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Air Quality Criteria for Ozone and Related Photochemical Oxidants (First External Review Draft)

Volume II of III

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Air Quality Criteria for Ozone and Related Photochemical Oxidants

Volume II

National Center for Environmental Assessment-RTP Office
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC

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PREFACE

National Ambient Air Quality Standards (NAAQS) are promulgated by the United States Environmental Protection Agency (EPA) to meet requirements set forth in Sections 108 and 109 of the U.S. Clean Air Act (CAA). Sections 108 and 109 require the EPA Administrator (1) to list widespread air pollutants that reasonably may be expected to endanger public health or welfare; (2) to issue air quality criteria for them that assess the latest available scientific information on nature and effects of ambient exposure to them; (3) to set "primary" NAAQS to protect human health with adequate margin of safety and to set "secondary" NAAQS to protect against welfare effects (e.g., effects on vegetation, ecosystems, visibility, climate, manmade materials, etc); and (5) to periodically review and revise, as appropriate, the criteria and NAAQS for a given listed pollutant or class of pollutants.

In 1971, the U.S. Environmental Protection Agency (EPA) promulgated National Ambient Air Quality Standards (NAAQS) to protect the public health and welfare from adverse effects of photochemical oxidants. The EPA promulgates the NAAQS on the basis of scientific information contained in air quality criteria issued under Section 108 of the Clean Air Act. Following the review of criteria as contained in the EPA document, Air Quality Criteria for Ozone and other Photochemical Oxidants published in 1978, the chemical designation of the standards was changed from photochemical oxidants to ozone (O_3) in 1979 and a 1-hour O_3 NAAQS was set. The 1978 document focused primarily on the scientific air quality criteria for O_3 and, to a lesser extent, on those for other photochemical oxidants such as hydrogen peroxide and the peroxyacyl nitrates, as have subsequent revised versions of the ozone document.

To meet Clean Air Act requirements noted above for periodic review of criteria and NAAQS, the O_3 criteria document, *Air Quality Criteria for Ozone and Other Photochemical*

Oxidants, was next revised and then released in August 1986; and a supplement, *Summary of Selected New Information on Effects of Ozone on Health and Vegetation*, was issued in January 1992. These documents were the basis for a March 1993 decision by EPA that revision of the existing 1-h NAAQS for O_3 was not appropriate at that time. That decision, however, did not take into account some of the newer scientific data that became available after completion of the 1986 criteria document. Such literature was assessed in the next periodic revision of the O_3 air quality criteria document, which was completed in 1996 and provided scientific bases supporting the setting by EPA in 1997 of an 8-h O_3 NAAQS that is currently in force together with the 1-h O_3 standard.

The purpose of this revised air quality criteria document for O_3 and related photochemical oxidants is to critically evaluate and assess the latest scientific information published since that assessed in the above 1996 Ozone Air Quality Criteria Document (O_3 AQCD), with the main focus being on pertinent new information useful in evaluating health and environmental effects data associated with ambient air O_3 exposures. However, some other scientific data are also presented and evaluated in order to provide a better understanding of the nature, sources, distribution, measurement, and concentrations of O_3 and related photochemical oxidants and their precursors in the environment. The document assesses pertinent literature available through 2004.

The present draft document (dated January 2005) is being released for public comment and review by the Clean Air Scientific Advisory Committee (CASAC) to obtain comments on the organization and structure of the document, the issues addressed, the approaches employed in assessing and interpreting the newly available information on O_3 exposures and effects, and the key findings and conclusions arrived at as a consequence of this assessment. Public comments and recommendations will be taken into account making any appropriate further revisions to this document for incorporation into a Second External Review Draft. That draft will be released for further public comment and CASAC review before last revisions are made in response and incorporated into a final version to be completed by early 2006. Evaluations contained in the present document will be drawn on to provide inputs to associated PM Staff Paper analyses prepared by EPA's Office of Air Quality Planning and Standards (OAQPS) to pose options for consideration by the EPA Administrator with regard to proposal and, ultimately, promulgation of decisions on potential retention or revision, as appropriate, of the current O_3 NAAQS.

Preparation of this document was coordinated by staff of EPA's National Center for Environmental Assessment in Research Triangle Park (NCEA-RTP). NCEA-RTP scientific staff, together with experts from other EPA/ORD laboratories and academia, contributed to writing of document chapters. Earlier drafts of document materials were reviewed by non-EPA experts in peer consultation workshops held by EPA. The document describes the nature, sources, distribution, measurement, and concentrations of O_3 in outdoor (ambient) and indoor environments. It also evaluates the latest data on human exposures to ambient O_3 and consequent health effects in exposed human populations, to support decision making regarding the primary, health-related O_3 NAAQS. The document also evaluates ambient O_3 environmental effects on vegetation and ecosystems, man-made materials, and surface level solar UV radiation flux and global climate change, to support decision making on secondary O_3 NAAQS.

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Air Quality Criteria for Ozone and Related Photochemical Oxidants (First External Review Draft)

VOLUME I

Air Quality Criteria for Ozone and Related Photochemical Oxidants (First External Review Draft)

(cont'd)

VOLUME II

Air Quality Criteria for Ozone and Related Photochemical Oxidants (First External Review Draft)

(cont'd)

VOLUME III

Table of Contents

Table of Contents
(cont'd)

Table of Contents
(cont'd)

Table of Contents
(cont'd)

Table of Contents
(cont'd)

Table of Contents
(cont'd)

Table of Contents
(cont'd)

Table of Contents
(cont'd)

Table of Contents
(cont'd)

Table of Contents
(cont'd)

List of Tables

List of Tables
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List of Tables
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List of Figures

List of Figures

List of Figures

List of Figures

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Abbreviations and Acronyms

AA	ascorbic acid
ACh	acetylcholine
ADs	alveolar entrance rings
ADSS	aged and diluted sidestream cigarette smoke
AED	aerodynamic diameter
AER	air exchange rate
AF	adsorbed fraction
AH_2	ascorbic acid
AHR	airway hyperreactivity
AHSMOG	Adventist Health Study on Smog
AIRS	Aerometric Information Retrieval System (U.S. Environmental Protection Agency)
ALI	air-liquid interface
AM	alveolar macrophage
A_p	cros-sectional area of peripheral lung
AP	alkaline phosphatase
APHEA	Air Pollution on Health: European Approach
AQCD	Air Quality Criteria Document
ATR	atrial natriuretic factor
BAL	bronchoalveolar lavage
BALF	bronchoalveolar lavage fluid
BALT	bronchus-associated lymphoid tissue
B[*a*]P	benzo[*a*]pyrene
BHR	bronchial hyperresponsiveness
b.i.d.	twice a day
BMZ	basement membrane zone
BP	blood pressure
BrdU	bromodeoxyuridine

BS	black smoke
BSA	body surface area
C	concentration
C × T	concentration times duration of exposure
C3a	complement protein fragment
CAPs	concentrated ambient particles
CAR	centriacinar region
CARB	California Air Resources Board
CAT	cell antioxidant capacity
CC16	Clara cell secretory protein
CCh	carbachol
CCSP	Clara cell secretory protein
Cdyn	dynamic lung compliance
CE	continuous exercise
CFD	computational fluid dynamics
CHO	Chinese hamster ovary
CI	confidence interval
CINC	cytokine-induced neutrophil chemoattractant
CIU, CBU	cumulative inhalation unit
CMD	count mean diameter
CO	carbon monoxide
CO_2	carbon dioxide
ConA	concanavalin A
COPD	chronic obstructive pulmonary disease
C_w	chest wall compliance
Cyt	cytochrome
Δ	mean change in a variable
DD	doubling dose
DHBA	2,3-dehydroxybenzoic acid

DNA	deoxyribonucleic acid
DPPC	dipalmitoylglycero-3-phosphocholine
DR	disulfide reductase
ϵ	convergence precision
EEG	electroencephalographic
ELF	epithelial lining fluid
EM	electron microscopy
ENA	epithelial cell-derived neutrophil-activating peptide
EPA	U.S. Environmental Protection Agency
ETS	environmental tobacco smoke
EU	
f, f_B	frequency of breathing
F	female
F344	Fischer 344
FA	filtered air
FA	fatty acid
FA	fractional absorption; absorbed fraction
FAA	Federal Aviation Administration
FEF	forced expiratory flow
FEF_{25}	forced expiratory flow after 25% vital capacity
FEF_{25-75}	forced expiratory flow between 25 and 75% of vital capacity
FEF_{50}	forced expiratory flow after 50% vital capacity
FEF_{6oP}	
FEF_{75}	forced expiratory flow after 75% vital capacity
$FEV_{0.75}$	forced expiratory volume in 0.75 s
FEV_1	forced expiratory volume in 1 s
FFA	free fatty acid
FGF	
FGFR	

FIVC	forced inspiratory vital capacity
Fn	fibronectin
FP	fluticasone propionate
FRC	functional residual capacity
FS	field stimulation
FVC	forced vital capacity
GAM	General Additive Model
GDT	glutathione-disulfide transhydrogenase
GEE	(model)
GLM	General Linear Model
GM-CSF	granulocyte-macrophage colony stimulating factor
G6PD	glucose-6-phosphate dehydrogenase
GR	glutathione reductase
GSH	glutathione
GSHPx, GPx	glutathione peroxidase
GST	glutathione-*S*-transferase
H^+	hydrogen ion
H_2CO, HCHO	formaldehyde
HDMA	house dust mite allergen
H_2O_2	hydrogen peroxide
H_2SO_4	sulfuric acid
HEI	Health Effects Institute
HHP-C9	1-hydroxy-1-hydroperoxynonane
HIST	histamine
HLA	human lymphocyte antigen
HNE	4-hydroxynonenal
HR	heart rate
HSP	heat shock protein
HSV	herpes simplex virus

5-HT	5-hydroxytryptamine
IAS	interalveolar septum
IC	inspiratory capacity
ICAM	intracellular adhesion molecule
ICRP	International Commission on Radiological Protection
ICS	inhaled steroids
IE	intermittent exercise
Ig	immunoglobulin (IgA, IgE, IgG, IgM)
IL	interleukin (IL-1, IL-6, IL-8)
IN	intranasal
INF	interferon
inh	inhalation
iNOS	inducible nitric oxide synthase
ip	intraperitoneal
IT	intratracheal
IU	International Units
iv	intravenous
K_a	intrinsic mass transfer coefficient/parameter
K_g	mass transfer coefficient for gas phase
K_{TB}	tracheobronchial region overall mass transfer coefficient
K_l	mass transfer coefficient for liquid phase
K_r	reaction rate constant
Λ	ozone uptake efficiency
LDH	lactate dehydrogenase
LIS	lateral intercellular space
LM	light microscopy
LOESS	locally estimated smoothing splines
LPS	lipopolysaccharide
LRT	lower respiratory tract

LT	leukotriene (LTB_4, LTC_4, LTD_4, LTE_4)
M	male
M	maximum number of iterations
MCB	monochlorobimane
MCh	methacholine
MCP	monocyte chemotactic protein
MDA	malondialdehyde
MHC	major histocompatibility
MIP	macrophage inflammatory protein
MLN	mediastinal lymph node
MMAD	mass median aerodynamic diameter
mRNA	messenger ribonucleic acid
MSA	metropolitan statistical area
Mt	metallothionein
n, N	number
NAAQS	National Ambient Air Quality Standards
NADH	reduced nicotinamide adenine dinucleotide
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NADPH-CR	cytochrome c reductase pertaining to nicotinamide adenine dinucleotide phosphate activity
NAG	N-acetyl-β-*d*-glucosamine
NB-κB	nuclear factor kappa B
NCEA-RTP	National Center for Environmental Assessment Division in Research Triangle Park, NC
NCICAS	National Cooperative Inner-City Asthma Study
NHBE	cultured human bronchial epithelial (cells)
$(NH_4)_2SO_4$	ammonium sulfate
NIH	National Institutes of Health
NIST	National Institute of Standards and Technology
NK	natural killer (cells)

NL	nasal lavage
NLF	nasal lavage fluid
NMMAPS	National Morbidity, Mortality and Air Pollution Study
NO	nitric oxide
NO_2	nitrogen dioxide
NO_x	nitrogen oxides
NOS	nitric oxide synthase
NS	nonsmoker
NSAID	non-steroidal anti-inflammatory agent
NSBR	nonspecific bronchial respnsiveness
NTP	National Toxicology Program
NTS	nucleus tractus solitarius
O_2^-	superoxide
O_3	ozone
OAQPS	Office of Air Quality Planning and Standards
8-OHdG	8-hydroxy-2′-deoxyguanosine
OVA	ovalbumin
p	probability
PAF	platelet-activating factor
PAN	peroxyacetyl nitrate
PAR	proximal alveolar region
PBPK	physiologically based pharmacokinetic
PC_{20}	provocative concentration that produces a 20% decrease in forced expiratory volume in 1 s
PC-ALF	1-palmitoyl-2-(9-oxononanoyl)-sn-glycero-3-phosphocholine
PCI	picryl chloride
PD	potential difference
PD_{100}	provocative dose that produces a 100% decrease in forced expiratory volume in 1 s

PD_{20}	provocative dose that produces a 20% decrease in forced expiratory volume in 1 s
PE	postexposure
PEF	peak expiratory flow
P_{enh}	enhanced pause
PG	prostaglandin (PGD_2, PGE, PGE_1, PGE_2 $PGF_{1\alpha}$, $PGF_{2\alpha}$)
6PGD	6-phosphogluconate dehydrogenase
PGP	protein gene product
pH	hydrogen ion concentration
PHA	phytohemagglutinin
PIF	peak inspiratory flow
PM	particulate matter
PM_{10}	particulate matter of mass median aerodynamic diameter ≤ 10 μm
PM_{15}	particulate matter of mass median aerodynamic diameter ≤ 15 μm
$PM_{2.5}$	particulate matter of mass median aerodynamic diameter ≤ 2.5 μm
PMN	polymorphonuclear neutrolphil leukocyte (also called neutrophil)
PND	post natal day
PPN	peroxypropionyl nitrate
PSA	picryl sulfonic acid
PUFA	polyunsaturated fatty acid
PUL	pulmonary
PWM	pokeweed mitogen
QCE	quasi continuous exercise
r	linear regression correlation coefficient
R	intraclass correlation coefficient
r^2	correlation coefficient
R^2	multiple correlation coefficient
rALP	recombinant antileukoprotease
RANTES	regulated on activation, normal T cell-expressed and -secreted
R_{aw}	airway resistance

RB	respiratory bronchiole
RER	rough endoplasmic reticulum
R_{FS}	
RH	relative humidity
R_L	total pulmonary resistance
ROI	reactive oxygen intermediate
ROS	reactive oxygen species
RT	respiratory tract
R_T	total respiratory resistance
σ_g	geometric standard deviation
S	smoker
SAC	*Staphylococcus aureus* Cowan 1 strain
SAW_{grp}	small airway function
sc	subcutaneous
SC	stratum corneum
SD, S-D	Sprague-Dawley
SD	standard deviation
SE	standard error
SES	socioeconomic status
SG_{aw}	specific airway conductance
SNPs	single nucleotide polymorphisms
SO_2	sulfur dioxide
SO_4^{2-}	sulfate ion
SO_4^{2-}	sulfate
SOA	secondary organic aerosol
SOD	superoxide dismutase
SP	substance P
SP	surfactant protein (SP-A, SP-D)
SR_{aw}	specific airway resistance

SRBC	sheep red blood cell
T	temperature
T	time (duration of exposure)
T_3	triiodothyronine
T_4	thyroxine
TB	tracheobronchial
TBA	thiobarbituric acid
^{99m}Tc-DTPA	radiolabeled diethylene triamine pentaacetic acid
T_{CO}	core body temperature
T_{CTL}	cytotoxic T-lymphocytes
Th	helper T-lymphocyte
TLC	total lung capacity
TLR	Toll-like receptor
TNF	tumor necrosis factor
TNFR	tumor necrosis factor receptor
TSH	thyroid-stimulating hormone
TSP	total suspended particulate
TWA	time-weighted average
TX	thromboxane (A_2, B_2)
URT	upper respiratory tract
UV	ultraviolet
V	volume
VC	vital capacity
VCAM	
V_D	dead space
V_E	minute ventilation; expired volume per minute
V_{Emax}	maximum minute ventilation
V_I	average inspiratory flow
$V_{max25\%}$	maximum expiratory flow at 25% of the vital capacity

$V_{max50\%}$	maximum expiratory flow at 50% of the vital capacity
$V_{max50\%TLC}$	maximum expiratory flow at 50% of the total lung capacity
$V_{max75\%}$	maximum expiratory flow at 75% of the vital capacity
VMD	volume mean diameter
VO_2	oxygen uptake by the body
VO_{2max}	maximal oxygen uptake (maximal aerobic capacity)
VOCs	volatile organic compounds
V_T	tidal volume
V_T	tracheal transepithelial potential
V_{TB}	tracheobronchial region volume
V_{Tmax}	maximum tidal volume
WT	wild-type

4. DOSIMETRY, SPECIES HOMOLOGY, SENSITIVITY, AND ANIMAL-TO-HUMAN EXTRAPOLATION

4.1 INTRODUCTION

The dosimetry of ozone (O_3) in humans has been examined in a series of studies published in the past decade. This important new information further characterizes the dose of O_3 delivered to various sites in the respiratory tract (RT). Ozone, classified as a reactive gas, interacts with surfactant, antioxidants, and other compounds in the epithelial lining fluid (ELF). Researchers have attempted to obtain a greater understanding of how these complex interactions affect uptake of O_3, which eventually link to O_3-induced injury. New work has also been completed evaluating species differences in responses to O_3 exposures, which allow more accurate quantitative extrapolation from animal to human.

