, GE; King, JS; Kopper, BJ; Kruger, EL; Kubiske, ME; Lindroth, RL; Mattson, WJ; McDonald, EP; Noormets, A; Oksanen, E; Parsons, WFJ; Percy, KE; Podila, GK; Riemenschneider, DE; Sharma, P; Thakur, R; Sober, A; Sober, J; Jones, WS; Anttonen, S; Vapaavuori, E; Mankovska, B; Heilman, W; Isebrands, JG. (2003). Tropospheric O3 moderates responses of temperate hardwood forests to elevated CO2: A synthesis of molecular to ecosystem results from the Aspen FACE project. Funct Ecol 17: 289-304.

Kasurinen, A; Keinanen, MM; Kaipainen, S; Nilsson, LO; Vapaavuori, E; Kontro, MH; Holopainen, T. (2005). Below-ground responses of silver birch trees exposed to elevated CO2 and O3 levels during three growing seasons. Global Change Biol 11: 1167-1179. http://dx.doi.org/10.1111/j.1365-2486.2005.00970.x

Kasurinen, A; Peltonen, PA; Julkunen-Tiitto, R; Vapaavuori, E; Nuutinen, V; Holopainen, T; Holopainen, JK. (2007). Effects of elevated CO2 and O3 on leaf litter phenolics and subsequent performance of litter-feeding soil macrofauna. Plant Soil 292: 25-43. http://dx.doi.org/10.1007/s11104-007-9199-3

Kasurinen, A; Riikonen, J; Oksanen, E; Vapaavuori, E; Holopainen, T. (2006). Chemical composition and decomposition of silver birch leaf litter produced under elevated CO2 and O3. Plant Soil 282: 261-280. http://dx.doi.org/10.1007/s11104-005-6026-6

Kats, G; Olszyk, DM; Thompson, CR. (1985). Open top experimental chambers for trees. J Air Waste Manag Assoc 35: 1298-1301.

Kats, G; Thompson, CR; Kuby, WC. (1976). Improved ventilation of open top greenhouses. J Air Pollut Control Assoc 26: 1089-1090.

Keller, T; Häsler, R. (1984). The influence of a fall fumigation with ozone on the stomatal behavior of spruce and fir. Oecologia 64: 284-286. http://dx.doi.org/10.1007/BF00376884

Kellomaki, S; Wang, KY. (1997). Effects of elevated O3 and CO2 concentrations on photosynthesis and stomatal conductance in Scots pine. Plant Cell Environ 20: 995-1006. http://dx.doi.org/10.1111/j.1365-3040.1997.tb00676.x

Kerner, R; Winkler, J; Dupuy, J; Jürgensen, M; Lindermayr, C; Ernst, D; Müller-starck, G. (2011). Changes in the proteome of juvenile European beech following three years exposure to free-air elevated ozone. iForest 4: 69-76. http://dx.doi.org/10.3832/ifor0570-004

Keutgen, AJ; Noga, G; Pawelzik, E. (2005). Cultivar-specific impairment of strawberry growth, photosynthesis, carbohydrate and nitrogen accumulation by ozone. Environ Exp Bot 53: 271-280. http://dx.doi.org/10.1016/j.envexpbot.2004.04.003

Keutgen, N; Keutgen, AJ; Janssens, MJJ. (2008). Sweet potato [Ipomoea batatas (L.) Lam.] cultivated as tuber or leafy vegetable supplier as affected by elevated tropospheric ozone. J Agric Food Chem 56: 6686-6690. http://dx.doi.org/10.1021/jf8006272

King, JS; Kubiske, ME; Pregitzer, KS; Hendrey, GR; McDonald, EP; Giardina, CP; Quinn, VS; Karnosky, DF. (2005). Tropospheric O3 compromises net primary production in young stands of trembling aspen, paper birch and sugar maple in response to elevated atmospheric CO2. New Phytol 168: 623-635. http://dx.doi.org/10.1111/j.1469-8137.2005.01557.x

King, JS; Pregitzer, KS; Zak, DR; Sober, J; Isebrands, JG; Dickson, RE; Hendrey, GR; Karnosky, DF. (2001). Fine-root biomass and fluxes of soil carbon in young stands of paper birch and trembling aspen as affected by elevated atmospheric CO2 and tropospheric O3. Oecologia 128: 237-250.

Kitao, M; Low, M; Heerdt, C; Grams, TEE; Haberle, KH; Matyssek, R. (2009). Effects of chronic elevated ozone exposure on gas exchange responses of adult beech trees (Fagus sylvatica) as related to the within-canopy light gradient. Environ Pollut 157: 537-544. http://dx.doi.org/10.1016/j.envpol.2008.09.016

Kline, LJ; Davis, DD; Skelly, JM; Decoteau, DR. (2009). Variation in ozone sensitivity within Indian hemp and common milkweed selections from the Midwest. Northeast Nat 16: 307-313. http://dx.doi.org/10.1656/045.016.0210

Kline, LJ; Davis, DD; Skelly, JM; Savage, JE; Ferdinand, J. (2008). Ozone sensitivity of 28 plant selections exposed to ozone under controlled conditions. Northeast Nat 15: 57-66. http://dx.doi.org/10.1656/1092-6194(2008)15[57:OSOPSE]2.0.CO;2

Kohut, R. (2007). Assessing the risk of foliar injury from ozone on vegetation in parks in the US National Park Service's Vital Signs Network. Environ Pollut 149: 348-357. http://dx.doi.org/10.1016/j.envpol.2007.04.022

Kolb, TE; Fredericksen, TS; Steiner, KC; Skelly, JM. (1997). Issues in scaling tree size and age responses to ozone: A review [Review]. Environ Pollut 98: 195-208. http://dx.doi.org/10.1016/S0269-7491(97)00132-2

Kollist, T; Moldau, H; Rasulov, B; Oja, V; Ramma, H; Huve, K; Jaspers, P; Kangasjarvi, J; Kollist, H. (2007). A novel device detects a rapid ozone-induced transient stomatal closure in intact Arabidopsis and its absence in abi2 mutant. Physiol Plant 129: 796-803. http://dx.doi.org/10.1111/j.1399-3054.2006.00851.x

Kostka-Rick, R; Hahn, HU. (2005). Biomonitoring using tobacco Bel W3 provides supplemental information for risk assessment of vegetation injury due to ozone. Gefahrstoffe Reinhaltung Der Luft 65: 485-491.

Kozovits, AR; Matyssek, R; Blaschke, H; Gottlein, A; Grams, TEE. (2005). Competition increasingly dominates the responsiveness of juvenile beech and spruce to elevated CO2 and/or O3 concentrations throughout two subsequent growing seasons. Global Change Biol 11: 1387-1401. http://dx.doi.org/10.1111/j.1365-2486.2005.00993.x

Krupa, SV; Grunhage, L; Jager, HJ; Nosal, M; Manning, WJ; Legge, AH; Hanewald, K. (1995). Ambient ozone (O3) and adverse crop response: A unified view of cause and effect. Environ Pollut 87: 119-126. http://dx.doi.org/10.1016/S0269-7491(99)80014-1

Krupa, SV; Nosal, M; Peterson, DL. (2001). Use of passive ozone O3 samplers in vegetation effects assessment. Environ Pollut 112: 303-309.

Kubiske, ME; Quinn, VS; Heilman, WE; McDonald, EP; Marquardt, PE; Teclaw, RM; Friend, AL; Karnoskey, DF. (2006). Interannual climatic variation mediates elevated CO2 and O3 effects on forest growth. Global Change Biol 12: 1054-1068. http://dx.doi.org/10.1111/j.1365-2486.2006.01152.x

Kubiske, ME; Quinn, VS; Marquardt, PE; Karnosky, DF. (2007). Effects of elevated atmospheric CO2 and/or O3 on intra- and interspecific competitive ability of aspen. Plant Biol (Stuttg) 9: 342-355. http://dx.doi.org/10.1055/s-2006-924760

Kurpius, MR; Goldstein, AH. (2003). Gas-phase chemistry dominates O3 loss to a forest, implying a source of aerosols and hydroxyl radicals to the atmosphere. Geophys Res Lett 30.

Laffray, X; Rose, C; Garrec, JP. (2007). Estimation of ozone concentration in a valley of the alps mountains based on bel-w3 tobacco leaf injury. Water Air Soil Pollut 186: 29-42. http://dx.doi.org/10.1007/s11270-007-9460-7

Lambers, H; Chapin, FS, III; Pons, TL. (1998). Plant physiological ecology. In H Lambers; FS Chapin, III; TL Pons (Eds.). New York: Springer.

Langebartels, C; Kerner, K; Leonardi, S; Schraudner, M; Trost, M; Heller, W; Sandermann, H, Jr. (1991). Biochemical plant responses to ozone: I. Differential induction of polyamine and ethylene biosynthesis in tobacco. J Plant Physiol 95: 882-889. http://dx.doi.org/10.1104/pp.95.3.882

Larson, JL; Zak, DR; Sinsabaugh, RL. (2002). Extracellular enzyme activity beneath temperate trees growing under elevated carbon dioxide and ozone. Soil Sci Soc Am J 66: 1848-1856.

Lawlor, DW. (1998). Plant responses to global change: Temperature and drought stress. In LJ De Kok; I Stulen (Eds.), Responses of plant metabolism to air pollution and global change. Leiden, The Netherlands: Backhuys Publishers.

Leakey, ADB; Bernacchi, CJ; Ort, DR; Long, SP. (2006). Long-term growth of soybean at elevated CO2 does not cause acclimation of stomatal conductance under fully open-air conditions. Plant Cell Environ 29: 1794-1800. http://dx.doi.org/10.1111/j.1365-3040.2006.01556.x

Lee, EH; Hogsett, WE. (1996). Methodology for calculating inputs for ozone secondary standard benefits anaylsis: Part II. Research Triangle Park, NC: U.S. Environmental Protection Agency.

Lee, EH; Hogsett, WE. (1999). Role of concentrations and time of day in developing ozone exposure indices for a secondary standard. J Air Waste Manag Assoc 49: 669-681.

Lee, EH; Hogsett, WE; Tingey, DT. (1994). Attainment and effects issues regarding alternative secondary ozone air quality standards. J Environ Qual 23: 1129-1140. http://dx.doi.org/10.2134/jeq1994.00472425002300060002x

Lee, EH; Tingey, DT; Hogsett, WE. (1987). Selection of the best exposure-response model using various 7-hour ozone exposure statistics. Research Triangle Park, NC: U.S. Environmental Protection Agency.

Lee, EH; Tingey, DT; Hogsett, WE. (1988a). Evaluation of ozone-exposure indices for relating exposure to plant production and for estimating agricultural losses. (EPA/600/3-88/039). Washington, DC: U.S. Environmental Protection Agency.

Lee, EH; Tingey, DT; Hogsett, WE. (1988b). Evaluation of ozone exposure indices in exposure-response modeling. Environ Pollut 53: 43-62. http://dx.doi.org/10.1016/0269-7491(88)90024-3

Lee, EH; Tingey, DT; Hogsett, WE. (1989). Interrelation of experimental exposure and ambient air quality data for comparison of ozone exposure indices and estimating agricultural losses. (EPA/600/3-89/047). Corvallis, OR: U.S. Environmental Protection Agency.

Lee, EH; Tingey, DT; Hogsett, WE; Laurence, JA. (2003a). History of tropospheric ozone for the San Bernardino Mountains of southern California, 1963-1999. Atmos Environ 37: 2705-2717. http://dx.doi.org/10.1016/S1352-2310(03)00203-6

Lee, EH; Tingey, DT; Waschmann, RS; Phillips, DL; Olszyk, DM; Johnson, MG; Hogsett, WE. (2009a). Seasonal and long-term effects of CO2 and O3 on water loss in ponderosa pine and their interaction with climate and soil moisture. Tree Physiol 29: 1381-1393. http://dx.doi.org/10.1093/treephys/tpp071

Lee, S; Yun, SC. (2006). The ozone stress transcriptome of pepper (Capsicum annuum L.). Molecules and Cells 21: 197-205.

Lee, WS; Chevone, BI; Seiler, JR. (1990). Growth and gas exchange of loblolly pine seedlings as influenced by drought and air pollutants. Water Air Soil Pollut 51: 105-116. http://dx.doi.org/10.1007/BF00211508

Lefohn, AS; Benedict, HM. (1982). Development of a mathematical index that describes ozone concentration, frequency and duration. Atmos Environ 16: 2529-2532. http://dx.doi.org/10.1016/0004-6981(82)90145-7

Lefohn, AS; Jackson, W; Shadwick, DS; Knudsen, HP. (1997). Effect of surface ozone exposures on vegetation grown in the southern Appalachian Mountains: Identification of possible areas of concern. Atmos Environ 31: 1695-1708. http://dx.doi.org/10.1016/S1352-2310(96)00258-0

Lefohn, AS; Laurence, JA; Kohut, RJ. (1988). A comparison of indices that describe the relationship between exposure to ozone and reduction in the yield of agricultural crops. Atmos Environ 22: 1229-1240. http://dx.doi.org/10.1016/0004-6981(88)90353-8

Lefohn, AS; Runeckles, VC. (1987). Establishing standards to protect vegetation - ozone exposure/dose considerations. Atmos Environ 21: 561-568. http://dx.doi.org/10.1016/0004-6981(87)90038-2

Lefohn, AS; Shadwick, DS. (2000). Differences in trending estimates in the United States using several ozone metrics. In Proceedings of the 93rd Air & Waste Management Association Annual Conference and Exhibition. Pittsburgh, PA: Air & Waste Management Association.

Legge, AH; Grunhage, L; Nosal, M; Jager, HJ; Krupa, SV. (1995). Ambient ozone and adverse crop response: An evaluation of North American and European data as they relate to exposure indices and critical levels. J Appl Bot Food Qual 69: 192-205.

Leitao, L; Bethenod, O; Biolley, JP. (2007a). The impact of ozone on juvenile maize (Zea mays L.) plant photosynthesis: Effects on vegetative biomass, pigmentation, and carboxylases (PEPc and Rubisco). Plant Biol (Stuttg) 9: 478-488. http://dx.doi.org/10.1055/s-2007-964942

Leitao, L; Delacote, E; Dizengremel, P; Le Thiec, D; Biolley, JP. (2007b). Assessment of the impact of increasing concentrations of ozone on photosynthetic components of maize (Zea mays L.), a C-4 plant. Environ Pollut 146: 5-8. http://dx.doi.org/10.1016/j.envpol.2006.05.019

Leitao, L; Maoret, JJ; Biolley, JP. (2007c). Changes in PEP carboxylase, rubisco and rubisco activase mRNA levels from maize (Zea mays) exposed to a chronic ozone stress. Biol Res 40: 137-153. http://dx.doi.org/10.4067/S0716-97602007000200005

Lesser, VM; Rawlings, JO; Spruill, SE; Somerville, MC. (1990). Ozone effects on agricultural crops: Statistical methodologies and estimated dose-response relationships. Crop Sci 30: 148-155.

Leuning, R; Unsworth, MH; Neumann, HN; King, KM. (1979). Ozone fluxes to tobacco and soil under field conditions. Atmos Environ 13: 1155-1163. http://dx.doi.org/10.1016/0004-6981(79)90039-8

Levine, JS; Pinto, JP. (1998). The production of CO by biomass burning. In MAK Khalil; JP Pinto; MJ Shearer (Eds.), Atmospheric carbon monoxide and its environmental effects: Proceedings of the international conference; December 1997; Portland, Oregon (pp. 251-256). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development.

Lewis, JS; Ditchkoff, SS; Lin, JC; Muntifering, RB; Chappelka, AH. (2006). Nutritive quality of big bluestem (Andropogon gerardii) and eastern gamagrass (Tripsacum dactyloides) exposed to tropospheric ozone. Rangeland Ecol Manag 59: 267-274.

Li, PH; Mane, SP; Sioson, AA; Robinet, CV; Heath, LS; Bohnert, HJ; Grene, R. (2006b). Effects of chronic ozone exposure on gene expression in Arabidopsis thaliana ecotypes and in Thellungielia halophila. Plant Cell Environ 29: 854-868. http://dx.doi.org/10.1111/j.1365-3040.2005.01465.x

Lin, JC; Nosal, M; Muntifering, RB; Krupa, SV. (2007). Alfalfa nutritive quality for ruminant livestock as influenced by ambient air quality in west-central Alberta. Environ Pollut 149: 99-103. http://dx.doi.org/10.1016/j.envpol.2006.12.009

Lindroth, RL. (2010). Impacts of elevated atmospheric CO2 and O3 on forests: Phytochemistry, trophic interactions, and ecosystem dynamics [Review]. J Chem Ecol 36: 21-Feb. http://dx.doi.org/10.1007/s10886-009-9731-4

Liu, L; King, J; Giardina, C. (2005). Effects of elevated concentrations of atmospheric CO2 and tropospheric O3 on leaf litter production and chemistry in trembling aspen and paper birch communities. Tree Physiol 25: 1511-1522.

Liu, L; King, JS; Giardina, CP. (2007a). Effects of elevated atmospheric CO2 and tropospheric O3 on nutrient dynamics: Decomposition of leaf litter in trembling aspen and paper birch communities. Plant Soil 299: 65-82. http://dx.doi.org/10.1007/s11104-007-9361-y

Liu, LL; King, JS; Giardina, CP; Booker, FL. (2009b). The influence of chemistry, production and community composition on leaf litter decomposition under elevated atmospheric CO2 and tropospheric O-3 in a northern hardwood ecosystem. Ecosystems 12: 401-416. http://dx.doi.org/10.1007/s10021-009-9231-y

Loats, KV; Rebbeck, J. (1999). Interactive effects of ozone and elevated carbon dioxide on the growth and physiology of black cherry, green ash, and yellow poplar seedlings. Environ Pollut 106: 237-248. http://dx.doi.org/10.1016/S0269-7491(99)00069-X

Long, SP. (1991). Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO2 concentrations: Has its importance been underestimated? Plant Cell Environ 14: 729-739. http://dx.doi.org/10.1111/j.1365-3040.1991.tb01439.x

Loranger, GI; Pregitzer, KS; King, JS. (2004). Elevated CO2 and O3t concentrations differentially affect selected groups of the fauna in temperate forest soils. Soil Biol Biochem 36: 1521-1524.

Lorenzini, G; Nali, C. (1995). Analysis of vertical ozone and nitrogen oxides profiles in a Prunus cerasifera canopy. Int J Biometeorol 39: 1-4. http://dx.doi.org/10.1007/BF01320885

Loreto, F; Fares, S. (2007). Is ozone flux inside leaves only a damage indicator? Clues from volatile isoprenoid studies. Plant Physiol 143: 1096-1100. http://dx.doi.org/10.1104/pp.106.091892

Loreto, F; Velikova, V. (2001). Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiol 127: 1781-1787. http://dx.doi.org/10.1104/pp.010497

Low, M; Herbinger, K; Nunn, AJ; Haberle, KH; Leuchner, M; Heerdt, C; Werner, H; Wipfler, P; Pretzsch, H; Tausz, M; Matyssek, R. (2006). Extraordinary drought of 2003 overrules ozone impact on adult beech trees (Fagus sylvatica). Trees Struct Funct 20: 539-548. http://dx.doi.org/10.1007/s00468-006-0069-z

Loya, WM; Pregitzer, KS; Karberg, NJ; King, JS; Giardina, CP. (2003). Reduction of soil carbon formation by tropospheric ozone under elevated carbon dioxide. Nature 425: 705-707.

Ludwikow, A; Gallois, P; Sadowski, J. (2004). Ozone-induced oxidative stress response in Arabidopsis: Transcription profiling by microarray approach. Cell Mol Biol Lett 9: 829-842.

Ludwikow, A; Kierzek, D; Gallois, P; Zeef, L; Sadowski, J. (2009). Gene expression profiling of ozone-treated Arabidopsis abi1td insertional mutant: Protein phosphatase 2C ABI1 modulates biosynthesis ratio of ABA and ethylene. Planta 230: 1003-1017. http://dx.doi.org/10.1007/s00425-009-1001-8

Ludwikow, A; Sadowski, J. (2008). Gene networks in plant ozone stress response and tolerance. J Integr Plant Biol 50: 1256-1267. http://dx.doi.org/10.1111/j.1744-7909.2008.00738.x

Luo, Y. (2001). Transient ecosystem response to free-air CO2 enrichment (FACE): Experimental evidence and methods of analysis. New Phytol 152: 3-8.

Luo, Y; Reynolds, JF. (1999). Validity of extrapolating field CO2 experiments to predict carbon sequestration in natural ecosystems. Ecology 80: 1568-1583.

Lyons, TM; Barnes, JD. (1998). Influence of plant age on ozone resistance in Plantago major. New Phytol 138: 83-89. http://dx.doi.org/10.1046/j.1469-8137.1998.00879.x

Maggio, A; Chiaranda, FQ; Cefariello, R; Fagnano, M. (2009). Responses to ozone pollution of alfalfa exposed to increasing salinity levels. Environ Pollut 157: 1445-1452. http://dx.doi.org/10.1016/j.envpol.2008.09.013

Mahalingam, R; Jambunathan, N; Gunjan, SK; Faustin, E; Weng, H; Ayoubi, P. (2006). Analysis of oxidative signalling induced by ozone in Arabidopsis thaliana. Plant Cell Environ 29: 1357-1371. http://dx.doi.org/10.1111/j.1365-3040.2006.01516.x

Mahalingam, R; Shah, N; Scrymgeour, A; Fedoroff, N. (2005). Temporal evolution of the Arabidopsis oxidative stress response. Plant Mol Biol 57: 709-730. http://dx.doi.org/10.1007/s11103-005-2860-4

Maier-Maercker, U. (1998). Predisposition of trees to drought stress by ozone. Tree Physiol 19: 71-78.

Mandl, RH; Laurence, JA; Kohut, RJ. (1989). Development and testing of open-top chambers for exposing large, perennial plants to air pollutants. J Environ Qual 18: 534-540. http://dx.doi.org/10.2134/jeq1989.00472425001800040026x

Mandl, RH; Weinstein, LH; McCune, DC; Keveny, M. (1973). A cylindrical, open-top chamber for the exposure of plants to air pollutants in the field. J Environ Qual 2: 371-376.

Maňkovská, B; Percy, KE; Karnosky, DF. (2005). Impacts of greenhouse gases on epicuticular waxes of Populus tremuloides Michx.: Results from an open-air exposure and a natural O3 gradient. Environ Pollut 137: 580-586. http://dx.doi.org/10.1016/j.envpol.2005.01.043

Manning, WJ. (2003). Detecting plant effects is necessary to give biological significance to ambient ozone monitoring data and predictive ozone standards. Environ Pollut 126: 375-379.

Manning, WJ; Krupa, SV. (1992). Experimental methodology for studying the effects of ozone on crops and trees. In AS Lefohn (Ed.), Surface level ozone exposures and their effects on vegetation (pp. 93-156). Chelsea, MI: Lewis Publishers.

Martin, MJ; Host, GE; Lenz, KE; Isebrands, JG. (2001). Simulating the growth response of aspen to elevated ozone: A mechanistic approach to scaling a leaf-level model of ozone effects on photosynthesis to a complex canopy architecture. Environ Pollut 115: 425-436.

Massman, WJ. (2004). Toward an ozone standard to protect vegetation based on effective dose: A review of deposition resistances and a possible metric [Review]. Atmos Environ 38: 2323-2337.

Massman, WJ; Grantz, DA. (1995). Estimating canopy conductance to ozone uptake from observations of evapotranspiration at the canopy scale and at the leaf scale. Global Change Biol 1: 183-198. http://dx.doi.org/10.1111/j.1365-2486.1995.tb00020.x

Massman, WJ; Musselman, RC; Lefohn, AS. (2000). A conceptual ozone dose-response model to develop a standard to protect vegetation. Atmos Environ 34: 745-759. http://dx.doi.org/10.1016/S1352-2310(99)00395-7

Matyssek, R; Gunthardt-Goerg, MS; Maurer, S; Keller, T. (1995). Nighttime exposure to ozone reduces whole-plant production in Betula pendula. Tree Physiol 15: 159-165.

Matyssek, R; Le Thiec, D; Low, M; Dizengremel, P; Nunn, AJ; Haberle, KH. (2006). Interactions between drought and O3 stress in forest trees. Plant Biol (Stuttg) 8: 11-17. http://dx.doi.org/10.1055/s-2005-873025

Matyssek, R; Sandermann, H; Wieser, G; Booker, F; Cieslik, S; Musselman, R; Ernst, D. (2008). The challenge of making ozone risk assessment for forest trees more mechanistic. Environ Pollut 156: 567-582. http://dx.doi.org/10.1016/j.envpol.2008.04.017

Matyssek, R; Wieser, G; Ceulemans, R; Rennenberg, H; Pretzsch, H; Haberer, K; Low, M; Nunn, AJ; Werner, H; Wipfler, P; Obwald, W; Nikolova, P; Hanke, DE; Kraigher, H; Tausz, M; Bahnweg, G; Kitao, M; Dieler, J; Sandermann, H; Herbinger, K; Grebenc, T; Blumenrother, M; Deckmyn, G; Grams, TEE; Heerdt, C; Leuchner, M; Fabian, P; Haberle, KH. (2010). Enhanced ozone strongly reduces carbon sink strength of adult beech (Fagus sylvatica): Resume from the free-air fumigation study at Kranzberg Forest. Environ Pollut 158: 2527-2532. http://dx.doi.org/10.1016/j.envpol.2010.05.009

Mautz, WJ; Dohm, MR. (2004). Respiratory and behavioral effects of ozone on a lizard and a frog. Comp Biochem Physiol A Mol Integr Physiol 139: 371-377. http://dx.doi.org/10.1016/j.cbpb.2004.10.004

McAinsh, MR; Evans, NH; Montgomery, LT; North, KA. (2002). Calcium signalling in stomatal responses to pollutants. New Phytol 153: 441-447.

McBride, JR; Laven, RD. (1999). Impact of oxidant air pollutants on forest succession in the mixed conifer forests of the San Bernardino Mountains. In PR Miller; JR McBride (Eds.), Oxidant air pollution impacts in the montane forests of southern California: A case study of the San Bernardino Mountains (pp. 338-352). New York, NY: Springer-Verlag.

McCarthy, HR; Oren, R; Johnsen, KH; Gallet-Budynek, A; Pritchard, SG; Cook, CW; LaDeau, SL; Jackson, RB; Finzi, AC. (2009). Re-assessment of plant carbon dynamics at the Duke free-air CO2 enrichment site: Interactions of atmospheric [CO2] with nitrogen and water availability over stand development. New Phytol 185: 514-528. http://dx.doi.org/10.1111/j.1469-8137.2009.03078.x

McCool, PM; Musselman, RC; Younglove, T; Teso, RR. (1988). Response of kidney bean to sequential ozone exposures. Environ Exp Bot 28: 307-313.

McFrederick, QS; Fuentes, JD; Roulston, T; Kathilankal, JC; Lerdau, M. (2009). Effects of air pollution on biogenic volatiles and ecological interactions. Oecologia 160: 411-420. http://dx.doi.org/10.1007/s00442-009-1318-9

McFrederick, QS; Kathilankal, JC; Fuentes, JD. (2008). Air pollution modifies floral scent trails. Atmos Environ 42: 2336-2348. http://dx.doi.org/10.1016/j.atmosenv.2007.12.033

McLaughlin, SB; Nosal, M; Wullschleger, SD; Sun, G. (2007a). Interactive effects of ozone and climate on tree growth and water use in a southern Appalachian forest in the USA. New Phytol 174: 109-124. http://dx.doi.org/10.1111/j.1469-8137.2007.02018.x

McLaughlin, SB; Wullschleger, SD; Sun, G; Nosal, M. (2007b). Interactive effects of ozone and climate on water use, soil moisture content and streamflow in a southern Appalachian forest in the USA. New Phytol 174: 125-136. http://dx.doi.org/10.1111/j.1469-8137.2007.01970.x

McLeod, AR; Long, SP. (1999). Free-air carbon dioxide enrichment (FACE) in global change research: A review. Adv Ecol Res 28: 1-56. http://dx.doi.org/10.1016/S0065-2504(08)60028-8

Medlyn, BE; Barton, CVM; Broadmeadow, MSJ; Ceulemans, R; De Angelis, P; Forstreuter, M; Freeman, M; Jackson, SB; Kellomaki, S; Laitat, E; Rey, A; Roberntz, P; Sigurdsson, BD; Strassemeyer, J; Wang, K; Curtis, PS; Jarvis, PG. (2001). Stomatal conductance of forest species after long-term exposure to elevated CO2 concentration: A synthesis. New Phytol 149: 247-264. http://dx.doi.org/10.1046/j.1469-8137.2001.00028.x

Meehan, TD; Crossley, MS; Lindroth, RL. (2010). Impacts of elevated CO2 and O3 on aspen leaf litter chemistry and earthworm and springtail productivity. Soil Biol Biochem 42: 1132-1137. http://dx.doi.org/10.1016/j.soilbio.2010.03.019

Menendez, AI; Romero, AM; Folcia, AM; Martinez-Ghersa, MA. (2009). Getting the interactions right: Will higher O3 levels interfere with induced defenses to aphid feeding? Basic Appl Ecol 10: 255-264. http://dx.doi.org/10.1016/j.baae.2008.03.010

Menendez, AI; Romero, AM; Folcia, AM; Martinez-Ghersa, MA. (2010). Aphid and episodic O3 injury in arugula plants (Eruca sativa Mill) grown in open-top field chambers. Agric Ecosyst Environ 135: 10-14. http://dx.doi.org/10.1016/j.agee.2009.08.005

Mereu, S; Gerosa, G; Finco, A; Fusaro, L; Muys, B; Manes, F. (2009). Improved sapflow methodology reveals considerable night-time ozone uptake by Mediterranean species. Biogeosciences 6: 3151-3162. http://dx.doi.org/10.5194/bg-6-3151-2009

Miles, GP; Samuel, MA; Zhang, YL; Ellis, BE. (2005). RNA interference-based (RNAi) suppression of AtMPK6, an Arabidopsis mitogen-activated protein kinase, results in hypersensitivity to ozone and misregulation of AtMPK3. Environ Pollut 138: 230-237. http://dx.doi.org/10.1016/j.envpol.2005.04.017

Miller, PL. (1973). Oxidant-induced community change in a mixed conifer forest. In JA Naegele (Ed.), Air pollution damage to vegetation (pp. 101-117). Washington, DC: American Chemical Society.