This chapter is not intended to be a complete overview of O_3 dosimetry and animal-to-human comparisons, but rather, it is an update of the dosimetry/extrapolation chapter from the last O_3 criteria document (U.S. Environmental Protection Agency, 1996), or 1996 O_3 AQCD, and other reviews of the earlier published literature. The framework for presenting this chapter is first a discussion in Section 4.2 of general concepts of the dosimetry of O_3 in the RT. Bolus-response studies are then presented in Section 4.2.1 followed by general uptake studies in Section 4.2.2. Dosimetry modeling is presented in Section 4.2.3 followed by the summary and conclusions for the dosimetry material in Section 4.2.4. The chapter continues in Section 4.3 with a discussion of species comparisons and ends with a discussion of animal-to-human extrapolation. More detailed discussions of the studies are presented in the supporting material to this chapter (Annex AX4). The toxicological effects of O_3 in laboratory animals and in vitro test systems are discussed in Chapter 5 and direct effects of O_3 in humans are discussed in Chapter 6. The historical O_3 literature is very briefly summarized in this chapter, providing a very concise overview of previous work. The reader is referred to the 1996 O_3 AQCD for more detailed discussion of the literature prior to the early 1990s.

4.2 DOSIMETRY OF OZONE IN THE RESPIRATORY TRACT

Dosimetry refers to measuring or estimating the quantity of or rate at which a chemical is absorbed by target sites within the RT. The compound most directly responsible for toxic effects may be the inhaled gas O_3 or one of its chemical reaction products. Complete identification of the actual toxic agents and their integration into dosimetry is a complex issue that has not been resolved. Thus, most dosimetry investigations are concerned with the dose of the primary inhaled chemical. The actual meaning of the word 'dose' is somewhat subjective. Dahl (1990) classified O_3 as a reactive gas and discussed dose in terms of: inhaled O_3 concentration; amount of O_3 inhaled as determined by minute volume, vapor concentration, and exposure duration; uptake or the amount of O_3 retained (i.e., not exhaled); O_3 or its active metabolites delivered to target cells or tissues; O_3 or its reactive metabolites delivered to target biomolecules or organelles; and O_3 or its metabolites participating in the ultimate toxic reactions - the effective dose. Understanding dosimetry as it relates to O_3-induced injury is complex due to the fact that O_3 interacts primarily with the ELF which contains surfactant and antioxidants. In the upper airways ELF is thick and highly protective against oxidant injury. In lower airways ELF is thinner, has lower levels of antioxidants, and thus, allows more cellular injury. Adding to the complexity is the fact that O_3 can react with molecules in the ELF to create even more reactive metabolites, which can then diffuse within the lung or be transported out of the lung to generate systemic effects.

Since the 1996 ozone criteria document (U.S. Environmental Protection Agency, 1996), all new experiments have been carried out in humans to obtain direct measurements of absorbed O_3 in the RT, the upper RT (URT) region proximal to the tracheal entrance, and in the tracheobronchial (TB) region; no uptake experiments have been identified as being performed using laboratory animals.

In vivo dosimetry studies described in the last criteria document estimate that uptake of inhaled O_3 in rat is 0.50 of inhaled O_3 concentration and of that about half is removed in the head, the just under half in the lungs, and only a small percentage removed in the larynx/trachea. There was no agreement as to whether uptake was dependent on flow. The uptake of O_3 in the RT of humans at rest was between 0.8 and 0.95 . Studies in humans have shown that increasing minute ventilation (V_E) with exercise (by increasing both breathing frequency and tidal volume) causes only a small decrease in uptake efficiency by the total RT. Mode of breathing had little

effect on uptake; oral breathing had approximately 10% greater uptake efficiency than nasal breathing. Comparing bronchoalveolar lavage (BAL) cells from rat and human, a 0.4 ppm exposure in exercising humans gave 4 to 5 times the dose of O_3 (dose retained) than a rat at rest exposed to the same concentration. Overall, uptake efficiency data between humans and other species had a great deal of consistency. Miller (1995) reviewed the major factors influencing RT uptake of O_3: (1) structure of the RT region, (2) ventilation, and (3) gas transport mechanisms. In comparing rats and humans, they differ greatly in URT structure, which imparts disparate airflow streams. Rate, depth, and route of breathing all influence the amount of O_3 inhaled. However, route of breathing has little biological significance. In humans at exposures to 0.5 ppm (resting) or 0.2 ppm (exercising), breathing frequency increases and tidal volume decreases such that minute ventilation is not altered. Miller further noted that local dose is the critical link between exposure and response, and that modeling of O_3 has typically discounted the mucociliary layer of the URT and tracheobronchial regions.

In vitro dosimetry studies using isolated lung preparations showed that uptake efficiency is chemical-reaction dependent, indicating the importance of reaction product formation. These reaction products, created mainly by the ozonolysis of polyunsaturated fatty acids (PUFA) , included hydrogen peroxide, aldehydes, and hydroxyhydroperoxides, which are mediators of O_3 toxicity. Other products are created by the reaction of O_3 with other ELF constituents, all of which must be considered in understanding the dosimetry of O_3.

The next two sections review the available new experimental studies on O_3 dosimetry, all of which were conducted by Ultman and colleagues. Table AX4-1 in Annex AX4 summarizes theses studies.

4.2.1 Bolus-Response Studies

The bolus-response method has been used by the Ultman group as an approach to explore the absorption of O_3 by humans. This non-invasive method consists of an injection of a known volume and concentration of O_3 during inspiration. Ozone uptake is the amount of O_3 absorbed during a single inspiration relative to the amount contained the inhaled bolus. Exposure to nitrogen dioxide (NO_2) or sulfur dioxide (SO_2) just prior to O_3 exposure has an effect on the amount of O_3 absorbed in conducting airways. Asplund et al. (1996) used continuous O_3 exposure, which consisted of 2 hours of O_3 at 0.0, 0.12, or 0.36 ppm. Rigas et al. (1997) used

continuous 2 h exposures of 0.0, 0.36, or 0.75 ppm NO_2, or 0.0 or 0.36 SO_2. In both experiments the continuous exposure was interrupted every 30 min by a series of bolus breaths of 2 ppm O_3 at 250 mL/s, targeting the lower conducting airways. With continuous O_3 exposure, the absorbed fraction (FA) of the bolus decreased, which suggested to the authors that the continuous O_3 exposure possibly depleted biochemical substrates from the airways, reducing their capacity to absorb more O_3. With NO_2 and SO_2 continuous exposures, the absorbed fraction of the bolus increased, which the authors contributed to the these same biochemical substrates being made available by the continuous exposure.

The bolus-response technique was also used to ascertain differences in lung anatomy and gender that can alter the exposure-dose cascade (Bush et al., 1996a). Forced vital capacity (FVC), total lung capacity (TLC) and anatomic dead space (V_D) were determined for ten male and ten female subjects, who were then exposed to a 20 ml bolus of 3 ppm O_3 injected into the airstream. In all subjects, dosimetry differences could be explained by differences in V_D. The investigators point out that the applicability of their results may be limited because of their assumptions that the intrinsic mass transfer parameter (K_a) was independent of location in the RT and that there was no mucous resistance. They further suggested that the dependence of K_a on flowrate and V_D be restricted to flowrates $\leq$ 1000 mL/s until studies at higher rates have been performed.

Nodelman and Ultman (1999a) used the bolus-response method to demonstrate that the uptake distributions of O_3 was sensitive to the mode of breathing and to the airflow rate. As flowrates increased from 150 to 1000 mL/s, O_3 penetrated deeper in to the lung and penetration was further increased with oral breathing. The authors suggest that the switch from nasal to oral breathing coupled with increases in respiratory flow as occurs during exercise causes a shift in the O_3 dose distribution, allowing O_3 to penetrate deeper into the lung, increasing the potential for damage to bronchiolar and alveolar tissues.

Very recent work by this group (Ultman et al., 2004) demonstrated that differences in regional O_3 uptake are due, in part, to differences in lung anatomy. Using 60 male and female subjects exposed to O_3 at a high minute ventilation, they measured the penetration volume at which 50% of the O_3 bolus was taken up. Very little O_3 was taken up in the upper airway, thus most of it was taken up in the lower conducting airways and peripheral airspaces. This significant difference in uptake suggests to the authors that in females the smaller airways,

and associated larger surface-to-volume ratio, enhance local O_3 uptake and cause reduced penetration of O_3 into the distal lung. Thus, these findings indicate that overall O_3 uptake is not related to airway size, but that the distribution of O_3 shifts distally as the size of the airway is increased.

These bolus- response studies suggest that prior continuous exposure to O_3 limits uptake from a bolus dose. This paradigm of exposure would have some relevance to environmental exposures in which humans may receive differing day and night concentrations of O_3. The lack of gender differences in O_3 dosimetry is an important finding that is in agreement with Sarangapani et al. (2003), discussed in Section4.2.3, who reported no gender differences in O_3 extraction. The new findings characterizing O_3 uptake as inversely related to airflow are in agreement with earlier animal studies. Discussions about estimating mass transfer coefficients and the "accuracy" of these models are contained in Annex AX4.

4.2.2 General Uptake Studies

Ultman and colleagues have recently completed some general uptake studies to determine the ratio of O_3 uptake to the quantity of O_3 inhaled. Fractional absorption (FA) was determined at exposures of 0.2 or 0.4 ppm O_3 while exercising at a minute volume of approximately 20 L/min for 60 minutes or 40 L/min for 30 minutes in both men and women (Rigas et al., 2000). Fractional absorption ranged from 0.56 to 0.98 with only large changes in concentration, minute volume, and exposure time having statistically significant effects on FA. Intersubject differences had the largest influence on FA, resulting in a variation of approximately 10%. As the quantity of O_3 retained by the RT is equal to FA times the quantity of O_3 inhaled, relatively large changes in concentration, minute volume, or exposure time may result in relatively large changes in the amount of O_3 retained by the RT or absorbed locally. The authors concluded that for exposure times < 2 h, inhaled dose is a reasonable predictor of actual uptake as long as there are fixed concentrations of O_3 and fixed levels of exercise. More importantly, individuals exposed to similar doses varied in the amount of actual dose received.

Santiago et al. (2001) studied the effects of airflow rate (3 to 15 L/min) and O_3 concentration (0.1, 0.2, or 0.4 ppm) on O_3 uptake in nasal cavities of males and females. The FA in the nose was inversely related to the flowrate and the concentration of O_3 in the inlet air. They computed a gas-phase diffusion resistance of < 24% of overall diffusion resistance which

suggested to them that simultaneously occurring diffusion and chemical reactions in the mucous layer were the limiting factors in O_3 uptake. Difference in O_3 uptake ranged from 0.63 to 0.97 at flowrates of 3 L/min and 0.25 to 0.50 at 15 L/min. The effect of flowrate, concentration, and subject on FA were statistically significant, but subject variability accounted for approximately half of the total variation in FA. Both these general uptake studies, done at environmentally relevant O_3 concentrations, indicate that inter-individual differences in fractional uptake are extremely important in O_3 dose-response relationships.

In the research mentioned above, Ultman et al. (2004) also completed continuous exposure studies. The same 60 subjects were exposed continuously for 1 h to either clean air or 0.25 ppm ozone while exercising at a target minute ventilation of 30 L/min. This is the first study to assess ventilatory and dosimetric parameters for an entire hour of exposure. Additionally they measured bronchial cross-sectional area available for gas diffusion in addition to other ventilatory parameters. The mean fractional O_3 uptake efficiency was 0.89 ± 0.06. They found an inverse correlation between uptake and breathing frequency and a direct correlation between uptake and tidal volume. The uptake efficiency decreased during the four sequential 15 minute intervals of the 1 h exposure, demonstrating a general decrease in uptake efficiency with increased breathing frequency and decreasing tidal volume. Ozone uptake rate correlated with individual bronchial cross-sectional area, but did not correlate with individual $\%FEV_1$. Neither of these parameters correlated with the penetration volume determined in the bolus studies mentioned above. The authors concluded that the intersubject differences in forced respiratory responses were not due to differences in O_3 uptake. However, these data did partially support the hypothesis that the differences in cross-sectional area available for gas diffusion induce differences in O_3 uptake.

4.2.3 Dosimetry Modeling

When all of the animal and human in vivo O_3 uptake efficiency data are compared, there is a good degree of consistency across data sets, which raises the level of confidence with which these data sets can be used to support dosimetric model formulations. Early models predicted that net O_3 dose to lung lining fluid plus tissue gradually decreases distally from the trachea toward the end of the TB, and then rapidly decreases in the pulmonary region. When the theoretical dose of O_3 to lung tissue is computed, it is low in the trachea, increases to a maximum

in the terminal bronchioles of the first generation of the pulmonary region, and then decreases rapidly distally into the pulmonary region. The increased V_T and flow, associated with exercise in humans or CO_2-stimulated ventilation increases in rats, shifts O_3 dose further into the periphery of the lung, causing a disproportionate increase in distal lung dose.

Localized damage to lung tissue has been modeled showing variation of O_3 dose among anatomically equivalent ventilatory units as a function of path length from the trachea with shorter paths showing greater damage. More recent data indicate that the primary site of acute cell injury occurs in the conducting airways (Postlethwait et al., 2000). These data must be considered when developing models that attempt to predict site-specific locations of O_3-induced injury. The early models computed relationships between delivered regional dose and response with the assumption that O_3 was the active agent responsible for injury. It is now known that reactive intermediates such as hydrohydroxyperoxides and aldehydes are important agents mediating the response to O_3 (further discussed in Section 5.3.1). Thus, models must consider O_3 reaction/diffusion in the ELF and ELF-derived reactions products.

Table AX4-2 in the annex presents a summary of new theoretical studies of the uptake of O_3 by the RTs (or regions) of humans and laboratory animals that have become available since the 1996 review. They are discussed below.

Overton and Graham (1995) created a rat model combining multiple path anatomic models and one-dimensional convection-dispersion equations which simulates transport and uptake of O_3 in airways and airspaces of the modeled TB region. Predictions from this model realistically detail O_3 transport and uptake of different but morphologically equivalent sites.

Using computational fluid dynamics (CFD), Cohen-Hubal et al. (1996) modeled the effect of the mucus layer thickness in the nasal passage of a rat. Predictions of overall uptake were within the range of measured uptake. Predicted regional O_3 flux was correlated with measured cell proliferation for the CFD simulation that incorporated two regions, each with a different mucus thickness. But using bolus-response data described above, Hu et al. (1994) and Bush et al. (2001) estimate a reaction rate constant that is more than 1000 times as large as that used by Cohen-Hubal et al. (1996).

The authors acknowledge that the reaction rate constant may be underestimated in this model due to the report by Pryor (1992) predicting that O_3 does not penetrate a liquid lining layer more than 0.1 μm thick.

With a RT dosimetry model, Overton et al. (1996) investigated the sensitivity of absorbed fraction (AF), proximal alveolar region (PAR) dose, and PAR dose ratio to TB region volume (V_{TB}) and TB region expansion in humans and rats. The PAR was defined as the first generation distal to terminal bronchioles and the PAR dose ratio was defined as the ratio of a rat's predicted PAR dose to a human's predicted PAR dose. This ratio relates human and rat exposure concentrations so that both species receive the same PAR dose. In rats, the PAR is a region of major damage from O_3. For each species, three values of V_{TB} were used: a mean value from the literature and the mean ± twice the SD. For both the rat and human simulations, there were several general findings: (1) AF and PAR both increased with decreasing V_{TB}, e.g., using the highest k_{TB}, the PAR for V_{TB}–2SD was five times greater than the PAR for V_{TB}+2SD, (2) AF and PAR both decreased with TB expansion relative to no expansion, 3) PAR increased with tidal volume, 4) PAR increased with decreasing k_{TB}, and 5) AF increased with k_{TB}.

Bush et al. (2001) modified their single-path model (Bush et al., 1996) so that simulations would coincide with experimental AF data for O_3 and Cl_2 during oral and nasal breathing. Relative to their original model, the Bush et al. (2001) model added lung expansion and modified the mass transfer coefficients for both the gas-phase (k_g) and the liquid-phase (k_l). Using k_l, a variable reaction rate constant (k_r) was defined as k_l divided by the mucus layer thickness which was assumed to decrease in thickness with progression from the trachea to the lower airways. Consistent with Overton et al. (1996), considering expansion of the TB airways reduced AF versus no expansion. As very little inhaled O_3 reaches the peripheral lung, it was not surprising that alveolar expansion had minimal affect on AF. Ignoring k_r, the simulations for O_3 and Cl_2 were nearly the same since the gas-phase diffusion coefficients of O_3 and Cl_2 are similar. But for a given volumetric depth into the TB airways of the lung, experimental AF data are always greater for O_3 than for Cl_2. The authors surmised that the difference between the AF for these gases could be explained adequately based solely on the diffusive resistance of O_3 in airways surface fluid (modeled by k_r). Interestingly, k_r was lower for oral than nasal breathing, implying less antioxidant capacity in the airway surface liquid of the oral versus the nasal cavity. Qualitatively, model simulations also agreed well with the experimental data of Gerrity et al. (1995).

Age- and gender-specific differences in both regional and systemic uptake in humans was modeled using physiologically-based pharmacokinetic (PBPK) approach (Sarangapani et al.,

2003). The model estimated that regional extraction of O_3 is relatively insensitive to age and gender and that the postnatal period is the age (in which extraction of O_3 in infants is 2- to 8-fold higher than is adults) at which the largest difference in pharmacokinetics exist.

A recent attempt was made (Mudway and Kelly, 2004) to model O_3 dose-inflammatory response using a meta-analysis of 23 exposures in published human chamber studies. The O_3 concentrations ranged from 0.08 to 0.6 ppm and the exposure durations ranged from 60 to 396 minutes. The analysis showed linear relationships between O_3 dose and neutrophilia in bronchoalveolar lavage fluid (BALF). Linear relationships were also observed between O_3 dose and protein leakage into BALF, which suggested to the authors that a large-scale study could determine a possible O_3 threshold level for these inflammatory responses.

4.2.4 Summary and Conclusions - Dosimetry

Ozone is a highly reactive gas and powerful oxidant with a short half-life. Uptake occurs in mucous membranes of the RT where O_3 immediately reacts with components of the ELF. Uptake efficiency is chemical-reaction dependent and the reaction products (hydrogen peroxide, aldehydes, and hydroxyhydroperoxides) created by ozonolysis of polyunsaturated fatty acids (PUFA) mediate of O_3 toxicity. The previous literature review found that uptake of O_3 in rat is about 0.50 and in humans at rest is about 0.8 to 0.95. About 0.07 of the O_3 is removed in the larynx/trachea, about 0.50 is removed in the head, and about 0.43 is removed in the lungs, where the primary site of damage is the centriacinar region (CAR). There was no agreement as to whether uptake was dependent on flow. Studies in humans have shown that increasing V_E with exercise (by increasing both breathing frequency and tidal volume) causes only a small decrease in uptake efficiency by the total RT. Mode of breathing had little effect on uptake, suggesting that O_3 is removed equally by both mouth and nose. Comparing BAL cells from rat and human, a 0.4 ppm exposure in exercising humans gave 4 to 5 times the dose of O_3 than a rat at rest exposed to the same concentration.

New research on O_3 uptake has been performed in humans, but not in laboratory animals. Bolus-response studies demonstrated that a previous continuous exposure to O_3 decreased the absorption of a bolus of O_3, probably due to depletion of compounds able to absorb O_3. Previous continuous exposure to NO_2 and SO_2 increased absorption of a bolus of O_3. These data are of some relevance to environmental exposures where humans may receive differing concentrations

of O_3 depending on time of day. Another bolus-response study showed that absorption of O_3 was dependent on V_D, but not height, weight, age, gender, FVC, or TLC. In contrast to earlier data, the bolus-response method was used to demonstrate that the uptake distributions of O_3 is sensitive to the mode of breathing and to the airflow rate. As flow rates increased from 150 to 1000 mL/s, O_3 penetrated deeper in to the lung and was further increased with oral breathing. This suggests that the switch from nasal to oral breathing coupled with increases in respiratory flow as occurs during exercise causes a shift in the O_3 dose distribution, allowing O_3 to penetrate deeper into the lung, increasing the potential of damage to bronchiolar and alveolar tissues. The finding that O_3 uptake is inversely related to airflow agrees with earlier animal studies.

New general uptake study data demonstrate that exercising men and women receiving 0.2 or 0.4 ppm O_3 at 20 L/min for 60 minutes or 40 L/min for 30 minutes absorb 0.56 to 0.98. The absorbed fraction or FA is affected only by large changes in concentration, minute volume, and exposure time. This suggests that for exposure times < 2 h, inhaled dose is a reasonable predictor of actual uptake as long as there are fixed concentrations of O_3 and fixed levels of exercise. Individuals exposed to similar concentrations vary considerably in the amount of actual dose received. This intersubject variability was also demonstrated in a study of O_3 uptake in nasal cavities of men and women. The FA in the nose was inversely related to the flowrate and the concentration of O_3, suggesting that simultaneously occurring diffusion and chemical reactions in the mucous layer were the limiting factors in O_3 uptake. Both these general uptake studies, done at environmentally relevant O_3 concentrations, indicate that inter-individual differences in fractional uptake, which can range from 0.25 to 0.97, are extremely important in O_3 dose-response relationships.

The consistency of uptake data generated in animal and human studies allow a high level of confidence in their use in dosimetry modeling. Early models predicted that net O_3 dose to ELF and tissue gradually decreases distally from the trachea toward the end of the TB, and then rapidly decreases in the pulmonary region. Exercise-induced or CO_2-stimulated increases in V_T and flow, shift O_3 dose further into the periphery of the lung, causing a disproportionate increase in distal lung dose. Localized damage to lung tissue has been modeled showing variation of O_3 dose among anatomically equivalent ventilatory units as a function of path length from the trachea with shorter paths showing greater damage.

New models have produced some refinements of earlier models such as: (1) the use of mucus resistance and thickness in describing O_3 dosimetry and determining the patterns of O_3-induced lesions; (2) the shape of the dose versus generation plot along any path from the trachea to alveoli is independent of path, with the tissue dose decreasing with increasing generation index; (3) simulations sensitive to conducting airway volume but relatively insensitive to characteristics of the respiratory airspace; (4) the importance of TB region expansion; (5) the importance of dose received in the PAR both inter-individual differences and extrapolations based on dose; (6) revaluation of mass transfer coefficients for conducting airways, and (7) extraction of O_3 in infants is 2- to 8-fold higher than in adults, but the differences leveled out by age 5. Additionally, more recent data indicate that the primary site of acute cell injury occurs in the conducting airways and that reactive intermediates in the ELF, rather than O_3 itself, are responsible for pulmonary injury. These data must be considered when developing new models.

4.3 SPECIES HOMOLOGY, SENSITIVITY, AND ANIMAL-TO-HUMAN EXTRAPOLATION

Basic similarities exist across human and other animals species with regard to basic anatomy, physiology, biochemistry, cell biology, and disease processes. This homology creates similarities in acute O_3-induced effects, especially in the respiratory tract and in lung defense mechanisms. Rodents appear to have a slightly higher tachypneic response to O_3, which is clearly concentration-dependent in most species and shows parallel exacerbation when hyperventilation (e.g., exercise or CO_2) is superimposed. What is not known is whether this is evidence of pulmonary irritant sensitivity, perhaps as a prelude to toxicity, or whether tachypnea is a defensive action taken by the respiratory system to minimize distal lung O_3 deposition. Airway or lung resistance in humans is not affected appreciably by acute exposure to O_3, except under conditions of heavy exercise; animals appear to need high-level exposures or special preparations that bypass nasal scrubbing. Dynamic lung compliance (Cdyn) has been shown to have small magnitude decreases in response to O_3 in some studies across species, but it is thought that these changes are of little biological significant for ambient exposures. Spirometric changes, the hallmark of O_3 response in humans, occur in rats, but to a lesser degree. It is

unclear, however, the degree to which anesthesia (rat) and the comparability of hyperventilation induced by CO_2 (rat) or exercise (human) may influence this difference in responsiveness. Collectively, the acute functional response of laboratory animals to O_3 appears quite homologous to that of the human.

Examination of BAL constituents show that the influx of inflammatory cells and protein from the serum is influenced by species, but perhaps to less extent than by ventilation and antioxidant status. Adjustment for these factors can modulate responses to approximate animal responses to those of humans. Unfortunately, these influential factors are rarely measured and, even less often, controlled. Increases in protein levels in BALF with O_3 exposures in guinea pigs are also a factor in the species' susceptibility to the effects of O_3. Species comparisons of acute O_3 exposures to mice, guinea pigs, rats, hamsters, and rabbits found that guinea pigs were the most responsive (to $\geq$ 0.2 ppm); rabbits were the least responsive (2.0 ppm only); and rats, hamsters, and mice were intermediate (effects at $\geq$ 1.0 ppm). Rats and humans have subtle species-specific differences in inflammatory responses to O_3 in terms of the timing of PMN influx in the nasal and bronchoalveolar regions.

When humans are exposed to O_3 repeatedly for several consecutive days, lung function decrements subside, and normal spirometric parameters are regained. This phenomenon of functional attenuation also has been demonstrated in rats, not only in terms of spirometry, but also in terms of the classic tachypneic ventilatory response. Full or partial attenuation of the BAL parameters also appears to occur in both rats and humans, but exposure scenario appears to play a role; other cellular changes in animals do not attenuate. Existing epidemiologic studies provide only suggestive evidence that persistent or progressive deterioration in lung function is associated with long-term oxidant-pollutant exposure (See Chapter 7). These long-term effects are thought to be expressed in the form of maximum airflow or spirometric abnormalities, but the foundation for this conclusion remains weak and hypothetical. Animal study data, although suggesting that O_3 has effects on lung function at near-ambient levels, present a variable picture of response that may or may not relate to technical conditions of exposure or some other, yet undiscovered variable of response. Thus, a cogent interpretation of the animal findings as definitive evidence of chronic deterioration of lung function would be difficult at this time. However, the subtle functional defects apparent after 12 to 18 mo of exposure and the detailed morphometric assessments of the O_3-induced lesions do appear consistent with the modicum of

studies focusing on long-term effects in human populations. Based on the apparent homology of these responses between humans and laboratory animals, animal studies may potentially provide a means to more directly assess such chronic health concerns.

A species' susceptibility to the effects of O_3 exposure may be due, in part, to biochemical differences among species. Evidence for this is provided by differences in activity of SD rat and rhesus monkey CYP moonoxygenases elicited by O_3 exposure (Lee et al., 1998). Additional characterization of species- and region-specific CYP enzymes will create a better understanding of the differences in response to O_3. This will allow more accurate extrapolation from animal exposures to human exposures and toxic effects.

Antioxidant metabolism varies widely among species, which can greatly influence the effects of O_3 (discussed in greater detail in 5.2.1.3). The guinea pig appears to be the species most susceptible to ozone. Early studies ranked mice > rats > guinea pigs in order of antioxidant responsiveness to O_3 challenge. Guinea pigs have been shown to have lower basal levels of GSH transferase activity, lower activity of GSH peroxidases, and lower levels of vitamin E compared to rats. These lower levels of antioxidants combined with increases in protein levels in BALF (discussed above) with O_3 exposures likely explain, at least in part, the species' susceptibility to the effects of O_3.

Because cytokine and chemokine responses are so important in an animal's defense against O_3 exposure, comparisons of differences in species expression and activity of these inflammatory mediators is necessary. Arsalane et al. (1995) compared guinea pig and human AM recovered in BALF and subsequently exposed in vitro to 0.1 to 1 ppm for 60 minutes. Measurement of inflammatory cytokines showed a peak at 0.4 ppm in both species. Guinea pig AM had an increase in IL-6 and TNFa while human AM had increases in TNFa, IL-1b, IL-6 and IL-8. This exposure also caused an increase in mRNA expression for TNFa, IL-1b, IL-6 and IL-8 in human cells. At 0.1 ppm exposures, only TNFa secretion was increased. These data suggest similar cytokine responses in guinea pigs and humans, both qualitatively and quantitatively.

Species differences in morphological responses to O_3 exposure have been characterized by Dormans et al. (1999), as discussed in previous sections. Dormans et al. (1999) continuously exposed rats, mice, and male guinea pigs to filtered air, 0.2, or 0.4 ppm O_3 for 3, 7, 28, and 56 days. The animals exposed for 28 days were examined at 3, 7, or 28 days PE. Depending

on the endpoint studied, the species varied in sensitivity. Greater sensitivity was shown in the mouse as determined by biochemical endpoints, persistence of bronchiolar epithelial hypertrophy, and recovery time. Guinea pigs were more sensitive in terms of the inflammatory response though all three species had increases in the inflammatory response after three days that did not decrease with exposure. These data on inflammation are in general agreement with Hatch et al., (1986), discussed above. In all species the longest exposure to the highest dose caused increased collagen in ductal septa and large lamellar bodies in Type II cells, but that response also occurred in rats and guinea pigs at 0.2 ppm. No fibrosis was seen at the shorter exposure times and the authors question whether fibrosis occurs in healthy humans after continuous exposure. The authors do not rule out the possibility that some of these differences may be attributable to differences in total inhaled dose or dose actually reaching a target site. Overall, the authors rated mice as most susceptible, followed by guinea pigs and rats.

Comparisons of airway effects in rats, monkeys and ferrets resulting from exposures of 1.0 ppm O_3 for 8 h (Sterner-Kock et al. 2000) demonstrated that monkeys and ferrets had a similar inflammatory responses and epithelial necrosis. The response of these two species was more severe than that seen in rats. These data suggest that ferrets are a good animal model for O_3-induced airway effects due to the similarities in pulmonary structure between primates and ferrets.

A number of species, including nonhuman primates, dogs, cats, rabbits, and rodents, have been used to study the effects of O_3 exposure on airway bronchoconstriction. A commonly used model of bronchospasm utilizes guinea pigs acutely exposed to high O_3 concentrations (2 to 3 ppm) to induce airway hyperreactivity (AHR). As mentioned earlier, the model is helpful for determining mechanistic aspects of AHR, but is not really relevant for extrapolation to potential airway responses in humans exposed to ambient levels of O_3. Additionally, guinea pigs have been shown to have AHR in other studies that is very similar to human asthmatics, but the utility of guinea pig data is somewhat limited by their disparity from other animal models.

The rat is a key species used in O_3 toxicological studies, but the rat has both behavioral and physiological mechanisms that can lower core temperature in response to acute exposures, thus limiting extrapolation of rat data to humans. Iwasaki et al. (1998) evaluated cardiovascular and thermoregulatory responses to O_3 at exposure of 0.1, 0.3, and 0.5 ppm O_3 8 hrs/day for 4 consecutive days. A dose-dependent disruption of HR and T_{co} were seen on the first and

second days of exposure, which then recovered to control values. Watkinson et al. (2003) exposed rats to 0.5 ppm O_3 and observed this hypothermic response, which included lowered HR, lowered T_{co}, and increased inflammatory components in BALF. The authors suggested that the response is an inherent reflexive pattern that can possibly attenuate O_3 toxicity in rodents. They discuss the cascade of effects created by decreases in T_{co}, which include: (1) lowered metabolic rate, (2) altered enzyme kinetics, (3) altered membrane function, (4) decreased oxygen consumption and demand, (5) reductions in minute ventilation, which would act to limit the dose of O_3 delivered to the lungs. These effects are concurrent with changes in HR which lead to: (1) decreased CO, (2) lowered BP, and (3) decreased tissue perfusion, all of which may lead to functional deficits. They hypothermic response has not been observed in humans except at very high exposures, which complicates extrapolation of results in rat studies to humans.

The importance of animal studies derives from their utilization in determining cause-effect relationships between exposure and health outcome, but the animal data must be integrated with epidemiological studies and controlled human clinical studies. Animal studies can corroborate both clinical and epidemiology studies and further provide important data that is impossible to collect in human studies. Toxic pulmonary and extrapulmonary effects following ozone exposure have been well-studied in rodents, nonhuman primates, and a few other species; so, extrapolation, both qualitative and quantitative, to human exposures and consequent health effects is possible. Quantitative extrapolation, required to determine what specific exposure is likely to cause an effect in humans, is theoretically founded on the equivalency of mechanisms across species. At the molecular level, O_3 acts on the carbon-carbon double bond in polyunsaturated fatty acids and on sulfhydryl groups in proteins, both of which are found within cell membranes in animals and humans. At higher levels of cellular organization, cells affected in animals (e.g., AMs, Type 1 cells) have similar functions in humans, and organ systems (e.g., respiratory system) have major interspecies similarities. However, interspecies differences do occur and confound extrapolation.

Quantitative extrapolation, which involves a combination of dosimetry and species sensitivity, still requires more research before it can be fully realized. Knowledge of dosimetric animal-to-human extrapolation is more advanced than that of species-sensitivity, but extrapolation models have not been completely validated, and therefore, significant uncertainties remain. Mathematical modeling of O_3 deposition in the lower respiratory tract (i.e., from the

trachea to alveoli) of several animal species and humans shows that the pattern of regional dose is similar, but that absolute values differ. In spite of structural and ventilatory differences between species, the greatest predicted tissue dose is to the CAR. Even though the CAR of rats has very rudimentary respiratory bronchioles, compared to well-developed ones in primates, the CAR of both rats and nonhuman primates respond similarly to O_3.