Miller, PR; McCutchan, MH; Ryan, BC. (1972). Influence of climate and topography on oxidant air pollution concentrations that damage conifer forests in southern California. Mitt Forstl Bundesversuchsanst Wien 97: 585-607.

Miller, PR; Parmeter, JR, Jr; Taylor, OC; Cardiff, EA. (1963). Ozone injury to the foliage of Pinus ponderosa. Phytopathology 53: 1072-1076.

Miller, PR; Rechel, J. (1999). Temporal changes in crown condition indices, needle litterfall, and collateral needle injuries of Ponderosa and Jeffrey pines. In PR Miller; JR McBride (Eds.), Oxidant air pollution impacts in the montane forests of southern California: A case study of the San Bernardino Mountains (pp. 164-178). New York, NY: Springer.

Mills, G. (2002). Modification of plant response by environmental conditions. In JNB Bell; M Treshow (Eds.), Air pollution and plant life (2nd ed., pp. 343-358). Chichester, United Kingdom: John Wiley & Sons.

Mills, G; Ball, G; Hayes, F; Fuhrer, J; Skarby, L; Gimeno, B; De Temmerman, L. (2000). Development of a multi-factor model for predicting the effects of ambient ozone on the biomass of white clover. Environ Pollut 109: 533-542. http://dx.doi.org/10.1016/S0269-7491(00)00057-9

Mills, G; Buse, A; Gimeno, B; Bermejo, V; Holland, M; Emberson, L; Pleijel, H. (2007a). A synthesis of AOT40-based response functions and critical levels of ozone for agricultural and horticultural crops. Atmos Environ 41: 2630-2643. http://dx.doi.org/10.1016/j.atmosenv.2006.11.016

Mills, G; Hayes, F; Jones, MLM; Cinderby, S. (2007b). Identifying ozone-sensitive communities of (semi-)natural vegetation suitable for mapping exceedance of critical levels. Environ Pollut 146: 736-743. http://dx.doi.org/10.1016/j.envpol.2006.04.005

Mills, G; Hayes, F; Wilkinson, S; Davies, WJ. (2009). Chronic exposure to increasing background ozone impairs stomatal functioning in grassland species. Global Change Biol 15: 1522-1533. http://dx.doi.org/10.1111/j.1365-2486.2008.01798.x

Mondor, EB; Awmack, CS; Lindroth, RL. (2010). Individual growth rates do not predict aphid population densities under altered atmospheric conditions. Agr Forest Entomol 12: 293-299. http://dx.doi.org/10.1111/j.1461-9563.2010.00478.x

Mondor, EB; Tremblay, MN; Awmack, CS; Lindroth, RL. (2004). Divergent pheromone-mediated insect behaviour under global atmospheric change. Global Change Biol 10: 1820-1824.

Mondor, EB; Tremblay, MN; Awmack, CS; Lindroth, RL. (2005). Altered genotypic and phenotypic frequencies of aphid populations under enriched CO2 and O3 atmospheres. Global Change Biol 11: 1990-1996. http://dx.doi.org/10.1111/j.1365-2486.2005.01054.x

Morgan, PB; Ainsworth, EA; Long, SP. (2003). How does elevated ozone impact soybean? A meta-analysis of photosynthesis, growth and yield. Plant Cell Environ 26: 1317-1328.

Morgan, PB; Bernacchi, CJ; Ort, DR; Long, SP. (2004). An in vivo analysis of the effect of season-long open-air elevation of ozone to anticipated 2050 levels on photosynthesis in soybean. J Plant Physiol 135: 2348-2357.

Morgan, PB; Mies, TA; Bollero, GA; Nelson, RL; Long, SP. (2006). Season-long elevation of ozone concentration to projected 2050 levels under fully open-air conditions substantially decreases the growth and production of soybean. New Phytol 170: 333-343. http://dx.doi.org/10.1111/j.1469-8137.2006.01679.x

Morison, JIL; Lawlor, DW. (1999). Interactions between increasing CO2 concentration and temperature on plant growth. Plant Cell Environ 22: 659-682. http://dx.doi.org/10.1046/j.1365-3040.1999.00443.x

Morsky, SK; Haapala, JK; Rinnan, R; Tiiva, P; Saarnio, S; Silvola, J; Holopainen, T; Martikainen, PJ. (2008). Long-term ozone effects on vegetation, microbial community and methane dynamics of boreal peatland microcosms in open-field conditions. Global Change Biol 14: 1891-1903. http://dx.doi.org/10.1111/j.1365-2486.2008.01615.x

Mudd, JB. (1996). Biochemical basis for the toxicity of ozone. In M Yunus; M Iqbal (Eds.), Plant response to air pollution (pp. 267-283). New York, NY: John Wiley & Sons.

Muntifering, RB; Chappelka, AH; Lin, JC; Karnosky, DF; Somers, GL. (2006a). Chemical composition and digestibility of Trifolium exposed to elevated ozone and carbon dioxide in a free-air (FACE) fumigation system. Funct Ecol 20: 269-275. http://dx.doi.org/10.1111/j.1365-2435.2006.01093.x

Muntifering, RB; Manning, WJ; Lin, JC; Robinson, GB. (2006b). Short-term exposure to ozone altered the relative feed value of an alfalfa cultivar. Environ Pollut 140: 1-3.

Musselman, RC; Lefohn, AS; Massman, WJ; Heath, RL. (2006). A critical review and analysis of the use of exposure- and flux-based ozone indices for predicting vegetation effects [Review]. Atmos Environ 40: 1869-1888. http://dx.doi.org/10.1016/j.atmosenv.2005.10.064

Musselman, RC; Massman, WJ. (1999). Ozone flux to vegetation and its relationship to plant response and ambient air quality standards. Atmos Environ 33: 65-73.

Musselman, RC; McCool, PM; Younglove, T. (1988). Selecting ozone exposure statistics for determining crop yield loss from air pollutants. Environ Pollut 53: 63-78. http://dx.doi.org/10.1016/0269-7491(88)90025-5

Musselman, RC; Minnick, TJ. (2000). Nocturnal stomatal conductance and ambient air quality standards for ozone. Atmos Environ 34: 719-733. http://dx.doi.org/10.1016/S1352-2310(99)00355-6

Nali, C; Balducci, E; Frati, L; Paoli, L; Loppi, S; Lorenzini, G. (2007). Integrated biomonitoring of air quality with plants and lichens: A case study on ambient ozone from central Italy. Chemosphere 67: 2169-2176. http://dx.doi.org/10.1016/j.chemosphere.2006.12.036

NAPAP (National Acid Precipitation Assessment Program). (1987). Diagnosing injury to Eastern forest trees: A manual for identifying damage caused by air pollution, pathogens, insects, and abiotic stresses. University Park, PA: Pennsylvania State University.

Nash, TH, III. (2008). Lichen sensitivity to air pollution. In TH Nash, III (Ed.), Lichen biology (2nd ed., pp. 301-316). Cambridge, UK: Cambridge University Press.

Neufeld, HS; Lee, EH; Renfro, JR; Hacker, WD. (2000). Seedling insensitivity to ozone for three conifer species native to Great Smoky Mountains National Park. Environ Pollut 108: 141-151. http://dx.doi.org/10.1016/S0269-7491(99)00247-X

Neufeld, HS; Lee, EH; Renfro, JR; Hacker, WD; Yu, BH. (1995). Sensitivity of seedlings of black cherry (Prunus serotina Ehrh) to ozone in Great Smoky Mountains National Park I Exposure-response curves for biomass. New Phytol 130: 447-459. http://dx.doi.org/10.1111/j.1469-8137.1995.tb01839.x

Neufeld, HS; Renfro, JR; Hacker, WD; Silsbee, D. (1992). Ozone in Great Smoky Mountains National Park: Dynamics and effects on plants. In RL Berglund (Ed.), Tropospheric ozone and the environment II: Effects, modeling and control (pp. 594-617). Pittsburgh, PA: Air & Waste Management Association.

Nikolova, PS; Andersen, CP; Blaschke, H; Matyssek, R; Häberle, KH. (2010). Belowground effects of enhanced tropospheric ozone and drought in a beech/spruce forest (Fagus sylvatica L./Picea abies [L.] Karst). Environ Pollut 158: 1071-1078. http://dx.doi.org/10.1016/j.envpol.2009.07.036

Noctor, G; Foyer, CH. (1998). Ascorbate and glutathione: Keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol 49: 249-279. http://dx.doi.org/10.1146/annurev.arplant.49.1.249

Norby, RJ; DeLucia, EH; Gielen, B; Calfapietra, C; Giardina, CP; King, JS; Ledford, J; McCarthy, HR; Moore, DJP; Ceulemans, R; De Angelis, P; Finzi, AC; Karnosky, DF; Kubiske, ME; Lukac, M; Pregitzer, KS; Scarascia-Mugnozza, GE; Schlesinger, WH; Oren, R. (2005). Forest response to elevated CO2 is conserved across a broad range of productivity. PNAS 102: 18052-18056. http://dx.doi.org/10.1073/pnas.0509478102

Novak, K; Cherubini, P; Saurer, M; Fuhrer, J; Skelly, JM; Kräuchi, N; Schaub, M. (2007). Ozone air pollution effects on tree-ring growth, delta(13)C, visible foliar injury and leaf gas exchange in three ozone-sensitive woody plant species. Tree Physiol 27: 941-949.

NPS (U.S. National Park Service). (2006). Ozone bioindicators. Washington, DC. http://www.nature.nps.gov/air/Pubs/bioindicators/index.cfm

NPS (U.S. National Park Service). (2007). Ozone effects studies. Washington, DC: U.S. Department of the Interior, National Park Service. http://www.nature.nps.gov/air/studies/ecoOzone.cfm

Nunn, AJ; Reiter, IM; Haberle, KH; Werner, H; Langebartels, C; Sandermann, H; Heerdt, C; Fabian, P; Matyssek, R. (2002). Free-air ozone canopy fumigation in an old-growth mixed forest: concept and observations in beech. Phyton (Buenos Aires) 42: 105-119.

Nussbaum, S; Geissmann, M; Fuhrer, J. (1995). Ozone exposure-response relationships for mixtures of perennial ryegrass and white clover depend on ozone exposure patterns. Atmos Environ 29: 989-995. http://dx.doi.org/10.1016/1352-2310(94)00368-U

O'Gara, PJ. (1922). Sulfur dioxide and fume problems and their solutions [Abstract]. The Journal of Industrial and Engineering Chemistry 14: 744.

O'Neill, BF; Zangerl, AR; Delucia, EH; Berenbaum, MR. (2008). Longevity and fecundity of Japanese beetle (Popillia japonica) on foliage grown under elevated carbon dioxide. Environ Entomol 37: 601-607.

O'Neill, BF; Zangerl, AR; Dermody, O; Bilgin, DD; Casteel, CL; Zavala, JA; DeLucia, EH; Berenbaum, MR. (2010). Impact of elevated levels of atmospheric CO2 and herbivory on flavonoids of soybean (Glycine max Linnaeus). J Chem Ecol 36: 35-45. http://dx.doi.org/10.1007/s10886-009-9727-0

Ogawa, D; Nakajima, N; Sano, T; Tamaoki, M; Aono, M; Kubo, A; Kanna, M; Ioki, M; Kamada, H; Saji, H. (2005). Salicylic acid accumulation under O-3 exposure is regulated by ethylene in tobacco plants. Plant Cell Physiol 46: 1062-1072. http://dx.doi.org/10.1093/pcp/pci118

Oksanen, E. (2003). Responses of selected birch (Betula pendula Roth) clones to ozone change over time. Plant Cell Environ 26: 875-886.

Oksanen, E; Holopainen, T. (2001). Responses of two birch (Betula pendula Roth) clones to different ozone profiles with similar AOT40 exposure. Atmos Environ 35: 5245-5254. http://dx.doi.org/10.1016/S1352-2310(01)00346-6

Olbrich, M; Betz, G; Gerstner, E; Langebartels, C; Sandermann, H; Ernst, D. (2005). Transcriptome analysis of ozone-responsive genes in leaves of European beech (Fagus sylvatica L.). Plant Biol (Stuttg) 7: 670-676. http://dx.doi.org/10.1055/s-2005-873001

Olbrich, M; Gerstner, E; Bahnweg, G; Haberle, KH; Matyssek, R; Welzl, G; Heller, W; Ernst, D. (2010). Transcriptional signatures in leaves of adult European beech trees (Fagus sylvatica L.) in an experimentally enhanced free air ozone setting. Environ Pollut 158: 977-982. http://dx.doi.org/10.1016/j.envpol.2009.08.001

Olbrich, M; Gerstner, E; Welzl, G; Winkler, JB; Ernst, D. (2009). Transcript responses in leaves of ozone-treated beech saplings seasons at an outdoor free air model fumigation site over two growing seasons. Plant Soil 323: 61-74. http://dx.doi.org/10.1007/s11104-009-0129-4

Ollinger, SV; Aber, JD; Reich, PB. (1997a). Simulating ozone effects on forest productivity: Interactions among leaf- and stand-level processes. Ecol Appl 123: 351-358.

Ollinger, SV; Aber, JD; Reich, PB. (1997b). Simulating ozone effects on forest productivity: Interactions among leaf-, canopy-, and stand-level processes. Ecol Appl 7: 1237-1251.

Ollinger, SV; Aber, JD; Reich, PB; Freuder, RJ. (2002). Interactive effects of nitrogen deposition, tropospheric ozone, elevated CO2 and land use history on the carbon dynamics of northern hardwood forests. Global Change Biol 8: 545-562. http://dx.doi.org/10.1046/j.1365-2486.2002.00482.x

Olszyk, DM; Kats, G; Dawson, PJ; Bytnerowicz, A; Wolf, J; Thompson, CR. (1986). Characteristics of air exclusion systems vs chambers for field air pollution studies. J Environ Qual 15: 326-334.

Omasa, K; Shimazaki, KI; Aiga, I; Larcher, W; Onoe, M. (1987). Image analysis of chlorophyll fluorescence transients for diagnosing the photosynthetic system of attached leaves. Plant Physiol 84: 748-752. http://dx.doi.org/10.1104/pp.84.3.748

Orendovici-Best, T; Skelly, JM; Davis, DD; Ferdinand, JA; Savage, JE; Stevenson, RE. (2008). Ozone uptake (flux) as it relates to ozone-induced foliar symptoms of Prunus serotina and Populus maximowizii x trichocarpa. Environ Pollut 151: 79-92. http://dx.doi.org/10.1016/j.envpol.2007.03.003

Orendovici, T; Skelly, JM; Ferdinand, JA; Savage, JE; Sanz, MJ; Smith, GC. (2003). Response of native plants of northeastern United States and southern Spain to ozone exposures; determining exposure/response relationships. Environ Pollut 125: 31-40.

Oshima, RJ; Braegelmann, PK; Baldwin, DW; Van Way, V; Taylor, OC. (1977). Reduction of tomato fruit size and yield by ozone. J Am Soc Hortic Sci 102: 289-293.

Oshima, RJ; Poe, MP; Braegelmann, PK; Baldwin, DW; Van Way, V. (1976). Ozone dosage-crop loss function for alfalfa: A standardized method for assessing crop losses from air pollutants. J Air Pollut Control Assoc 26: 861-865.

Overmyer, K; Brosche, M; Pellinen, R; Kuittinen, T; Tuominen, H; Ahlfors, R; Keinanen, M; Saarma, M; Scheel, D; Kangasjarvi, J. (2005). Ozone-induced programmed cell death in the Arabidopsis radical-induced cell death1 mutant. Plant Physiol 137: 1092-1104. http://dx.doi.org/10.1104/pp.104.055681

Overmyer, K; Kollist, H; Tuominen, H; Betz, C; Langebartels, C; Wingsle, G; Kangasjarvi, S; Brader, G; Mullineaux, P; Kangasjarvi, J. (2008). Complex phenotypic profiles leading to ozone sensitivity in Arabidopsis thaliana mutants. Plant Cell Environ 31: 1237-1249. http://dx.doi.org/10.1111/j.1365-3040.2008.01837.x

Overmyer, K; Tuominen, H; Kettunen, R; Betz, C; Langebartels, C; Sandermann, H, Jr; Kangasjarvi, J. (2000). Ozone-sensitive Arabidopsis rcd1 mutant reveals opposite roles for ethylene and jasmonate signaling pathways in regulating superoxide-dependent cell death. Plant Cell 12: 1849-1862. http://dx.doi.org/10.1105/tpc.12.10.1849

Pan, YD; Birdsey, R; Hom, J; McCullough, K. (2009). Separating effects of changes in atmospheric composition, climate and land-use on carbon sequestration of US Mid-Atlantic temperate forests. For Ecol Manage 259: 151-164. http://dx.doi.org/10.1016/j.foreco.2009.09.049

Panek, J; Kurpius, MR; Goldstein, AH. (2002). An evaluation of ozone exposure metrics for a seasonally drought-stressed ponderosa pine ecosystem. Environ Pollut 117: 93-100. http://dx.doi.org/10.1016/S0269-7491(01)00155-5

Panek, JA. (2004). Ozone uptake, water loss and carbon exchange dynamics in annually drought-stressed Pinus ponderosa forests: Measured trends and parameters for uptake modeling. Tree Physiol 24: 277-290.

Panek, JA; Goldstein, AH. (2001). Responses of stomatal conductance to drought in ponderosa pine: Implications for carbon and ozone uptake. Tree Physiol 21: 337-344.

Paolacci, AR; Miraldi, C; Tanzarella, OA; Badiani, M; Porceddu, E; Nali, C; Lorenzini, G; Ciaffi, M. (2007). Gene expression induced by chronic ozone in the Mediterranean shrub Phillyrea latifolia: Analysis by cDNA-AFLP. Tree Physiol 27: 1541-1550. http://dx.doi.org/10.1093/treephys/27.11.1541

Paoletti, E; Grulke, NE. (2005). Does living in elevated CO2 ameliorate tree response to ozone? A review on stomatal responses [Review]. Environ Pollut 137: 483-493. http://dx.doi.org/10.1016/j.envpol.2005.01.035

Paoletti, E; Grulke, NE. (2010). Ozone exposure and stomatal sluggishness in different plant physiognomic classes. Environ Pollut 158: 2664-2671. http://dx.doi.org/10.1016/j.envpol.2010.04.024

Paoletti, E; Manning, WJ. (2007). Toward a biologically significant and usable standard for ozone that will also protect plants. Environ Pollut 150: 85-95. http://dx.doi.org/10.1016/j.envpol.2007.06.037

Paoletti, E; Seufert, G; Della Rocca, G; Thomsen, H. (2007). Photosynthetic responses to elevated CO2 and O-3 in Quercus ilex leaves at a natural CO2 spring. Environ Pollut 147: 516-524. http://dx.doi.org/10.1016/j.envpol.2006.08.039

Parsons, WFJ; Bockheim, JG; Lindroth, RL. (2008). Independent, interactive, and species-specific responses of leaf litter decomposition to elevated CO2 and O3 in a northern hardwood forest. Ecosystems 11: 505-519.

Pearson, M; Mansfield, TA. (1993). Interacting effects of ozone and water stress on the stomatal resistance of beech (Fagus sylvatica L). New Phytol 123: 351-358. http://dx.doi.org/10.1111/j.1469-8137.1993.tb03745.x

Pearson, S; Davison, AW; Reiling, K; Ashenden, T; Ollerenshaw, JH. (1996). The effects of different ozone exposures on three contrasting populations of Plantago major. New Phytol 132: 493-502. http://dx.doi.org/10.1111/j.1469-8137.1996.tb01869.x

Pei, ZM; Murata, Y; Benning, G; Thomine, S; Klüsener, B; Allen, GJ; Grill, E; Schroeder, JI. (2000). Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. Nature 406: 731-734. http://dx.doi.org/10.1038/35021067

Pell, EJ; Sinn, JP; Brendley, BW; Samuelson, L; Vinten-Johansen, C; Tien, M; Skillman, J. (1999). Differential response of four tree species to ozone-induced acceleration of foliar senescence. Plant Cell Environ 22: 779-790. http://dx.doi.org/10.1046/j.1365-3040.1999.00449.x

Peltonen, PA; Julkunen-Tiitto, R; Vapaavuori, E; Holopainen, JK. (2006). Effects of elevated carbon dioxide and ozone on aphid oviposition preference and birch bud exudate phenolics. Global Change Biol 12: 1670-1679. http://dx.doi.org/10.1111/j.1365-2486.2006.01226.x

Peltonen, PA; Vapaavuori, E; Heinonen, J; Julkunen-Tiitto, R; Holopainen, JK. (2010). Do elevated atmospheric CO2 and O-3 affect food quality and performance of folivorous insects on silver birch? Global Change Biol 16: 918-935. http://dx.doi.org/10.1111/j.1365-2486.2009.02073.x

Percy, KE; Nosal, M; Heilman, W; Dann, T; Sober, J; Legge, AH; Karnosky, DF. (2007). New exposure-based metric approach for evaluating O3 risk to North American aspen forests. Environ Pollut 147: 554-566. http://dx.doi.org/10.1016/j.envpol.2006.10.009

Peterson, DL; Arbaugh, MJ; Wakefield, VA; Miller, PR. (1987). Evidence of growth reduction in ozone-injured Jeffrey pine (Pinus jeffreyi Grev and Balf) in Sequoia and Kings Canyon National Parks. J Air Waste Manag Assoc 37: 906-912.

Pfleeger, TG; Plocher, M; Bichel, P. (2010). Response of pioneer plant communities to elevated ozone exposure. Agric Ecosyst Environ 138: 116-126. http://dx.doi.org/10.1016/j.agee.2010.04.009

Phillips, DL; Johnson, MG; Tingey, DT; Storm, MJ. (2009). Elevated CO2 and O3 effects on fine-root survivorship in ponderosa pine mesocosms. Oecologia 160: 827-837. http://dx.doi.org/10.1007/s00442-009-1339-4

Phillips, RL; Zak, DR; Holmes, WE; White, DC. (2002). Microbial community composition and function beneath temperate trees exposed to elevated atmospheric carbon dioxide and ozone. Oecologia 131: 236-244.

Piikki, K; De Temmerman, L; Hogy, P; Pleijel, H. (2008a). The open-top chamber impact on vapour pressure deficit and its consequences for stomatal ozone uptake. Atmos Environ 42: 6513-6522. http://dx.doi.org/10.1016/j.atmosenv.2008.04.014

Piikki, K; De Temmerman, L; Ojanpera, K; Danielsson, H; Pleijel, H. (2008b). The grain quality of spring wheat (Triticum aestivum L.) in relation to elevated ozone uptake and carbon dioxide exposure. Eur J Agron 28: 245-254. http://dx.doi.org/10.1016/j.eja.2007.07.004

Piikki, K; Vorne, V; Ojanpera, K; Pleijel, H. (2007). Impact of elevated O3 and CO2 exposure on potato (Solanum tuberosum L. cv. Bintje) tuber macronutrients (N, P, K, Mg, Ca). Agric Ecosyst Environ 118: 55-64. http://dx.doi.org/10.1016/j.agee.2006.04.012

Pinto, DM; Blande, JD; Nykanen, R; Dong, WX; Nerg, AM; Holopainen, JK. (2007a). Ozone degrades common herbivore-induced plant volatiles: Does this affect herbivore prey location by predators and parasitoids? J Chem Ecol 33: 683-694. http://dx.doi.org/10.1007/s10886-007-9255-8

Pinto, DM; Blande, JD; Souza, SR; Nerg, AM; Holopainen, JK. (2010). Plant volatile organic compounds (VOCs) in ozone (O3) polluted atmospheres: The ecological effects [Review]. J Chem Ecol 36: 22-34. http://dx.doi.org/10.1007/s10886-009-9732-3

Pinto, DM; Himanen, SJ; Nissinen, A; Nerg, AM; Holopainen, JK. (2008). Host location behavior of Cotesia plutellae Kurdjumov (Hymenoptera: Braconidae) in ambient and moderately elevated ozone in field conditions. Environ Pollut 156: 227-231. http://dx.doi.org/10.1016/j.envpol.2007.12.009

Pinto, DM; Nerg, AM; Holopainen, JK. (2007b). The role of ozone-reactive compounds, terpenes, and green leaf volatiles (GLVs), in the orientation of Cotesia plutellae. J Chem Ecol 33: 2218-2228. http://dx.doi.org/10.1007/s10886-007-9376-0

Pinto, J. (2009). Wyoming winter smog. Nat Geosci 2: 88-90. http://dx.doi.org/10.1038/ngeo430

Pleijel, H; Danielsson, H; Gelang, J; Sild, E; Sellden, G. (1998). Growth stage dependence of the grain yield response to ozone in spring wheat (Triticum aestivum L). Agric Ecosyst Environ 70: 61-68. http://dx.doi.org/10.1016/S0167-8809(97)00167-9

Pleijel, H; Danielsson, H; Ojanpera, K; De Temmerman, L; Hogy, P; Badiani, M; Karlsson, PE. (2004a). Relationships between ozone exposure and yield loss in European wheat and potato--a comparison of concentration- and flux-based exposure indices. Atmos Environ 38: 2259-2269.

Pleijel, H; h, D; Ojanpera, K; De Temmerman, L; Hogy, P. (2004b). Relationships between ozone exposure and yield loss in wheat and potato - Suggestions of critical levels for ozone effects on crops. Atmos Environ 38: 2259-2269. http://dx.doi.org/10.1016/j.atmosenv.2003.09.076

Pleijel, H; Ojanpera, K; Mortensen, L. (1997). Effects of tropospheric ozone on the yield and grain protein content of spring wheat (Triticum aestivum L) in the nordic countries. Acta Agric Scand B Soil Plant Sci 47: 20-25. http://dx.doi.org/10.1080/09064719709362434

Plessl, M; Elstner, EF; Rennenberg, H; Habermeyer, J; Heiser, I. (2007). Influence of elevated CO2 and ozone concentrations on late blight resistance and growth of potato plants. Environ Exp Bot 60: 447-457. http://dx.doi.org/10.1016/j.envexpbot.2007.01.003

Plochl, M; Lyons, T; Ollerenshaw, J; Barnes, J. (2000). Simulating ozone detoxification in the leaf apoplast through the direct reaction with ascorbate. Planta 210: 454-467. http://dx.doi.org/10.1007/PL00008153

Pollastrini, M; Desotgiu, R; Cascio, C; Bussotti, F; Cherubini, P; Saurer, M; Gerosa, G; Marzuoli, R. (2010). Growth and physiological responses to ozone and mild drought stress of tree species with different ecological requirements. Trees Struct Funct 24: 695-704. http://dx.doi.org/10.1007/s00468-010-0439-4

Polle, A; Pell, EJ. (1999). Role of carbon dioxide in modifying the plant response to ozone. In Y Luo; HA Mooney (Eds.), Carbon dioxide and environmental stress (pp. 193-213). San Diego, CA: Academic Press.