Experimental measurement of delivered O_3 doses estimate that total respiratory uptake is ~47% in laboratory animals and ~87% in exercising humans, while nasopharyngeal removal is ~17% in rats and ~40% in humans. The previous O_3 AQCD (U.S. Environmental Protection Agency, 1996) provided the first quantitative animal-to-human extrapolation of morphological changes in the proximal alveolar region using rat and monkey studies. The extrapolation predicted that a 9-year-old child would have a 20% or 75% increase in PAR tissue thickness if their sensitivity to O_3 was equal to that of a rat or monkey, respectively. Adults would have 15 or 70% increase, suggesting the potential for chronic effects in humans. In spite of the significant uncertainties, this extrapolation raises concern about the potential for chronic effects in humans

Experiments using 0.4 ppm $^{18}O_3$ suggested that exercising humans received a 4- to 5-fold higher $^{18}O_3$ concentrations in BAL than resting rats (Hatch et al., 1994). That level of exposure increased BAL protein and PMNs in humans, while a concentration of 2.0 ppm in rats was necessary for similar effects. Caveats in the interpretation of $^{18}O_3$ studies include: (1) only a very small portion of the labeled compound is recoverable to assess incorporation; and (2) if species being compared differ in physiocochemical factors controlling mass transfer and downstream O_3 metabolism, it could cause significant differences in the amount of inhaled $^{18}O_3$ that is detected during subsequent tissue analysis. Further, species differences in pulmonary anatomy, ventilation, antioxidants, and susceptibility all influence dose, repair processes, and tolerance to subsequent O_3 exposure. Important differences between exercising humans and resting rats that can affect tissue O_3 dose include: (1) increased ventilation and O_3 delivery with exercise; (2) decreased pulmonary ventilation and body temperature during O_3 exposure in rats; (3) diminished dose received in rats due to their burying their noses in their fur during exposure; and (4) increased concentration of antioxidants in ELF in rats compared to humans. These antioxidants are important for converting O_3 to inactive products before toxicity occurs (Kari et al., 1997; Gunnison and Hatch, 1999; Plopper et al., 1998), though this quenching is not

quantitative. These and possibly other differences between rats and humans suggest that a 2 ppm exposure in nonexercising rats approximates a 0.4 ppm exposure in exercising humans. Further comparisons of exercising human exposure to 0.1 ppm for 6 hours (Devlin et al., 1991) and resting rat exposure to 0.3 ppm show inflammatory and permeability changes in humans but not rats.

4.3.1 Summary and Conclusions: Species Homology, Sensitivity, and Animal-to-Human Extrapolation

Comparisons of acute exposures in rat and human suggest that, though both species have similar qualitative responses to O_3 exposure, there are interspecies mechanistic disparities that necessitate careful comparisons of dose-response relationships. There is no perfect nonhuman species with which to model O_3 toxicity. All have limitations that must be considered when attempting to extrapolate to human exposures. Awareness of these limitations, even at the level of subtle strain differences within a test species, is extremely important. The currently available data suggest that LOELs in resting rats are approximately 4- to-5 fold higher than for exercising humans for toxicological endpoints including BAL protein and BAL PMNs. Studies comparing species-specific differences in O_3-induced effects showed that guinea pigs were the most susceptible, rabbits the least susceptible, and rodents intermediate in susceptibility. The recent work being done utilizing various mouse strains with differing sensitivities to O_3 will help us to understand the extremely complex inter-individual differences in human sensitivity to O_3.

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CHAPTER 4 ANNEX. DOSIMETRY OF OZONE IN THE RESPIRATORY TRACT

AX4.1 INTRODUCTION

This annex serves to provide supporting material for Chapter 4 - Dosimetry, Species, Homology, Sensitivity, and Animal-to-Human Extrapolation. It includes tables that summarize new literature published since the last O_3 criteria documents (U.S. Environmental Protection Agency, 1996). In addition, it provides descriptions of those new findings, in many cases, with more detail than is provided in the chapter.

Dosimetry refers to measuring or estimating the quantity of or rate at which a chemical is absorbed by target sites within the respiratory tract (RT). The compound most directly responsible for toxic effects may be the inhaled gas O_3 or one of its chemical reaction products. Complete identification of the actual toxic agents and their integration into dosimetry is a complex issue that has not been resolved. Thus, most dosimetry investigations are concerned with the dose of the primary inhaled chemical. In this context, a further confounding aspect can be the units of dose (e.g., mass retained per breath, mass retained per breath per body weight, mass retained per breath per respiratory tract surface area). That is, when comparing dose between species, what is the relevant measure of dose? This question has not been answered; units are often dictated by the type of experiment or by a choice made by the investigators. There is also some lack of agreement as to what constitutes "dose." Dahl's seminal paper (1990) classified O_3 as a reactive gas and discussed the characterization of dose measurement by parameters including: (1) inhaled O_3 concentration; (2) amount of O_3 inhaled as determined by minute volume, vapor concentration, and exposure duration; (3) uptake or the amount of O_3 retained (i.e., not exhaled); (4) O_3 or its active metabolites delivered to target cells or tissues; (5) O_3 or its reactive metabolites delivered to target biomolecules or organelles; and (6) O_3 or its metabolites participating in the ultimate toxic reactions - the effective dose. This characterization goes from least complex to greatest, culminating in measurement of the fraction of the inhaled O_3 that participates in the effects of cellular perturbation and/or injury. Understanding dosimetry as it relates to O_3-induced injury is complex due to the fact that O_3 interacts primarily with the epithelial lining fluid (ELF) which contains surfactant and

antioxidants. In the upper airways ELF is thick and highly protective against oxidant injury. In lower airways ELF is thinner, has lower levels of antioxidants, and thus, allows more cellular injury. Adding to the complexity is the fact that O_3 can react with molecules in the ELF to create even more reactive metabolites, which can then diffuse within the lung or be transported out of the lung to generate systemic effects. Section 5.3.1 contains further information on the cellular targets of O_3 interactions and antioxidants.

Experimental dosimetry studies in laboratory animals and humans, and theoretical (dosimetry modeling) studies, have been used to obtain information on O_3 dose. Since the last ozone criteria document (U.S. Environmental Protection Agency, 1996), all new experiments have been carried out in humans to obtain direct measurements of absorbed O_3 in the RT, the upper RT (URT) region proximal to the tracheal entrance, and in the tracheobronchial (TB) region; no uptake experiments have been performed using laboratory animals. Experimentally obtaining dosimetry data is extremely difficult in smaller regions or locations, such as specific airways or the centriacinar region (CAR; junction of conducting airways and gas exchange region), where lesions caused by O_3 occur. Nevertheless, experimentation is important for determining dose, making dose comparisons between subpopulations and between different species, assessing hypotheses and concepts, and validating mathematical models that can be used to predict dose at specific respiratory tract sites and under more general conditions.

Theoretical studies are based on the use of mathematical models developed for the purposes of simulating the uptake and distribution of absorbed gases in the tissues and fluids of the RT. Because the factors affecting the transport and absorption of gases are applicable to all mammals, a model that uses appropriate species or disease-specific anatomical and ventilatory parameters can be used to describe absorption in the species and in different-sized, aged, or diseased members of the same species. More importantly, models also may be used to make interspecies and intraspecies dose comparisons, to compare and reconcile data from different experiments, to predict dose in conditions not possible or feasible experimentally, and to better understand the processes involved in toxicity.

A review (Miller, 1995) of the factors influencing RT uptake of O_3 stated that structure of the RT region, ventilation, and gas transport mechanisms were important. Additionally, local dose is the critical link between exposure and response. A criticism of previous models of O_3

uptake is that they have typically discounted the mucociliary layer of the URT and tracheobronchial regions.

For a more detailed discussion on experimental and theoretical dosimetry studies the reader is referred to the previous O_3 criteria document (Volume III, Chapter 8, U.S. Environmental Protection Agency, 1996).

AX4.2 EXPERIMENTAL OZONE DOSIMETRY INVESTIGATIONS

There have been some advances in understanding human O_3 dosimetry that better enable quantitative extrapolation from laboratory animal data. The next two sections review the available new experimental studies on O_3 dosimetry, which involve only human subjects and are all from the same laboratory. Of the studies considered in the following discussion, five involved the use of the bolus response method as a probe to obtain information about the mechanism of O_3 uptake in the URT and TB regions. Of the remaining two investigations, one focused on total uptake by the RT and the other on uptake by the nasal cavities. Table AX4-1 provides a summary of the newer studies.

AX4.2.1 Bolus-Response Studies

The bolus-response method has been used as a probe to explore the effects of physiological and anatomical differences or changes on the uptake of O_3 by human beings.

Asplund et al. (1996) studied the effects of continuous O_3 inhalation on O_3 uptake and Rigas et al. (1997) investigated the potential effects of continuous coexposure to O_3, nitrogen dioxide (NO_2), or sulfur dioxide (SO_2) on O_3 absorption. In both of these studies, subjects were exposed "continuously" to a gas for 2 h. Every 30 min, breathing at 250 mL/s, a series of bolus test breaths was performed targeted at the lower conducting airways. Differences in bolus-response absorbed fraction from an established baseline indicated the degree to which the "continuous" gas exposure affected the absorption of O_3. Depending on the gas and concentration, changes in absorbed fraction ranged from −3 to +7 % (see Table 5-1). Continuous O_3 exposure lowered the uptake of O_3, whereas NO_2 and SO_2 increased the uptake of O_3. The investigators concluded that in the tested airways, NO_2 and SO_2 increased the capacity to absorb O_3 because more of the compounds oxidized by O_3 were made available. On the other

Table AX4-1. New Experimental Human Studies on Ozone Dosimetry [a]

Purpose/Objective	Subject Characteristics	Region of Interest	Breathing Patterns/Exposure	Results	Reference
Determine the effect of continuous O_3 inhalation on O_3 uptake	8 male, 3 female, 22-31 years old, 166-186 cm, 64-93 kg	Central conducting airways (70-120 mL from lips)	2 h of continuous exposure at rest: 0.0, 0.12, and 0.36 ppm O_3. Spontaneous breathing. Bolus test breaths every 30 minutes using 250 mL/sec constant flow rate.	Averaged over all subjects and the 4 measurement intervals, the absorbed fraction (AF) changed +0.04, -0.005, and -0.03 for the 0, 0.12, and 0.36 ppm continuous exposures, respectively. These changes are approximately +6, -1, and –4 % based on an average AF value of 0.7 in the range 70 –120 ml.[b] Both non zero exposures were significantly different than the air exposure.	Asplund et al. (1996)
Evaluate the influence of V_D on intersubject variation of O_3 dose.	10 male, 22-30 years old, 163-186 cm, 64-92 kg; 10 female, 22-35 years old, 149-177 cm, 48-81 kg	Conducting airways	Bolus-response test (V_T = 500ml at 250 mL/sec constant flow rate). Fowler single-breath N_2 washout method to determine V_D.	On average, for the same V_P , women had a larger AF than men; women had a smaller V_D than men. However, for the same value of V_P/V_D, AF for men and women were indistinguishable. Further analysis indicated "that previously reported gender differences may be due to a failure in properly accounting for tissue surface within the conducting airways".	Bush et al. (1996a)
Investigate the effect of continuous exposure to O_3, nitrogen dioxide and sulfur dioxide on O_3 absorption.	6 male, 21-29 years old, 165 185 cm, 60-92 kg; 6 female, 19-33 years old, 152-173 cm, 48-61 kg	Lower conducting airways (70-120 mL from lips)	2 h of continuous exposure at rest: O_3 (0, 0.36 ppm), SO_2 (0, 0.36 ppm), or NO_2 (0, 0.36, 0.75 ppm). 5-min Bolus test every 30 minutes: V_T = 500 ml; 250 mL/sec constant flow rate.	Averaging over all subjects or by gender, all exposures except O_3 resulted in an increase of AF. Based on an AF reference value[b], the change in AF ranged from –3 to +7 %. Only the O_3 and the NO_2 (0.36 ppm) exposures were significantly different from the air exposures.	Rigas et al. (1997)

Table AX4-1 (cont'd). New Experimental Human Studies on Ozone Dosimetry [a]

Purpose/Objective	Subject Characteristics	Region of Interest	Breathing Patterns/Exposure	Results	Reference
Compare the absorption of chlorine and O_3. Determine how the physical-chemical properties of these compounds affect their uptake distribution in the RT	5 male, 21-26 years old, 168-198 cm, 64-95 kg; 5 female, 18-28 years old, 162-178 cm, 55-68 kg[c]	Conducting airways. Nasal & oral routes	Bolus-response technique; V_T = 500 ml; 3 constant flow rates: 150, 250, and 1000 mL/sec.	Ozone dose to the URT was sensitive to the mode of breathing and to the respiratory rate. With increased airflow rate, O_3 retained by the upper airways decreased from 95 to 50%. TB region dose ranged from 0 to 35%. Mass transfer theory indicated that the diffusion resistance of the tissue phase is important for O_3. The gas phase resistances were the same for O_3 and Cl_2.	Nodelman and Ultman (1999a)[c]
To determine O_3 uptake relative to inhaled O_3 dose.	5 male, 5 female, 18-35 years old, 175 ± 13 (SD) cm, 72 ± 13 (SD) kg	Respiratory tract; oral breathing	Breath-by-breath calculation of O_3 retention based on data from fast response analyzers for O_3 and airflow rates. Oral breathing: 0.2 or 0.4 ppm O_3 at V_E of approximately 20 L/min for 60 min or 40 L/min for 30 min.	The FA for all breaths was 0.86. Concentration, minute volume, and time have small but statistically significant effects on AF when compared to intersubject variability. The investigators concluded: for a given subject, constant O_3 exposure, a given exercise level, and time < 2 h, inhaled dose is a reasonable surrogate for the actual uptake of O3. However, the actual doses may vary considerably among individuals who are exposed to similar inhaled doses.	Rigas et al. (2000)

Table AX4-1 (cont'd). New Experimental Human Studies on Ozone Dosimetry [a]

Purpose/Objective	Subject Characteristics	Region of Interest	Breathing Patterns/Exposure	Results	Reference
Study the effect of gas flow rate and O_3 concentration on O_3 uptake in the nose.	7 male, 3 female, 26 ± years, 170 ± 11 (SD) cm, 75 ± 20 (SD) kg	Nasal cavities	For a given flow rate and exposure concentration, the subjects inhaled through one nostril and exhale through the other. For two 1-h sessions, a series of 9-12 measurements of AF were carried out for 10 s each: (1) O_3 exposure concentration = 0.4 ppm; flow rates = 3, 5, 8, and 15 L/min. (2) O_3 exposure = 0.1, 0.2, and 0.4 ppm; flow rate = 15 L/min.(3) O_3 exposure = 0.4 ppm, flow rate = 15 L/min; AF was measured every 5 min for 1 h.	(1) With the exposure concentration at 0.4 ppm O_3, AF decreased from 0.80 to 0.33 when the flow rate was increased from 3 to 15 L/min. (2) At a flow rate of 15 L/min, the AF changed from 0.36 to 0.32 when the exposure concentration increased from 0.1 to 0.4 ppm O_3. (3) Statistical analysis indicated that the AF was not associated with the time at which the measurement was taken.	Santiago et al. (2001)
Evaluate intersubject variability in O_3 uptake; correlate differences in breathing pattern and lung anatomy with O_3 uptake	nonsmokers, 32 male, 22.9 ± 0.8 years old,178±1 cm, 80.6 ± 2.5kg; 28 female, 22.4 ± 0.9 years old, 166 ± 1 cm, 62.1 ± 2.2 kg	Respiratory tract; oral breathing	Continuous: 1 h exposure to 0.25 ppm, exercising at 30L/min. Bolus: breath-by- breath calculation of O_3 retention. Timing of bolus varied to create penetration volumes of 10 to 250 ml. Peak inhaled bolus of ~1 ppm.	Continuous: Fractional O_3 uptake efficiency ranged from 0.70 to 0.98 (mean 0.89 ± 0.06). Inverse correlation between uptake and breathing frequency. Direct correlation between uptake and tidal volume. Intersubject differences in forced respiratory responses not due to differences in O_3 uptake. Bolus: The penetration volume at which 50% of the bolus was taken up was 90.4 ml in females and 107 ml in males. Distribution of O_3 shifts distally as the size of the airway increases.	Ultman et al. (2004)

[a] See Appendix A for abbreviations and acronyms.
[b] Fig. 4, Hu et al. (1994), for the 250 mL/s curve and penetration volume range of 70 – 120 ml; the average AF is approximately 0.7.
[c] Subject characteristics are from Nodelman and Ultman (1999b).

hand, they conjectured that continuous O_3 exposure depleted these compounds, thereby reducing O_3 uptake.

Bush et al. (1996a) investigated the effect of lung anatomy and gender on O_3 absorption in the conducting airways during oral breathing using the bolus-response technique. Absorption was measured using this technique applied to 10 men and 10 women. Anatomy was defined in terms of forced vital capacity (FVC), total lung capacity (TLC), and dead space (V_D). The absorbed fraction data were analyzed in terms of a function of penetration volume, airflow rate, and an "intrinsic mass transfer parameter (K_a)", which was determined for each subject and found to be highly correlated with V_D, but not with height, weight, age, gender, FVC, or TLC. That is, in all subjects, whether men or women, dosimetry differences could be explained by differences in V_D. Based on Hu et al. (1994), where absorbed fraction was determined for several flow rates, Bush et al. (1996a) inferred that K_a was proportional to flow rate/V_D. The investigators point out that the applicability of their results may be limited because of their assumptions that K_a was independent of location in the RT and that there was no mucous resistance. They also suggested that the dependence of K_a on flow rate and V_D be restricted to flow rates $\leq$ 1000 mL/s until studies at higher rates have been performed.

With flow rates of 150, 250, and 1000 mL/s, Nodelman and Ultman (1999b) used the bolus-response technique to compare the uptake distributions of O_3 and chlorine gas (Cl_2), and to investigate how their uptakes were affected by their physical and chemical properties. Ozone dose to the URT was found to be sensitive to the mode of breathing and to the airflow rate. With increased rate, O_3 retained by the upper airways decreased from 95 to 50% and TB region dose increased from 0 to 35%. At the highest flow rate only 10% of the O_3 reached the pulmonary region. Mass transfer theory indicated that the diffusion resistance of the tissue phase is important for O_3. The gas phase resistances were found to be the same for O_3 and Cl_2 as expected. These resistances were inversely related to the volumes of the oral and nasal cavities during oral and nasal breathing, respectively.

Ultman et al. (2004) used both bolus and continuous exposures to test the hypotheses that differences in O_3 uptake in lungs are responsible for variation in O_3-induced changes in lung function parameters and that differences in O_3 uptake are due to variations in breathing patterns and lung anatomy. Thirty-two males and 28 female nonsmokers were exposed to bolus penetration volumes ranging from 10 to 250 ml, which was determined by the timing of the

bolus injection. The subjects controlled their breathing to generate a target respired flow of 1000ml/sec. At this high minute ventilation, there was very little uptake in the upper airway and most of the O_3 reached areas where gas exchange takes place. To quantify intersubject differences in O_3 bolus uptake, they measured the penetration volume at which 50% of the O_3 was taken up. Values for penetration volume ranged from 69 to 134 ml and were directly correlated with the subjects' values for anatomic dead space volume. A better correlation was seen when the volume of the upper airways was subtracted. The penetration volume at which 50% of the bolus was taken up was 90.4 ml in females and 107 ml in males. This significant difference in uptake suggests to the authors that in females the smaller airways, and associated larger surface-to-volume ratio, enhance local O_3 uptake and cause reduced penetration of O_3 into the distal lung. Thus, these findings indicate that overall O_3 uptake is not related to airway size, but that the distribution of O_3 shifts distally as the size of the airway in increased.

General comment on estimating mass transfer coefficients: Bush et al. (1996b) and Nodelman and Ultman (1999a) used a simple model to analyze their bolus-response data. This model presented by Hu et al. (1992, 1994) assumed steady-state mass transfer by convection (but no dispersion) and the mass transfer of O_3 to the walls of a tube of uniform cross-sectional area. These assumptions led to an analytical solution (for the absorbed fraction) which was a function of an "overall mass transfer coefficient," penetration volume, and airflow rate. As the investigators have shown, the model is very useful for statistical analysis and hypothesis testing. Given the absorbed fraction data, the model overall mass transfer coefficients were estimated for each flow rate. In those bolus-response studies that used this method to analyze data, there was no discussion of the models' "accuracy" in representing mass transfer in the human respiratory tract with respect to omitting dispersion. In addition, the formulation of the gas phase mass transfer coefficient does not take into account that it has a theoretical lower limit greater than zero as the airflow rate goes to zero (Miller et al., 1985; Bush et al., 2001). As a consequence, there is no way to judge the usefulness of the values of the estimated mass transfer coefficients for dosimetry simulations that are based on convection-dispersion equations, or whether or not the simple model's mass transfer coefficients, as well as other parameters derived using these coefficients, are the same as actual physiological parameters.

AX4.2.2 General Uptake Studies

Rigas et al. (2000) performed an experiment to determine the ratio of O_3 uptake to the quantity of O_3 inhaled (fractional absorption, FA). Five men and five women were exposed orally to 0.2 or 0.4 ppm O_3 while exercising at a minute volume of approximately 20 L/min for 60 minutes or 40 L/min for 30 minutes. Ozone retention was calculated from breath-by-breath data taken from fast response analyzers of O_3 and airflow rates. The FA was statistically analyzed in terms of subject, exposure concentration, minute volume, and exposure time.

Fractional absorption ranged from 0.56 to 0.98 with a mean ± SD of 0.85 ± 0.06 for all recorded breaths. Intersubject differences had the largest influence on FA, resulting in a variation of approximately 10%. Statistical analysis indicated that concentration, minute volume, and exposure time had statistically significant effects on FA. However, relatively large changes in these variables were estimated to result in relatively small changes in FA. Note: the quantity of O_3 retained by the RT is equal to FA times the quantity of O_3 inhaled; thus, relatively large changes in concentration, minute volume, or exposure time may result in relatively large changes in the amount of O_3 retained by the RT or absorbed locally. Also, according to Overton et al. (1996), difference in PAR dose due to anatomical variability may be considerably larger than corresponding small changes in FA would indicate.

Santiago et al. (2001) studied the effects of airflow rate and O_3 concentration on O_3 uptake in the nasal cavities of three women and seven men. Air was supplied at a constant flow rate to one nostril and exited from the other nostril while the subject kept the velopharyngeal aperture closed by raising the soft palate. Thus, a constant unidirectional flow of air plus O_3 was restricted to the nasal cavities. The fraction of O_3 absorbed was calculated using the inlet and outlet concentrations. Inlet concentration and airflow rate were varied in order to determine their effect on O_3 uptake.

The mean FA decreased from 0.80 to 0.33 with an increase in flow rate from 3 to 15 L/min. The effect of both flow rate and subject on FA was statistically significant. Further analysis indicated that the overall mass transfer coefficient was highly correlated with the flow rate and that the gas phase resistance contributed from 6.3% (15 L/min) to 23% (3 L/min) of the total resistance to O_3 transfer to the nasal cavity surface. Concentration had a small, but statistically significant effect on FA, when the inlet concentration was increased from 0.1 to 0.4 ppm O_3, FA decreased from 0.36 to 0.32. The investigators observed that differences in FA

among subjects were important; generally, subject variability accounted for approximately half of the total variation in FA.

As mentioned above Ultman et al. (2004) tested hypotheses that differences in O_3 uptake in lungs are responsible for variation in O_3-induced changes in lung function parameters and that differences in O_3 uptake are due to variations in breathing patterns and lung anatomy. Thirty-two males and 28 female nonsmokers were exposed continuously for 1 h to either clean air or 0.25 ppm ozone while exercising at a target minute ventilation of 30 L/min. They first determined the forced expiratory response to clean air, then evaluated O_3 uptake measuring dead space volume, cross-sectional area of peripheral lung (A_p) for CO_2 diffusion, FEV_1, FVC, and $FEF_{25\%-75\%}$. The fractional O_3 uptake efficiency ranged from 0.70 to 0.98, with a mean of 0.89 ± 0.06. They found an inverse correlation between uptake and breathing frequency and a direct correlation between uptake and tidal volume. The uptake efficiency decreased during the four sequential 15 minute intervals of the 1 h exposure (0.906 ± 0.058 to 0.873 ± 0.088, first and last, respectively), demonstrating a general decrease in uptake efficiency with increased breathing frequency and decreasing tidal volume. Ozone uptake rate correlated with individual $\%A_p$, but did not correlate with individual $\%FEV_1$. Neither of these parameters correlated with the penetration volume determined in the bolus studies mentioned above. The authors concluded that the intersubject differences in forced respiratory responses were not due to differences in O_3 uptake. However, these data did partially support the second hypothesis, i.e., that the differences in cross-sectional area available for gas diffusion induce differences in O_3 uptake.

AX4.3 DOSIMETRY MODELING

When all of the animal and human in vivo O_3 uptake efficiency data are compared, there is a good degree of consistency across data sets (U.S. Environmental Protection Agency, 1996). This agreement raises the level of confidence with which these data sets can be used to support dosimetric model formulations.

Recent data indicate that the primary site of acute cell injury occurs in the conducting airways (Postlethwait et al., 2000). These data must be considered when developing models that attempt to predict site-specific locations of O_3-induced injury. The early models computed relationships between delivered regional dose and response with the assumption that O_3 was the

active agent responsible for injury. It is now known that reactive intermediates such as hydrohydroxyperoxides and aldehydes are important agents mediating the response to O_3 (further discussed in Section 5.3.1). Thus, models must consider O_3 reaction/diffusion in the epithelial lining fluid (ELF) and ELF-derived reactions products.

Table AX4-2 presents a summary of new theoretical studies on the uptake of O_3 by the RTs (or regions) of humans and laboratory animals that have become available since the 1996 review. They are discussed below.

Overton and Graham (1995) described the development and simulation results of a dosimetry model that was applied to a TB region anatomical model that had branching airways, but which had identical single-path pulmonary units distal to each terminal bronchiole. The anatomical model of the TB region was based on Raabe et al. (1976), which reported lung cast data for the TB region of a 330 g rat.

Rat effects data (from the PAR) are available that are identified with the lobe and the generation in the lobe from which tissue samples were obtained (Pinkerton et al., 1995, 1998). Models, like Overton et al. (1995), can be helpful in understanding the distribution of the magnitude of such effects as well as suggesting sampling sites for future experiments.

Using computational fluid dynamics (CFD), Cohen-Hubal et al. (1996) explored the effect of the mucus layer thickness in the nasal passage of a rat. The nasal lining was composed of mucus and tissue layers in which mass transfer was by molecular diffusion with first order chemical reaction. Physicochemical parameters for O_3 were obtained from the literature. Three scenarios were considered: 10 μm thick mucus layer, no mucus layer, and two nasal passage regions each with a different mucus layer thickness. Predictions of overall uptake were within the range of measured uptake. Predicted regional O_3 flux was correlated with measured cell proliferation for the CFD simulation that incorporated two regions, each with a different mucus thickness.

The reaction rate constant used by Cohen-Hubal and co-workers may be too low. Using bolus-response data, Hu et al. (1994) and Bush et al. (2001) estimated a reaction rate constant that is more than a 1000 times as large as that used by Cohen-Hubal et al. (1996). A rate constant this large could result in a conclusion different than those based on the smaller constant.

With an RT dosimetry model, Overton et al. (1996) investigated the sensitivity of absorbed fraction (AF), proximal alveolar region (PAR) dose, and PAR dose ratio to TB region volume

Table AX4-2. New Ozone Dosimetry Model Investigations[a]

Purpose/Objective	Type of mass transport model/Anatomical model [b]	Species/ RT region of interest/Regional anatomical models	Ventilation and Exposure	Results	Reference
To describe an RT dosimetry model that uses a branching TB region anatomical model and to illustrate the results of its application to a rat exposed to O_3.	One-dimensional (along axis of airflow), time-dependent, convection-dispersion equation of mass transport applied to each airway or model segment. URT: single path; TB: asymmetric branching airways. PUL: single path anatomical model distal to each terminal bronchiole.	Rat/ RT/URT: Patra et al. (1987). TB: multiple path model of Raabe et al. (1976). PUL: Mercer et al. (1991).	f = 150 bpm; V_T = 1.5, 2.0 , 2.5 mL. One constant concentration.	(1) For V_T = 2.0 mL, f = 150 bpm: The general shape of the dose versus generation plot along any path from the trachea to a sac is independent of path: generally the tissue dose decreases with increasing generation index. In the TB region, the coefficient of variation for dose ranges from 0 to 34 %, depending on generation. The maximum ratio of the largest to smallest dose in the same generation is 7; the average ratio being 3. In the first PUL region model segment, the coefficient of variation for the dose is 29 %. (2) The average dose to the first PUL region model segment increases with increasing V_T.	Overton and Graham (1995)
To incorporate into the CFD model of Kimbell et al. (1993) resistance to mass transfer in the nasal lining and to investigate the effects of this lining on O_3 uptake.	Three dimensional steady-state Navier-Stokes equations for solving air velocity flow field. Three dimensional steady-state convection-diffusion equation for O_3 transport. Three-dimensional CFD model of the nasal passages of a rat.	Rat/nasal passages Nasal passages: Kimbell et al. (1993).	Steady-state unidirectional ("inhalation") flow rate = 576 mL1/min. One constant concentration.	Predictions of overall uptake were within the range of measured uptake. Results suggest that mucus resistance is important for describing O_3 dosimetry and this thickness may play a role for determining patterns of O_3-induced lesions in the rat nasal passage.	Cohen-Hubal et al. (1996)
To determine if the single-path model is able to simulate bolus inhalation data recorded during oral breathing at quiet respiratory flow.	Single-path, one-dimensional (along axis of airflow), time-dependent, convection-dispersion equation of mass transport. Single-path anatomical model	Human/ RT/URT (oral): Olson et al. (1973). LRT: Weibel (1963).	V_T = 500 mL, f = 15 bpm, constant flow rate = 250 mL/s. Bolus-response simulations. (protocol used is described by Hu et al., 1992).	Simulations are sensitive to conducting airway volume but are relative insensitive to characteristics of the respiratory airspace. Although the gas-phase resistance to lateral diffusion limits O_3 absorption during quiet breathing, diffusion through mucus may become important at the large respiratory flows that are normally associated with exercise. The single-path convection-diffusion model was a reasonable approach /to simulate the bolus-response data.	Bush et al. (1996b)

Table AX4-2 (cont'd). New Ozone Dosimetry Model Investigations [a]

Purpose/Objective	Type of mass transport model/Anatomical model [b]	Species/ RT region of interest/Regional anatomical models	Ventilation and Exposure	Results	Reference
To assess age- and gender-specific differences in regional and systemic uptake.	PBPK, at ages 1, 3, 6, months and 1, 5, 10, 15, 25, 50, and 75 years	Human/ET/TB	Pulmonary ventilation ranged from 34 mL/s (in 1-month-old) to 190 mL/s (in 15-year-old). V_T varied with age	Regional extraction is insensitive to age. Extraction per unit surface area is 2- to 8-fold higher in infants compared to adults. PU and ET regions have a large increase in unit extraction with increasing age. Early postnatal period is time of largest differences in PK, due to immaturity of metabolic enzymes.	Sarangapani et al. (2003)
To examine the impact on predictions due to the value used for the TB region volume at FRC and due to TB region volume change during respiration.	Single-path, one-dimensional (along axis of airflow), time-dependent, convection-dispersion equation of mass transport. Single-path anatomical model	Human /RT/ URT: Nunn et al. (1959) LRT: Weibel (1963) Rat / RT/ URT: Patra et al. (1987) TB: Yeh et al. (1979) PUL: Mercer et al. (1991)	Human: V_T = 500, 2250 mL; f = 15, 30 bpm. Rat: V_T = 1.4, 2.4 ml; f = 96, 157 bpm. One constant concentration.	(1) A better understanding and characterization of the role of TB region expansion (mainly the rat) and volume is important for an improved understanding of respiratory-tract dosimetry modeling of reactive gases. (2) Extrapolations based on dose in the PAR can differ significantly from those based on exposure concentration or total uptake. (3) Human subjects who appear similar outwardly may have very different PAR doses and potentially different responses to the same exposure.(Uptake by the URT was not considered.)	Overton et al. (1996)
To make parameter modifications so that a single-path model would simulate AF from bolus-response experiments involving O_3 (and Cl_2).	Single-path, one-dimensional (along axis of airflow), time-dependent, convection-dispersion equation of mass transport. Single-path anatomical model	Human/ RT/URT (oral): Olson et al. (1973). URT (nasal): Olson et al. (1973) and Guilmette et al. (1989) LRT: Weibel (1963).	Oral & nasal breathing. Flow rates = 150, 250, 1000 mL/s, V_T = 500 mLl. Bolus-response simulations	(Simulation results for O_3 only) (1) Using parameter values from the literature and assuming that absorption was gas-phase controlled, the simulations of O_3 data were realistic at flow rate = 250 mL/s, but not realistic at 1000 mL/s.(2) Accurate simulations at 250 mL/s required modification of mass transfer coefficients reported in the literature for the conducting airways.(3) It was necessary to include a diffusion resistance for the epithelial lining fluid based on an assumed O_3 reaction rate constant that was much greater than in in vitro estimates.(4) Partial validation of the final parameters (determined at 250 mL/s) was obtained by simulations of bolus-response data at flow rates of 150 and 1000 mL/s. Validation was obtained also by simulating internal measurements of O_3 in subjects exposed during quiet breathing.	Bush et al. (2001)

[a]See Appendix A for abbreviations and acronyms.
[b]The anatomical models used in an investigation generally differ from those described in the references, e.g., dimensions are often scaled to dimensions appropriate to the dosimetry investigation; or the original structure may be simplified, keeping or scaling the original dimensions.

(V_{TB}) and TB region expansion in human beings and rats. The PAR was defined as the first generation distal to terminal bronchioles and the PAR dose ratio was defined as the ratio of a rat's predicted PAR dose to a human's predicted PAR dose. This ratio relates human and rat exposure concentrations so that both species receive the same PAR dose. In rats the PAR is a region of major damage from O_3. For each species, three literature values of V_{TB} were used: a mean value and the mean ± twice the SD. The following predictions were obtained:

(1) The sensitivity of AF and PAR dose to V_{TB} depends on species, ventilation, TB region overall mass transfer coefficient (k_{TB}), and expansion. Depending on these latter four parameters, AF was predicted to be 1 to 25% smaller and 1 to 40% larger than the values predicted for the mean V_{TB}, given the range of V_{TB}. However, AF can be insensitive to V_{TB} and PAR dose very sensitive. For k_{TB} = 0.26 cm/s and quiet breathing, AF was predicted to vary by less than 3% for the ±2 SD range of V_{TB}; in contrast, the PAR dose predicted using the smallest V_{TB} is five times larger than the PAR dose predicted with the largest V_{TB}. The effect of V_{TB} is much less during heavy exercise: the ratio of maximum to minimum PAR dose was approximately 1.5. In any case, the simulations predicted that fractional changes in AF due to different V_{TB} are not, in general, a good predictor of the fractional changes in PAR doses.