Pregitzer, K; Loya, W; Kubiske, M; Zak, D. (2006). Soil respiration in northern forests exposed to elevated atmospheric carbon dioxide and ozone. Oecologia 148: 503-516. http://dx.doi.org/10.1007/s00442-006-0381-8

Pregitzer, KS; Burton, AJ; King, JS; Zak, DR. (2008). Soil respiration, root biomass, and root turnover following long-term exposure of northern forests to elevated atmospheric Co-2 and tropospheric O-3. New Phytol 180: 153-161. http://dx.doi.org/10.1111/j.1469-8137.2008.02564.x

Pretzsch, H; Dieler, J; Matyssek, R; Wipfler, P. (2010). Tree and stand growth of mature Norway spruce and European beech under long-term ozone fumigation. Environ Pollut 158: 1061-1070. http://dx.doi.org/10.1016/j.envpol.2009.07.035

Pritsch, K; Esperschuetz, J; Haesler, F; Raidl, S; Winkler, B; Schloter, M. (2009). Structure and activities of ectomycorrhizal and microbial communities in the rhizosphere of Fagus sylvatica under ozone and pathogen stress in a lysimeter study. Plant Soil 323: 97-109. http://dx.doi.org/10.1007/s11104-009-9972-6

Puckette, MC; Tang, YH; Mahalingam, R. (2008). Transcriptomic changes induced by acute ozone in resistant and sensitive Medicago truncatula accessions. BMC Plant Biol 8: 46. http://dx.doi.org/10.1186/1471-2229-8-46

Pujol Pereira, EI; Chung, H; Scow, K; Sadowsky, MJ; van Kessel, C; Six, J. (2011). Soil nitrogen transformations under elevated atmospheric CO and O during the soybean growing season. Environ Pollut 159: 401-407. http://dx.doi.org/10.1016/j.envpol.2010.10.033

Ramo, K; Kanerva, T; Ojanpera, K; Manninen, S. (2007). Growth onset, senescence, and reproductive development of meadow species in mesocosms exposed to elevated O3 and CO2. Environ Pollut 145: 850-860. http://dx.doi.org/10.1016/j.envpol.2006.03.054

Rao, MV; Hale, BA; Ormrod, DP. (1995). Amelioration of ozone-induced oxidative damage in wheat plants grown under high carbon dioxide: Role of antioxidant enzymes. J Plant Physiol 109: 421-432.

Rawlings, JO; Cure, WW. (1985). The Weibull function as a dose-response model to describe ozone effects on crop yields. Crop Sci 25: 807-814.

Reich, PB. (1987). Quantifying plant response to ozone: A unifying theory. Tree Physiol 3: 63-91. http://dx.doi.org/10.1093/treephys/3.1.63

Reich, PB; Lassoie, JP. (1984). Effects of low level O3 exposure on leaf diffusive conductance and water-use efficiency in hybrid poplar. Plant Cell Environ 7: 661-668. http://dx.doi.org/10.1111/1365-3040.ep11571645

Reid, CD; Fiscus, EL. (2008). Ozone and density affect the response of biomass and seed yield to elevated CO2 in rice. Global Change Biol 14: 60-76. http://dx.doi.org/10.1111/j.1365-2486.2007.01472.x

Reiling, K; Davison, AW. (1992). Effects of a short ozone exposure given at different stages in the development of Plantago major L. New Phytol 121: 643-647. http://dx.doi.org/10.1111/j.1469-8137.1992.tb01135.x

Reiling, K; Davison, AW. (1994). Effects of exposure to ozone at different stages in the development of Plantago major L on chlorophyll fluorescence and gas exchange. New Phytol 128: 509-514. http://dx.doi.org/10.1111/j.1469-8137.1994.tb02998.x

Reinert, RA; Eason, G; Barton, J. (1997). Growth and fruiting of tomato as influenced by elevated carbon dioxide and ozone. New Phytol 137: 411-420. http://dx.doi.org/10.1046/j.1469-8137.1997.00846.x

Reinert, RA; Ho, MC. (1995). Vegetative growth of soybean as affected by elevated carbon dioxide and ozone. Environ Pollut 89: 89-96. http://dx.doi.org/10.1016/0269-7491(94)00039-G

Ren, W; Tian, H; Chen, G; Liu, M; Zhang, C; Chappelka, AH; Pan, S. (2007a). Influence of ozone pollution and climate variability on net primary productivity and carbon storage in China's grassland ecosystems from 1961 to 2000. Environ Pollut 149: 327-335. http://dx.doi.org/10.1016/j.envpol.2007.05.029

Ren, W; Tian, H; Tao, B; Chappelka, A; Sun, G; Lu, C; Liu, M; Chen, G; Xu, X. (2011). Impacts of tropospheric ozone and climate change on net primary productivity and net carbon exchange of China's forest ecosystems. Global Ecology and Biogeography 20: 391-406. http://dx.doi.org/10.1111/j.1466-8238.2010.00606.x

Ren, W; Tian, HQ; Liu, ML; Zhang, C; Chen, GS; Pan, SF; Felzer, B; Xu, XF. (2007b). Effects of tropospheric ozone pollution on net primary productivity and carbon storage in terrestrial ecosystems of China. J Geophys Res 112: D22S09. http://dx.doi.org/10.1029/2007jd008521

Rhea, L; King, J; Kubiske, M; Saliendra, N; Teclaw, R. (2010). Effects of elevated atmospheric CO2 and tropospheric O3 on tree branch growth and implications for hydrologic budgeting. Environ Pollut 158: 1079-1087. http://dx.doi.org/10.1016/j.envpol.2009.08.038

Riddell, J; Padgett, PE; Nash, TH, III. (2010). Responses of the lichen Ramalina menziesii Tayl. to ozone fumigations. In TH Nash, III (Ed.), Biology of lichens: Symbiosis, ecology, environmental monitoring, systematics, cyber applications (pp. 113-123). Stuttgart: J. Cramer in der Gebrüder Borntraeger Verlagsbuchhandlung.

Riikonen, J; Kets, K; Darbah, J; Oksanen, E; Sober, A; Vapaavuori, E; Kubiske, ME; Nelson, N; Karnosky, DF. (2008). Carbon gain and bud physiology in Populus tremuloides and Betula papyrifera grown under long-term exposure to elevated concentrations of CO2 and O3. Tree Physiol 28: 243-254. http://dx.doi.org/10.1093/treephys/28.2.243

Riikonen, J; Maenpaa, M; Alavillamo, M; Silfver, T; Oksanen, E. (2009). Interactive effect of elevated temperature and O3 on antioxidant capacity and gas exchange in Betula pendula saplings. Planta 230: 419-427. http://dx.doi.org/10.1007/s00425-009-0957-8

Rizzo, M; Bernardi, R; Salvini, M; Nali, C; Lorenzini, G; Durante, M. (2007). Identification of differentially expressed genes induced by ozone stress in sensitive and tolerant poplar hybrids. J Plant Physiol 164: 945-949. http://dx.doi.org/10.1016/j.jplph.2006.07.012

Rodenkirchen, H; Gottlein, A; Kozovits, AR; Matyssek, R; Grams, TEE. (2009). Nutrient contents and efficiencies of beech and spruce saplings as influenced by competition and O3/CO2 regime. European Journal of Forest Research 128: 117-128. http://dx.doi.org/10.1007/s10342-008-0221-y

Rogers, A; Allen, DJ; Davey, PA; Morgan, PB; Ainsworth, EA; Bernacchi, CJ; Cornic, G; Dermody, OC; Dohleman, FG; Heaton, EA; Mahoney, J; Zhu, XG; Delucia, EH; Ort, DR; Long, SP. (2004). Leaf photosynthesis and carbohydrate dynamics of soybean grown throughout their life-cycle under free-air carbon dioxide enrichment. Plant Cell Environ 27: 449-458.

Rowland-Bamford, AJ. (2000). Plant responses to changing carbon dioxide and temperature. In SN Singh (Ed.), Trace gas emissions and plants (pp. 63-74). Dordecht, The Netherlands: Kluwer Academic Publishers.

Ryan, A; Cojocariu, C; Possell, M; Davies, WJ; Hewitt, CN. (2009). Defining hybrid poplar (Populus deltoides x Populus trichocarpa) tolerance to ozone: Identifying key parameters. Plant Cell Environ 32: 31-45. http://dx.doi.org/10.1111/j.1365-3040.2008.01897.x

Ryang, SZ; Woo, SY; Kwon, SY; Kim, SH; Lee, SH; Kim, KN; Lee, DK. (2009). Changes of net photosynthesis, antioxidant enzyme activities, and antioxidant contents of Liriodendron tulipifera under elevated ozone. Photosynthetica 47: 19-25. http://dx.doi.org/10.1007/s11099-009-0005-8

Samuel, MA; Ellis, BE. (2002). Double jeopardy: Both overexpression and suppression of a redox-activated plant mitogen-activated protein kinase render tobacco plants ozone sensitive. Plant Cell 14: 2059-2069. http://dx.doi.org/10.1105/tpc.002337

Samuel, MA; Miles, GP; Ellis, BE. (2000). Ozone treatment rapidly activates MAP kinase signalling in plants. Plant J 22: 367-376. http://dx.doi.org/10.1046/j.1365-313x.2000.00741.x

Samuel, MA; Walia, A; Mansfield, SD; Ellis, BE. (2005). Overexpression of SIPK in tobacco enhances ozone-induced ethylene formation and blocks ozone-induced SA accumulation. J Exp Bot 56: 2195-2201. http://dx.doi.org/10.1093/jxb/eri219

Samuelson, LJ; Kelly, JM. (1997). Ozone uptake in Prunus serotina, Acer rubrum and Quercus rubra forest trees of different sizes. New Phytol 136: 255-264. http://dx.doi.org/10.1046/j.1469-8137.1997.00734.x

Sánchez, MJS; Peña, GS; Lorente, VC; Gallego, TM; Albert, JC. (2001). La contaminación atmosférica en los bosques: Guía para la identificacíon de daños visibles causados por Ozono. Madrid, Spain: Ministerio de Medio Ambiente. http://www.ceam.es/VentaLibros/guia_O3/index.htm

Sandermann, H. (2008). Ecotoxicology of ozone: Bioactivation of extracellular ascorbate. Biochem Biophys Res Commun 366: 271-274. http://dx.doi.org/10.1016/j.bbrc.2007.12.018

Sanz, J; Bermejo, V; Gimeno, BS; Elvira, S; Alonso, R. (2007). Ozone sensitivity of the Mediterranean terophyte trifolium striatum is modulated by soil nitrogen content. Atmos Environ 41: 8952-8962. http://dx.doi.org/10.1016/j.atmosenv.2007.08.016

Sanz, J; Muntifering, RB; Bermejo, V; Gimeno, BS; Elvira, S. (2005). Ozone and increased nitrogen supply effects on the yield and nutritive quality of Trifolium subterraneum. Atmos Environ 39: 5899-5907. http://dx.doi.org/10.1016/j.atmosenv.2005.06.022

Sarkar, A; Rakwal, R; Agrawal, SB; Shibato, J; Ogawa, Y; Yoshida, Y; Agrawal, GK; Agrawal, M. (2010). Investigating the impact of elevated levels of ozone on tropical wheat using integrated phenotypical, physiological, biochemical, and proteomics approaches. J Proteome Res 9: 4565-4584. http://dx.doi.org/10.1021/Pr1002824

Saviranta, NMM; Julkunen-Tiitto, R; Oksanen, E; Karjalainen, RO. (2010). Leaf phenolic compounds in red clover (Trifolium pratense L.) induced by exposure to moderately elevated ozone. Environ Pollut 158: 440-446. http://dx.doi.org/10.1016/j.envpol.2009.08.029

Sawada, H; Kohno, Y. (2009). Differential ozone sensitivity of rice cultivars as indicated by visible injury and grain yield. Plant Biol (Stuttg) 11: 70-75. http://dx.doi.org/10.1111/j.1438-8677.2009.00233.x

Scebba, F; Giuntini, D; Castagna, A; Soldatini, G; Ranieri, A. (2006). Analysing the impact of ozone on biochemical and physiological variables in plant species belonging to natural ecosystems. Environ Exp Bot 57: 89-97. http://dx.doi.org/10.1016/j.envexpbot.2005.04.005

Schaub, M; Skelly, JM; Zhang, JW; Ferdinand, JA; Savage, JE; Stevenson, RE; Davis, DD; Steiner, KC. (2005). Physiological and foliar symptom response in the crowns of Prunus serotina, Fraxinus americana and Acer rubrum canopy trees to ambient ozone under forest conditions. Environ Pollut 133: 553-567. http://dx.doi.org/10.1016/j.envpol.2004.06.012

Schraudner, M; Moeder, W; Wiese, C; Van Camp, W; Inze, D; Langebartels, C; Sandermann, H, Jr. (1998). Ozone-induced oxidative burst in the ozone biomonitor plant, tobacco Bel W3. Plant J 16: 235-245. http://dx.doi.org/10.1046/j.1365-313x.1998.00294.x

Severino, JF; Stich, K; Soja, G. (2007). Ozone stress and antioxidant substances in Trifolium repens and Centaurea jacea leaves. Environ Pollut 146: 707-714. http://dx.doi.org/10.1016/j.envpol.2006.04.006

Sharkey, TD; Wiberley, AE; Donohue, AR. (2008). Isoprene emission from plants: Why and how. Ann Bot 101: 5-18. http://dx.doi.org/10.1093/aob/mcm240

Singh, E; Tiwari, S; Agrawal, M. (2009). Effects of elevated ozone on photosynthesis and stomatal conductance of two soybean varieties: A case study to assess impacts of one component of predicted global climate change. Plant Biol (Stuttg) 11: 101-108. http://dx.doi.org/10.1111/j.1438-8677.2009.00263.x

Singh, E; Tiwari, S; Agrawal, M. (2010a). Variability in antioxidant and metabolite levels, growth and yield of two soybean varieties: An assessment of anticipated yield losses under projected elevation of ozone. Agric Ecosyst Environ 135: 168-177. http://dx.doi.org/10.1016/j.agee.2009.09.004

Sitch, S; Cox, PM; Collins, WJ; Huntingford, C. (2007). Indirect radiative forcing of climate change through ozone effects on the land-carbon sink. Nature 448: 791-794. http://dx.doi.org/10.1038/nature06059

Skarby, L; Ottosson, S; Karlsson, PE; Wallina, G; Sellden, G; Medina, EL; Pleijel, H. (2004). Growth of Norway spruce (Picea abies) in relation to different ozone exposure indices: A synthesis. Atmos Environ 38: 2225-2236.

Skarby, L; Troeng, E; Bostrom, CA. (1987). Ozone uptake and effects on transpiration, net photosynthesis, and dark respiration in Scots pine. Forest Sci 33: 801-808.

Smith, G. (2012). Ambient ozone injury to forest plants in Northeast and North Central USA: 16 years of biomonitoring. Environ Monit Assess 184: 4049-4065. http://dx.doi.org/10.1007/s10661-011-2243-z

Smith, G; Coulston, J; Jepsen, E; Prichard, T. (2003). A national ozone biomonitoring program: Results from field surveys of ozone sensitive plants in northeastern forests (1994-2000). Environ Monit Assess 87: 271-291.

Soja, G; Barnes, JD; Posch, M; Vandermeiren, K; Pleijel, H; Mills, G. (2000). Phenological weighting of ozone exposures in the calculation of critical levels for wheat, bean and plantain. Environ Pollut 109: 517-524. http://dx.doi.org/10.1016/S0269-7491(00)00055-5

Soja, G; Reichenauer, TG; Eid, M; Soja, AM; Schaber, R; Gangl, H. (2004). Long-term ozone exposure and ozone uptake of grapevines in open-top chambers. Atmos Environ 38: 2313-2321.

Somers, GL; Chappelka, AH; Rosseau, P; Renfro, JR. (1998). Empirical evidence of growth decline related to visible ozone injury. For Ecol Manage 104: 129-137. http://dx.doi.org/10.1016/S0378-1127(97)00252-1

Souza, L; Neufeld, HS; Chappelka, AH; Burkey, KO; Davison, AW. (2006). Seasonal development of ozone-induced foliar injury on tall milkweed (Asclepias exaltata) in Great Smoky Mountains National Park. Environ Pollut 141: 175-183. http://dx.doi.org/10.1016/j.envpol.2005.07.022

Stampfli, A; Fuhrer, J. (2010). Spatial heterogeneity confounded ozone-exposure experiment in semi-natural grassland. Oecologia 162: 515-522. http://dx.doi.org/10.1007/s00442-009-1462-2

Stewart, CA. (1998) Impact of ozone on the reproductive biology of Brassica campestris L and Plantago major L. (Doctoral Dissertation). Loughborough University of Technology, England. Retrieved from http://ethos.bl.uk/OrderDetails.do?did=1&uin=uk.bl.ethos.299673

Stewart, CA; Black, VJ; Black, CR; Roberts, JA. (1996). Direct effects of ozone on the reproductive development of Brassica species. J Plant Physiol 148: 172-178.

Stoelken, G; Pritsch, K; Simon, J; Mueller, CW; Grams, TEE; Esperschuetz, J; Gayler, S; Buegger, F; Brueggemann, N; Meier, R; Zeller, B; Winkler, JB; Rennenberg, H. (2010). Enhanced ozone exposure of European beech (Fagus sylvatica) stimulates nitrogen mobilization from leaf litter and nitrogen accumulation in the soil. Plant Biosystems 144: 537-546. http://dx.doi.org/10.1080/11263500903429346

Street, NR; James, TM; James, T; Mikael, B; Jaakko, K; Mark, B; Taylor, G. (2011). The physiological, transcriptional and genetic responses of an ozone-sensitive and an ozone tolerant poplar and selected extremes of their F2 progeny. Environ Pollut 159: 45-54. http://dx.doi.org/10.1016/j.envpol.2010.09.027

Summers, CG; Retzlaff, WA; Stephenson, S. (1994). The effect of ozone on the mean relative growth-rate of Diuraphis-noxia (Mordvilko) (Homoptera, Aphididae). J Agr Entomol 11: 181-187.

Talhelm, AF; Pregitzer, KS; Zak, DR. (2009). Species-specific responses to atmospheric carbon dioxide and tropospheric ozone mediate changes in soil carbon. Ecol Lett 12: 1219-1228. http://dx.doi.org/10.1111/j.1461-0248.2009.01380.x

Tamaoki, M; Nakajima, N; Kubo, A; Aono, M; Matsuyama, T; Saji, H. (2003). Transcriptome analysis of O3-exposed Arabidopsis reveals that multiple signal pathways act mutually antagonistically to induce gene expression. Plant Mol Biol 53: 443-456. http://dx.doi.org/10.1023/B:PLAN.0000019064.55734.52

Temple, PJ; Kupper, RS; Lennox, RW; Rohr, K. (1988). Injury and yield responses of differentially irrigated cotton to ozone. Agron J 80: 751-755. http://dx.doi.org/10.2134/agronj1988.00021962008000050011x

Temple, PJ; Riechers, GH; Miller, PR. (1992). Foliar injury responses of ponderosa pine seedlings to ozone, wet and dry acidic deposition, and drought. Environ Exp Bot 32: 101-113. http://dx.doi.org/10.1016/0098-8472(92)90035-Z

Theis, N; Raguso, RA. (2005). The effect of pollination on floral fragrance in thistles. J Chem Ecol 31: 2581-2600.

Thomas, VFD; Braun, S; Fluckiger, W. (2005). Effects of simultaneous ozone exposure and nitrogen loads on carbohydrate concentrations, biomass, and growth of young spruce trees (Picea abies). Environ Pollut 137: 507-516. http://dx.doi.org/10.1016/j.envpol.2005.02.002

Thomas, VFD; Braun, S; Fluckiger, W. (2006). Effects of simultaneous ozone exposure and nitrogen loads on carbohydrate concentrations, biomass, growth, and nutrient concentrations of young beech trees (Fagus sylvatica). Environ Pollut 143: 341-354. http://dx.doi.org/10.1016/j.envpol.2005.11.036

Tian, H; Melillo, J; Lu, C; Kicklighter, D; Liu, M; Ren, W; Xu, X; Chen, G; Zhang, C; Pan, S; Liu, J; Running, S. (2011). China's terrestrial carbon balance: Contributions from multiple global change factors. Global Biogeochem Cycles 25: GB1007. http://dx.doi.org/10.1029/2010GB003838

Tingey, DT; Hogsett, WE; Lee, EH; Herstrom, AA; Azevedo, SH. (1991). An evaluation of various alternative ambient ozone standards based on crop yield loss data. In RL Berglund; DR Lawson; DJ McKee (Eds.), Tropospheric Ozone and the Environment (pp. 272-288). Pittsburgh, PA: Air & Waste Management Association.

Tingey, DT; Hogsett, WE; Lee, EH; Laurence, JA. (2004). Stricter ozone ambient air quality standard has beneficial effect on ponderosa pine in California. J Environ Manage 34: 397-405.

Tingey, DT; Johnson, MG; Lee, EH; Wise, C; Waschmann, R; Olszyk, DM; Watrud, LS; Donegan, KK. (2006). Effects of elevated CO2 and O3 on soil respiration under ponderosa pine. Soil Biol Biochem 38: 1764-1778. http://dx.doi.org/10.1016/j.soilbio.2005.12.003

Tingey, DT; McVeety, BD; Waschmann, R; Johnson, MG; Phillips, DL; Rygiewicz, PT; Olszyk, DM. (1996). A versatile sun-lit controlled-environment facility for studying plant and soil processes. J Environ Qual 25: 614-625.

Tingey, DT; Rodecap, KD; Lee, EH; Hogsett, WE; Gregg, JW. (2002). Pod development increases the ozone sensitivity of Phaseolus vulgaris. Water Air Soil Pollut 139: 325-341.

Tissue, DT; Griffin, KL; Ball, T. (1999). Photosynthetic adjustment in field-grown ponderosa pine trees after six years of exposure to elevated CO2. Tree Physiol 19: 221-228.

Tissue, DT; Thomas, RB; Strain, BR. (1997). Atmospheric CO2 enrichment increases growth and photosynthesis of Pinus taeda: A 4 year experiment in the field. Plant Cell Environ 20: 1123-1134. http://dx.doi.org/10.1046/j.1365-3040.1997.d01-140.x

Tjoelker, MG; Volin, JC; Oleksyn, J; Reich, PB. (1995). Interaction of ozone pollution and light effects on photosynthesis in a forest canopy experiment. Plant Cell Environ 18: 895-905. http://dx.doi.org/10.1111/j.1365-3040.1995.tb00598.x

Tobiessen, P. (1982). Dark opening of stomata in successional trees. Oecologia 52: 356-359. http://dx.doi.org/10.1007/BF00367959

Toet, S; Ineson, P; Peacock, S; Ashmore, M. (2011). Elevated ozone reduces methane emissions from peatland mesocosms. Global Change Biol 17: 288-296. http://dx.doi.org/10.1111/j.1365-2486.2010.02267.x

Tong, D; Mathur, R; Schere, K; Kang, D; Yu, S. (2007). The use of air quality forecasts to assess impacts of air pollution on crops: Methodology and case study. Atmos Environ 41: 8772-8784. http://dx.doi.org/10.1016/j.atmosenv.2007.07.060

Tong, DQ; Mauzerall, DL. (2008). Summertime state-level source-receptor relationships between nitrogen oxides emissions and surface ozone concentrations over the continental United States. Environ Sci Technol 42: 7976-7984. http://dx.doi.org/10.1021/es7027636

Topa, MA; Vanderklein, DW; Corbin, A. (2001). Effects of elevated ozone and low light on diurnal and seasonal carbon gain in sugar maple. Plant Cell Environ 24: 663-677.

Torsethaugen, G; Pell, EJ; Assmann, SM. (1999). Ozone inhibits guard cell K+ channels implicated in stomatal opening. PNAS 96: 13577-13582.

Tosti, N; Pasqualini, S; Borgogni, A; Ederli, L; Falistocco, E; Crispi, S; Paolocci, F. (2006). Gene expression profiles of O3-treated Arabidopsis plants. Plant Cell Environ 29: 1686-1702. http://dx.doi.org/10.1111/j.1365-3040.2006.01542.x

Turnipseed, AA; Burns, SP; Moore, DJP; Hu, J; Guenther, AB; Monson, RK. (2009). Controls over ozone deposition to a high elevation subalpine forest. Agr Forest Meteorol 149: 1447-1459. http://dx.doi.org/10.1016/j.agrformet.2009.04.001

U.S. EPA (U.S. Environmental Protection Agency). (1977). Photochemical oxidant air pollutant effects on a mixed conifer forest ecosystem: A progress report [EPA Report]. (EPA-600/3-77-104). Corvallis, OR. http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=20015IYP.txt

U.S. EPA (U.S. Environmental Protection Agency). (1978a). Air quality criteria for ozone and other photochemical oxidants [EPA Report]. (EPA/600/8-78/004). Washington, DC.

U.S. EPA (U.S. Environmental Protection Agency). (1984). Air quality criteria for ozone and other photochemical oxidants, volume III of V (review draft) [EPA Report]. (EPA-600/8-84-020A3). Research Triangle Park, NC. http://www.ntis.gov/search/product.aspx?ABBR=PB85126050

U.S. EPA (U.S. Environmental Protection Agency). (1986). Air quality criteria for ozone and other photochemical oxidants [EPA Report]. (EPA-600/8-84-020aF - EPA-600/8-84-020eF). Research Triangle Park, NC. http://www.ntis.gov/search/product.aspx?ABBR=PB87142949

U.S. EPA (U.S. Environmental Protection Agency). (1996b). Air quality criteria for ozone and related photochemical oxidants, Vol. II of III [EPA Report]. (EPA/600/P-93/004BF). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (1996c). Air quality criteria for ozone and related photochemical oxidants, Vol. III of III [EPA Report]. (EPA/600/P-93/004cF). Research Triangle Park, NC. http://www.ntis.gov/search/product.aspx?ABBR=PB96185608

U.S. EPA (U.S. Environmental Protection Agency). (1996e). Review of national ambient air quality standards for ozone: Assessment of scientific and technical information: OAQPS staff paper [EPA Report]. (EPA/452/R-96/007). Research Triangle Park, NC. http://www.ntis.gov/search/product.aspx?ABBR=PB96203435

U.S. EPA (U.S. Environmental Protection Agency). (2006b). Air quality criteria for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-05/004AF). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=149923

U.S. EPA (U.S. Environmental Protection Agency). (2007b). Review of the national ambient air quality standards for ozone: Policy assessment of scientific and technical information: OAQPS staff paper [EPA Report]. (EPA/452/R-07/003). Research Triangle Park, NC. http://www.epa.gov/ttn/naaqs/standards/ozone/data/2007_01_ozone_staff_paper

U.S. EPA (U.S. Environmental Protection Agency). (2008b). Integrated science assessment for oxides of nitrogen and sulfur: Ecological criteria [EPA Report]. (EPA/600/R-08/082F). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=201485

U.S. Forest Service, National Park Service,, and U.S. Fish and Wildlife Service, . (2000). Federal land managers air quality related values work group (FLAG): Phase I report. Denver, CO: U.S. Forest Service. http://www.nature.nps.gov/air/permits/flag/index.cfm

Uddling, J; Hogg, AJ; Teclaw, RM; Carroll, MA; Ellsworth, DS. (2010). Stomatal uptake of O3 in aspen and aspen-birch forests under free-air CO2 and O3 enrichment. Environ Pollut 158: 2023-2031. http://dx.doi.org/10.1016/j.envpol.2009.12.001

Uddling, J; Teclaw, RM; Kubiske, ME; Pregitzer, KS; Ellsworth, DS. (2008). Sap flux in pure aspen and mixed aspen-birch forests exposed to elevated concentrations of carbon dioxide and ozone. Tree Physiol 28: 1231-1243.

Uddling, J; Teclaw, RM; Pregitzer, KS; Ellsworth, DS. (2009). Leaf and canopy conductance in aspen and aspen-birch forests under free-air enrichment of carbon dioxide and ozone. Tree Physiol 29: 1367-1380. http://dx.doi.org/10.1093/treephys/tpp070

UIUC (University of Illinois at Urbana-Champain). (2010). SoyFACE. Available online at http://soyface.illinois.edu/ (accessed December 8, 2010).

UNECE (United Nations Economic Commission for Europe). (1988). ECE critical levels workshop; March; Bad Harzburg, Germany. Geneva, Switzerland.

UNEP (United Nations Environment Programme). (2003). Ecosystems and human well-being: A framework for assessment. Washington, DC: Island Press.

Unsworth, MH; Heagle, AS; Heck, WW. (1984a). Gas exchange in open-top field chambers: I. Measurement and analysis of atmospheric resistances to gas exchange. Atmos Environ 18: 373-380. http://dx.doi.org/10.1016/0004-6981(84)90111-2

Unsworth, MH; Heagle, AS; Heck, WW. (1984b). Gas exchange in open-top field chambers: II. Resistances to ozone uptake by soybeans. Atmos Environ 18: 381-385. http://dx.doi.org/10.1016/0004-6981(84)90112-4

USDA (U.S. Department of Agriculture). (2011). Ozone biomonitoring program. Available online at http://www.nrs.fs.fed.us/fia/topics/ozone/ (accessed January 28, 2011).