(2) Relative to no expansion in the TB region, expansion decreases both AF and PAR dose. The largest effect of including expansion in the human simulations was to decrease the AF by ≈8%; in rats, the maximum decrease was ≈45%. The PAR doses decreased relatively more, 25 and 65% in human beings and rat, respectively.

(3) The authors attempted to obtain an understanding as to uncertainty or variability in estimates of exposure concentrations (that give the same PAR dose in both species) if the literature mean value of V_{TB} was used. For various values of f, V_T, k_{TB}, and expansion, the PAR dose ratios at upper and lower values of V_{TB} deviated in absolute values from the PAR dose ratio calculated at the mean values of V_{TB} by as little as 10% to as large as 310%. The smallest deviation occurred at the largest V_T and smallest k_{TB} for both species; whereas, the largest deviation occurred at the smallest V_T and largest k_{TB} for both species.

Bush et al. (2001) modified the single-path model of Bush et al. (1996b) in order to be able to simulate absorbed fraction data for O_3 (and Cl_2, which is not considered) for three airflow rates and for oral and nasal breathing. By adjusting several parameters a reasonable agreement between predicted and experimental values was obtained. On the other hand, the O_3 plots of the

experimental and predicted values of absorbed fraction versus penetration volume (e.g., Figures 4 and 5 of Bush et al., 2001) show sequential groups composed of only positive or only negative residuals, indicating a lack of fit. Possibly adjusting other parameters would eliminate this. To obtain an independent validation of the model, Bush et al. (2001) simulated measurements of O_3 concentrations made by Gerrity et al. (1995) during both inhalation and exhalation at four locations between the mouth and the bronchus intermedius of human subjects. Simulated and experimental values obtained are in close agreement. Note, however, that Bush et al. made no quantitative assessment of how well their simulations agreed with the experimental data; assessments were made on the basis of visual inspection of experimental and simulated values plotted on the same figure. Thus, evaluation of the model was, or is, subjective.

Recently Sarangapani et al. (2003) used physiologically based pharmacokinetic (PBPK) modeling to characterize age- and gender-specific differences in both regional and systemic uptake of O_3 in humans. This model indicated that regional extraction of O_3 is relatively insensitive to age, but extraction per unit surface area is 2- to 8-fold higher in infants compared to adults, due to the region-specific mass transfer coefficient not varying with age. The PU and ET regions have a large increase in unit extraction with increasing age because both regions increase in surface area. Males and females in this model have similar trends in regional extraction and regional unit extraction. In early childhood, dose metrics were as much as 12 times higher than adult levels, but these differences leveled out with age, such that inhalation exposures varied little after age 5. These data suggest that the early postnatal period is the time of the largest difference in pharmacokinetics observed, and this difference is primarily due to the immaturity of the metabolic enzymes used to clear O_3 from the respiratory tract.

Mudway and Kelly (2004) attempted to model O_3 dose-inflammatory response using a metaanalysis of 23 exposures in published human chamber studies. The O_3 concentrations ranged from 0.08 to 0.6 ppm and the exposure durations ranged from 60 to 396 minutes. The analysis showed linear relationships between O_3 dose and neutrophilia in bronchoalveolar lavage fluid (BALF). Linear relationships were also observed between O_3 dose and protein leakage into BALF.

AX4.4 SPECIES HOMOLOGY, SENSITIVITY AND ANIMAL-TO-HUMAN EXTRAPOLATION

Biochemical differences among species are becoming increasingly apparent and these differences may factor into a species' susceptibility to the effects of O_3 exposure. Lee et al. (1998) compared SD rats and rhesus monkeys to ascertain species differences in the various isoforms of CYP moonoxygenases in response to O_3 exposure (discussed in more detail in Section 5.3.1.2). Differences in activities between rat and monkey were 2- to 10-fold, depending on the isoform and the specific lung region assayed. This study supports the view that differential expression of CYPs is a key factor in determining the toxicity of O_3. As further characterization of species- and region-specific CYP enzymes occurs, a greater understanding of the differences in response may allow more accurate extrapolation from animal exposures to human exposures and toxic effects.

Arsalane et al. (1995) compared guinea pig and human AM recovered in BALF and subsequently exposed in vitro to 0.1 to 1 ppm for 60 minutes. Measurement of inflammatory cytokines showed a peak at 0.4 ppm in both species. Guinea pig AM had an increase in IL-6 and TNF-α while human AM had increases in TNF-α, IL-1b, IL-6 and IL-8. This exposure also caused an increase in mRNA expression for TNF-α, IL-1b, IL-6 and IL-8 in human cells. At 0.1 ppm exposures, only TNF-α secretion was increased. These data suggest similar cytokine responses in guinea pigs and humans, both qualitatively and quantitatively.

Dormans et al. (1999) continuously exposed rats, mice, male guinea pigs to filtered air, 0.2, or 0.4 ppm for 3, to 56 days or to 28 days with 3, 7, and 28 days PE. Depending on the endpoint studied, the species varied in sensitivity. Greater sensitivity was shown in the mouse as determined by biochemical endpoints, persistence of bronchiolar epithelial hypertrophy, and recovery time. Guinea pigs were more sensitive in terms of the inflammatory response though all three species had increases in the inflammatory response after three days that did not decrease with exposure. In all species the longest exposure to the highest dose caused increased collagen in ductal septa and large lamellar bodies in Type II cells, but that response also occurred in rats and guinea pigs at 0.2 ppm. No fibrosis was seen at the shorter exposure times and the authors question whether fibrosis occurs in healthy humans after continuous exposure. The authors do not rule out the possibility that some of these differences may be attributable to differences in

total inhaled dose or dose actually reaching a target site. Overall, the authors rated mice as most susceptible, followed by guinea pigs and rats.

Comparisons of airway effects in rats, monkeys and ferrets resulting from exposures of 1.0 ppm O_3 for 8 h (Sterner-Kock et al. 2000) demonstrated that monkeys and ferrets had a similar inflammatory responses and epithelial necrosis. The response of these two species was more severe than that seen in rats. These data suggest that ferrets are a good animal model for O_3-induced airway effects due to the similarities in pulmonary structure between primates and ferrets.

The rat is a key species used in O_3 toxicological studies, but Watkinson and Gordon, (1993) suggest that, because the rat has both behavioral and physiological mechanisms that can lower core temperature in response to acute exposures, extrapolation of these exposure data to humans may be limited. Another laboratory (Iwasaki et al., 1998) has demonstrated both cardiovascular and thermoregulatory responses to O_3 at exposure to 0.1, 0.3, and 0.5 ppm O_3 8 h/day for 4 consecutive days. A dose-dependent disruption of HR and T_{co} were seen on the first and second days of exposure, which then recovered to control values. Watkinson et al. (2003) exposed rats to 0.5 ppm O_3 and observed this hypothermic response which included lowered HR, lowered T_{co}, and increased inflammatory components in BALF. The authors suggest that the response is an inherent reflexive pattern that can possibly attenuate O_3 toxicity in rodents. They discuss the cascade of effects created by decreases in T_{co}, which include: (1) lowered metabolic rate, (2) altered enzyme kinetics, (3) altered membrane function, (4) decreased oxygen consumption and demand, (5) reductions in minute ventilation, which would act to limit the dose of O_3 delivered to the lungs. These effects are concurrent with changes in HR which lead to: (1) decreased CO, (2) lowered BP, (3) decreased tissue perfusion, all of which may lead to functional deficits. The hypothermic response has not been observed in humans except at very high exposures.

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5. TOXICOLOGICAL EFFECTS OF OZONE AND RELATED PHOTOCHEMICAL OXIDANTS IN LABORATORY ANIMALS AND IN VITRO TEST SYSTEMS

5.1 INTRODUCTION

A wide range of effects of ozone (O_3) has been demonstrated in laboratory animals. The major research findings are that environmentally relevant levels of O_3 cause lung inflammation; decreases in host defenses against infectious lung disease; acute changes in lung function, structure, and metabolism; chronic lung disease, some elements of which are irreversible; and systemic effects on target organs (e.g., brain, heart, liver, immune system) distant from the lung. The research also has served to expand the understanding of mechanisms of O_3 toxicity and the relationships between concentration and duration of exposure.

The framework for presenting the health effects of O_3 in animals begins with a presentation of respiratory tract effects, followed by systemic effects, and then interactions of O_3 with other common co-occurring pollutants. The information discussed in this chapter is founded on a very wide body of literature on studies in laboratory animals and on in vitro test systems of animal cell lines and organ systems that may mimic responses in intact animals. The direct effects of O_3 in humans are discussed in the following chapter (Chapter 6).

This chapter is not intended to be a compendium of all that is known about O_3; rather, it is an update of the toxicology chapter from the last O_3 criteria document (U.S. Environmental Protection Agency, 1996), or 1996 O_3 CD, and other reviews of the earlier published literature. The historical O_3 literature is very briefly summarized in an opening paragraph of each section or subsection. This paragraph is intended as a very concise overview of previous work, and the reader is referred to the 1996 O_3 CD for more detailed discussion of the literature prior to the early 1990's. Each section then continues with brief discussions of the key new studies (or somewhat older studies that were not included in the previous CD). Longer discussions of new studies are included where warranted. Sections are ended with comparisons of data from the

previous CD with new data and basic conclusions are drawn. More detailed descriptive summaries of new studies and results are provided in text and tables in Annex AX5.

Except for nitrogen dioxide (NO_2), the subject of another criteria document (U.S. Environmental Protection Agency, 1993), there is very little relevant information on other photochemical oxidants in the published literature. What is known about the effects of these other oxidants is also summarized briefly in this chapter.

5.2 RESPIRATORY TRACT EFFECTS OF OZONE

5.2.1 Biochemical Effects

Biochemically detected effects of O_3 are integrally involved in effects on both structure and function (respiratory and nonrespiratory) of the respiratory tract. Changes in xenobiotic metabolism, antioxidant metabolism and oxygen consumption, lipids and arachidonic acid metabolism, and collagen metabolism are all observed with O_3 exposure, though the mechanisms and associations are not fully understood.

5.2.1.1 Cellular Targets of O_3 Interaction

Ozone has the potential to interact with a wide range of different cellular components that include polyunsaturated fatty acids (PUFAs); some protein amino acid residues (cysteine, histidine, methionine, and tryptophan); and some low-molecular-weight compounds that include glutathione (GSH), urate, vitamins C and E, and free amino acids. Early work demonstrated that O_3, being a highly reactive compound, does not penetrate much beyond the epithelial lining fluid (ELF). Ozone-induced cell damage most likely results from its reactions with PUFAs to form stable but less reactive ozonide, aldehyde, and hydroperoxide reaction products (the reactions are summarized in Figure 5-1 of Annex AX5). These reaction products (Crigee ozonides and hydroxyhydroperoxides) may act as signal transduction molecules involved in signaling of cellular responses such as inflammation, and thus mediate O_3 toxicity.

These recent reports combined with observations reported in the previous O_3 CD (US EPA, 1996) suggest that interactions of O_3 with cellular components and ELF generate toxic ozonation products and mediate toxic effects through these products.

5.2.1.2 Monooxygenases

Both short- and long-term exposures to O_3 have been shown to enhance lung xenobiotic metabolism, possibly as a result of changes in the number and function of bronchiolar epithelial Clara cells and alveolar epithelial Type 2 cells. Studies of the effects of O_3 on lung monooxygenases are listed in Table 5-1. Early studies showed that exposure to O_3 increased CYP 2B1 (the major CYP isoform in rat lung) content and activity in rat lung. Ozone exposures also caused hypertrophy and hyperplasia of CYP 2B1-immunoreactive Clara cells. Comparisons of rat and rhesus monkey CYP isoforms demonstrated species-specific and region-specific (e.g., trachea, parenchyma, differences in the activities of P450 isoforms (Lee et al., 1998)

Watt et al. (1998) found that 1 ppm O_3 in both short and long-term exposures in rat increased CYP 2E1 in a region-specific manner. Paige et al. (2000) showed that a long term exposure (0.8 ppm 8h/day for 90 days) increased the activity of CYP 2B in distal lung, but not trachea or intrapulmonary airways. Studies have focused on P450 gene expression to examine possible genetic mechanisms that may explain differential O_3-sensitivity (Mango et al., 1998). Mice (129 strain) deficient in Clara cell secretory protein (CCSP-/-), which are oxidant-sensitive, were exposed to 1 ppm O_3 for 2 hours. The CCSP null mice demonstrated increases in IL-6 and metallothionein (Mt) mRNA that preceded decreases in Clara cell CYP2F2 mRNA (normally expressed at high levels in mouse lung) levels. In 129 strain wildtype (WT) mice, RNA levels changed similarly, to a lesser degree. These data suggest a protective role against oxidant damage for CSPP, and further, that genetic susceptibility to oxidant stress may be mediated, in part, by the gene coding for CSPP.

5.2.1.3 Antioxidants, Antioxidant Metabolism, and Mitochondrial Oxygen Consumption

Ozone also undergoes reactions with AA, GSH, and uric acid, all antioxidants present in ELF. This is a protective interaction, but even with environmentally relevant exposures to O_3, the reactivity of O_3 is not quantitatively quenched. Antioxidants offer some protection from O_3 exposure, but often do not maintain sufficiently high concentration to fully protect the lung. Thus, O_3- induced cell injury occurs in both the lower and upper respiratory tract. Early work has shown that short-term exposures to < 1 ppm O_3 increase antioxidant metabolism, including levels of cytosolic enzymes G6PD, 6PGD, GR, and GSHPx. Re-exposure after a recovery

period causes increases equivalent to first-time exposures, thus previous exposure appears to not be protective.

Increases in enzyme activity appear to increase as a function of age, suggesting that O_3 exposure can cause greater lung injury in the older animal. Species differences exist in antioxidant metabolism, with guinea pigs being very sensitive due to their diminished increases in antioxidants and antioxidant enzymes. Long term exposures of rats to urban patterns of O_3 (daily peaks of 0.25 ppm) caused increases in GSHPx and GR, but not superoxide dismutase (SOD). The enzyme changes could be accounted for by changes in the steady-state cell population or in cellular antioxidant capacity.

Ozone induced both site- and cell-specific changes in copper-zinc (Cu-Zn) and manganese (Mn) SOD in rats exposed to 1.0 ppm O_3 for up to 3 months (Weller et al., 1997). Cu-Zn SOD labeling was decreased in epithelial cells in airways and parenchyma. Mn SOD labeling was increased in both AM and epithelial type II cells of the centriacinar region (CAR), which the authors suggest may allow these cells to tolerate further O_3 exposure.

In a recent report Freed et al. (1999) evaluated the role of antioxidants in O_3- induced oxidant stress in dogs (exposed to 0.2 ppm in a 6-h exposure) by inhibiting the antioxidant transport using probenencid (an anion-transport inhibitor). Blocking antioxidant transport caused heterogeneously distributed increases in peripheral airway resistance and reactivity, supporting the hypothesis that in the lung periphery, endogenous antioxidants moderate the effects of O_3 and that this exposure is a subthreshold stimulus for producing effects on peripheral airway resistance and reactivity in dogs. The authors further found that treatment with probenecid also inhibited O_3-induced neutrophilic inflammation, providing evidence for a dissociation between airway function and inflammation, and suggesting that O_3-induced inflammation and airway hyperreactivity (AHR) are independent phenomena.

Mudway and Kelly (1998) modeled the interactions of O_3 with ELF antioxidants using a continually mixed, interfacial exposure set up with O_3 concentrations of 0 to 1.5 ppm. Uric acid was ranked the most O_3-reactive, AA the second most reactive, and GSH the least reactive. Thus, they concluded that GSH is not an important substrate for O_3, while uric acid appeared to be the most important substrate which confers protection from O_3 by removing it from inhaled air and limiting the amount that reaches the distal lung. The authors acknowledge limitations in extrapolating these data to in vivo O_3 exposures.

5.2.1.4 Lipid Metabolism and Content of the Lung

One of the major postulated molecular mechanisms of action of O_3 is peroxidation of unsaturated fatty acids in the lung. In both acute and short-term studies, a variety of lung lipid changes occur, including an increase in arachidonic acid. Metabolism of arachidonic acid produces a variety of biologically active mediators that can, in turn, affect host defenses, lung function, the immune system, and other functions. The protein A component of surfactant is also a primary target of O_3 interaction. During the first few days of O_3 exposure, the changes in lung lipid biosynthesis can be accounted for by the alveolar epithelial proliferative repair. With longer exposures (eg 0.12 ppm for 90 days), an increases in PUFAs and a decrease in cholesterol-esters are seen, indicative of long-term alterations of surfactant lipid composition.

Several new studies examined the effects of O_3 exposure on phospholipids in lung tissue. Ozonation of PUFAs is has been shown to generate other aldehydes such as nonanal and hexanal in rat (Pryor et al., 1996; Frampton et al., 1999). These aldehydes are short-lived and found to not affect lung function (Frampton et al., 1999). These observations suggest that levels of these aldehydes are dependent on a dynamic relationship between their production and their disappearance from the ELF.

Pryor et al. (1995) proposed a cascade mechanism whereby ozonation products cause activation of specific lipases, which then trigger the activation of second messenger pathways (e.g., phospholipase A_2 or phospholipase C). This group (Kafoury et al., 1999) showed that exposure of cultured human bronchial epithelial cells to the lipid ozonation product 1 -palmitoyl-2-(9-oxononanoyl)-sn-glycero-3-phosphocholine elicited release of platelet-activating factor (PAF) and prostaglandin E2, but not IL-6. The lipid ozonation product 1-hydroxy-1-hydroperoxynonane caused release of PAF and IL-6 in these cells, but not prostaglandin E2. These results suggest to the authors that O_3-induced production of lipid ozonation products causes release of proinflammatory mediators that then generate an early inflammatory response. Long et al. (2001) exposed hamsters to 0.12, 1.0 or 3.0 ppm O_3 to evaluate lipid peroxidation and antioxidant depletion. Six hour exposures to the two higher levels resulted in increased BALF neutrophil numbers and F_2-isoprostanes. Exposures to 1.0 ppm O_3 with 1 h of exercise caused increased levels of F_2-isoprostanes.

Postlethwait et al. (1998) utilized three biologically relevant models, isolated epithelial lining fluid, intact lung, and liposome suspensions to determine the O_3-induced production of

heptanal, nonanal and hexanal. Data obtained from these studies suggested that PUFAs directly react with O_3 and the amount of bioactive lipids produced is inversely related to ascorbic acid (AA) availability. The authors caution that there are limitations to the use of measurements of these reactions products in determining O_3 dose-response relationships due to the heterogenous nature of O_3 reactions in the ELF. Connor et al. (2004) have recently reported that interfacial phospholipids may modulate the distribution of inhaled O_3 and the extent of site-specific cell injury. They utilized interfacial films composed of dipalmitoylglycero-3-phosphocholine (DPPC) with rat lung lavage fluid and human fibroblast cell culture systems.

Hamilton et al. (1998) reported increased protein adducts in human AM exposed to 0.4 ppm O_3 for 1 h with exercise. These adducts were found to be created due to the formation of one of the most toxic ozonation products, 4-hydroxynonenal (HNE). Using human AM in vitro cultures treated with HNE they also demonstrated a potential role of HNE in acute cellular toxic effect of O_3.

Uhlson et al. (2002) reacted O_3 with calf lung surfactant which resulted in the production of 1-palmitoyl-2-(9'-oxo-nonanoyl)-glycerophosphocholine (16:0a/9-al-GPCho). The biological activity of this oxidized phospholipid included: (1) decreased macrophage viability, (2) induction of apoptosis in pulmonary epithelial-like A549 cells, (3) and release of IL-8 from A549 cells. Exposures levels of 0.125 ppm O_3 in this in vivo system were capable of generating biologically active phospholipids that were capable of mediating toxic effects of O_3.

Thus, new work has attempted to elucidate the mechanisms by which reactions of O_3 with lipids create phospholipids that then mediate downstream toxic effects. However, it is uncertain whether these described changes in lipid content and/or metabolism lead to significant changes in surface tension or compliance properties of the lung, and thus are biologically relevant and affect human health.

5.2.1.5 Protein Synthesis

Collagen, a structural protein involved in fibrosis, increases with O_3 exposure. Some studies have shown that this increase persists after exposure stops. The increased collagen has been correlated with structural changes in the lung. Rats exposed to an urban pattern of O_3 with daily peaks of 0.25 ppm for 38 weeks displayed extracellular matrix thickening. Increased levels of collagen in CAR were demonstrated in female rats exposed to 0.5 to 1.0 ppm O_3 for 6 h/day

for 20 months and in monkeys exposed to 0.61 ppm for 1 year. Both increased age and health status (e.g., emphysemic) were implicated in the increased collagen formation in response to O_3 exposure. A recent time-course study (van Bree et al., 2001) evaluating the lung injury and changes in collagen content in rats exposed acutely or subchronically to 0.4 ppm O_3 demonstrated CAR thickening of septa which progressed from 7 through 56 days of exposure. Though collagen content decreased with PE recovery, the structural fibrotic changes in ductular septa and respiratory bronchioles persisted, suggesting that subchronic O_3 exposures in rats creates a progression of structural lung injury that can evolve to a more chronic form, which included fibrosis. As with changes in lung lipids, the biological relevance and adverse health effects of altered protein synthesis and collagen accumulation are uncertain.

5.2.1.6 Gene Expression

Gohil et al. (2003) examined differential gene expression in C57BL/6 mice exposed to 1 ppm O_3 for three consecutive nights for 8 hours. Ozone exposure induced changes in expression of 260 genes (80% repressed and 20% induced). These included genes involved in progression of the cell cycle, several NF-κB-activated genes, and genes invoved in xenobiotic and major histocompatibility complex, suggesting that O_3 exposure suppresses immune function and xenobiotic metabolism and enhances cellular proliferation.

5.2.1.7 Summary and Conclusions - Biochemical Effects

Ozone has been shown to interact with a wide range of different cellular components including PUFAs, amino acid residues, and some low-molecular-weight compounds (GSH, urate, vitamins C and E). As O_3 does not penetrate much beyond the ELF, damage likely results from its PUFA ozonation products (mostly hydroxyhydroperoxides) involvement in signaling of cellular responses such as inflammation. New work has shown that ozonation of PUFA also forms the aldehydes nonanal, heptanal, and hexanal, the production of which is dependent on AA availability. Saturated phospholipids are thought to reduce the local dose and limit site-specific cell injury from O_3 exposure. Another ozonation product HNE creates protein adducts that have been linked to apoptosis and heat shock proteins in vitro.

Both short- and long-term exposures to O_3 have been shown to enhance lung xenobiotic metabolism, possibly as a result of changes in the number and function of bronchiolar epithelial

Clara cells and alveolar epithelial Type 2 cells. This modulation is both species- and region-specific and includes the isoforms CYP 2B1, CYP 2E1.CCSP is also involved in inflammatory responses to O_3 exposure. Mice strains with differing sensitivities to O_3 show that responses in protein, LDH and inflammatory cell influx are due to CCSP levels and changes in lung epithelium permability.

Reactions of O_3 with AA, GSH, and uric acid (all antioxidants present in ELF) are a protective mechanism. But even with environmentally relevant exposures, the reactivity of O_3 is not quantitatively quenched and cell injury occurs in both the lower and upper respiratory tract. Early work has shown that short-term exposures to < 1 ppm O_3 increase antioxidant metabolism. Re-exposure after a recovery period causes increases equivalent to first-time exposures, suggesting that previous exposure is not protective. Increases in enzyme activity appear to increase as a function of age, suggesting that O_3 exposure can cause greater lung injury in the older animal. Long-term urban patterns of exposure to O_3 (daily peaks of 0.25 ppm) caused increases in GSHPx and GR, but not SOD. Recent work has suggested that endogenous antioxidants moderate the effects of O_3 and that this exposure is a subthreshold stimulus for producing effects on peripheral airway resistance and reactivity, thus indicating a dissociation between airway function and inflammation.

In both acute and short-term studies, a variety of lung lipid changes occur with O_3 exposure, including an increase in AA. With longer exposures (e.g., 0.12 ppm for 90 days), an increase in PUFAs and a decrease in cholesterol-esters are seen, indicative of long-term alterations of surfactant lipid composition. Whether these changes in lipid content and/or metabolism lead to significant changes in surface tension or compliance properties of the lung remains unknown. New studies evaluating O_3-induced alterations in lipid metabolism have not been completed.

Collagen, a structural protein involved in fibrosis, increases with O_3 exposure, and some studies have shown that this increase persists after exposure stops. Urban patterns of exposure (daily peaks of 0.25 ppm for 38 weeks) created extracellular matrix thickening. Increases in centriacinar collagen were demonstrated in female rats exposed to 0.5 to 1.0 ppm O_3 for 6 h/day for 20 months and in monkeys exposed to 0.61 ppm for 1 year. New work examining the time course of lung injury and changes in collagen content in rats exposed acutely or subchronically to 0.4 ppm O_3 showed centriacinar thickening of septa. Collagen content decreased with PE

recovery but not the structural fibrotic changes in ductular septa and respiratory bronchioles, which suggests that subchronic O_3 exposures in rats creates a progression of structural lung injury that can evolve to a more chronic form, which includes fibrosis.

5.2.2 Lung Host Defenses

Defense mechanisms, including the mucociliary clearance system, AMs, and humoral- and cell-mediated immune system, exist in the lung to protect it from infectious and neoplastic disease and inhaled particles. Summaries of key new animal studies examining the effects of O_3 on lung host defenses are presented in Table AX5-2 of Annex AX5. Acute human exposures to O_3 result in similar effects on AMs (see Chapter 6).

5.2.2.1 Clearance

Early studies of the effect of O_3 on the mucociliary escalator showed morphological damage to ciliated epithelial cells of the tracheobronchial tree at doses of < 1 ppm. Functionally, O_3 slowed particle clearance in rats at doses of 0.8 ppm for 4 h and in rabbits at 0.6 ppm for 2 h exposures. Acute exposures at 0.5 ppm O_3 in sheep caused increased basal secretion of glycoproteins, while longer exposures reduced tracheal glycoprotein secretions, both of which can alter the effectiveness of the mucociliary escalator. Early postnatal exposures of sheep to 1 ppm O_3 caused retardation of normal morphologic development of the tracheal epithelium, decreased epithelial mucosia density, decreased tracheal mucous velocity, and delayed development of carbohydrate composition. Conversely , alveolar clearance in rabbits after acute exposure (0.1 ppm, 2 h/day, for 1 to 4 days) is increased. Longer exposures showed no effect and increased O_3 (1.2 ppm) slowed clearance. This pattern of clearance occurs in rats also. A study using rat tracheal explants exposed to O_3 (Churg et al., 1996) showed that uptake of TiO_2 and asbestos was enhanced at 0.01 and 0.1 ppm, respectively. The authors attribute the increased uptake as a direct effect of O_3, suggesting mediation by H_2O_2 or hydroxyl radical. Studies of the clearance of the radiolabled chelate ^{99m}Tc diethylenetriamine pentaacetic acid (Tc-DTPA) have shown that clearance is significantly increased following a 3h exposure to 0.8 ppm O_3 in SD rats (Pearson and Bhalla,1997). Examination of regional clearance of ^{99m}Tc-DTPA in dogs following a 6 h isolated sublobar exposure to 0.4 ppm O_3 or air showed that O_3 decreased the clearance halftime by 50% at 1 day following exposure (Foster and Freed,

1999). Clearance was still elevated at 7 d PE but had recovered by 14 d. So, a single local exposure to O_3 increases transepithelial clearance but without any influence on contralateral segements, i.e., only for epithelia directly exposed to O_3.

Alveolar clearance is slower than tracheobronchial clearance and involves particle movement through interstitial pathways to the lymphatic system or movement of particle-laden AMs to the bottom of the mucociliary escalator. Exposures of rabbits to 0.1 ppm accelerated clearance while 1.2 ppm slowed clearance. A chronic exposure has been shown to slow clearance. New evaluations of the effects of O_3 on alveolar clearance have not been performed.

5.2.2.2 Alveolar Macrophages

A primary function of AMs is to clear the lung of infectious and non-infectious particles by phagocytosis, detoxification, and removal. Further, AMs secrete cellular mediators that recruit and activate inflammatory cells in the lungs. Ozone has been shown to inhibit phagocytosis at 0.1 ppm for 2 h in rabbits. This inhibition returns to control levels if exposures are repeated for several days. The production of superoxide anion radicals and the activity AM lysosomal enzymes (both involved in bactericidal activity) are inhibited by 3 h exposures to 0.4 and 0.25 ppm O_3 in rodents and rabbits, respectively. Production of IFNγ was decreased in rabbit AM by 1 ppm O_3 for 3 h.

New studies have shown that O_3 affects AM chemotaxis, cell adhesion, and surface expression of cell adhesion molecules (Bhalla, 1996). AM from SD rats exposed to 0.8 ppm O_3 for 3 h showed greater mobility and greater adhesion than air exposed controls. This increased mobility and adhesion were attenuated by CD16b and ICAM-1 antibodies, suggesting these adhesion molecules modulate O_3-induced inflammation. Antibodies to TNFα and IL1α also mitigated AM adherence, suggesting further that the inflammatory response to O_3 is mediated by these cytokines (Pearson and Bhalla, 1997). Cohen et al. (1996) showed that O_3 reduces binding of INF γ to AM in WEHI-3 cells, and additionally reduces phagocytic activity, production of reactive oxygen intermediates, and elevation of intracellular Ca^{++}. Glutathione content in AM is reduced by a 2 ppm 3 h exposure to O_3, possibly due to its interaction with ozonation products from O_3-induced lipid peroxidation (Pendino et al., 1996).

Cohen et al. (2001, 2002) exposed male F-344 rats to either 0.1 or 0.3 ppm O_3 for 4 h/day, 5 days/week or either 1 or 3 weeks. In this study, superoxide anion production was increased at

1 week. Hydrogen peroxide production was reduced at both exposure concentrations and durations and was further reduced with INFγ stimulation, suggesting that one effect of O_3 is compromised killing of bacteria by AM due to the reduction in hydrogen peroxide production.

Ozone treatment (2 ppm O_3, 3 h in female SD rats) caused a time-dependent increase in NO levels in both AM and type II epithelial cells that was correlated with increased expression of iNOS mRNA and protein (Laskin et al., 1998). Inhibition of NF-κB, caused a dose-dependent inhibition of NO and iNOS production. Additionally, O_3 caused a time-dependent increase in NF-κB binding activity in the nucleus of both cell types. The authors hypothesize that O_3 exposure causes the cytokines TNFα and IL-1βα to bind to surface receptors and initiate intracellular signaling pathways in AM leading to activation of NF-κB, its entry into the nucleus, and its binding to the regulatory sequences of genes such as iNOS to allow their transcription. Additional studies (Laskin et al., 2002) using AM isolated from C57Bl6x129 mice with a targeted disruption of the gene for iNOS showed no toxicity to 0.8 ppm O_3 for 3h, as measured by BALF protein levels and nitrotyrosine staining of the lung. Additionally, mice overexpressing human Cu, Zn superoxide dismutase (SOD) and mice with a targeted disruption of p50 NF-κB were also resistant to O_3 toxicity. WT mice exposed to O_3 showed an increase in expression of STAT-1, a protein that binds to the regulatory region of iNOS. Taken together, these results suggest to the authors that a number of proteins including NF-κB, phosphoinoside 3-kinase, and STAT-1 that bind to and regulate expression of iNOS are modulated by O_3 exposure. The same iNOS knockout mice strain exposed to O_3 (Fakhrzadeh et al., 2002) showed no increase in AM superoxide anion and prostaglandin. These data provide further evidence the NO and its reactive oxidative product peroxynitrite are important in O_3-induced lung injury. Further discussions of the role of nitric oxide synthase/reactive nitrogen and cytokines/chemokines in O_3-induced inflammation are provided in Section 5.2.3.

5.2.2.3 Immune System

The effects of O_3 on the immune system are complex and depend on the exposure parameters and observation periods. T-cell-dependent functions appear to be more affected than B-cell-dependent functions. Generally, there is an early immunosuppressive effect that can, with continued exposure, either return to normal or actually enhance immunity. Changes in immune cell population occur with O_3 exposure including T:B-cell ratios in the MLN. Natural killer

(NK) cell activity increases with 1 week exposures of 0.2 to 0.4 ppm O_3 but decreases with exposures to 0.82 ppm. Ozone exposure has also shown to be responsible for enhancement of allergic sensitization at levels of 0.5 to 0.8 ppm for 3 days. Studies of the effects of O_3 on the immune system are summarized in Table AX5-2.

Recent work examining immune system responses to O_3, Garssen et al. (1997) have shown that BALB/c mice sensitized with PCI are hyperreactive to carbachol after a PSA challenge, but not if exposed to 0.4 to 1.6 mg/m^3 O_3. The sensitized mice also demonstrated a suppressed inflammatory reaction (PMN) with 1.6 mg O_3 exposure. These results are opposite to the effect on type I (IgE-mediated) allergic reactions, which the authors suggest is due to activation of Th-2 cell-dependent reactions that are possibly potentiated by O_3 or to a direct effect by O_3 on Th-1 cells or other cells that are crucial for the tracheal hyperreactivity and inflammation seen in this mouse model.

Kleeberger et al. (2000, 2001b) have recently demonstrated a potential interaction between the innate and acquired immune system with O_3 exposure. Using O_3-susceptible (C57BL/6J) and O_3-resistant (C3H/HeJ) mice, they identified a candidate gene on chromosome 4, Toll-like receptor 4 (*Tlr4*). Ozone exposure (0.3ppm for 24 to 72 hours) of C3H/HeJ and C3H/HeOuJ mice, the latter differing from the O_3-resistant strain by a polymorphism in the coding region of *Tlr4*, then demonstrated greater protein concentrations in the OuJ strain. The two strains exhibited differential expression of *Tlr4* mRNA with O_3 exposure. Thus, a quantitative trait locus on chromosome 4 appears to be responsible for a significant portion of the genetic variance in O_3 -induced lung hyperpermeability. In these mouse strains lavageable protein concentration was lowered by inhibition of inducible nitric oxide synthase (iNOS) and by targeted disruption of *Nos2*. Comparisons of C3H/HeJ and C3H/HeOuJ O_3 exposures demonstrated reduced *Nos2* and *Tlr4* mRNA levels in the O_3-resistant C3H/HeJ mice. These data are consistent with the hypothesis that O_3 -induced lung hyperpermeability is mediated by iNOS. These studies suggest a role for TLR4 in the host response to O_3 similar to the role it has demonstrated in lipopolysaccharide (LPS) sensitivity (Schwartz 2002; Wells et al. 2003). TLR4 signaling is thought to be critical to linking the innate and acquired immune system through antigen presenting cells and Th1/Th2 differentiation.