Vahisalu, T; Kollist, H; Wang, YF; Nishimura, N; Chan, WY; Valerio, G; Lamminmäki, A; Brosché, M; Moldau, H; Desikan, R; Schroeder, JI; Kangasjärvi, J. (2008). SLAC1 is required for plant guard cell S-type anion channel function in stomatal signalling. Nature 452: 487-491. http://dx.doi.org/10.1038/nature06608

Valkama, E; Koricheva, J; Oksanen, E. (2007). Effects of elevated O3, alone and in combination with elevated CO2, on tree leaf chemistry and insect herbivore performance: A meta-analysis. Global Change Biol 13: 184-201. http://dx.doi.org/10.1111/j.1365-2486.01284.x

van Buuren, ML; Guidi, L; Fornale, S; Ghetti, F; Franceschetti, M; Soldatini, GF; Bagni, N. (2002). Ozone-response mechanisms in tobacco: Implications of polyamine metabolism. New Phytol 156: 389-398. http://dx.doi.org/10.1046/j.1469-8137.2002.00539.x

Van Dingenen, R; Dentener, FJ; Raes, F; Krol, MC; Emberson, L; Cofala, J. (2009). The global impact of ozone on agricultural crop yields under current and future air quality legislation. Atmos Environ 43: 604-618. http://dx.doi.org/10.1016/j.atmosenv.2008.10.033

Vandermeiren, K; Black, C; Pleijel, H; de Temmerman, L. (2005). Impact of rising tropospheric ozone on potato: Effects on photosynthesis, growth, productivity and yield quality. Plant Cell Environ 28: 982-996. http://dx.doi.org/10.1111/j.1365-3040.2005.01316.x

Velikova, V; Pinelli, P; Pasqualini, S; Reale, L; Ferranti, F; Loreto, F. (2005). Isoprene decreases the concentration of nitric oxide in leaves exposed to elevated ozone. New Phytol 166: 419-426. http://dx.doi.org/10.1111/j.1469-8137.2005.01409.x

Vickers, CE; Possell, M; Cojocariu, CI; Velikova, VB; Laothawornkitkul, J; Ryan, A; Mullineaux, PM; Hewitt, CN. (2009). Isoprene synthesis protects transgenic tobacco plants from oxidative stress. Plant Cell Environ 32: 520-531. http://dx.doi.org/10.1111/j.1365-3040.2009.01946.x

Vigue, LM; Lindroth, RL. (2010). Effects of genotype, elevated CO2 and elevated O3 on aspen phytochemistry and aspen leaf beetle Chrysomela crotchi performance. Agr Forest Entomol 12: 267-276. http://dx.doi.org/10.1111/j.1461-9563.2010.00475.x

Volk, M; Bungener, P; Contat, F; Montani, M; Fuhrer, J. (2006). Grassland yield declined by a quarter in 5 years of free-air ozone fumigation. Global Change Biol 12: 74-83. http://dx.doi.org/10.1111/j.1365-2486.2005.01083.x

Volk, M; Geissmann, M; Blatter, A; Contat, F; Fuhrer, J. (2003). Design and performance of a free-air exposure system to study long-term effects of ozone on grasslands. Atmos Environ 37: 1341-1350.

Volk, M; Obrist, D; Novak, K; Giger, R; Bassin, S; Fuhrer, J. (2011). Subalpine grassland carbon dioxide fluxes indicate substantial carbon losses under increased nitrogen deposition, but not at elevated ozone concentration. Global Change Biol 17: 366-376. http://dx.doi.org/10.1111/j.1365-2486.2010.02228.x

Vollenweider, P; Woodcock, H; Kelty, MJ; Hofer, RM. (2003). Reduction of stem growth and site dependency of leaf injury in Massachusetts black cherries exhibiting ozone symptoms. Environ Pollut 125: 467-480.

Vollsnes, AV; Kruse, OMO; Eriksen, AB; Oxaal, U; Futsaether, CM. (2010). In vivo root growth dynamics of ozone exposed Trifolium subterraneum. Environ Exp Bot 69: 183-188. http://dx.doi.org/10.1016/j.envexpbot.2010.03.007

von Tiedemann, A. (1996). Single and combined effects of nitrogen fertilization and ozone on fungal leaf diseases on wheat. Z Pflanzenkrankh Pflanzenschutz 103: 409-419.

Vuorinen, T; Nerg, AM; Holopainen, JK. (2004). Ozone exposure triggers the emission of herbivore-induced plant volatiles, but does not disturb tritrophic signalling. Environ Pollut 131: 305-311. http://dx.doi.org/10.1016/j.envpol.2004.02.027

Wallin, G; Skärby, L. (1992). The influence of ozone on the stomatal and non-stomatal limitation of photosynthesis in Norway spruce, Picea abies (L.) Karst, exposed to soil moisture deficit. Trees Struct Funct 6: 128-136. http://dx.doi.org/10.1007/BF00202428

Wang, L; He, X; Chen, W. (2009b). Effects of elevated ozone on photosynthetic CO2 exchange and chlorophyll a fluorescence in leaves of Quercus mongolica grown in urban area. Bull Environ Contam Toxicol 82: 478-481. http://dx.doi.org/10.1007/s00128-008-9606-3

Wang, X; Mauzerall, DL. (2004). Characterizing distributions of surface ozone and its impact on grain production in China, Japan and South Korea: 1990 and 2020. Atmos Environ 38: 4383-4402. http://dx.doi.org/10.1016/j.atmosenv.2004.03.067

Wang, X; Taub, DR. (2010). Interactive effects of elevated carbon dioxide and environmental stresses on root mass fraction in plants: A meta-analytical synthesis using pairwise techniques. Oecologia 163: 1-11. http://dx.doi.org/10.1007/s00442-010-1572-x

Wang, X; Zheng, Q; Feng, Z; Xie, J; Feng, Z; Ouyang, Z; Manning, WJ. (2008). Comparison of a diurnal vs steady-state ozone exposure profile on growth and yield of oilseed rape (Brassica napus L.) in open-top chambers in the Yangtze Delta, China. Environ Pollut 156: 449-453. http://dx.doi.org/10.1016/j.envpol.2008.01.027

Watanabe, M; Yamaguchi, M; Tabe, C; Iwasaki, M; Yamashita, R; Funada, R; Fukami, M; Matsumura, H; Kohno, Y; Izuta, T. (2007). Influences of nitrogen load on the growth and photosynthetic responses of Quercus serrata seedlings to O3. Trees Struct Funct 21: 421-432. http://dx.doi.org/10.1007/s00468-007-0134-2

Weinstein, DA; Gollands, B; Retzlaff, WA. (2001). The effects of ozone on a lower slope forest of the Great Smoky Mountain National Park: Simulations linking an individual tree model to a stand model. Forest Sci 47: 29-42.

Weinstein, DA; Laurence, JA; Retzlaff, WA; Kern, JS; Lee, EH; Hogsett, WE; Weber, J. (2005). Predicting the effects of tropospheric ozone on regional productivity of ponderosa pine and white fir. For Ecol Manage 205: 73-89. http://dx.doi.org/10.1016/j.foreco.2004.10.007

Werner, H; Fabian, P. (2002). Free-air fumigation of mature trees: A novel system for controlled ozone enrichment in grown-up beech and spruce canopies. Environ Sci Pollut Res Int 9: 117-121.

Wesely, ML; Hicks, BB. (2000). A review of the current status of knowledge on dry deposition [Review]. Atmos Environ 34: 2261-2282. http://dx.doi.org/10.1016/S1352-2310(99)00467-7

Whitfield, CP; Davison, AW; Ashenden, TW. (1996). Interactive effects of ozone and soil volume on Plantago major. New Phytol 134: 287-294. http://dx.doi.org/10.1111/j.1469-8137.1996.tb04633.x

Whitfield, CP; Davison, AW; Ashenden, TW. (1997). Artificial selection and heritability of ozone resistance in two populations of Plantago major. New Phytol 137: 645-655.

Wieser, G; Havranek, WM. (1995). Environmental control of ozone uptake in Larix decidua Mill: A comparison between different altitudes. Tree Physiol 15: 253-258.

Wieser, G; Manning, WJ; Tausz, M; Bytnerowicz, A. (2006). Evidence for potential impacts of ozone on Pinus cembra L. at mountain sites in Europe: An overview. Environ Pollut 139: 53-58. http://dx.doi.org/10.1016/j.envpol.2005.04.037

Wilkinson, S; Davies, WJ. (2010). Drought, ozone, ABA and ethylene: New insights from cell to plant to community [Review]. Plant Cell Environ 33: 510-525. http://dx.doi.org/10.1111/j.1365-3040.2009.02052.x

Will, RE; Ceulemans, R. (1997). Effects of elevated CO2 concentration on photosynthesis, respiration and carbohydrate status of coppice Populus hybrids. Physiol Plant 100: 933-939. http://dx.doi.org/10.1111/j.1399-3054.1997.tb00020.x

Winner, WE; Lefohn, AS; Cotter, IS; Greitner, CS; Nellessen, J; McEvoy, LR, Jr; Olson, RL; Atkinson, CJ; Moore, LD. (1989). Plant responses to elevational gradients of O3 exposures in Virginia. PNAS 86: 8828-8832.

Wittig, VE; Ainsworth, EA; Long, SP. (2007). To what extent do current and projected increases in surface ozone affect photosynthesis and stomatal conductance of trees? A meta-analytic review of the last 3 decades of experiments [Review]. Plant Cell Environ 30: 1150-1162. http://dx.doi.org/10.1111/j.1365-3040.2007.01717.x

Wittig, VE; Ainsworth, EA; Naidu, SL; Karnosky, DF; Long, SP. (2009). Quantifying the impact of current and future tropospheric ozone on tree biomass, growth, physiology and biochemistry: A quantitative meta-analysis. Global Change Biol 15: 396-424. http://dx.doi.org/10.1111/j.1365-2486.2008.01774.x

Woo, SY; Hinckley, TM. (2005). The effects of ozone on growth and stomatal response in the F-2 generation of hybrid poplar (Populus trichocarpa x Populus deltoides). Biol Plantarum 49: 395-404. http://dx.doi.org/10.1007/s10535-005-0014-9

Wright, GA; Lutmerding, A; Dudareva, N; Smith, BH. (2005). Intensity and the ratios of compounds in the scent of snapdragon flowers affect scent discrimination by honeybees (Apis mellifera). J Comp Physiol A Neuroethol Sens Neural Behav Physiol 191: 105-114.

Yamaguchi, M; Watanabe, M; Iwasaki, M; Tabe, C; Matsumura, H; Kohno, Y; Izuta, T. (2007). Growth and photosynthetic responses of Fagus crenata seedlings to O3 under different nitrogen loads. Trees Struct Funct 21: 707-718. http://dx.doi.org/10.1007/s00468-007-0163-x

Yan, K; Chen, W; He, XY; Zhang, GY; Xu, S; Wang, LL. (2010). Responses of photosynthesis, lipid peroxidation and antioxidant system in leaves of Quercus mongolica to elevated O3. Environ Exp Bot 69: 198-204. http://dx.doi.org/10.1016/j.envexpbot.2010.03.008

Yoshida, S; Tamaoki, M; Ioki, M; Ogawa, D; Sato, Y; Aono, M; Kubo, A; Saji, S; Saji, H; Satoh, S; Nakajima, N. (2009). Ethylene and salicylic acid control glutathione biosynthesis in ozone-exposed Arabidopsis thaliana. Physiol Plant 136: 284-298. http://dx.doi.org/10.1111/j.1399-3054.2009.01220.x

Younglove, T; McCool, PM; Musselman, RC; KahI, ME. (1994). Growth-stage dependent crop yield response to ozone exposure. Environ Pollut 86: 287-295. http://dx.doi.org/10.1016/0269-7491(94)90169-4

Yuan, JS; Himanen, SJ; Holopainen, JK; Chen, F; Stewart, CN, Jr. (2009). Smelling global climate change: Mitigation of function for plant volatile organic compounds [Review]. Trends Ecol Evol 24: 323-331. http://dx.doi.org/10.1016/j.tree.2009.01.012

Yun, SC; Laurence, JA. (1999). The response of sensitive and tolerant clones of Populus tremuloides to dynamic ozone exposure under controlled environmental conditions. New Phytol 143: 305-313.

Zak, DR; Holmes, WE; Pregitzer, KS. (2007). Atmospheric CO2 and O3 alter the flow of N15 in developing forest ecosystems. Ecology 88: 2630-2639.

Zhang, C; Tian, HQ; Chappelka, AH; Ren, W; Chen, H; Pan, SF; Liu, ML; Styers, DM; Chen, GS; Wang, YH. (2007a). Impacts of climatic and atmospheric changes on carbon dynamics in the Great Smoky Mountains National Park. Environ Pollut 149: 336-347. http://dx.doi.org/10.1016/j.envpol.2007.05.028

Zhang, J; Schaub, M; Ferdinand, JA; Skelly, JM; Steiner, KC; Savage, JE. (2010a). Leaf age affects the responses of foliar injury and gas exchange to tropospheric ozone in Prunus serotina seedlings. Environ Pollut 158: 2627-2634. http://dx.doi.org/10.1016/j.envpol.2010.05.003

Zheng, F; Wang, X; Lu, F; Hou, P; Zhang, W; Duan, X; Zhou, X; Ai, Y; Zheng, H; Ouyang, Z; Feng, Z. (2011). Effects of elevated ozone concentration on methane emission from a rice paddy in Yangtze River Delta, China. Global Change Biol 17: 898-910. http://dx.doi.org/10.1111/j.1365-2486.2010.02258.x

# 10 THE ROLE OF TROPOSPHERIC OZONE IN CLIMATE CHANGE AND UV-B SHIELDING EFFECTS

## 10.1 Introduction

Atmospheric $O_3$ plays an important role in the Earth's energy budget by interacting with incoming solar radiation and outgoing infrared radiation. Over mid-latitudes, approximately 90% of the total atmospheric $O_3$ column is located in the stratosphere (Kar et al., 2010; Crist et al., 1994). Therefore, tropospheric $O_3$ makes up a relatively small portion (~10%) of the total column of $O_3$ over mid-latitudes, but it does play an important role in the overall radiation budget. The next section (Section 10.2) briefly describes the physics of the earth's radiation budget, providing background material for the subsequent two sections assessing how perturbations in tropospheric $O_3$ concentrations might affect (1) climate through its role as a greenhouse gas (Section 10.3), and (2) health, ecology and welfare through its role in shielding the earth's surface from solar ultraviolet radiation (Section 10.4). The concluding section in this chapter (Section 10.5) includes a summary of effects assessed in this chapter along with their associated causal determinations.

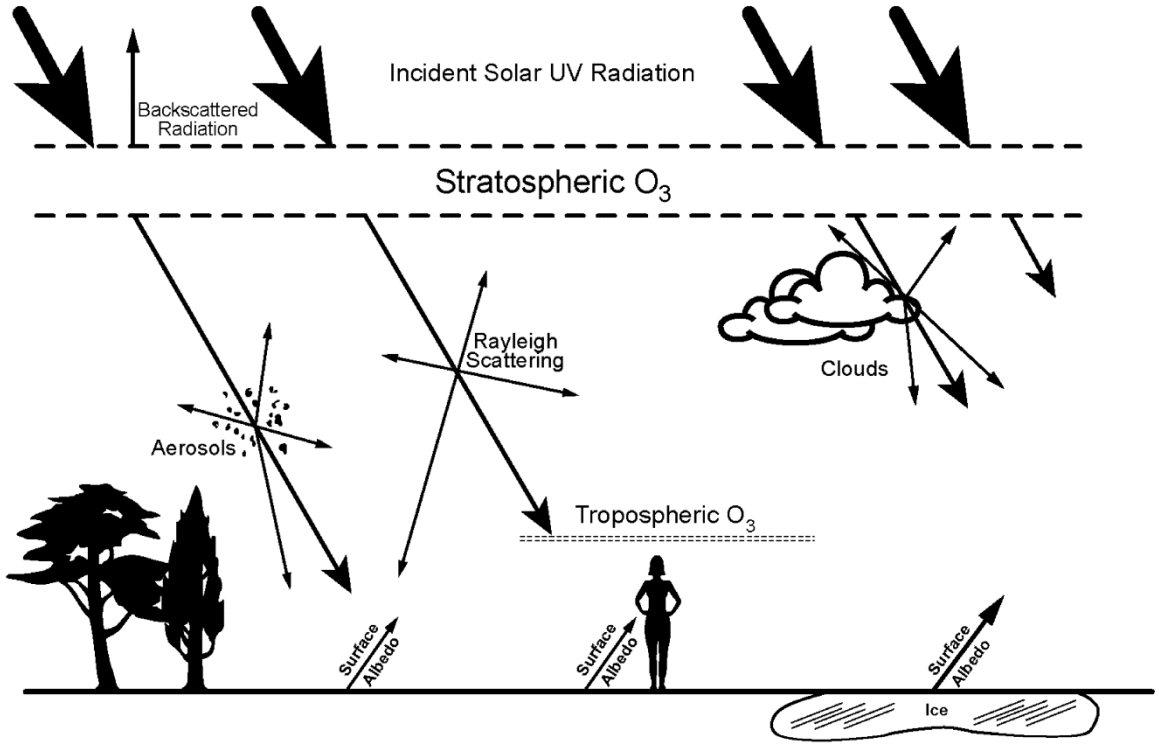
## 10.2 Physics of the Earth's Radiation Budget

Radiant energy from the sun enters the atmosphere in a range of wavelengths, but peaks strongly in the visible (400-750 nm) part of the spectrum. Longer wavelength infrared (750 nm-1 mm) and shorter wavelength ultraviolet (100-400 nm) radiation are also present in the solar electromagnetic spectrum. Since the energy possessed by a photon is inversely proportional to its wavelength, infrared (IR) radiation carries the least energy per photon, and ultraviolet (UV) radiation carries the most energy per photon. Ultraviolet radiation is further subdivided into classes (bands) based on wavelength: UV-A refers to wavelengths from 400-315 nm; UV-B from 315-280 nm; and UV-C from 280-100 nm. Within the UV spectrum, UV-A radiation is the least energetic band and UV-C is the most energetic band.

The wavelength of radiation also determines how the photons interact with the complex mixture of gases, clouds, and particles present in the atmosphere (see Figure 10-1). UV-A radiation can be scattered but is not absorbed to any meaningful degree by atmospheric gases including $O_3$. UV-B radiation is absorbed and scattered in part within the atmosphere. UV-C is almost entirely blocked by the Earth's upper atmosphere, where it participates in photoionization and photodissociation processes including absorption by stratospheric $O_3$. Since UV-A radiation is less energetic and does not interact with $O_3$ in the troposphere or the stratosphere and UV-C radiation is almost entirely blocked by stratospheric $O_3$, UV-B radiation is the most important band to consider in relation to tropospheric $O_3$ shielding.

Tropospheric $O_3$ plays a "disproportionate" role in absorbing UV-B radiation compared with stratospheric $O_3$ on a molecule per molecule basis (Balis et al., 2002; Zerefos et al., 2002; Crist et al., 1994; Bruhl and Crutzen, 1989). This effect results from the higher atmospheric pressure present in the troposphere, resulting in higher concentrations of gas molecules present that can absorb or scatter radiation. For this reason, the troposphere is referred to as a "multiple scattering" regime for UV absorption, compared to the "single scattering" regime in the stratosphere. Thus, careful quantification of atmospheric absorbers and scatterers, along with a well-resolved description of the physics of these interactions, is necessary for predicting the effects of tropospheric $O_3$ on UV-B flux at the surface.

Solar flux at all wavelengths has a temporal dependence, while radiative scattering and absorption have strong wavelength, path length, and gas/particle concentration dependencies. These combine to create nonlinear effects on UV flux at the Earth's surface. Chapter 10 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b) describes in detail several key factors that influence the spatiotemporal distribution of ground-level UV radiation flux, including: (1) long-term solar activity including sunspot cycle; (2) solar rotation; (3) the position of the Earth in its orbit around the sun; (4) atmospheric absorption and scattering of UV radiation by gas molecules and aerosol particles; (5) absorption and scattering by stratospheric and tropospheric clouds; and (6) surface albedo. The efficiencies of absorption and scattering are highly dependent on the concentration of the scattering medium, particle size (for aerosols and clouds), and the altitude at which these processes are occurring. These properties are sensitive to meteorology, which introduces additional elements of spatial and temporal dependency in ground-level UV radiation flux.




Source: 2006 $O_3$ AQCD (U.S. EPA, 2006b).

**Figure 10-1 Diagram of the factors that determine human exposure to ultraviolet radiation.**

About 30% of incoming solar radiation is directly reflected back to space, mainly by clouds or surfaces with high albedo (reflectivity), such as snow, ice, and desert sand. Radiation that does penetrate to the Earth's surface and is absorbed can be re-emitted in the longwave (infrared) portion of the spectrum; the rest goes into evaporating water or soil moisture or emerges as sensible heat. The troposphere is opaque to the outgoing longwave radiation. Polyatomic gases such as water vapor, $CO_2$, $CH_4$, and $O_3$ absorb and re-emit the radiation upwelling from the Earth's surface, reducing the efficiency with which that energy returns to space. In effect, these gases act as a blanket warming the Earth's surface. This phenomenon, known as the "Greenhouse Effect," was first quantified in the 19th century (Arrhenius, 1896), and gives rise to the term "greenhouse gas." The most important greenhouse gas is water vapor.

## 10.3 Effects of Tropospheric $O_3$ on Climate

### 10.3.1 Background

As a result of its interaction with incoming solar radiation and outgoing longwave radiation, tropospheric $O_3$ plays a major role in determining climate, and increases in its abundance may contribute to climate change (IPCC, 2007c). Models estimate that the global average concentration of $O_3$ in the troposphere has increased 30-70% since the pre-industrial era (Gauss et al., 2006), while observations indicate that in some regions tropospheric $O_3$ concentrations may have increased by factors as great as 4 or 5 (Marenco et al., 1994; Staehelin et al., 1994). These increases are tied to the rise in emissions of $O_3$ precursors from human activity, mainly fossil fuel consumption and agricultural processes.

The effect on climate of the tropospheric $O_3$ concentration change since pre-industrial times has been estimated to be about 25-40% of the anthropogenic $CO_2$ effect and about 75% of the anthropogenic $CH_4$ effect (IPCC, 2007c), ranking it third in importance behind these two major greenhouse gases. In the 21st century, as the Earth's population continues to grow and energy technology spreads to developing countries, a further rise in the global concentration of tropospheric $O_3$ is likely, with associated consequences for human health and ecosystems relating to climate change.

To examine the science of a changing climate and to provide balanced and rigorous information to policy makers, the World Meteorological Organization (WMO) and the United Nations Environment Programme (UNEP) formed the Intergovernmental Panel on Climate Change (IPCC) in 1988. The IPCC supports the work of the Conference of Parties (COP) to the United Nations Framework Convention on Climate Change (UNFCCC). The IPCC periodically brings together climate scientists from member countries of WMO and the United Nations to review knowledge of the physical climate system, past and future climate change, and evidence of human-induced climate change. IPCC climate assessment reports are issued every five to seven years.

This section draws in part on the fourth IPCC Assessment Report (AR4) (IPCC, 2007c), as well as other peer-reviewed published research. Section 10.3.2 reviews evidence of climate change in the recent past and projections of future climate change. It also offers a brief comparison of tropospheric $O_3$ relative to other greenhouse gases. Section 10.3.3 describes factors that influence the magnitude of tropospheric $O_3$ effects on climate. Section 10.3.4 considers the competing effects of $O_3$ precursors on climate. Finally, Section 10.3.5 and Section 10.3.6 describe the effects of changing tropospheric $O_3$ concentrations on past and future climate. Downstream effects resulting from climate change, such as ecosystem responses, are outside the scope of this assessment, which focuses rather on the effects of changes in tropospheric $O_3$ concentrations on radiative forcing and climate.

## 10.3.2 Climate Change Evidence and the Influence of Tropospheric $O_3$

### 10.3.2.1 Climate Change in the Recent Past

From the end of the last ice age 12,000 years ago until the mid-1800s, observations from ice cores show that concentrations of the long-lived greenhouse gases $CO_2$, $CH_4$, and $N_2O$ have been relatively stable. Unlike these greenhouse gases, $O_3$ is not preserved in ice, and no record of it before the late 1800s exists. Models, however, suggest that it, too, has remained relatively constant during this time period (Thompson et al., 1993; Thompson, 1992). The stable mix of these greenhouse gases in the atmosphere, together with water vapor, has kept the global mean temperature of the Earth close to 15ºC. Without the presence of greenhouse gases in the atmosphere, the Earth's global mean temperature would be about 30ºC cooler, or -15ºC.

Since the start of the Industrial Revolution, human activity has led to observable increases of greenhouse gases in the atmosphere, mainly through fossil fuel combustion. According to the IPCC AR4 (IPCC, 2007c), there is now "very high confidence" that the net effect of anthropogenic greenhouse gas emissions since 1750 has led to warming, and it is "very likely" that human activity contributed to the 0.76ºC rise in global mean temperature observed over the last century. The increase of tropospheric $O_3$ abundance may have contributed 0.1-0.3ºC warming to the global climate during this time period (Hansen et al., 2005; Mickley et al., 2004). Global cooling due to anthropogenic aerosols (IPCC, 2007c) has likely masked the full warming effect of the anthropogenic greenhouse gases on a global scale.

### 10.3.2.2 Projections of Future Climate Change

The IPCC AR4 projects a warming of ~0.2ºC per decade for the remainder of the 21st century (IPCC, 2007c). Even at constant concentrations of greenhouse gases in the atmosphere, temperatures are expected to increase by about 0.1ºC per decade, due to the slow response of oceans to the warming applied so far. It is likely that the Earth will experience longer and more frequent heat waves in the 21st century, together with more frequent droughts and/or heavy precipitation events in some regions, due to perturbations in the hydrological cycle that result from changing temperatures. Sea levels could increase by 0.3-0.8 meters by 2300 due to thermal expansion of the oceans. The extent of Arctic sea ice is expected to decline, and contraction of the Greenland ice sheet could further contribute to the sea level rise (IPCC, 2007c).

Projections of future climate change are all associated with some degree of uncertainty. A major uncertainty involves future trends in the anthropogenic emissions of greenhouse gases or their precursors. For the IPCC AR4 climate projections, a set of distinct "storylines" or emission pathways was developed (IPCC,

2000). Each storyline took into account factors such as population growth, mix of energy technologies, and the sharing of technology between developed and developing nations, and each resulted in a different scenario for anthropogenic emissions. When these trends in emissions are applied to models, these scenarios yield a broad range of possible climate trajectories for the 21st century.

A second factor bringing large uncertainty to model projections of future climate is the representation of climate and, especially, climate feedbacks. A rise in surface temperatures would perturb a suite of other processes in the earth-atmosphere-ocean system, which may in turn either amplify the temperature increase (positive feedback) or diminish it (negative feedback). One important feedback involves the increase of water vapor content of the atmosphere that would accompany higher temperatures (Bony et al., 2006). Water vapor is a potent greenhouse gas; accounting for the water vapor feedback may increase the climate sensitivity to a doubling of $CO_2$ by nearly a factor of two (Held and Soden, 2000). The ice-albedo feedback is also strongly positive; a decline in snow cover and sea ice extent would diminish the Earth's albedo, allowing more solar energy to be retained at the surface (Holland and Bitz, 2003; Rind et al., 1995). A final example of a climate feedback involves the effects of changing cloud cover in a warming atmosphere. Models disagree on the magnitude and even the sign of the cloud cover feedback on surface temperatures (Soden and Held, 2006).

#### 10.3.2.3 Metrics of Potential Climate Change

Two metrics frequently used to estimate the potential climate effect of some perturbation such as a change in greenhouse gas concentration are: (1) radiative forcing; and (2) global warming potential (GWP). These metrics differ in a fundamental way as described below.

Radiative forcing is a change in the radiative balance at a particular level of the atmosphere or at the surface when a perturbation is introduced in the earth-atmosphere-ocean system. In the global mean, radiative forcing of greenhouse gases at the tropopause (top of the troposphere) is roughly proportional to the surface temperature response (Hansen et al., 2005; NRC, 2005). It thus provides a useful metric for policymakers for assessing the response of the earth's surface temperature to a given change in the concentration of a greenhouse gas. Positive values of radiative forcing indicate warming in a test case relative to the control; negative values indicate cooling. The units of radiative forcing are energy flux per area, or $W/m^2$.

Radiative forcing requires just a few model years to calculate, and it shows consistency from model to model. However, radiative forcing does not take into account the climate feedbacks that could amplify or dampen the actual surface temperature response, depending on region. Quantifying the change in surface temperature requires a climate simulation in which all important feedbacks are accounted for. As some of these processes are not well understood, the surface

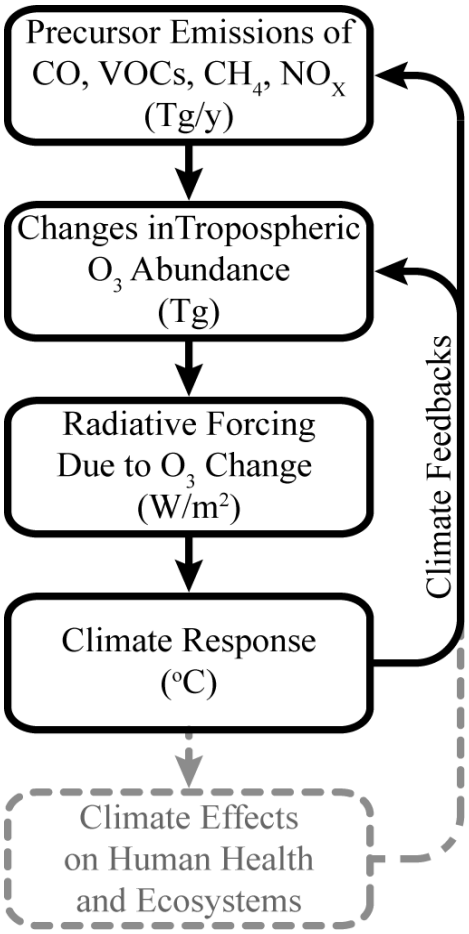
temperature response to a given radiative forcing can be highly uncertain and can vary greatly among models and even from region to region within the same model.

GWP indicates the integrated radiative forcing over a specified period (usually 100 years) from a unit mass pulse emission of a greenhouse gas or its precursor, and is reported as the magnitude of this radiative forcing relative to that of $CO_2$. GWP is most useful for comparing the potential climate effects of long-lived gases, such as $N_2O$ or $CH_4$. Since tropospheric $O_3$ has a lifetime on the order of weeks to months, GWP is not seen as a valuable metric for quantifying the importance of $O_3$ on climate (Forster et al., 2007). Thus, this assessment focuses on radiative forcing as the metric of climate influence resulting from changes in tropospheric $O_3$ concentrations.

#### 10.3.2.4 Tropospheric $O_3$ as a Greenhouse Gas

Tropospheric $O_3$ differs in important ways from other greenhouse gases. It is not emitted directly, but is produced through photochemical oxidation of CO, $CH_4$, and nonmethane volatile organic compounds (VOCs) in the presence of nitrogen oxide radicals ($NO_X$ = NO + $NO_2$; see Chapter 3, Section 3.2 for further details on the chemistry of $O_3$ formation). It is also supplied by vertical transport from the stratosphere. The lifetime of $O_3$ in the troposphere is typically a few weeks, resulting in an inhomogeneous distribution that varies seasonally; the distribution of the long-lived greenhouse gases like $CO_2$ and $CH_4$ are much more uniform. The longwave radiative forcing by $O_3$ is mainly due to absorption in the 9.6 μm window, where absorption by water vapor is weak. It is therefore less sensitive to local humidity than the radiative forcing by $CO_2$ or $CH_4$, for which there is much more overlap with the water absorption bands (Lenoble, 1993). And unlike other major greenhouse gases, $O_3$ absorbs in the shortwave as well as the longwave part of the spectrum.

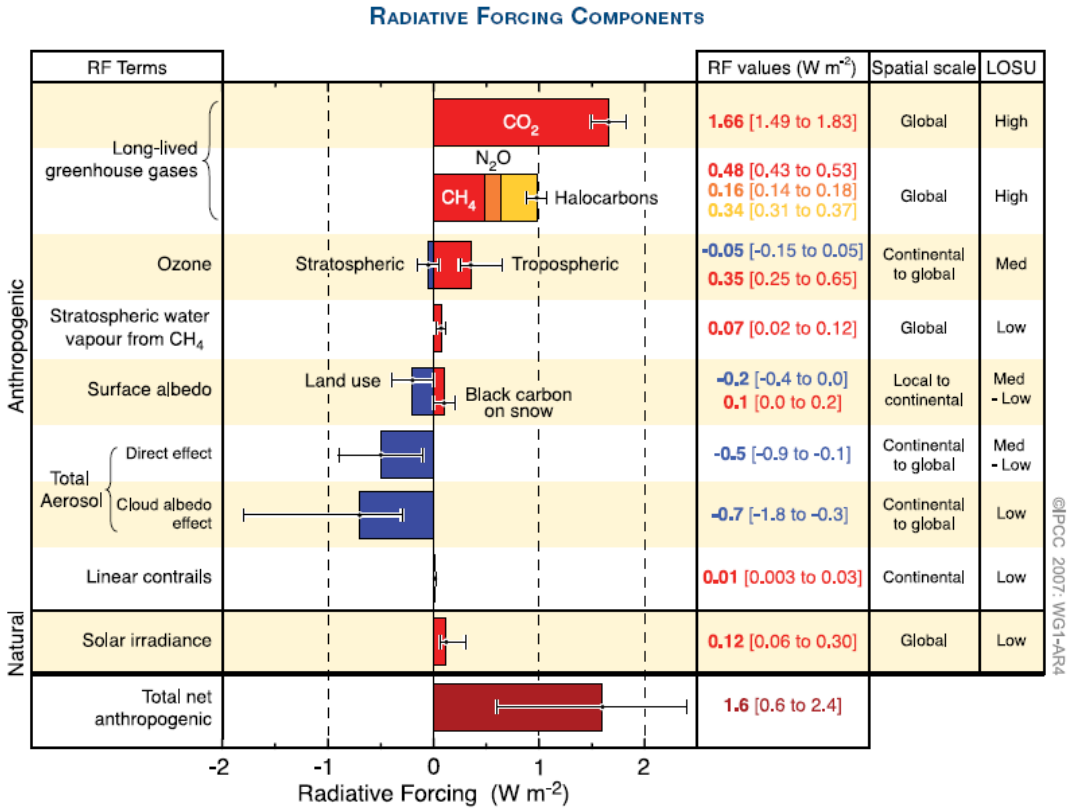
Figure 10-2 shows the main steps involved in the influence of tropospheric $O_3$ on climate. Emissions of $O_3$ precursors including CO, VOCs, $CH_4$, and $NO_X$ lead to production of tropospheric $O_3$. A change in the abundance of tropospheric $O_3$ perturbs the radiative balance of the atmosphere, an effect quantified by the radiative forcing metric. The earth-atmosphere-ocean system responds to the radiative forcing with a climate response, typically expressed as a change in surface temperature. Finally, the climate response causes downstream climate-related health and ecosystem effects, such as redistribution of diseases or ecosystem characteristics due to temperature changes. Feedbacks from both the climate response and downstream effects can, in turn, affect the abundance of tropospheric $O_3$ and $O_3$ precursors through multiple mechanisms. Direct feedbacks are discussed further in Section 10.3.3.4; the downstream climate effects and their long-term feedbacks are extremely complex and outside the scope of this assessment.




Note: This figure includes the relationship between precursor emissions, changes in tropospheric $O_3$ abundance, radiative forcing, climate response, and climate effects. Units shown are those typical for each quantity illustrated. Feedbacks from both the climate response and climate effects can, in turn, affect the abundance of tropospheric $O_3$ and $O_3$ precursors through multiple feedback mechanisms. Climate effects and their feedbacks are deemphasized in the figure since these downstream effects are extremely complex and outside the scope of this assessment.

**Figure 10-2 Schematic illustrating the effects of tropospheric $O_3$ on climate.**

The IPCC (2007c) reported a radiative forcing of 0.35 $W/m^2$ for the change in tropospheric $O_3$ abundance since the pre-industrial era, ranking it third in importance after the greenhouse gases $CO_2$ (1.66 $W/m^2$) and $CH_4$ (0.48 $W/m^2$). Figure 10-3 shows the global average radiative forcing estimates and uncertainty ranges in 2005 for anthropogenic $CO_2$, $CH_4$, $O_3$ and other important agents and mechanisms. The error bars encompassing the tropospheric $O_3$ radiative forcing estimate in the figure range from 0.25 to 0.65 $W/m^2$, making it relatively more uncertain than the long-lived greenhouse gases.




Note: This figure shows the typical geographical extent (spatial scale) of the radiative forcing and the assessed level of scientific understanding (LOSU). The net anthropogenic radiative forcing and its range are also shown. These require summing asymmetric uncertainty estimates from the component terms, and cannot be obtained by simple addition. Additional radiative forcing factors not included here are considered to have a very low LOSU.

Source: Reprinted with permission of Cambridge University Press (IPCC, 2007c).

**Figure 10-3 Global average radiative forcing (RF) estimates and uncertainty ranges in 2005 for anthropogenic $CO_2$, $CH_4$, $O_3$, and other important agents and mechanisms.**

## 10.3.3 Factors that Influence the Effect of Tropospheric $O_3$ on Climate

This section describes the main factors that influence the magnitude of the climate response to changes in tropospheric $O_3$ abundance. They include: (1) trends in the concentration of tropospheric $O_3$; (2) the effect of surface albedo on $O_3$ radiative forcing; (3) the effect of vertical distribution on $O_3$ radiative forcing; (4) feedback factors that can alter the climate response to $O_3$ radiative forcing; and (5) the indirect effects of tropospheric $O_3$ on the carbon cycle. Trends in stratospheric $O_3$ abundance may also affect temperatures at the Earth's surface, but aside from issues relating to stratospheric-tropospheric exchange discussed in Chapter 3, Section 3.4.1.1, stratospheric $O_3$ assessment is beyond the scope of this document.

### 10.3.3.1 Trends in the Concentration of Tropospheric $O_3$

To first order, the effect of tropospheric $O_3$ on global surface temperature is proportional to the change in tropospheric $O_3$ concentration. The earth's surface temperatures are most sensitive to $O_3$ abundance perturbations in the mid to upper troposphere. This section therefore focuses mainly on observed $O_3$ concentration trends in the free troposphere or in regions far from $O_3$ sources, where a change in $O_3$ concentrations may indicate change throughout the troposphere. Data from ozonesondes, mountaintops, and remote surface sites are discussed, as well as satellite data.

#### Observed Trends in $O_3$ Concentrations since the Pre-Industrial Era

Measurements of $O_3$ concentrations at two European mountain sites dating from the late 1800s to early 1900s show values at about 10 ppb, about one-fifth the values observed today at similar sites (Pavelin et al., 1999; Marenco et al., 1994). The accuracy of these early measurements is questionable however, in part because they exhibit $O_3$ concentrations equivalent to or only a couple of parts per billion greater than those observed at nearby low-altitude sites during the same time period (Mickley et al., 2001; Volz and Kley, 1988). A larger vertical gradient in tropospheric $O_3$ concentration would be expected because of its stratospheric source and its longer lifetime aloft. In another study, Staehelin et al. (1994) revisited observations made in the Swiss mountains during the 1950s and found a doubling in $O_3$ concentrations from that era to 1989-1991.

Routine observations of $O_3$ in the troposphere began in the 1970s with the use of balloon-borne ozonesondes, but even this record is sparse. Trends from ozonesondes have been highly variable and dependent on region (Logan et al., 1999). Over most sites in the U.S., ozonesondes reveal little trend. Over Canada, observations show a decline in $O_3$ concentrations between 1980 and 1990, then a rebound in the following decade (Tarasick et al., 2005). Ozonesondes over Europe give a mixed picture. European ozonesondes showed increases in the 1970s and 1980s, with smaller

increases or even declines since then (Oltmans et al., 2006; Logan et al., 1999). Over Japan, $O_3$ concentrations in the lower troposphere increased about 0.2-0.4 ppb/year during the 1990s (Naja and Akimoto, 2004).

Ground-based measurements in remote regions provide a record of tropospheric $O_3$ concentrations, but like ozonesonde data, such measurements are sparse before the 1970s. Springtime $O_3$ observations from several mountain sites in the western U.S. show a positive trend of about of 0.5-0.7 ppb/year since the 1980s (Cooper et al., 2010; Jaffe et al., 2003). Ship-borne $O_3$ measurements for the time period 1977 to 2002 indicate increases of 0.1-0.7 ppb/year over much of the Atlantic south of 40ºN, but no appreciable change north of 40ºN (Lelieveld et al., 2004). The lack of trend for the North Atlantic would seem at odds with $O_3$ observations at Mace Head (53ºN) on the west coast of Ireland, which show a significant positive trend of about 0.5 ppb/year from 1987 to 2003 (Simmonds et al., 2004). Over Japan, $O_3$ concentrations at a remote mountain site have increased 1 ppb/year from 1998 to 2003 (Tanimoto, 2009), a rate more than double that recorded by ozonesondes in the lower troposphere over Japan during the 1990s (Naja and Akimoto, 2004).
At Zugspitze, a mountain site in Germany, $O_3$ concentrations increased by 12% per decade during the 1970s and 1980s, consistent with European ozonesondes (Oltmans et al., 2006). Since then, $O_3$ concentrations continue to increase at Zugspitze, but more slowly. What little data exist for the Southern Hemisphere point to measurable increases in tropospheric $O_3$ concentrations in recent decades, as much as ~15% at Cape Grim in the 1989-2004 time period (Oltmans et al., 2006).

The satellite record is now approaching a length that can be useful for diagnosing trends in the total tropospheric $O_3$ column (details on the use of satellites to measure tropospheric $O_3$ concentrations are covered in Chapter 3, Section 3.5.5.5). In contrast to the surface data from ships, tropospheric $O_3$ columns from the Total Ozone Mapping Spectrometer (TOMS) show no trend over the tropical Atlantic for the period 1980-1990 (Thompson and Hudson, 1999). Over the Pacific Ocean, a longer, 25 year record of TOMS data again reveals no trend over the tropics, but shows increases in tropospheric column $O_3$ of about 2-3 Dobson Units (DU)[1] at mid-latitudes in both hemispheres (Ziemke et al., 2005).

Interpreting these recent trends in tropospheric $O_3$ concentrations is challenging. The first difficulty is reconciling apparently contradictory trends in the observations, e.g., over tropical oceans. A second difficulty is that the $O_3$ concentration trends depend on several factors, not all of which can be well characterized. These factors include (1) trends in emissions of $O_3$ precursors, (2) variation in the stratospheric source of $O_3$, (3) changes in solar radiation resulting from stratospheric $O_3$ depletion, and (4) trends in tropospheric temperatures (Fusco and Logan, 2003). Recent positive trends in the western U.S. and over Japan are consistent with the rapid increase in emissions of $O_3$ precursors from mainland Asia and transport of pollution across the

[1] The Dobson Unit is a typical unit of measure for the total $O_3$ in a vertical column above the Earth's surface. One DU is equivalent to the amount of $O_3$ that would exist in a 1 μm ($10^{-5}$ meter) thick layer of pure $O_3$ at standard temperature (0ºC) and pressure (1 atm), and corresponds to a column of $O_3$ containing $2.69 \times 10^{20}$ molecules/$m^2$. A typical value for the amount of $O_3$ in a column of the Earth's atmosphere, although highly variable, is 300 DU and approximately 10% (30 DU) of that exists in the troposphere at mid latitudes.

Pacific Ocean (Cooper et al., 2010; Tanimoto, 2009). The satellite trends over the northern mid-latitudes are consistent with this picture as well (Ziemke et al., 2005). Increases in tropospheric $O_3$ concentrations in the Southern Hemisphere are also likely due to increased anthropogenic $NO_X$ emissions, especially from biomass burning (Fishman et al., 1991). Recent declines in summertime $O_3$ concentrations over Europe can be partly explained by decreases in $O_3$ precursor emissions there (Jonson et al., 2005), while springtime increases at some European sites are likely linked to changes in stratospheric dynamics (Ordonez et al., 2007). Over Canada, Fusco and Logan (2003) found that $O_3$ depletion in the lowermost stratosphere may have reduced the stratospheric flux of $O_3$ into the troposphere by as much as 30% from the early 1970s to the mid 1990s, consistent with the trends in ozonesondes there.

### Calculation of $O_3$ Concentration Trends for the Recent Past

Simulations of trends in tropospheric $O_3$ concentrations provide a means for testing current knowledge of $O_3$ processes and predicting with greater confidence trends in future $O_3$ concentrations. Time-dependent emission inventories of $O_3$ precursors have also been developed for 1850-2000 (Lamarque et al., 2010) and for 1890-1990 (Van Aardenne et al., 2001). These inventories allow for the calculation of changing $O_3$ concentration over time.

One recent multi-model study calculated an increase in the $O_3$ concentration since pre-industrial times of 8-14 DU, or about 30-70% (Gauss et al., 2006). The large spread in modeled estimates reveals the limitations in knowledge of processes in the pristine atmosphere. Models typically overestimate the late nineteenth and early twentieth century observations available in surface air and at mountain sites by 50-100% (Lamarque et al., 2005; Shindell et al., 2003; Mickley et al., 2001; Kiehl et al., 1999). Reconciling the differences between models and measurements will require more accurate simulation of the natural sources of $O_3$ (Mickley et al., 2001) and/or implementation of novel sinks such as bromine radicals, which may reduce background $O_3$ concentrations in the pristine atmosphere by as much as 30% (Yang et al., 2005c).

For the more recent past (since 1970), application of time-dependent emissions reveals an equatorward shift in the distribution of tropospheric $O_3$ in the Northern Hemisphere due to the industrialization of societies at low-latitudes (Lamarque et al., 2005; Berntsen et al., 2000). By constraining a model with historical (1950s-2000) observations, Shindell and Faluvegi (2002) calculated a large increase of 8.2 DU in tropospheric $O_3$ abundance over polluted continental regions since 1950. This trend is not captured in standard chemistry models, but is consistent with the change in tropospheric $O_3$ concentrations since pre-industrial times implied by the observations from the late 1800s (Pavelin et al., 1999; Marenco et al., 1994).

### 10.3.3.2 The Effect of Surface Albedo on $O_3$ Radiative Forcing

The Earth's surface albedo plays a role in $O_3$ radiative forcing. Through most of the troposphere, absorption of incoming shortwave solar radiation by $O_3$ is small relative to its absorption of outgoing longwave terrestrial radiation. However, over surfaces characterized by high albedo (e.g., over snow, ice, or desert sand), incoming radiation is more likely to be reflected than over darker surfaces, and the probability that $O_3$ will absorb shortwave solar radiation is therefore larger. In other words, energy that would otherwise return to space may instead be retained in the atmosphere. Several studies have shown that transport of $O_3$ to the Arctic from mid-latitudes leads to radiative forcing estimates greater than 1.0 $W/m^2$ in the region, especially in summer (Shindell et al., 2006; Liao et al., 2004b; Mickley et al., 1999). Both the high surface albedo of the Arctic and the large solar zenith angles there (which increase the path length of incoming sunlight) lead to strong shortwave radiative forcing in the region. Because the Arctic is especially sensitive to radiative forcing through the ice-albedo feedback, the large contribution in the shortwave solar spectrum to the total radiative forcing in the region may be important.

### 10.3.3.3 The Effect of Vertical Distribution on $O_3$ Radiative Forcing

In the absence of feedbacks, $O_3$ increments near the tropopause produce the largest increases in surface temperature (Lacis et al., 1990; Wang et al., 1980). This is a result of the colder temperature of the tropopause relative to the rest of the troposphere and stratosphere. Since radiation emitted by the atmosphere is approximately proportional to the fourth power of its temperature[1], the colder the added $O_3$ is relative to the earth's surface, the weaker the radiation emitted and the greater the "trapping" of longwave radiation in the troposphere.

### 10.3.3.4 Feedback Factors that Alter the Climate Response to Changes in $O_3$ Radiative Forcing

Estimates of radiative forcing provide a first-order assessment of the effect of tropospheric $O_3$ on climate. In the atmosphere, climate feedbacks and transport of heat alter the sensitivity of Earth's surface temperature to addition of tropospheric $O_3$. Assessment of the full climate response to increases in tropospheric $O_3$ concentrations requires use of a climate model to simulate these interactions.

Due to its short lifetime, $O_3$ is heterogeneously distributed through the troposphere. Sharp horizontal gradients exist in the radiative forcing of $O_3$, with the greatest radiative forcing since pre-industrial times occurring over the northern mid-latitudes (more on this in Section 10.3.5 and Section 10.3.6). If climate feedbacks are

[1] As described by the Stefan-Boltzmann law, an ideal blackbody--which the atmosphere approximates--absorbs at all wavelengths and re-radiates proportional to the fourth power of its temperature.

particularly powerful, they may obscure or even erase the correlation between regional radiative forcing and climate response (Harvey, 2004; Boer and Yu, 2003). The transport of heat through the atmosphere, though not technically a feedback, may also weaken the correlation between radiative forcing and climate response. Several model studies have reported that the horizontal pattern of surface temperature response from 2000-2100 trends in predicted short-lived species (including $O_3$) closely matches the pattern from the trends in the long-lived greenhouse gases over the same time period (Levy et al., 2008; Shindell et al., 2008; Shindell et al., 2007). This correspondence occurs even though the patterns of radiative forcing for the short-lived and long-lived species differ substantially. In a separate paper, Shindell et al. (2007) found that Arctic temperatures are especially sensitive to the mid-latitude radiative forcing from tropospheric $O_3$.

Other studies have found that the signature of warming due to tropospheric $O_3$ does show some consistency with the $O_3$ radiative forcing. For example, Mickley et al. (2004) examined the change in $O_3$ concentrations since pre-industrial times and found greater warming in the Northern Hemisphere than in the Southern Hemisphere (+0.4ºC versus +0.2ºC), as well as higher surface temperatures downwind of Europe and Asia and over the North American interior in summer. For an array of short-lived species including $O_3$, Shindell and Faluvegi (2009) found that radiative forcing applied over northern mid-latitudes yield more localized responses due to local cloud, water vapor, and albedo feedbacks than radiative forcing applied over the tropics.

Climate feedbacks can also alter the sensitivity of surface temperature to the vertical distribution of tropospheric $O_3$. The previous section (Section 10.3.3.3) described the greater effect of $O_3$ added to the upper troposphere (near the tropopause) on radiative forcing, relative to additions in the mid- to lower troposphere. However, warming induced by increased $O_3$ concentrations in the upper troposphere could stabilize the atmosphere to some extent, limiting the transport of heat to the Earth's surface and mitigating the effect of the added $O_3$ on surface temperature (Joshi et al., 2003; Christiansen, 1999). Hansen et al. (1997) determined that allowing cloud feedbacks in a climate model meant that $O_3$ enhancements in the mid-troposphere had the greatest effect on surface temperature.

Finally, climate feedbacks can amplify or diminish the climate response of one greenhouse gas relative to another. For example, Mickley et al. (2004) found a greater temperature response to $CO_2$ radiative forcing than to an $O_3$ radiative forcing of similar global mean magnitude, due in part to the relatively weak ice-albedo feedback for $O_3$ radiative forcing. Since $CO_2$ absorbs in the same bands as water vapor, $CO_2$ radiative forcing saturates in the middle troposphere and is also shifted toward the drier poles. A poleward shift in radiative forcing amplifies the ice-albedo feedback in the case of $CO_2$, and the greater mid-troposphere radiative forcing allows for greater surface temperature response, relative to that for $O_3$.

### 10.3.3.5 Indirect Effects of Tropospheric $O_3$ on the Carbon Cycle

A proposed indirect effect of tropospheric $O_3$ on climate involves the carbon cycle. By directly damaging plant life in ways discussed in Chapter 9, increases in tropospheric $O_3$ concentrations may depress the land-carbon sink of $CO_2$, leading to accumulation of $CO_2$ in the atmosphere and ultimately warming of the Earth's surface. Sitch et al. (2007) calculated that this indirect warming effect of $O_3$ on climate has about the same magnitude as the $O_3$ direct effect. Their results suggest a doubled sensitivity of surface temperatures to $O_3$ radiative forcing, compared to current model estimates.

A large array of additional indirect effects involving biospheric responses to tropospheric $O_3$ concentrations are possible. For example, increasing temperature due to increases in tropospheric $O_3$ concentration may alter biodiversity, species migration, and consequent impacts on surface albedo. Such long-term feedbacks may play an important role in the eventual climate response to changes in tropospheric $O_3$ abundance, but a full evaluation of such long-term feedbacks on climate change is outside the scope of this assessment.

## 10.3.4 Competing Effects of $O_3$ Precursors on Climate

Changes in concentrations of $O_3$ precursors can affect the radiative balance of the atmosphere through multiple (and sometimes competing) mechanisms. For example, the $O_3$ precursor $CH_4$ is itself a powerful greenhouse gas. Ozone and its other precursors also exert a strong control on the oxidizing capacity of the troposphere, and so can affect the lifetime of gases such as $CH_4$ (Derwent et al., 2001). For example, an increase in CO or VOCs would lead to a decrease in hydroxyl (OH) concentrations. Since OH is a major sink for $CH_4$, a decline in OH would lengthen the $CH_4$ lifetime, enhance the $CH_4$ concentration, and amplify surface warming. A rise in $NO_X$ emissions, on the other hand, could lead to an increase in OH in certain locations, shortening the $CH_4$ lifetime and causing surface cooling (Fuglestvedt et al., 1999). Ozone can itself generate OH through (1) photolysis leading to excited oxygen atoms followed by reaction with water vapor and (2) reaction with $HO_2$.

Figure 10-4 shows the radiative forcing associated with a suite of anthropogenic emissions, including $O_3$ precursors (IPCC, 2007b). The emission-based radiative forcing for $CH_4$, which includes the $CH_4$ effect on $O_3$ production, is +0.9 W/m$^2$, or nearly double that of the $CH_4$ abundance-based radiative forcing shown in Figure 10-3. Figure 10-4 also shows a warming from anthropogenic CO and VOC emissions of +0.27 W/m$^2$ and a net cooling of -0.21 W/m$^2$ for $NO_X$ emissions. The net cooling for $NO_X$ occurs mainly due to the links between $NO_X$ and $CH_4$. Consistent with these results, Shindell and Faluvegi (2009) calculated positive (+0.25 W/m$^2$) radiative forcing from the increase in anthropogenic emissions of CO and VOCs since pre-industrial times, as well as for $CH_4$ (+1 W/m$^2$). In contrast,

Shindell and Faluvegi (2009) found negative (-0.29 W/m$^2$) radiative forcing from anthropogenic emissions of $NO_X$. Other studies have found a near cancellation of the positive $O_3$ radiative forcing and the negative $CH_4$ radiative forcing that arise from an incremental increase in anthropogenic $NO_X$ emissions (Naik et al., 2005; Fiore et al., 2002; Fuglestvedt et al., 1999). The net effect of aircraft $NO_X$ on climate is especially complex (Isaksen et al., 2001; Wild et al., 2001). Stevenson (2004) calculated that aircraft $NO_X$ leads to short-term net warming via $O_3$ production in the cool upper troposphere, but long-term net cooling because of $CH_4$ loss.

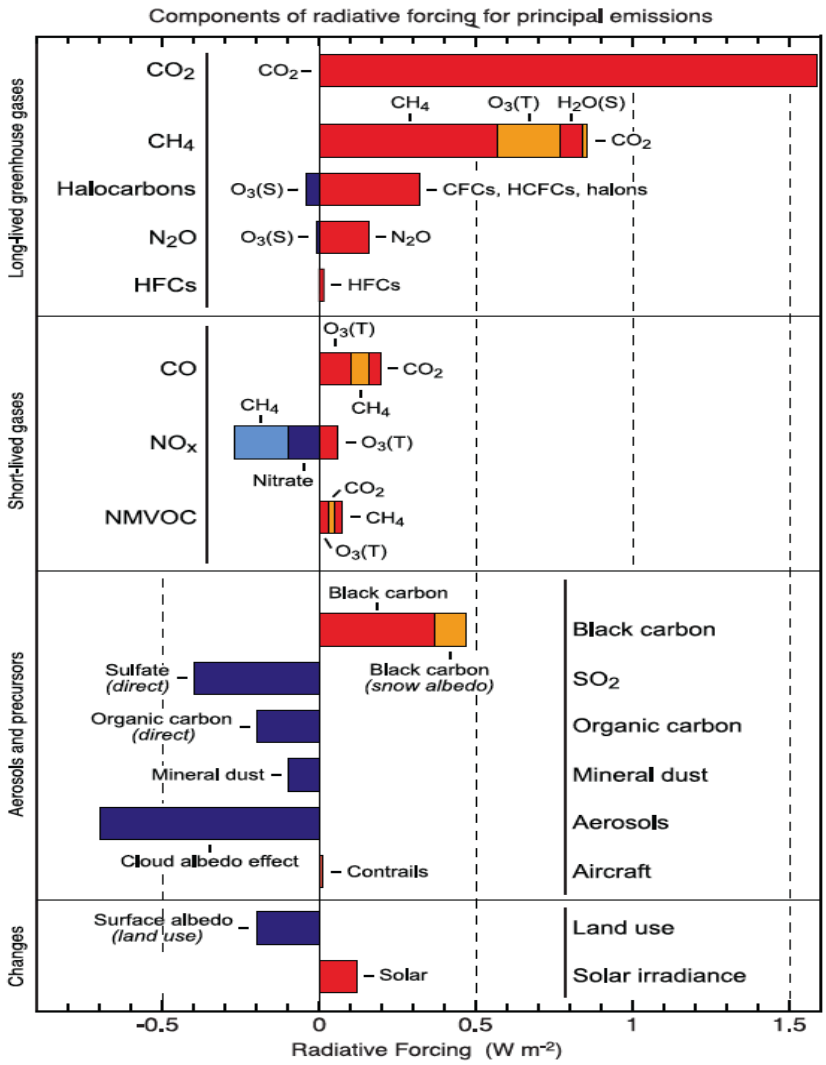
OH production from $O_3$ precursors can also affect regional sulfate air quality and climate by increasing gas-phase oxidation rates of $SO_2$. Using the A1B scenario in the IPCC AR4, Unger (2006) reported that by 2030, enhanced OH from the A1B $O_3$ precursors may increase surface sulfate aerosol concentrations by up to 20% over India and China, relative to the present-day, with a corresponding increase in radiative cooling over these regions. In this way, $O_3$ precursors may impose an indirect cooling via sulfate (Unger, 2006).

Taken together, these results point out the need for careful assessment of net radiative forcing involving multiple pollutants in developing climate change policy (Unger et al., 2008). Many studies point to $CH_4$ as a particularly attractive target for emissions control since $CH_4$ is itself an important precursor of $O_3$ (West et al., 2007; Fiore et al., 2002). Fiore et al. (2002) found that reducing anthropogenic $CH_4$ emissions by 50% would lead to a global negative (-0.37 W/m$^2$) radiative forcing, mostly from $CH_4$. In later research, Fiore et al. (2008) reported that $CH_4$ reductions would most strongly affect tropospheric $O_3$ column amounts in regions of strong downwelling from the upper troposphere (e.g., around 30ºN) and in regions of $NO_X$-saturated conditions.

The magnitude of the radiative forcing from the change in tropospheric $O_3$ abundance since the pre-industrial era is uncertain. This uncertainty derives in part from the scarcity of early measurements and in part from limited knowledge regarding processes in the natural atmosphere. As noted previously, the IPCC AR4 reports a radiative forcing of 0.35 W/m$^2$ from the change in tropospheric $O_3$ abundance since 1750 (Forster et al., 2007), ranking it third in importance behind the greenhouse gases $CO_2$ and $CH_4$. The $O_3$ radiative forcing could, in fact, be as large as 0.7 W/m$^2$, if reconstructions of pre-industrial and mid-20th century $O_3$ concentrations based on the measurement record are valid (Shindell and Faluvegi, 2002; Mickley et al., 2001). In any event, Unger et al. (2010) showed that present-day $O_3$ radiative forcing can be attributed to emissions from many economic sectors, including on-road vehicles, household biofuel, power generation, and biomass burning. As much as one-third of the radiative forcing from the 1890 to 1990 change in tropospheric $O_3$ concentration could be due to increased biomass burning (Ito et al., 2007a).

These calculated radiative forcing estimates can be compared to those obtained from satellite data. Using data from TOMS, Worden et al. (2008) estimated a reduction in clear-sky outgoing longwave radiation of 0.48 W/m$^2$ by $O_3$ in the upper troposphere over oceans in 2006. This radiative forcing includes contributions from both

anthropogenic and natural sources of $O_3$. Assuming that the concentration of $O_3$ has roughly doubled since pre-industrial times (Gauss et al., 2006), the total $O_3$ radiative forcing estimated with TOMS is consistent with that obtained from models estimating just the anthropogenic contribution.




Note: Values represent radiative forcing in 2005 due to emissions and changes since 1750. (S) and (T) next to gas species represent stratospheric and tropospheric changes, respectively.

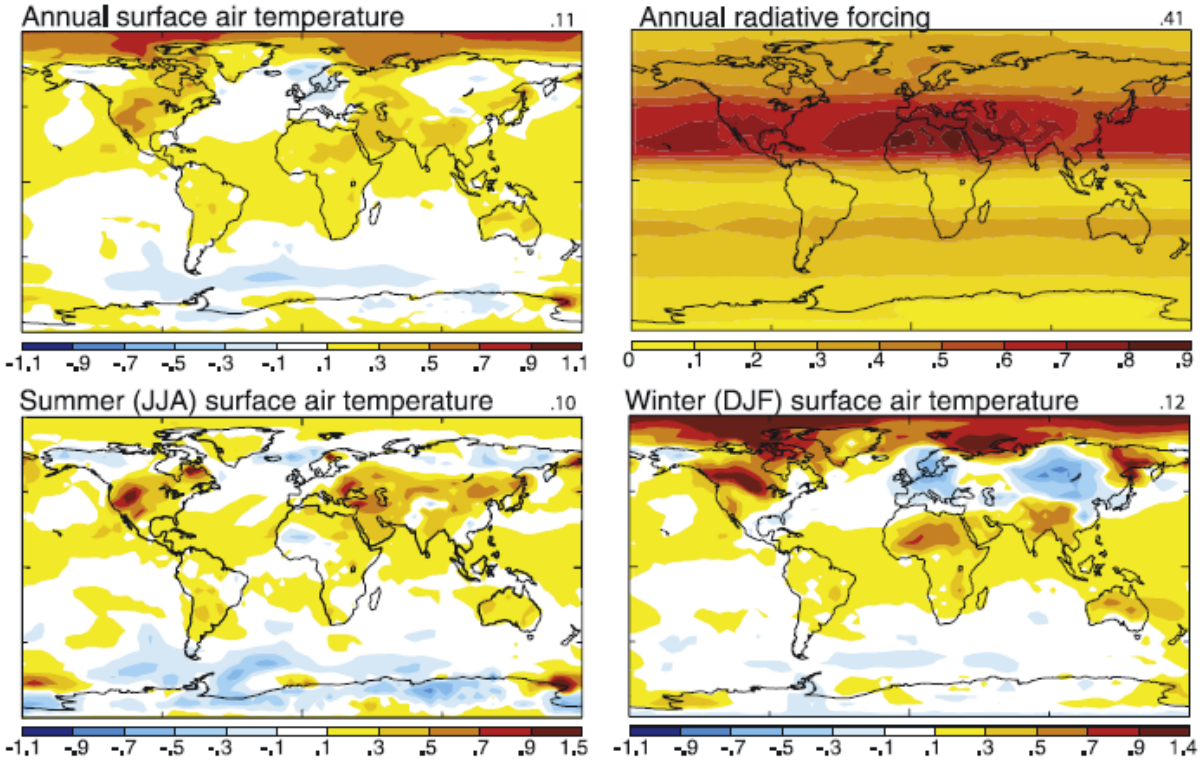
Source: Reprinted with permission of Cambridge University Press (IPCC, 2007b).

**Figure 10-4 Components of radiative forcing for emissions of principal gases, aerosols, aerosol precursors, and other changes.**

### 10.3.5 Calculating Radiative Forcing and Climate Response to Past Trends in Tropospheric $O_3$ Concentrations

Calculation of the climate response to the $O_3$ radiative forcing is challenging due to complexity of feedbacks, as mentioned in Section 10.3.2.2 and Section 10.3.3.4. In their modeling study, Mickley et al. (2004) reported a global mean increase of 0.28ºC since pre-industrial times, with values as large as 0.8ºC in continental interiors. For the time period since 1870, Hansen et al. (2005) estimated a much smaller increase in global mean surface temperature (0.11ºC), but they implemented 1880s anthropogenic emissions in their base simulation and also took into account trends in both stratospheric and tropospheric $O_3$ concentrations. The modeled decline of lower stratospheric $O_3$ concentrations, especially over polar regions, cooled surface temperatures in this study, counteracting the warming effect of increasing tropospheric $O_3$ concentrations.

Figure 10-5 shows the Hansen et al. (2005) results as reported in Shindell et al. (2006). In that figure, summertime $O_3$ has the largest radiative effect over the continental interiors of the Northern Hemisphere. Shindell et al. (2006) estimated that the change in tropospheric $O_3$ concentration over the 20th century could have contributed about 0.3ºC to annual mean Arctic warming and as much as 0.4-0.5ºC during winter and spring. Over eastern China, Chang et al. (2009) calculated a surface temperature increase of 0.4ºC to the 1970-2000 change in tropospheric $O_3$ concentration. It is not clear, however, to what degree regional changes in $O_3$ concentration influenced this response, as opposed to more global changes.




Note: This figure includes the input radiative forcing ($W/m^2$), as computed by the NASA GISS chemistry-climate model. Values are surface temperature trends for the annual average (top left), June–August (bottom left), and December-February (bottom right) and annual average tropopause instantaneous radiative forcing from 1880 to 1990 (top right). Temperature trends greater than about 0.1ºC are significant over the oceans, while values greater than 0.3ºC are typically significant over land, except for northern middle and high latitudes during winter where values in excess of about 0.5ºC are significant. Values in the top right corner give area-weighted global averages in the same units as the plots.

Source: Reprinted with permission of American Geophysical Union (Shindell et al., 2006).

**Figure 10-5 Ensemble average 1900-2000 radiative forcing and surface temperature trends (ºC per century) in response to tropospheric $O_3$ concentration changes.**

### 10.3.6 Calculating Radiative Forcing and Climate Response to Future Trends in Tropospheric $O_3$ Concentrations

Future trends in tropospheric $O_3$ concentrations depend in large part on what pathways in energy technology the world's societies will follow in coming decades. The trends in $O_3$ concentration will also depend on the changes in a suite of climate-sensitive factors, such as the water vapor content of the atmosphere. This section describes the following issues: (1) projected trends in the anthropogenic emissions of $O_3$ precursors; (2) the effects of these emissions on the tropospheric $O_3$ concentrations; (3) the effects of changing climate on tropospheric $O_3$ concentrations; and (4) radiative forcing and climate response to 21st century trends in tropospheric $O_3$ concentrations.

### 10.3.6.1 Emissions of Anthropogenic $O_3$ Precursors Across the 21st Century

The IPCC SRES effort devised scenarios for short-lived $O_3$ precursors as well as the well-mixed greenhouse gases including $NO_X$, CO, and VOCs (IPCC, 2000). Using the IMAGE socioeconomic model, Streets et al. (2004) provided speciation for $NO_X$ and VOCs and allocated the trends in emissions over 17 regions and 8 economic sectors for the 2000-2050 time period. The worst-case IPCC scenario, A2, features continued dependence on fossil fuels, rapid population growth, and little sharing of technology between developed and developing nations. By 2100 in this scenario, global $NO_X$, CO and $CH_4$ emissions increase by a factor of 3.5, 2.6, and 2.9, respectively, relative to 2000 (IPCC, 2000). Most of these increases in emissions occur over developing countries. For example over Asia, $NO_X$ emissions in the A2 scenario increase by more than a factor of four by 2100. The more moderate A1B scenario has global $NO_X$ and CO emissions increasing by 25% and 90%, respectively by 2100, but global $CH_4$ emissions decreasing by 10%. In the B1 scenario, with its emphasis on clean and efficient technologies, global emissions of $NO_X$, CO, and $CH_4$ all decrease by 2100, relative to the present day (-40%, -60%, and -30%, respectively).

Other emissions scenarios have been recently developed to describe trends in the short-term (up to 2030). The Current Legislation (CLE) scenario provides trends consistent with existing air quality regulations; the Maximum Feasible Reduction (MFR) scenario seeks to reduce emissions of $O_3$ precursors to the maximum extent possible. Emission source changes relative to the present day for CLE, MFR, and A2 are given in Stevenson et al. (2006).

For the Fifth Assessment Report (IPCC AR5), a new set of climate futures has been developed: the Representative Concentration Pathways (RCPs) (Moss et al., 2010). The RCPs will explore for the first time approaches to climate change mitigation. The RCPs are designed to achieve radiative forcing targets of 2.6, 4.5, 6.0 and 8.5 $W/m^2$ by 2100, and have been designated RCP 2.6, RCP 4.5, RCP 6.0, and RCP 8.5, respectively (RCP 2.6 is also known as RCP3-PD.) The trends in $O_3$ precursors for the RCP scenarios were determined by climate policies implicit in each scenario and by plausible assumptions regarding future air quality regulations. These scenarios were chosen to map the wide range of climate outcomes presented in the literature and represent only four of many possible scenarios that would lead to the specific radiative forcing targets; a wide range of socioeconomic conditions could be consistent with each radiative forcing pathway (Moss et al., 2010). Therefore, they should not be interpreted as forecasts of future conditions, but rather as plausible climate and socio-economic futures.

Plots and comparisons of the RCP trends are available on the RCP website (RCP, 2009). In all RCPs, global anthropogenic $NO_X$ emissions decline 30-50% during the 21st century, though RCP 8.5 shows a peak during the 2020s at a value ~15% greater than that of 2000. Global anthropogenic VOC and CO emissions are relatively flat during the 2000-2050 time range, and then decline by 30-50% by the end of the

century. For $CH_4$, global mean emission trends for the four RCP projections differ substantially across the 21st century, with RCP 8.5 showing a tripling of emissions by 2100, and RCP 2.6 showing the emissions cut by half in this time range. RCP 4.5 and 6.0 show a peak in $CH_4$ emissions in the middle of the century before dropping by the end of the century to just below 2000 emission levels. All these global trends, however, contain some regional variation. For example, Asian emissions of both $NO_X$ and VOCs show large increases in the near term (2030s to 2050s).

### 10.3.6.2 Impact of 21st Century Trends in Emissions on Tropospheric $O_3$ Concentrations

Due to its short lifetime, tropospheric $O_3$ concentrations will respond readily to changes in anthropogenic emissions of $O_3$ precursors. As shown in Table 10-1, a recent multi-model study found increases in the tropospheric $O_3$ concentration of 15% and 6% for the IPCC A2 and CLE scenarios respectively for the 2000-2030 time period, and a decrease for the MFR scenario of 5% (Stevenson et al., 2006). These results indicate that the growth in tropospheric $O_3$ concentrations between 2000 and 2030 could be reduced or even reversed, depending on emission controls. For the relatively moderate A1B emissions scenario over the 2000-2050 time period, Wu et al. (2008a) calculated a change in $O_3$ concentration of about 20%.

As noted above, the RCP scenarios show large variations in their future projections of global mean $CH_4$ emissions, but mainly declines in the emissions of the other $O_3$ precursors across the 21st century. In one of the first efforts to assess the effect of these emission trends on global $O_3$ abundances, Lamarque et al. (2011) found that the large $CH_4$ increase in the RCP 8.5 scenario would drive a 15% enhancement of the tropospheric $O_3$ abundance by 2100, relative to the present-day, leading to a global mean radiative forcing of +0.2 W/m$^2$. By contrast, the global $O_3$ abundance would decrease in the other three RCPs, with declines in radiative forcing ranging from -0.07 to -0.2 W/m$^2$.

**Table 10-1 Changes in anthropogenic emissions, $CH_4$ and tropospheric $O_3$ concentrations between 2000 and 2030, and the associated tropospheric $O_3$ radiative forcing for three scenarios.**

| Scenario | IPCC A2[a] | Current Legislation (CLE)[a] | Maximum Feasible Reduction (MFR)[a] |
|---|---|---|---|
| Percent change in $NO_X$ emissions | +96% | +18% | -53% |
| Percent change in CO emissions | +62% | -16% | -53% |
| Percent change in $CH_4$ concentration | +23% | +19% | 0% |
| Percent change in tropospheric $O_3$ concentration | +15% | +6% | -5% |
| Radiative forcing due to $O_3$ concentration change[b] ($W/m^2$) | 0.3 | 0.18 | -0.05 |

[a]Values are ensemble means.

[b]Includes radiative forcing due to corresponding $CH_4$ change.

Source: Adapted from Stevenson et al. (2006).

### 10.3.6.3 Impact of 21st Century Climate on Tropospheric $O_3$ Concentrations

For the time period from the 1800s to the present-day, most of the increase in the concentration of tropospheric $O_3$ can be traced to changing emissions. Model studies show that climate change so far has likely had little effect on the tropospheric $O_3$ concentrations (e.g., Grenfell et al., 2001). In the future, however, climate change is expected to bring large changes in a suite of variables that could affect $O_3$ production, loss, and transport. For example, increased water vapor in a warming atmosphere is expected to enhance OH concentrations, which in remote, $NO_X$-poor regions will accelerate $O_3$ loss rates (Johnson et al., 1999).

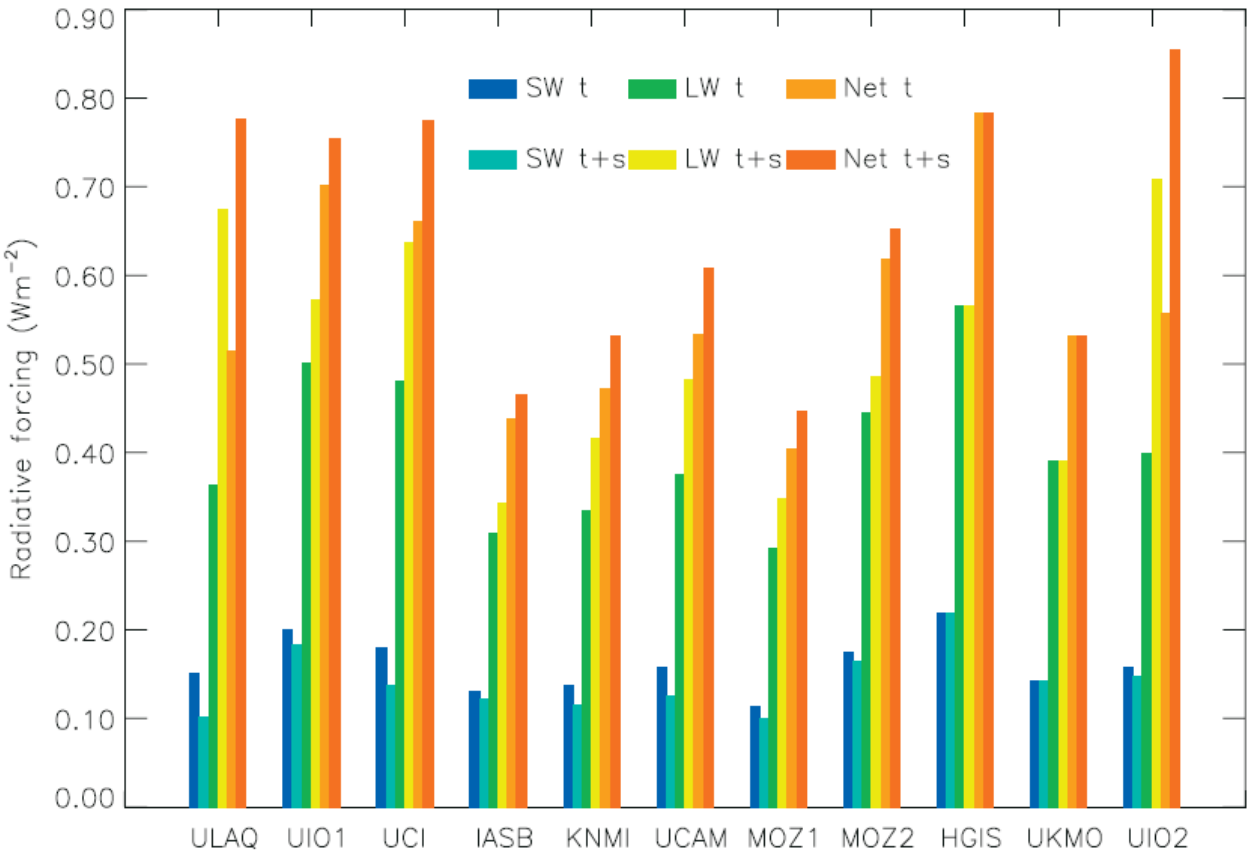
In the 2050s A1B climate, Wu et al. (2008b) calculated a 5 ppb decrease in surface $O_3$ concentrations over oceans. A rise in temperatures will also likely promote emissions of isoprene, an important biogenic precursor of $O_3$. Model studies have calculated 21st-century increases in isoprene emissions ranging from 25-50%, depending on climate scenario and time horizon (Wu et al., 2008a and references therein). These studies however did not take into account the effects of changing climate and $CO_2$ concentration on vegetation extent, which could have large consequences for biogenic emissions (Heald et al., 2008; Sanderson et al., 2003). In any event, enhanced isoprene emissions will increase $O_3$ concentrations in VOC-limited regions, but decrease $O_3$ concentrations in $NO_X$-limited regions (Wu et al., 2008a; Pyle et al., 2007; Sanderson et al., 2003). Convection frequencies and lightning flash rates will also likely change in a changing climate, with consequences for lightning $NO_X$ emissions and $O_3$ concentrations in the upper troposphere (Sinha and Toumi, 1997; Price and Rind, 1994). While Wu et al. (2008a) calculated an

increase in lightning $NO_X$ by 2050 due to enhanced deep convection, Jacobson and Streets (2009) projected a decrease in lightning $NO_X$ due to a declining cloud ice in their future atmosphere. Finally, changes in transport processes will almost certainly accompany global climate change. For the 2050 A1B climate, Wu et al. (2008b) showed that flattening of the meridional temperature gradient in a warming world would lead to slower intercontinental transport of tropospheric $O_3$. For the A2 climate in 2100, Zeng and Pyle (2003) projected an 80% increase in the flux of stratospheric $O_3$ into the troposphere, relative to the present-day.

Taken together, these climate-driven processes could have appreciable effects on the concentration and distribution of tropospheric $O_3$. As shown in Wu et al. (2008b), model projections of the change in $O_3$ concentration due solely to future climate change range from -12% to +3%, depending on the model, scenario, and time horizon.

#### 10.3.6.4 Radiative Forcing and Climate Response from 21st Century Trends in Tropospheric $O_3$ Concentrations

In the near term (2000-2030), Stevenson et al. (2006) estimated an $O_3$ radiative forcing of near zero for MFR, 0.18 W/m$^2$ for CLE, and 0.3 W/m$^2$ for the A2 scenario (Table 10-1). Menon et al. (2008), following the moderate A1B scenario, calculated a radiative forcing of 0.12 W/m$^2$ from the 2000-2030 change in tropospheric $O_3$ concentrations, about the same as that derived by Stevenson et al. (2006) for the CLE scenario. Over the longer term (2000 to 2100) for the A1B scenario, Gauss et al. (2003) reported large positive radiative forcing (0.40 to 0.78 W/m$^2$) due to the change in tropospheric $O_3$ concentrations, as shown in Figure 10-6. Normalized radiative forcing for these model calculations fell within a relatively narrow range, 0.032 to 0.040 W/m$^2$ DU, indicating that the largest uncertainty lies in the model-calculated changes in $O_3$ concentration. Applying the A2 scenario, Chen et al. (2007b) estimated a global mean radiative forcing of 0.65 W/m$^2$ from tropospheric $O_3$ by 2100, consistent with the Gauss et al. (2003) results. These studies took into account only the effect of changing emissions on tropospheric $O_3$ concentrations. In their calculations of the 2000-2100 radiative forcing from $O_3$ in the A2 scenario, Liao et al. (2006) found that inclusion of climate effects on tropospheric $O_3$ reduced their radiative forcing estimate by 20%.




Note: Shown are the components of radiative forcing in W/m$^2$. SW = shortwave component; LW = longwave component; Net = total radiative forcing; t = tropospheric $O_3$ changes only; and t+s = both tropospheric and stratospheric changes.

Source: Reprinted from Gauss et al. (2003), American Geophysical Union.

**Figure 10-6 Global mean radiative forcing estimates calculated by a set of models for the 2000-2100 change in tropospheric $O_3$ concentrations.**

Several studies have included tropospheric $O_3$ in their investigations of the response in the future atmosphere to a suite of short-lived species (e.g., Levy et al., 2008; Shindell et al., 2008; Shindell et al., 2007). Few studies, however, have calculated the climate response to changes in tropospheric $O_3$ concentrations alone in the future atmosphere. For the A2 atmosphere, Chen et al. (2007b) estimated a global mean surface temperature increase of +0.34ºC by 2100 in response to the change in $O_3$ concentration. The largest temperature increases in this study, as much as 5ºC, occurred over the populous regions of Asia and the Middle East and downwind of biomass burning regions in South Africa and South America.

## 10.4 UV-B Shielding Effects and Tropospheric $O_3$

### 10.4.1 Background

UV radiation emitted from the Sun contains sufficient energy when it reaches the Earth to break (photolyze) chemical bonds in molecules, thereby leading to damaging effects on living organisms and materials. Atmospheric $O_3$ plays a crucial role in reducing exposure to solar UV radiation at the Earth's surface. Stratospheric $O_3$ is responsible for the majority of this shielding effect, as approximately 90% of total atmospheric $O_3$ is located there over mid-latitudes (Kar et al., 2010; Crist et al., 1994). Investigation of the supplemental shielding of UV-B radiation provided by tropospheric $O_3$ is necessary for quantifying UV-B exposure and the incidence of related human health effects, ecosystem effects, and materials damage. The role of tropospheric $O_3$ in shielding of UV-B radiation is discussed in this section.

### 10.4.2 Human Exposure and Susceptibility to Ultraviolet Radiation

The factors that potentially influence UV radiation exposure were discussed in detail in Chapter 10 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b) and are summarized here. These factors included outdoor activity, occupation, age, sex, geography, and protective behavior. Outdoor activity and occupation both influenced the amount of time people spend outdoors during daylight hours, the predominant factor for exposure to solar UV radiation. Age and sex were found to be factors that influence human exposure to UV radiation, particularly by influencing other factors of exposure such as outdoor activity and risk behavior. Studies indicated that females generally spent less time outdoors and, consequently, had lower UV radiation exposure on average compared to males. Geography influences the degree of solar UV flux to the surface, and hence exposure to UV radiation. Higher solar flux at lower latitudes increased the annual UV radiation dose for people living in southern states relative to northern states. Altitude was also found to influence personal exposure to UV radiation. Protective behaviors such as using sunscreen, wearing protective clothing, and spending time in shaded areas were shown to reduce exposure to UV radiation. Given these and other factors that potentially influence UV radiation exposure, the 2006 $O_3$ AQCD (U.S. EPA, 2006b) listed the following subpopulations potentially at risk for higher exposures to UV radiation:

- Individuals who engage in high-risk behavior (e.g., sunbathing);
- Individuals who participate in outdoor sports and activities;
- Individuals who work outdoors with inadequate shade (e.g., farmers, construction workers, etc.);
- Individuals living in geographic areas with higher solar flux including lower latitudes (e.g., Honolulu, HI) and higher altitudes (e.g., Denver, CO).

The risks associated with all these factors are, of course, highly dependent on season and region (Sliney and Wengraitis, 2006).

### 10.4.3 Human Health Effects due to UV-B Radiation

Chapter 10 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b) covered in detail the human health effects associated with solar UV-B radiation exposure. These effects include erythema, skin cancer, ocular damage, and immune system suppression. These adverse effects, along with protective effects of UV radiation through increased production of vitamin D are summarized in this section. For additional details, the reader is referred to Chapter 10 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b) and references therein.

The most conspicuous and well-recognized acute response to UV radiation is erythema, or the reddening of the skin. Erythema is likely caused by direct damage to DNA by UV radiation. Many studies discussed in Chapter 10 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b) found skin type to be a significant risk factor for erythema. Skin cancer is another prevalent health effect associated with UV radiation. Exposure to UV radiation is considered to be a major risk factor for all forms of skin cancer. Ocular damage from UV radiation exposure includes effects on the cornea, lens, iris, and associated epithelial and conjunctival tissues. The region of the eye affected by exposure to UV radiation depends on the wavelength of the incident UV radiation. Depending on wavelength, common health effects associated with UV radiation include photokeratitis (snow blindness; short wavelengths) and cataracts (opacity of the lens; long wavelengths).

Experimental studies reviewed in Chapter 10 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b) have shown that exposure to UV radiation may suppress local and systemic immune responses to a variety of antigens. Results from controlled human exposure studies suggest that immune suppression induced by UV radiation may be a risk factor contributing to skin cancer induction. There is also evidence that UV radiation has indirect involvement in viral oncogenesis through the human papillomavirus, dermatomyositis, human immunodeficiency virus, and other forms of immunosuppression.

A potential health benefit of increased UV-B exposure relates to the production of vitamin D in humans. Most humans depend on sun exposure to satisfy their requirements for vitamin D. Vitamin D deficiency can cause metabolic bone disease among children and adults, and also may increase the risk of many common chronic diseases, including type I diabetes mellitus and rheumatoid arthritis. Substantial in vitro and toxicological evidence also support a role for vitamin D activity against the incidence or progression of various forms of cancer. In some studies, UV-B related production of vitamin D had potential beneficial immunomodulatory effects on multiple sclerosis, insulin-dependent diabetes mellitus, and rheumatoid arthritis. More details on UV-B protective studies are provided in Chapter 10 of the 2006 $O_3$ AQCD (U.S. EPA, 2006b).

In establishing guidelines on limits of exposure to UV radiation, the International Commission on Non-Ionizing Radiation Protection (ICNIRP) agreed that some low-level exposure to UV radiation has health benefits (ICNIRP, 2004). However, the adverse health effects of higher UV exposures necessitated the development of exposure limits for UV radiation. The ICNIRP recognized the challenge in establishing exposure limits that would achieve a realistic balance between beneficial and adverse health effects. As concluded by ICNIRP (2004), "[t]he present understanding of injury mechanisms and long-term effects of exposure to [UV radiation] is incomplete, and awaits further research."

### 10.4.4 Ecosystem and Materials Damage Effects Due to UV-B Radiation

A 2009 progress report on the environmental effects of $O_3$ depletion from the UNEP, Environmental Effects Assessment Panel (UNEP, 2009) lists many ecosystem and materials damage effects from UV-B radiation. An in-depth assessment of the global ecosystem and materials damage effects from UV-B radiation per se is out of the scope of this assessment. However, a brief summary of some mid-latitude effects is provided in this section to provide context for UV-B related issues pertaining to tropospheric $O_3$. The reader is referred to the UNEP report (UNEP, 2009) and references therein for further details. All of these UV-B related ecosystem and materials effects can also be influenced by climate change through temperature and other meteorological alterations, making quantifiable predictions of UV-B shielding effects difficult.

**Terrestrial ecosystem effects** from increased UV-B radiation include reduced plant productivity and plant cover, changes in biodiversity, susceptibility to infection, and increases in natural UV protective responses. In general, however, these effects are small for moderate UV-B increases at mid-latitudes. A field study on wheat in southern Chile found no substantial changes in crop yield with moderate increases in UV-B radiation (Calderini et al., 2008). Similarly, field studies on silver birch (*Betula pendula*) in Finland found no measurable effects in photosynthetic function with increases in UV-B radiation (Aphalo et al., 2009). Subtle, but important, changes in habitat and biodiversity have also been linked to increases in UV-B radiation (Mazza et al., 2010; Obara et al., 2008; Wahl, 2008). Some plants have natural coping mechanisms for dealing with changes in UV-B radiation (Favory et al., 2009; Jenkins, 2009; Brown and Jenkins, 2008; Ioki et al., 2008), but these defenses may have costs in terms of reduced growth (Snell et al., 2009; Clarke and Robinson, 2008; Semerdjieva et al., 2003; Phoenix et al., 2000).

**Aquatic ecosystem effects** from increased UV-B radiation include sensitivity in growth, immune response, and behavioral patterns of aquatic organisms. One study looking at coccolithophores, an abundant phytoplankton group, found a 25% reduction in cellular growth with UV-B exposure (Gao et al., 2009a). Exposure to relevant levels of UV-B radiation has been shown to modify immune response, blood chemistry, and behavior in certain species of fish (Markkula et al., 2009; Holtby and

Bothwell, 2008; Jokinen et al., 2008). Adverse effects on growth and development from UV-B radiation have also been observed for amphibians, sea urchins, mollusks, corals, and zooplankton (Garcia et al., 2009b; Romansic et al., 2009; Croteau et al., 2008b; Croteau et al., 2008a; Marquis et al., 2008; Marquis and Miaud, 2008; Oromi et al., 2008). Increases in the flux of UV-B radiation may also result in an increase in the catalysis of trace metals including mercury, particularly in clear oligotrophic lakes with low levels of dissolved organic carbon to stop the penetration of UV-B radiation (Schindler et al., 1996). This could then alter the mobility of trace metals including the potential for increased mercury volatilization and transport within and among ecosystems.

**Biogeochemical cycles**, particularly the carbon cycle, can also be influenced by increased UV-B radiation. A study on high latitude wetlands found UV-induced increases in $CO_2$ uptake through soil respiration (Haapala et al., 2009) while studies on arid terrestrial ecosystems found evidence for UV-induced release of $CO_2$ through photodegradation of above-ground plant litter (Brandt et al., 2009; Henry et al., 2008; Caldwell et al., 2007; Zepp et al., 2007). Changes in solar UV radiation may also have effects on carbon cycling and $CO_2$ uptake in the oceans (Brewer and Peltzer, 2009; Meador et al., 2009; Fritz et al., 2008; Zepp et al., 2008; Hader et al., 2007) as well as release of dissolved organic matter from sediment and algae (Mayer et al., 2009; Riggsbee et al., 2008). Additional studies showing effects on these and additional biogeochemical cycles including the water cycle and halocarbon cycle can be found in the UNEP report (UNEP, 2009) and references therein.

**Materials damage** from increased UV-B radiation include UV-induced photodegradation of wood (Kataoka et al., 2007) and plastics (Pickett et al., 2008). These studies and others summarizing photo-resistant coatings and materials designed to reduce photodegradation of materials are summarized in the UNEP report (UNEP, 2009) and references therein.

The ecosystem, carbon cycle, and materials effects described in this section are for UV-B exposure in general. Only a small fraction of these effects would be offset by incremental decreases in UV-B exposure resulting from increases in tropospheric $O_3$ concentrations. Attribution of UV-B shielding effects to changes in tropospheric $O_3$ concentrations is a highly complex problem as discussed in the next section.

### 10.4.5 UV-B Shielding Effects Associated with Changes in Tropospheric $O_3$ Concentrations

There are multiple complexities in attempting to quantify the relationship between changes in tropospheric $O_3$ concentrations and UV-B exposure. The 2006 $O_3$ AQCD (U.S. EPA, 2006b) described a handful of studies addressing this relationship, but none reported quantifiable effects of tropospheric $O_3$ concentration fluctuations on UV-B exposure at the surface. Further quantifying the relationship between UV-B exposure and health or welfare effects is complicated by the uncertainties involved in the selection of an action spectrum and appropriate characterization of dose

(e.g., peak or cumulative levels of exposure, timing of exposures, etc.) The lack of published studies that critically examined these issues together--that is the incremental health or welfare effects attributable specifically to UV-B changes resulting from changes in tropospheric $O_3$ concentrations--lead to the prior conclusion that the effect of changes in surface-level $O_3$ concentrations on UV-induced health outcomes could not be critically assessed within reasonable uncertainty (U.S. EPA, 2006b).[1]

A recent study by Madronich et al. (2011) used CMAQ to estimate UV radiation response to changes in tropospheric $O_3$ concentrations under different control scenarios projected out to 2020. This study focused on southeastern U.S. and accounted for spatial and temporal variation in tropospheric $O_3$ concentration reductions, an important consideration since most controls are focused on reducing $O_3$ concentrations in populated urban areas. The contrasting control strategies considered in this study included a historical scenario designed to meet an 84 ppb 8-h daily max standard and a reduced scenario designed to bring areas predicted to exceed a similarly designed 70 ppb standard into attainment. A biologically effective irradiance was estimated by multiplying the modeled UV irradiance by a sensitivity function (action spectrum) for the induction of nonmelanoma skin cancer in mice corrected for human skin transmission, then integrating over UV wavelengths. The average relative change in skin cancer-weighted surface UV radiation between the two scenarios was 0.11 ± 0.03% over June, July and August. Weighting by population, this estimate increased to 0.19 ± 0.06%. Madronich et al. (2011) report that their estimated UV radiation increment is an order of magnitude less than that reported in an earlier study by Lutter and Wolz (1997) with the main reason for the discrepancy coming from the overly-simplified uniform 10 ppb reduction in $O_3$ concentrations assumed in the former study. Madronich et al. (2011) did not attempt to link their predicted increase in UV radiation to a predicted increase in skin cancer incidence, however, due to several remaining and substantial uncertainties.

Quantitatively estimating human health and welfare effects directly attributed to changes in UV-B penetration resulting from changes in ground-level $O_3$ concentrations will require both (a) a solid understanding of the multiple factors that define the extent of exposure to UV-B, and (b) well-defined and quantifiable links between UV-B exposure and human disease and welfare effects. Detailed information does not exist regarding the relevant type (e.g., peak or cumulative) and time period (e.g., developmental, lifetime, or current) of exposure, wavelength dependency of biological responses, and inter-individual variability in UV resistance.

[1] The reader is referred to the U.S. EPA 2003 Final Response to Court Remand (U.S. EPA, 2003) for detailed discussions of the data and scientific issues associated with the determination of public health benefits resulting from the attenuation of UV-B by surface-level $O_3$.

Although the UV-B related health effects attributed to marginal reductions in tropospheric or ground-level $O_3$ concentrations have not been directly assessed to date, they would be expected to be small based on current information indicating a negligibly small effect of potential future changes in tropospheric $O_3$ concentrations on ground-level UV-B radiation. In conclusion, the effect of changes in surface-level $O_3$ concentrations on UV-induced health and welfare outcomes cannot yet be critically assessed within reasonable uncertainty.

## 10.5 Summary and Causal Determinations

### 10.5.1 Summary of the Effects of Tropospheric $O_3$ on Climate

Radiative forcing by a greenhouse gas or aerosol is a metric used to quantify the change in balance between radiation coming into and going out of the atmosphere caused by the presence of that substance. Tropospheric $O_3$ is a major greenhouse gas and radiative forcing agent; evidence from satellite data shows a sharp dip in the outgoing infrared radiation in the 9.6 μm $O_3$ absorption band. Models calculate that the global average concentration of tropospheric $O_3$ has doubled since the pre-industrial era, while observations indicate that in some regions $O_3$ may have increased by factors as great as 4 or 5. These increases are tied to the rise in emissions of $O_3$ precursors from human activity, mainly fossil fuel consumption and agricultural processes. Overall, the evidence supports **a causal relationship between changes in tropospheric $O_3$ concentrations and radiative forcing.**

While the developed world has successfully reduced emissions of $O_3$ precursors in recent decades, many developing countries have experienced large increases in precursor emissions and these trends are expected to continue, at least in the near term. Projections of radiative forcing due to changing $O_3$ concentrations over the 21st century show wide variation, due in large part to the uncertainty of future emissions of source gases. In the near-term (2000-2030), projections of $O_3$ radiative forcing range from near zero to +0.3 W/m$^2$, depending on the emissions scenario (Stevenson et al., 2006).

The impact of the tropospheric $O_3$ change since pre-industrial times on climate has been estimated to be about 25-40% of the anthropogenic $CO_2$ impact and about 75% of the anthropogenic $CH_4$ impact according to the IPCC, ranking it third in importance after $CO_2$ and $CH_4$. There are large uncertainties in the magnitude of the radiative forcing estimate attributed to tropospheric $O_3$, making the impact of tropospheric $O_3$ on climate more uncertain than the effect of the longer-lived greenhouse gases. Furthermore, radiative forcing does not take into account the climate feedbacks that could amplify or dampen the actual climate response (e.g., surface temperature change) that would result from a change in tropospheric $O_3$ concentrations. Quantifying the change in surface temperature requires a complex climate simulation in which all important feedbacks and interactions are accounted

for. As these processes are not well understood or easily modeled, the surface temperature response to a given radiative forcing is highly uncertain and can vary greatly among models and from region to region within the same model. Even with these these uncertainties, global climate models indicate that tropospheric $O_3$ has contributed to observed changes in global mean and regional surface temperatures. As a result of such evidence presented in climate modeling studies, there **is likely to be a causal relationship between changes in tropospheric $O_3$ concentrations and effects on climate** as quantified through surface temperature response.

Reduction of tropospheric $O_3$ concentrations could therefore provide an important means to slow climate change in addition to the added benefit of improving surface air quality. However the precursors of $O_3$ also have competing effects on the greenhouse gas $CH_4$, complicating emissions reduction strategies. A decrease in CO or VOC emissions would enhance OH concentrations, shortening the lifetime of $CH_4$, while a decrease in $NO_X$ emissions could depress OH concentrations in certain regions and lengthen the $CH_4$ lifetime. Abatement of $CH_4$ emissions would likely provide the most straightforward means to address climate change since $CH_4$ is itself an important $O_3$ precursor (West et al., 2007; West et al., 2006; Fiore et al., 2002). A reduction of $CH_4$ emissions would also improve air quality on its own right. A set of global abatement measures identified by West and Fiore (2005) could reduce $CH_4$ emissions by 10% at a cost savings, decrease background $O_3$ concentrations by about 1 ppb in the Northern Hemisphere summer, and lead to a global net cooling of 0.12 W/m$^2$. West et al. (2007) explored further the benefits of $CH_4$ abatement, finding that a 20% reduction in global $CH_4$ emissions would lead to greater cooling per unit reduction in surface $O_3$ concentration, compared to 20% reductions in VOCs or CO.

Important uncertainties remain regarding the effect of tropospheric $O_3$ on future climate change. To address these uncertainties, further research is needed to: (1) improve knowledge of the natural atmosphere; (2) interpret observed trends in $O_3$ concentrations in the free troposphere and remote regions; (3) improve understanding of the $CH_4$ budget, especially emissions from wetlands and agricultural sources, (4) understand the relationship between regional $O_3$ radiative forcing and regional climate change; and (5) determine the optimal mix of emissions reductions that would act to limit future climate change.

### 10.5.2 Summary of UV-B Related Effects on Human Health, Ecosystems, and Materials Relating to Changes in Tropospheric $O_3$ Concentrations

UV radiation emitted from the Sun contains sufficient energy when it reaches the Earth to break (photolyze) chemical bonds in molecules, thereby leading to damaging effects on living organisms and materials. Atmospheric $O_3$ plays a crucial role in reducing exposure to solar UV radiation at the Earth's surface. Ozone in the stratosphere is responsible for the majority of this shielding effect, as approximately 90% of total atmospheric $O_3$ is located there over mid-latitudes. Ozone in the

troposphere provides supplemental shielding of radiation in the wavelength band from 280-315 nm, referred to as UV-B radiation. UV-B radiation has important effects on human health and ecosystems, and is associated with materials damage.

EPA has found no published studies that adequately examine the incremental health or welfare effects (adverse or beneficial) attributable specifically to changes in UV-B exposure resulting from perturbations in tropospheric $O_3$ concentrations. While the effects are expected to be small, they cannot yet be critically assessed within reasonable uncertainty. Overall, the evidence **is inadequate to determine if a causal relationship exists between changes in tropospheric $O_3$ concentrations and effects on health and welfare related to UV-B shielding.**

### 10.5.3 Summary of $O_3$ Causal Determinations

The evidence reviewed in this chapter describes the recent findings regarding the climate and UV-B shielding effects of changes in tropospheric $O_3$ concentrations. Table 10-2 provides an overview of the causal determinations for each of the categories evaluated including the effect of tropospheric $O_3$ on radiative forcing, climate change, and health and welfare effects related to UV-B shielding.

**Table 10-2 Summary of $O_3$ causal determinations for climate and UV-B shielding effects.**

| Effects | Causal Determination |
|---|---|
| Radiative Forcing | Causal relationship |
| Climate Change | Likely to be a causal relationship |
| Health and Welfare Effects Related to UV-B Shielding | Inadequate to determine if a causal relationship exists |

## References


Aphalo, PJ; Vapaavuori, EM; de la Rosa, TM; Lehto, T. (2009). Does supplemental UV-B radiation affect gas exchange and RuBisCO activity of Betula pendula Roth. seedlings grown in forest soil under greenhouse conditions? Plant Ecol Divers 2: 37-43. http://dx.doi.org/10.1080/17550870902780299

Arrhenius, S. (1896). On the influence of carbonic acid in the air upon the temperature of the ground. Philos Mag 41: 237-276.

Balis, DS; Zerefos, CS; Kourtidis, K; Bais, AF; Hofzumahaus, A; Kraus, A; Schmitt, R; Blumthaler, M; Gobbi, GP. (2002). Measurements and modeling of photolysis rates during the photochemical activity and ultraviolet radiation (PAUR) II campaign. J Geophys Res 107: 8138. http://dx.doi.org/10.1029/2000JD000136

Berntsen, TK; Myhre, G; Stordal, F; Isaksen, ISA. (2000). Time evolution of tropospheric ozone and its radiative forcing. J Geophys Res 105: 8915-8930. http://dx.doi.org/10.1029/1999JD901139

Boer, GJ; Yu, B. (2003). Climate sensitivity and response. Clim Dynam 20: 415-429. http://dx.doi.org/10.1007/s00382-002-0283-3

Bony, S; Colman, R; Kattsov, VM; Allan, RP; Bretherton, CS; Dufresne, JL; Hall, A; Hallegatte, S; Holland, MM; Ingram, W; Randall, DA; Soden, BJ; Tselioudis, G; Webb, MJ. (2006). How well do we understand and evaluate climate change feedback processes? J Clim 19: 3445-3482.

Brandt, LA; Bohnet, C; King, JY. (2009). Photochemically induced carbon dioxide production as a mechanism for carbon loss from plant litter in arid ecosystems. J Geophys Res 114: G02004. http://dx.doi.org/10.1029/2008jg000772

Brewer, PG; Peltzer, ET. (2009). Limits to marine life. Science 324: 347-348. http://dx.doi.org/10.1126/science.1170756

Brown, BA; Jenkins, GI. (2008). UV-B signaling pathways with different fluence-rate response profiles are distinguished in mature Arabidopsis leaf tissue by requirement for UVR8, HY5, and HYH. Plant Physiol 146: 576-588. http://dx.doi.org/10.1104/pp.107.108456

Bruhl, C; Crutzen, PJ. (1989). On the disproportionate role of tropospheric ozone as a filter against solar UV-B radiation. Geophys Res Lett 16: 703-706. http://dx.doi.org/10.1029/GL016i007p00703

Calderini, DF; Lizana, XC; Hess, S; Jobet, CR; Zuniga, JA. (2008). Grain yield and quality of wheat under increased ultraviolet radiation (UV-B) at later stages of the crop cycle. J Agr Sci 146: 57-64. http://dx.doi.org/10.1017/S0021859607007447

Caldwell, MM; Bornman, JF; Ballare, CL; Flint, SD; Kulandaivelu, G. (2007). Terrestrial ecosystems, increased solar ultraviolet radiation, and interactions with bother climate change factors. Photochem Photobiol Sci 6: 252-266. http://dx.doi.org/10.1039/B700019g

Chang, W; Liao, H; Wang, H. (2009). Climate responses to direct radiative forcing of anthropogenic aerosols, tropospheric ozone, and long-lived greenhouse gases in eastern China over 1951-2000. Adv Atmos Sci 26: 748-762. http://dx.doi.org/10.1007/s00376-009-9032-4

Chen, WT; Liao, H; Seinfeld, JH. (2007b). Future climate impacts of direct radiative forcing of anthropogenic aerosols, tropospheric ozone, and long-lived greenhouse gases. J Geophys Res 112: D14209. http://dx.doi.org/10.1029/2006JD008051

Christiansen, B. (1999). Radiative forcing and climate sensitivity: The ozone experience. Q J Roy Meteorol Soc 125: 3011-3035. http://dx.doi.org/10.1002/qj.49712556011

Clarke, LJ; Robinson, SA. (2008). Cell wall-bound ultraviolet-screening compounds explain the high ultraviolet tolerance of the Antarctic moss, Ceratodon purpureus. New Phytol 179: 776-783. http://dx.doi.org/10.1111/j.1469-8137.2008.02499.x

Cooper, OR; Parrish, DD; Stohl, A; Trainer, M; Nedelec, P; Thouret, V; Cammas, JP; Oltmans, SJ; Johnson, BJ; Tarasick, D; Leblanc, T; Mcdermid, IS; Jaffe, D; Gao, R; Stith, J; Ryerson, T; Aikin, K; Campos, T; Weinheimer, A; Avery, MA. (2010). Increasing springtime ozone mixing ratios in the free troposphere over western North America. Nature 463: 344-348. http://dx.doi.org/10.1038/nature08708

Crist, KC; Carmichael, GR; John, K. (1994). UV-B exposure and atmospheric ozone - Evaluation of radiative flux to changes in ambient ozone levels. J Hazard Mater 37: 527-538. http://dx.doi.org/10.1016/0304-3894(93)E0096-K

Croteau, MC; Davidson, MA; Lean, DRS; Trudeau, VL. (2008a). Global increases in ultraviolet B radiation: Potential impacts on amphibian development and metamorphosis [Review]. Physiol Biochem Zool 81: 743-761. http://dx.doi.org/10.1086/591949

Croteau, MC; Martyniuk, CJ; Trudeau, VL; Lean, DRS. (2008b). Chronic exposure of rana pipiens tadpoles to uvb radiation and the estrogenic chemical 4-tert-octylphenol. J Toxicol Environ Health A 71: 134-144.

Derwent, RG; Collins, WJ; Johnson, CE; Stevenson, DS. (2001). Transient behaviour of tropospheric ozone precursors in a global 3-D CTM and their indirect greenhouse effects. Clim Change 49: 463-487.

Favory, JJ; Stec, A; Gruber, H; Rizzini, L; Oravecz, A; Funk, M; Albert, A; Cloix, C; Jenkins, GI; Oakeley, EJ; Seidlitz, HK; Nagy, F; Ulm, R. (2009). Interaction of COP1 and UVR8 regulates UV-B-induced photomorphogenesis and stress acclimation in Arabidopsis. EMBO J 28: 591-601. http://dx.doi.org/10.1038/emboj.2009.4

Fiore, AM; Jacob, DJ; Field, BD; Streets, DG; Fernandes, SD; Jang, C. (2002). Linking ozone pollution and climate change: The case for controlling methane. Geophys Res Lett 29: 1919. http://dx.doi.org/10.1029/2002GL015601

Fiore, AM; West, JJ; Horowitz, LW; Naik, V; Schwartzkopf, MD. (2008). Characterizing the tropospheric ozone response to methane emission controls and the benefits to climate and air quality. J Geophys Res 113: D08307. http://dx.doi.org/10.1029/2007JD009162

Fishman, J; Fakhruzzaman, K; Cros, B; Nganga, D. (1991). Identification of widespread pollution in the Southern Hemisphere deduced from satellite analyses. Science 252: 1693-1696. http://dx.doi.org/10.1126/science.252.5013.1693

Forster, P; Ramaswamy, V; Artaxo, P; Berntsen, T; Betts, R; Fahey, DW; Haywood, J; Lean, J; Lowe, DC; Myhre, G; Nganga, J; Prinn, R; Raga, G; Schultz, M; Van Dorland, R. (2007). Changes in atmospheric constituents and in radiative forcing. In Climate Change 2007: The physical science basis: Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (pp. 129-234). Cambridge, UK: Cambridge University Press. http://www.ipcc.ch/pdf/assessment-report/ar4/wg1/ar4-wg1-chapter2.pdf

Fritz, JJ; Neale, PJ; Davis, RF; Peloquin, JA. (2008). Response of Antarctic phytoplankton to solar UVR exposure: Inhibition and recovery of photosynthesis in coastal and pelagic assemblages. Mar Ecol Prog Ser 365: 1-16. http://dx.doi.org/10.3354/Meps07610

Fuglestvedt, JS; Berntsen, TK; Isaksen, ISA; Mao, H; Liang, XZ; Wang, WC. (1999). Climatic forcing of nitrogen oxides through changes in tropospheric ozone and methane: Global 3D model studies. Atmos Environ 33: 961-978. http://dx.doi.org/10.1016/S1352-2310(98)00217-9

Fusco, AC; Logan, JA. (2003). Analysis of 1970-1995 trends in tropospheric ozone at Northern Hemisphere midlatitudes with the GEOS-CHEM model. J Geophys Res 108: 4449. http://dx.doi.org/10.1029/2002JD002742

Gao, KS; Ruan, ZX; Villafane, VE; Gattuso, JP; Helbling, EW. (2009a). Ocean acidification exacerbates the effect of UV radiation on the calcifying phytoplankter Emiliania huxleyi. Limnol Oceanogr 54: 1855-1862.

Garcia, TS; Paoletti, DJ; Blaustein, AR. (2009b). Correlated trait responses to multiple selection pressures in larval amphibians reveal conflict avoidance strategies. Freshw Biol 54: 1066-1077. http://dx.doi.org/10.1111/j.1365-2427.2008.02154.x

Gauss, M; Myhre, G; Isaksen, ISA; Grewe, V; Pitari, G; Wild, O; Collins, WJ; Dentener, FJ; Ellingsen, K; Gohar, LK; Hauglustaine, DA; Iachetti, D; Lamarque, JF; Mancini, E; Mickley, LJ; Prather, MJ; Pyle, JA; Sanderson, MG; Shine, KP; Stevenson, DS; Sudo, K; Szopa, S; Zeng, G. (2006). Radiative forcing since preindustrial times due to ozone change in the troposphere and the lower stratosphere. Atmos Chem Phys 6: 575-599.

Gauss, M; Myhre, G; Pitari, G; Prather, MJ; Isaksen, ISA; Berntsen, TK; Brasseur, GP; Dentener, FJ; Derwent, RG; Hauglustaine, DA; Horowitz, LW; Jacob, DJ; Johnson, M; Law, KS; Mickley, LJ; Müller, JF; Plantevin, PH; Pyle, JA; Rogers, HL; Stevenson, DS; Sundet, JK; Van Weele, M; Wild, O. (2003). Radiative forcing in the 21st century due to ozone changes in the troposphere and the lower stratosphere. J Geophys Res 108: 4292. http://dx.doi.org/10.1029/2002JD002624

Grenfell, JL; Shindell, DT; Koch, D; Rind, D. (2001). Chemistry-climate interactions in the Goddard Institute for Space Studies general circulation model 2. New insights into modeling the preindustrial atmosphere. J Geophys Res 106: 33435-33451.

Haapala, JK; Morsky, SK; Saarnio, S; Rinnan, R; Suokanerva, H; Kyr, E; Latola, K; Martikanen, PJ; Holopainen, T; Silvola, J. (2009). Carbon dioxide balance of a fen ecosystem in northern Finland under elevated UV-B radiation. Global Change Biol 15: 943-954. http://dx.doi.org/10.1111/j.1365-2486.2008.01785.x

Hader, DP; Kumar, HD; Smith, RC; Worrest, RC. (2007). Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. Photochem Photobiol Sci 6: 267-285. http://dx.doi.org/10.1039/B700020k

Hansen, J; Sato, M; Ruedy, R; Nazarenko, L; Lacis, A; Schmidt, GA; Russell, G; Aleinov, I; Bauer, M; Bauer, S; Bell, N; Cairns, B; Canuto, V; Chandler, M; Cheng, Y; Del Genio, A; Faluvegi, G; Fleming, E; Friend, A; Hall, T; Jackman, C; Kelley, M; Kiang, N; Koch, D; Lean, J; Lerner, J; Lo, K; Menon, S; Miller, R; Minnis, P; Novakov, T; Oinas, V; Perlwitz, J; Perlwitz, J; Rind, D; Romanou, A; Shindell, D; Stone, P; Sun, S; Tausnev, N; Thresher, D; Wielicki, B; Wong, T; Yao, M; Zhang, S. (2005). Efficacy of climate forcings. J Geophys Res 110: D18104. http://dx.doi.org/10.1029/2005JD005776

Hansen, JE; Sato, M; Ruedy, R. (1997). Radiative forcing and climate response. J Geophys Res 102: 6831-6864. http://dx.doi.org/10.1029/96JD03436

Harvey, LDD. (2004). Characterizing the annual-mean climatic effect of anthropogenic CO2 and aerosol emissions in eight coupled atmosphere-ocean GCMs. Clim Dynam 23: 569-599. http://dx.doi.org/10.1007/s00382-004-0455-4

Heald, CL; Henze, DK; Horowitz, LW; Feddema, J; Lamarque, JF; Guenther, A; Hess, PG; Vitt, F; Seinfeld, JH; Goldstein, AH; Fung, I. (2008). Predicted change in global secondary organic aerosol concentrations in response to future climate, emissions, and land use change. J Geophys Res 113: D05211. http://dx.doi.org/10.1029/2007jd009092

Held, IM; Soden, BJ. (2000). Water vapor feedback and global warming. Annual Review of Energy and the Environment 25: 441-475.

Henry, HAL; Brizgys, K; Field, CB. (2008). Litter decomposition in a california annual grassland: Interactions between photodegradation and litter layer thickness. Ecosystems 11: 545-554. http://dx.doi.org/10.1007/s10021-008-9141-4

Holland, MM; Bitz, CM. (2003). Polar amplification of climate change in coupled models. Clim Dynam 21: 221-232. http://dx.doi.org/10.1007/s00382-003-0332-6

Holtby, LB; Bothwell, ML. (2008). Effects of solar ultraviolet radiation on the behaviour of juvenile coho salmon (Oncorhynchus kisutch): Avoidance, feeding, and agonistic interactions. Can J Fish Aquat Sci 65: 701-711. http://dx.doi.org/10.1139/F08-013

ICNIRP (International Commission on Non-Ionizing Radiation Protection). (2004). Guidelines on limits of exposure to ultraviolet radiation of wavelengths between 180 nm and 400 nm (incoherent optical radiation). In ICNIRP Guidelines. Oberschleissheim, Germany.

Ioki, M; Takahashi, S; Nakajima, N; Fujikura, K; Tamaoki, M; Saji, H; Kubo, A; Aono, M; Kanna, M; Ogawa, D; Fukazawa, J; Oda, Y; Yoshida, S; Watanabe, M; Hasezawa, S; Kondo, N. (2008). An unidentified ultraviolet-B-specific photoreceptor mediates transcriptional activation of the cyclobutane pyrimidine dimer photolyase gene in plants. Planta 229: 25-36. http://dx.doi.org/10.1007/s00425-008-0803-4

IPCC (Intergovernmental Panel on Climate Change). (2000). Special report on emissions scenarios: A special report of Working Group III of the Intergovernmental Panel on Climate Change. Cambridge, UK: Intergovernmental Panel on Climate Change; Cambridge University Press. http://www.grida.no/climate/ipcc/emission/

IPCC (Intergovernmental Panel on Climate Change). (2007b). Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge, United Kingdom: Cambridge University Press. http://www.ipcc.ch/publications_and_data/ar4/wg1/en/contents.html

IPCC (Intergovernmental Panel on Climate Change). (2007c). Summary for policymakers. In: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press. http://www.ipcc.ch/publications_and_data/ar4/wg2/en/spm.html

Isaksen, ISA; Berntsen, TK; Wang, WC. (2001). NOx emissions from aircraft: Its impact on the global distribution of CH4 and O3 and on radiative forcing. Terr Atmos Ocean Sci 12: 63-78.

Ito, A; Sudo, K; Akimoto, H; Sillman, S; Penner, JE. (2007a). Global modeling analysis of tropospheric ozone and its radiative forcing from biomass burning emissions in the twentieth century. J Geophys Res 112: D24307. http://dx.doi.org/10.1029/2007JD008745

Jacobson, MZ; Streets, DG. (2009). Influence of future anthropogenic emissions on climate, natural emissions, and air quality. J Geophys Res 114: D08118. http://dx.doi.org/10.1029/2008JD011476

Jaffe, D; Price, H; Parrish, D; Goldstein, A; Harris, J. (2003). Increasing background ozone during spring on the west coast of North America. Geophys Res Lett 30: 1613. http://dx.doi.org/10.1029/2003GL017024

Jenkins, GI. (2009). Signal transduction in responses to UV-B radiation. Annu Rev Plant Biol 60: 407-431. http://dx.doi.org/10.1146/annurev.arplant.59.032607.092953

Johnson, CE; Collins, WJ; Stevenson, DS; Derwent, RG. (1999). The relative roles of climate and emissions changes on future tropospheric oxidant concentrations. J Geophys Res 104: 18631-18645. http://dx.doi.org/10.1029/1999JD900204

Jokinen, IE; Markkula, ES; Salo, HM; Kuhn, P; Nikoskelainen, S; Arts, MT; Browman, HI. (2008). Exposure to increased ambient ultraviolet B radiation has negative effects on growth, condition and immune function of juvenile Atlantic salmon (Salmo salar). Photochem Photobiol 84: 1265-1271. http://dx.doi.org/10.1111/j.1751-1097.2008.00358.x

Jonson, JE; Simpson, D; Fagerli, H; Solberg, S. (2005). Can we explain the trends in European ozone levels? Atmos Chem Phys 6: 51-66. http://dx.doi.org/10.5194/acp-6-51-2006

Joshi, M; Shine, KP; Ponater, M; Stuber, N; Sausen, R; Li, L. (2003). A comparison of climate response to different radiative forcings in three general circulation models: Towards an improved metric of climate change. Clim Dynam 20: 843-854. http://dx.doi.org/10.1007/s00382-003-0305-9

Kar, J; Fishman, J; Creilson, JK; Richter, A; Ziemke, J; Chandra, S. (2010). Are there urban signatures in the tropospheric ozone column products derived from satellite measurements? Atmos Chem Phys 10: 5213-5222. http://dx.doi.org/10.5194/acp-10-5213-2010

Kataoka, Y; Kiguchi, M; Williams, RS; Evans, PD. (2007). Violet light causes photodegradation of wood beyond the zone affected by ultraviolet radiation. Holzforschung und Holzverwertung 61: 23-27. http://dx.doi.org/10.1515/HF.2007.005

Kiehl, JT; Schneider, TL; Portmann, RW; Solomon, S. (1999). Climate forcing due to tropospheric and stratospheric ozone. J Geophys Res 104: 31239-31254. http://dx.doi.org/10.1029/1999JD900991

Lacis, AA; Wuebbles, DJ; Logan, JA. (1990). Radiative forcing of climate by changes in the vertical distribution of ozone. J Geophys Res 95: 9971-9981. http://dx.doi.org/10.1029/JD095iD07p09971

Lamarque, JF; Bond, TC; Eyring, V; Granier, C; Heil, A; Klimont, Z; Lee, D; Liousse, C; Mieville, A; Owen, B; Schultz, MG; Shindell, D; Smith, SJ; Stehfest, E; Van Aardenne, J; Cooper, OR; Kainuma, M; Mahowald, N; Mcconnell, J. R.; Naik, V; Riahi, K; Van Vuuren, DP. (2010). Historical (1850-2000) gridded anthropogenic and biomass burning emissions of reactive gases and aerosols: Methodology and application. Atmos Chem Phys Discuss 10: 4963-5019. http://dx.doi.org/10.5194/acpd-10-4963-2010

Lamarque, JF; Hess, P; Emmons, L; Buja, L; Washington, W; Granier, C. (2005). Tropospheric ozone evolution between 1890 and 1990. J Geophys Res 110: D08304. http://dx.doi.org/10.1029/2004JD005537

Lamarque, JF; Kyle, GP; Meinshausen, M; Riahi, K; Smith, SJ; Vuuren, DP; Conley, AJ; Vitt, F. (2011). Global and regional evolution of short-lived radiatively-active gases and aerosols in the Representative Concentration Pathways. Clim Change 109: 191-212. http://dx.doi.org/10.1007/s10584-011-0155-0

Lelieveld, J; van Aardenne, J; Fischer, H; de Reus, M; Williams, J; Winkler, P. (2004). Increasing ozone over the Atlantic Ocean. Science 304: 1483-1487. http://dx.doi.org/10.1126/science.1096777

Lenoble, J. (1993). Atmospheric radiative transfer. Hampton, VA: A. Deepak Publishing. http://www.worldcat.org/title/atmospheric-radiative-transfer/oclc/27769441

Levy, H; Schwaarzkopf, MD; Horowitz, L; Ramaswamy, V; Findell, KL. (2008). Strong sensitivity of late 21st century climate to projected changes in short-lived air pollutants. J Geophys Res 113: D06102. http://dx.doi.org/10.1029/2007JD009176

Liao, H; Chen, WT; Seinfeld, JH. (2006). Role of climate change in global predictions of future tropospheric ozone and aerosols. J Geophys Res 111: D12304. http://dx.doi.org/10.1029/2005jd006852

Liao, H; Seinfeld, JH; Adams, PJ; Mickley, LJ. (2004b). Global radiative forcing of coupled tropospheric ozone and aerosols in a unified general circulation model. J Geophys Res 109: D16207. http://dx.doi.org/10.1029/2003JD004456

Logan, JA; Megretskaia, IA; Miller, AJ; Tiao, GC; Choi, D; Zhang, L; Stolarski, RS; Labow, GJ; Hollandsworth, SM; Bodeker, GE; Claude, H; De Muer, D; Kerr, JB; Tarasick, DW; Oltmans, SJ; Johnson, B; Schmidlin, F; Staehelin, J; Viatte, P; Uchino, O. (1999). Trends in the vertical distribution of ozone: A comparison of two analyses of ozonesonde data. J Geophys Res 104: 26373-26399. http://dx.doi.org/10.1029/1999JD900300

Lutter, R; Wolz, C. (1997). UV-B screening by tropospheric ozone: Implications for the national ambient air quality standard. Environ Sci Technol 31: 142A-146A.

Madronich, S; Wagner, M; Groth, P. (2011). Influence of tropospheric ozone control on exposure to ultraviolet radiation at the surface. Environ Sci Technol 45: 6919-6923. http://dx.doi.org/10.1021/es200701q

Marenco, A; Gouget, H; Nédélec, P; Pagés, JP; Karcher, F. (1994). Evidence of a long-term increase in tropospheric ozone from Pic du Midi data series: Consequences: Positive radiative forcing. J Geophys Res 99: 16617-16632. http://dx.doi.org/10.1029/94JD00021

Markkula, E; Salo, HM; Rikalainen, K; Jokinen, IE. (2009). Long-term UVB irradiation affects the immune functions of carp (Cyprinus carpio) and rainbow trout (Oncorhynchus mykiss). Photochem Photobiol 85: 347-352. http://dx.doi.org/10.1111 j.1751-1097.2008.00446.x

Marquis, O; Miaud, C. (2008). Variation in UV sensitivity among common frog Rana temporaria populations along an altitudinal gradient. Zoology (Jena) 111: 309-317. http://dx.doi.org/10.1016/j.zool.2007.09.003

Marquis, O; Miaud, C; Lena, JP. (2008). Developmental responses to UV-B radiation in common frog Rana temporaria embryos from along an altitudinal gradient. Population Ecology 50: 123-130. http://dx.doi.org/10.1007/s10144-007-0071-3

Mayer, LM; Schick, LL; Hardy, KR; Estapa, ML. (2009). Photodissolution and other photochemical changes upon irradiation of algal detritus. Limnol Oceanogr 54: 1688-1698.

Mazza, CA; Izaguirre, MM; Curiale, J; Ballare, CL. (2010). A look into the invisible: Ultraviolet-B sensitivity in an insect (Caliothrips phaseoli) revealed through a behavioural action spectrum. Proc Biol Sci 277: 367-373. http://dx.doi.org/10.1098/rspb.2009.1565

Meador, JA; Baldwin, AJ; Catala, P; Jeffrey, WH; Joux, F; Moss, JA; Pakulski, JD; Stevens, R; Mitchell, DL. (2009). Sunlight-induced DNA damage in marine micro-organisms collected along a latitudinal gradient from 70 degrees N to 68 degrees S. Photochem Photobiol 85: 412-421. http://dx.doi.org/10.1111/j.1751-1097.2008.00462.x

Menon, S; Unger, N; Koch, D; Francis, J; Garrett, T; Sednev, I; Shindell, D; Streets, D. (2008). Aerosol climate effects and air quality impacts from 1980 to 2030. Environmental Research Letters 3: 024004. http://dx.doi.org/10.1088/1748-9326/3/2/024004

Mickley, LJ; Jacob, DJ; Field, BD; Rind, D. (2004). Climate response to the increase in tropospheric ozone since preindustrial times: A comparison between ozone and equivalent CO2 forcings. J Geophys Res 109: D05106. http://dx.doi.org/10.1029/2003JD003653

Mickley, LJ; Jacob, DJ; Rind, D. (2001). Uncertainty in preindustrial abundance of tropospheric ozone: Implications for radiative forcing calculations. J Geophys Res 106: 3389-3399. http://dx.doi.org/10.1029/2000JD900594

Mickley, LJ; Murti, PP; Jacob, DJ; Logan, JA; Koch, DM; Rind, D. (1999). Radiative forcing from tropospheric ozone calculated with a unified chemistry-climate model. J Geophys Res 104: 30153-30172. http://dx.doi.org/10.1029/1999JD900439

Moss, RH; Edmonds, JA; Hibbard, KA; Manning, MR; Rose, SK; van Vuuren, DP; Carter, TR; Emori, S; Kainuma, M; Kram, T; Meehl, GA; Mitchell, JF; Nakicenovic, N; Riahi, K; Smith, SJ; Stouffer, RJ; Thomson, AM; Weyant, JP; Wilbanks, TJ. (2010). The next generation of scenarios for climate change research and assessment. Nature 463: 747-756. http://dx.doi.org/10.1038/nature08823

Naik, V; Mauzerall, D; Horowitz, L; Schwarzkopf, MD; Ramaswamy, V; Oppenheimer, M. (2005). Net radiative forcing due to changes in regional emissions of tropospheric ozone precursors. J Geophys Res 110: D24306. http://dx.doi.org/10.1029/2005JD005908

Naja, M; Akimoto, H. (2004). Contribution of regional pollution and long-range transport to the Asia-Pacific region: Analysis of long-term ozonesonde data over Japan. J Geophys Res 109: D21306. http://dx.doi.org/10.1029/2004JD004687

NRC (National Research Council). (2005). Radiative forcing of climate change: Expanding the concept and addressing uncertainties. Washington, DC: The National Academies Press. http://books.nap.edu/openbook.php?record_id=11175&page=R1

Obara, Y; Koshitaka, H; Arikawa, K. (2008). Better mate in the shade: Enhancement of male mating behaviour in the cabbage butterfly, Pieris rapae crucivora, in a UV-rich environment. J Exp Biol 211: 3698-3702. http://dx.doi.org/10.1242/jeb.021980

Oltmans, SJ; Lefohn, AS; Harris, JM; Galbally, I; Scheel, HE; Bodeker, G; Brunke, E; Claude, H; Tarasick, D; Johnson, BJ; Simmonds, P; Shadwick, D; Anlauf, K; Hayden, K; Schmidlin, F; Fujimoto, T; Akagi, K; Meyer, C; Nichol, S; Davies, J; Redondas, A; Cuevas, E. (2006). Long-term changes in tropospheric ozone. Atmos Environ 40: 3156-3173. http://dx.doi.org/10.1016/j.atmosenv.2006.01.029

Ordonez, C; Brunner, D; Staehelin, J; Hadjinicolaou, P; Pyle, JA; Jonas, M; Wernli, H; Prevot, ASH. (2007). Strong influence of lowermost stratospheric ozone on lower tropospheric background ozone changes over Europe. Geophys Res Lett 34: L07805. http://dx.doi.org/10.1029/2006GL029113

Oromi, N; Marquis, O; Miaud, C; Sanuy, D. (2008). Influence of ambient ultraviolet radiation on Bufo calamita egg development in a semiarid zone (Catalonia, Spain). J Environ Biol 29: 135-137.

Pavelin, EG; Johnson, CE; Rughooputh, S; Toumi, R. (1999). Evaluation of pre-industrial surface ozone measurements made using Schonbein's method. Atmos Environ 33: 919-929. http://dx.doi.org/10.1016/S1352-2310(98)00257-X

Phoenix, GK; Gwynn-Jones, D; Lee, JA; Callaghan, TV. (2000). The impacts of UV-B radiation on the regeneration of a sub-arctic heath community. Plant Ecol 146: 67-75. http://dx.doi.org/10.1023/A:1009839506658

Pickett, JE; Gibson, DA; Gardner, MM. (2008). Effects of irradiation conditions on the weathering of engineering thermoplastics. Polym Degrad Stabil 93: 1597-1606. http://dx.doi.org/10.1016/j.polymdegradstab.2008.02.009

Price, C; Rind, D. (1994). Possible implications of global climate change on global lightning distributions and frequencies. J Geophys Res 99: 10823-10831. http://dx.doi.org/10.1029/94JD00019

Pyle, JA; Warwick, N; Yang, X; Young, PJ; Zeng, G. (2007). Climate/chemistry feedbacks and biogenic emissions. Philos Transact A Math Phys Eng Sci 365: 1727-1740. http://dx.doi.org/10.1098/rsta.2007.2041

RCP (Representative Concentration Pathways). (2009). RCP Database (version 2.0). Available online at http://iiasa.ac.at/web-apps/tnt/RcpDb/dsd?Action=htmlpage&page=about (accessed January 28, 2011).

Riggsbee, JA; Orr, CH; Leech, DM; Doyle, MW; Wetzel, RG. (2008). Suspended sediments in river ecosystems: Photochemical sources of dissolved organic carbon, dissolved organic nitrogen, and adsorptive removal of dissolved iron. J Geophys Res 113: G03019. http://dx.doi.org/10.1029/2007jg000654

Rind, D; Healy, R; Parkinson, C; Martinson, D. (1995). The role of sea ice in 2CO2 climate model sensitivity. Part I: The total influence of sea ice thickness and extent. J Clim 8: 449-463. http://dx.doi.org/10.1175/1520-0442(1995)008<0449:TROSII>2.0.CO;2

Romansic, JM; Waggener, AA; Bancroft, BA; Blaustein, AR. (2009). Influence of ultraviolet-B radiation on growth, prevalence of deformities, and susceptibility to predation in Cascades frog (Rana cascadae) larvae. Hydrobiologia 624: 219-233. http://dx.doi.org/10.1007/s10750-009-9703-2

Sanderson, MG; Jones, CD; Collins, WJ; Johnson, CE; Derwent, RG. (2003). Effect of climate change on isoprene emissions and surface ozone levels. Geophys Res Lett 30: 1936. http://dx.doi.org/10.1029/2003GL017642

Schindler, DW; Curtis, PJ; Parker, BR; Stainton, MP. (1996). Consequences of climate warming and lake acidification for UV-B penetration in North American boreal lakes. Nature 379: 705-708. http://dx.doi.org/10.1038/379705a0

Semerdjieva, SI; Phoenix, GK; Hares, D; Gwynn-Jones, D; Callaghan, TV; Sheffield, E. (2003). Surface morphology, leaf and cuticle thickness of four dwarf shrubs from a sub-Arctic heath following long-term exposure to enhanced levels of UV-B. Physiol Plant 117: 289-294. http://dx.doi.org/10.1034/j.1399-3054.2003.00006.x

Shindell, D; Faluvegi, G. (2009). Climate response to regional radiative forcing during the twentieth century. Nat Geosci 2: 294-300. http://dx.doi.org/10.1038/ngeo473

Shindell, D; Faluvegi, G; Lacis, A; Hansen, J; Ruedy, R; Aguilar, E. (2006). Role of tropospheric ozone increases in 20th-century climate change. J Geophys Res 111: D08302. http://dx.doi.org/10.1029/2005JD006348

Shindell, DT; Faluvegi, G. (2002). An exploration of ozone changes and their radiative forcing prior to the chlorofluorocarbon era. Atmos Chem Phys Discuss 2: 363-374. http://dx.doi.org/10.5194/acp-2-363-2002

Shindell, DT; Faluvegi, G; Bauer, SE; Koch, DM; Unger, N; Menon, S; Miller, RL; Schmidt, GA; Streets, DG. (2007). Climate response to projected changes in short-lived species under an A1B scenario from 2000-2050 in the GISS climate model. J Geophys Res 112: D20103. http://dx.doi.org/10.1029/2007jd008753

Shindell, DT; Faluvegi, G; Bell, N. (2003). Preindustrial-to-present-day radiative forcing by tropospheric ozone from improved simulations with the GISS chemistry-climate GCM. Atmos Chem Phys 3: 1675-1702. http://dx.doi.org/10.5194/acp-3-1675-2003

Shindell, DT; Levy H, II; Schwarzkopf, MD; Horowitz, LW; Lamarque, JF; Faluvegi, G. (2008). Multimodel projections of climate change from short-lived emissions due to human activities. J Geophys Res 113: D11109. http://dx.doi.org/10.1029/2007JD009152

Simmonds, PG; Derwent, RG; Manning, AL; Spain, G. (2004). Significant growth in surface ozone at Mace Head, Ireland, 1987-2003. Atmos Environ 38: 4769-4778. http://dx.doi.org/10.1016/j.atmosenv.2004.04.036

Sinha, A; Toumi, R. (1997). Tropospheric ozone, lightning, and climate change. J Geophys Res 102: 10667-10672. http://dx.doi.org/10.1029/96JD03710

Sitch, S; Cox, PM; Collins, WJ; Huntingford, C. (2007). Indirect radiative forcing of climate change through ozone effects on the land-carbon sink. Nature 448: 791-794. http://dx.doi.org/10.1038/nature06059

Sliney, DH; Wengraitis, S. (2006). Is a differentiated advice by season and region necessary? Prog Biophys Mol Biol 92: 150-160. http://dx.doi.org/10.1016/j.pbiomolbio.2006.02.007

Snell, KRS; Kokubun, T; Griffiths, H; Convey, P; Hodgson, DA; Newsham, KK. (2009). Quantifying the metabolic cost to an Antarctic liverwort of responding to an abrupt increase in UVB radiation exposure. Global Change Biol 15: 2563-2573. http://dx.doi.org/10.1111/j.1365-2486.2009.01929.x

Soden, BJ; Held, IM. (2006). An assessment of climate feedbacks in coupled ocean-atmosphere models. J Clim 19: 3354-3360.

Staehelin, J; Thudium, J; Buehler, R; Volz-Thomas, A; Graber, W. (1994). Trends in surface ozone concentrations at Arosa (Switzerland). Atmos Environ 28: 75-87. http://dx.doi.org/10.1016/1352-2310(94)90024-8

Stevenson, DS. (2004). Radiative forcing from aircraft NO emissions: Mechanisms and seasonal dependence. J Geophys Res 109: D17307. http://dx.doi.org/10.1029/2004JD004759

Stevenson, DS; Dentener, FJ; Schultz, MG; Ellingsen, K; Van Noije, TPC; Wild, O; Zeng, G; Amann, M; Atherton, CS; Bell, N; Bergmann, DJ; Bey, I; Butler, T; Cofala, J; Collins, WJ; Derwent, RG; Doherty, RM; Drevet, J; Eskes, HJ; Fiore, AM; Gauss, M; Hauglustaine, DA; Horowitz, LW; Isaksen, ISA; Krol, MC; Lamarque, JF; Lawrence, MG; Montanaro, V; Muller, JF; Pitari, G; Prather, MJ; Pyle, JA; Rast, S; Rodriguez, JM; Sanderson, MG; Savage, NH; Shindell, DT; Strahan, SE; Sudo, K; Szopa, S. (2006). Multimodel ensemble simulations of present-day and near-future tropospheric ozone. J Geophys Res 111: D08301. http://dx.doi.org/10.1029/2005JD006338

Streets, D; Bond, T; Lee, T; Jang, C. (2004). On the future of carbonaceous aerosol emissions. J Geophys Res 109: D24212. http://dx.doi.org/10.1029/2004JD004902

Tanimoto, H. (2009). Increase in springtime tropospheric ozone at a mountainous site in Japan for the period 1998-2006. Atmos Environ 43: 1358-1363. http://dx.doi.org/10.1016/j.atmosenv.2008.12.006

Tarasick, DW; Fioletov, VE; Wardle, DI; Kerr, JB; Davies, J. (2005). Changes in the vertical distribution of ozone over Canada from ozonesondes: 19802001. J Geophys Res 110: D02304. http://dx.doi.org/10.1029/2004JD004643

Thompson, AM. (1992). The oxidizing capacity of the Earth's atmosphere: Probable past and future changes. Science 256: 1157-1165. http://dx.doi.org/10.1126/science.256.5060.1157

Thompson, AM; Chappellaz, JA; Fung, IY; Kucsera, TL. (1993). The atmospheric CH4 increase since the last glacial maximum (2) Interactions with oxidants. Tellus B Chem Phys Meteorol 45: 242-257. http://dx.doi.org/10.1034/j.1600-0889.1993.t01-2-00003.x

Thompson, AM; Hudson, RD. (1999). Tropical tropospheric ozone (TTO) maps from Nimbus 7 and Earth Probe TOMS by the modified-residual method: Evaluation with sondes, ENSO signals, and trends from Atlantic regional time series. J Geophys Res 104: 26961-26975. http://dx.doi.org/10.1029/1999JD900470

U.S. EPA (U.S. Environmental Protection Agency). (2003). National ambient air quality standards for ozone: Final response to remand. Fed Reg 68: 614-645.

U.S. EPA (U.S. Environmental Protection Agency). (2006b). Air quality criteria for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-05/004AF). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=149923

UNEP (United Nations Environment Programme). (2009). Environmental effects of ozone depletion and its interactions with climate change. Nairobi, Kenya. http://ozone.unep.org/Assessment_Panels/EEAP/EEAP-Progress-report-2009.pdf

Unger, N. (2006). Cross influences of ozone and sulfate precursor emissions changes on air quality and climate. PNAS 103: 4377-4380. http://dx.doi.org/10.1073/pnas.0508769103

Unger, N; Bond, TC; Wang, JS; Koch, DM; Menon, S; Shindell, DT; Bauer, S. (2010). Attribution of climate forcing to economic sectors. PNAS 107: 3382-3387. http://dx.doi.org/10.1073/pnas.0906548107

Unger, N; Shindell, DT; Koch, DM; Streets, DG. (2008). Air pollution radiative forcing from specific emissions sectors at 2030. J Geophys Res 113: D02306. http://dx.doi.org/10.1029/2007JD008683

Van Aardenne, JA; Dentener, FJ; Olivier, JGJ; Klein Goldewijk, CGM; Lelieveld, J. (2001). A 11 resolution data set of historical anthropogenic trace gas emissions for the period 1890-1990. Global Biogeochem Cycles 15: 909-928. http://dx.doi.org/10.1029/2000GB001265

Volz, A; Kley, D. (1988). Evaluation of the Montsouris series of ozone measurements made in the nineteenth century. Nature 332: 240-242. http://dx.doi.org/10.1038/332240a0

Wahl, M. (2008). Ecological modulation of environmental stress: Interactions between ultraviolet radiation, epibiotic snail embryos, plants and herbivores. J Anim Ecol 77: 549-557. http://dx.doi.org/10.1111/j.1365-2656.2007.01352.x

Wang, WC; Pinto, JP; Yung, YL. (1980). Climatic effects due to halogenated compounds in the earth's atmosphere. J Atmos Sci 37: 333-338. http://dx.doi.org/10.1175/1520-0469(1980)037<0333:CEDTHC>2.0.CO;2

West, JJ; Fiore, AM. (2005). Management of tropospheric ozone by reducing methane emissions [Review]. Environ Sci Technol 39: 4685-4691.

West, JJ; Fiore, AM; Horowitz, LW; Mauzerall, DL. (2006). Global health benefits of mitigating ozone pollution with methane emission controls. PNAS 103: 3988-3993. http://dx.doi.org/10.1073/pnas.0600201103

West, JJ; Fiore, AM; Naik, V; Horowitz, LW; Schwarzkopf, MD; Mauzerall, DL. (2007). Ozone air quality and radiative forcing consequences of changes in ozone precursor emissions. Geophys Res Lett 34: L06806. http://dx.doi.org/10.1029/2006GL029173

Wild, O; Prather, MJ; Akimoto, H. (2001). Indirect long-term global radiative cooling from NOX emissions. Geophys Res Lett 28: 1719-1722. http://dx.doi.org/10.1029/2000GL012573

Worden, HM; Bowman, KW; Worden, JR; Eldering, A; Beer, R. (2008). Satellite measurements of the clear-sky greenhouse effect from tropospheric ozone. Nat Geosci 1: 305-308. http://dx.doi.org/10.1038/ngeo182

Wu, S; Mickley, LJ; Jacob, DJ; Rind, D; Streets, DG. (2008a). Effects of 2000-2050 changes in climate and emissions on global tropospheric ozone and the policy-relevant background surface ozone in the United States. J Geophys Res 113: D18312. http://dx.doi.org/10.1029/2007JD009639

Wu, S; Mickley, LJ; Leibensperger, EM; Jacob, DJ; Rind, D; Streets, DG. (2008b). Effects of 2000-2050 global change on ozone air quality in the United States. J Geophys Res 113: D06302. http://dx.doi.org/10.1029/2007JD008917

Yang, X; Cox, RA; Warwick, NJ; Pyle, JA; Carver, GD; O'connor, FM; Savage, NH. (2005c). Tropospheric bromine chemistry and its impacts on ozone: A model study. J Geophys Res 110: D23311. http://dx.doi.org/10.1029/2005JD006244

Zeng, G; Pyle, JA. (2003). Changes in tropospheric ozone between 2000 and 2100 modeled in a chemistry-climate model. Geophys Res Lett 30: 1392. http://dx.doi.org/10.1029/2002GL016708

Zepp, RG; Erickson, DJ; Paul, ND; Sulzberger, B. (2007). Interactive effects of solar UV radiation and climate change on biogeochemical cycling. In The Environmental Effects of Ozone Depletion and its Interactions with Climate Change: 2006 Assessment (pp. 135-164). Nairobi, Kenya: United Nations Environment Programme.

Zepp, RG; Shank, GC; Stabenau, E; Patterson, KW; Cyterski, M; Fisher, W; Bartels, E; Anderson, SL. (2008). Spatial and temporal variability of solar ultraviolet exposure of coral assemblages in the Florida Keys: Importance of colored dissolved organic matter. Limnol Oceanogr 53: 1909-1922.

Zerefos, CS; Kourtidis, KA; Melas, D; Balis, D; Zanis, P; Katsaros, L; Mantis, HT; Repapis, C; Isaksen, I; Sundet, J; Herman, J; Bhartia, PK; Calpini, B. (2002). Photochemical activity and solar ultraviolet radiation (PAUR) modulation factors: An overview of the project. J Geophys Res 107: 8134. http://dx.doi.org/10.1029/2000JD000134

Ziemke, JR; Chandra, S; Bhartia, PK. (2005). A 25-year data record of atmospheric ozone in the Pacific from Total Ozone Mapping Spectrometer (TOMS) cloud slicing: Implications for ozone trends in the stratosphere and troposphere. J Geophys Res 110: D15105. http://dx.doi.org/10.1029/2004JD005687