Ozone exposure has been shown to affect IgE responses in both in vitro and in mice. Becker et al. (1991) demonstrated changes in IgG production in cultured human lymphocytes

with O_3 exposures of 1.0, 0.5, and 0.1 ppm for 2 h. Subsequent to O_3 exposure, cells were stimulated with pokeweed mitogen (PWM, a T-cell-dependent stimulus) or Staphylococcus aureus Cowan 1 strain (SAC, a T-cell-independent stimulus). Both B and T cells were affected by O_3. T cells also demonstrated an increase in IL-6 and a decrease in IL-2, which suggested to the authors that O_3 may have direct effects on IgG producing cells and concurrently an effect that is mediated by altered production of T cell immunoregulatory molecules. Responses to repeated O_3 (0.08 - 0.25 ppm) and OVA (1%) exposures were compared in "IgE-high responder" (BALB/c) and "IgE-low responder" (C57BL/6) mice (Neuhaus-Steinmetz et al., 2000). Ozone appeared to shift the immune response toward a Th2-like pattern in the two mouse strains with differing potentials for developing allergic reactions.

Surfactant protein A and D (SP-A and SP-D) were shown to create an inflammatory feedback loop with perturbations in lung immune defenses (reviewed in Hawgood and Poulain, 2001). Earlier studies suggested that SP-A is a target for O_3 toxicity by causing inhibition of SP-A self-association and SP-A-mediated lipid vesicle aggregation. Further, O_3 reduced the ability of SP-A to inhibit phospholipid secretion by alveolar type II cells O_3 and reduced the capacity of SP-A to induce superoxide anion production and enhance phagocytosis of herpes simplex virus. Bridges et al. (2000) reported that both SP-A and SP-D directly protect surfactant phospholipids and macrophages from oxidative damage by blocking accumulation of TBARS and conjugated dienes.

Eight human variants of PS-A in CHO cells exposed to O_3 (1ppm for 4 hr) showed decreased ability to stimulate cytokine (TNF- and IL-8) production in THP-1 cells, a macrophage-like cell line (Wang et al., 2002). Each variant had a unique time- and dose-dependent pattern of stimulation of cytokine production with O_3 exposure which the authors attribute to possible differences in susceptibility to O_3 oxidation. Targeted disruption of mouse SP- A and SP-D (Hawgood et al, 2002) caused increases in BAL phospholipid, macrophage, and protein through 24 weeks of age. Further, the deficient mice developed patchy lung inflammation and air space enlargement consistent with emphysema. Future experiments using these null mice will help to establish the role of SP-A and SP-D in pulmonary host defense to O_3 exposure.

5.2.2.4 Interactions with Infectious Microorganisms

Ozone-induced dysfunction of host defense systems results in enhanced susceptibility to bacterial lung infections. Acute exposures of 0.08 ppm (3h) O_3 can overcome the ability of mice to resist infection (by decreasing lung bactericidal activity) with Streptococcal bacteria, resulting in mortality. Changes in antibacterial defenses are dependent on exposure regimens, species and strain of test animal, species of bacteria, and age of animal, with young mice more susceptible to the effects of O_3. The effect of O_3 exposure on antibacterial host defenses appears to be concentration- and time-dependent. Early studies using the mouse "infectivity model," consisting of exposure to clean air or O_3 followed by exposure to an aerosolized microorganism, showed that the difference in mortality between O_3-exposed groups and controls is concentration-related. Chronic exposures (weeks, months) of 0.1 ppm do not cause greater effects on infectivity than short exposures, due to defense parameters becoming reestablished with prolonged exposures.

More recent studies of O_3-induced modulation of cell-mediated immune responses showed effects on the onset and persistence of infection. Cohen et al. (2001,2002) exposed male F-344 rats subchronically to either 0.1 or 0.3 ppm O_3. Subsequent exposure with viable Listeria monocytogenes demonstrated no observed effect on cumulative mortality, but did show a concentration-related effect on morbidity onset and persistence. These data suggest that O_3 may cause a possible imbalance between Th-1 and Th-2 cells, which can subsequently lead to suppression of the resistance to intracellular pathogens.

Effects of O_3 on viral infections are dependent on the temporal relationship between O_3 exposure and viral infection. Only high concentrations (1.0 ppm O_3, 3 h/day, 5 days, mice) increased viral-induced mortality. 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. But there were O_3-enhanced postifluenzal alveolitis and lung parenchymal changes. As O_3 does not affect lung influenza viral titers, it apparently does not impact antiviral clearance mechanisms. In general, the evidence suggests that O_3 can enhance both bacterial and viral lung infections, but the key mechanisms have not yet been identified. New studies on the interactions of O_3 and viral infections have not been published.

5.2.2.5 Summary and Conclusions - Lung Host Defenses

New data on lung host defenses support earlier work which suggests that mucociliary clearance is affected in most test species at just under 1ppm, with lower levels (~0.1 ppm) increasing clearance and somewhat higher levels decreasing clearance. These data also propose mechanisms whereby O_3 affects clearance, which include uptake being a direct effect of O_3, but modulated by ROS and hydroxyl radicals.

Alveolar macrophage function is disrupted by O_3 as shown by a number of studies demonstrating inhibition of phagocytosis at concentrations ranging from 0.1 to 1.2 ppm. This inhibition returns to control levels if exposures are repeated for several days. Two new studies corroborate earlier findings of increases in AM number in that same exposure range. In this environmentally relevant exposure range, new studies support older findings of decreased resistance to microbial pathogens as shown by the endpoints examining superoxide radical formation, altered chemotaxis/motility, decreased INFγ, levels, decreased lysosomal activity, increased PGE levels, and increased NO mRNA and protein.

New research evaluating the effects of O_3 on immune function advances previous work that has shown that exposures can enhance or suppress immune responsiveness depending on the species studied, concentration of O_3, route of exposure of allergen, and timing of exposure. 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 a suppression of responses to antigen in O_3 exposures post-immunization. The use of mouse strains with genetically determined sensitivity or resistance to O_3 indicated a possible interaction between the innate and acquired immune system, and further, that O_3 may shift the immune response towards a Th-2-like pattern. Work has also focused the deleterious effects of O_3 exposure on SP-A and SP-D and their immunomodulatory function in protecting against oxidative stress.

Several new studies evaluating the effects of O_3 exposures on infectious microorganisms are in concurrence with previous studies which showed, in general, increased mortality and morbidity, decreased clearance, increased bacterial growth, and increased severity of infection at exposure levels of 0.1 to 1 ppm O_3 for 1 week.

5.2.3 Inflammation and Lung Permeability Changes

Lung inflammation and increased permeability, which are distinct events controlled by independent mechanisms, are two well-characterized effects of O_3 exposure. Disruption of the lung barrier leads to leakage of serum proteins, influx of polymorphonuclear leukocytes (PMNs), release of bioactive mediators, and movement of compounds from the airspaces into the blood. Increases in permeability and inflammation have been observed at levels as low as 0.1 ppm O_3 for 2 h/day for 6 days in rabbit and 0.12 ppm in mice (24-h exposure) and rats (6-h exposure). After acute exposures, the influence of the time of exposure increases as the concentration of O_3 increases. The exact role of inflammation in causation of lung disease is not known, nor is the relationship between inflammation and changes in lung function. Table AX5-3 in Annex AX5 summarizes new key studies describing the potential for O_3 exposure affect lung permeability and inflammation. Controlled human exposure studies discussed in Chapter 6 indicate that the majority of acute responses in humans are similar to those observed in animals.

5.2.3.1 Time Course of Inflammation and Lung Permeability Changes

The maximal increase in BALF protein, albumin, and PMN occurs in 8 to 18 h after the cessation of acute exposure. A recent study of OVA-sensitized male Dunkin-Hartley guinea pigs exposed to 1.0 ppm O_3 for 3 h showed that PMN levels were significantly increased at 3 h PE, but BAL protein levels were not, suggesting a lack of correlation between the two endpoints (Sun et al., 1997). Increased PMN without a concordant increase in BAL protein levels were found when the guinea pigs were exposed to 1.0 ppm O_3 for 1 h and evaluated 24-h PE. The first group also had an increase in AHR, but not the second group, which suggests a dissociation between PMN levels and AHR.

Earlier work demonstrated that O_3 exposures of 0.8 to 1 ppm transiently increase the permeability from the air to the blood compartment. This permeability is greatest in trachea and bronchoalveolar zone, and may allow increased entry of antigens and other bioactive compounds (e.g., bronchoconstrictors) into lung tissues. The time course of the influx of PMNs into the lung and the BALF fluid levels of macrophage inflammatory protein-2 (MIP-2) were found to be roughly similar to that for proteins (Bhalla and Gupta, 2000). Adherence of neutrophils to pulmonary vascular endothelium is maximal within 2 h after exposure and returns to control levels by 12 h PE (Lavnikova et al., 1998). In an *in vitro* system utilizing rat alveolar type II cell

monolayers, O_3 produced a dose-dependent increase in permeability (Cheek et al., 1995). At higher O_3 levels, neutrophils exacerbated the injury, but their presence after the exposure expedited restoration of epithelial barrier. Vesely et al. (1999) have demonstrated that neutrophils contribute to the repair process in O_3 -injured airway epithelium.

Subchronic exposures (3 to 7 days) have been found to cause increases in BALF protein and PMNs that typically peak after a few days (depending upon species tested and exposures) and return towards control even with continuing exposure. Van Bree et al. (2002) observed lower BALF levels of protein, fibronectin, IL-6 and inflammatory cells in rats exposed for 5 days than in rats exposed for 1 day, suggesting adaptation to O_3 exposure. Postexposure challenge with single O_3 exposures at different time points showed recovery of susceptibility to O_3. McKinney et al. (1998) observed differences in IL-6 levels due to repetitive exposures and demonstarted a role of IL-6 in the adaptive response induced by repeated O_3 exposures.

5.2.3.2 Concentration and Time of Exposure

Analysis of the relative influence of concentration and duration of exposure (i.e., C×T) of O_3 has shown that concentration generally dominates the response. The impact of T was C-dependent (at higher Cs, the impact of T was greater); at the lowest C and T values, this dependence appeared to be lost. New studies evaluating C×T relationships in animal models have not been found.

5.2.3.3 Susceptibility Factors

Factors that have been studied for potential impact on the effects of O_3 exposure include age, gender, nutritional status, exposure to co-pollutants, exercise, and genetic variability. A full characterization of the effects of age on O_3 responses has not been completed. Data available indicate that effects of age on O_3 responses are endpoint-dependent, with young mice, rats and rabbits having greater prostaglandin levels with exposure and senescent rats having greater IL-6 and N-acetyly-β-D-glucosaminidase levels with exposure.

A new study (Johnston et al., 2000b) compared gene expression of chemokines and cytokine in newborn and 8-week-old C57Bl/6J mice exposed to 1.0 or 2.5 ppm for 4, 20, or 24 h. The newborn mice displayed increased levels of Mt mRNA only, while the 8-week-old mice had increases in MIP-1α, MIP-2, IL-6, and Mt mRNA.Comparisons were made with mice of the

same age groups with exposures to endotoxin (10 min). Both age groups displayed similar cytokine/chemokine profiles with endotoxin exposure. This suggested to the authors that the responses to endotoxin, which does not cause epithelial injury, and the responses to O_3, which does, demonstrate that differences in inflammatory control between newborn and adult mice is secondary to epithelial injury.

Pregnancy and lactation increased the susceptibility of rats to acute O_3, but no clear effects of gender have been identified. The effects of vitamin C deficiency on O_3 responses are unclear. Ascorbate-deficient guinea pigs exposed to O_3 demonstrated only minimal effects on injury and inflammation (Kodavanti et al., 1995). Utilizing a diet-restricted (20% of the freely-fed diet) rat model, Elsayed (2001) demonstrated higher survivability on exposure to higher O_3 (0.8 ppm continuously for 3 d) compared to freely-fed rats. Pre-exposure to sidestream cigarette smoke had been found to cause increased lung injury (Yu et al., 2002). In vitro studies on the macrophages from smoke + O_3- exposed animals responded by a greater release of TNF-α following LPS stimulation when compared to macrophages exposed to air, smoke or O_3 alone.

Recent lines of evidence illustrate that genetic background is an extremely important determinant of susceptibility to O_3. In earlier studies using inflammation-prone (susceptible) C57BL/6J (B6) and inflammation-resistant C3H/HeJ (C3) mouse strains and high doses of O_3 (2ppm for 3 hours) identified *Inf*-2 as a locus controlling susceptibility. Further studies in these two strains of mice identified that the acute and subacute exposures are controlled by two distinct genes, referred to as *Inf*-1 and *Inf*-2, respectively (Tankersley and Kleeberger, 1994). Kleeberger et al. (1997) also identified another potential susceptibility gene, tumor necrosis factor (*Tnf,* which codes for the pro-inflammatory cytokine TNF-α) on a qualitative trait locus on mouse chromosome 17. By neutralizing the function of TNF-α with a specific antibody, they were able to confer protection against O_3 injury in susceptible mice. The group then demonstrated a role for TNF receptor 1 and 2 (TNFR1 and TNFR2, respectively) signaling in subacute (0.3 ppm for 48 hrs) O_3-induced pulmonary epithelial injury and inflammation (Cho et al., (2001). TNFR1 and TNFR2 knockouts were less sensitive to subacute O_3 exposure than WT C57BL/6J mice. Further studies using these knockouts by Shore et al. (2001) indicated a role of TNF-α in AHR but not in O_3-induced infiltration of PMN, and provided evidence for the mechanistic separation of hyperresponsiveness and PMN infiltration.

In studies to evaluate differences in susceptibility to death from O_3 exposure, Prows et al. (1997; 1999) exposed A/J and C57BL/6J mice exposed to 10 ppm O_3. The A/J strain is more sensitive to O_3-induced death, while the C57BL/6J strains is resistant to O_3-induced death. They identified two loci (acute lung injury-1 and -3, Ali-1 and Ali-3, respectively) on chromosome 11 that appear to control susceptibility to death after O_3 exposure.

An integrated and more comprehensive effort to identify the genetic basis for the susceptibility to O_3-induced lung injury was reported by Savov et al. (2004). In this report, summarized in Table AX5-4 of Annex AX5, acute lung injury to high dose of O_3 (2 ppm for 3h) was assessed and integrated with physiological, biochemical, and genetic observations using 9 inbred mouse strains. This work indicated the presence of genetic loci on chromosomes 1, 7, 15 associated with phenotypic characteristics for resistance to acute O_3-induced lung injury. They identified C3H/HeJ and A/J as consistently O_3-resistant,C57BL/6J and 129/SvIm as consistently O_3-vulnerable, and CAST/Ei, BTBR, DBA/2J, FVB/NJ, and BALB/cJ as intermediate in response to O_3.

A similar comparative study by Wattiez et al. (2003) using five inbred mouse strains to characterize the molecular basis for O_3-induced lung injury identified differential expression of CCSP isoform CC16a in BALF of C57BL/6J (O_3-sensitive) and C3H/HeJ (O_3-resistant) strains. Ozone-induced changes in CCSP expression were evaluated in five inbred mouse strains: C57BL/6J and CBA both considered sensitive to acute O_3-induced inflammation, C3H/HeJ and AKR/J both considered resistant, and SJL/J considered intermediate (Broeckaert et al., 2003). Two exposure paradigms (1.8 ppm O_3 for 3 h or 0.11 ppm O_3, 24/h day for up to 3 days) were used, and BALF and serum were assayed immediately after exposure or at 6 h. Both exposure levels caused a transient increase in CC16 in serum that correlated with BALF changes in protein, LDH, and inflammatory cells. There was an inverse relationship between preexposure levels of CC16 in BALF and epithelial damage based on serum CC16 levels and BALF markers of inflammation. There was also an inverse relationship between preexposure levels of albumin in BALF and lung epithelium damage. These results suggest to the authors that a major determinant of susceptibility to O_3 is basal lung epithelium permeability. I.e., the leakiness of the epithelium allows CC16 to enter the blood and protein and inflammatory cells to enter the lung. As all of the mouse strains had similar levels of preexposure CC16 mRNA, they conclude that strain differences in the basal permeability of the airway epithelium is responsible for lung

differences in basal CC16 among strains. This group also used 2-dimensional protein electrophoresis to examine differences in BALF protein between C57BL/6J and C3H/HeJ mice before and after O_3 exposure. They found the CC16 monomer, a 7kD protein, in two isoforms with differing p*I* values, CC16a (4.9) and CC16b (5.2). C57BL/6J mice had lower levels of CC16a (the more acidic form) than the C3H/HeJ mice, but both strain had similar levels of CC16b. They hypothesize that the C57BL/6J strain has greater epithelial permeability, and thus allows more leakage of CC16a. These data taken together suggest a protective role against oxidant damage for CSPP, and further that genetic susceptibility to oxidant stress may be moderated, in part, by the gene coding for CSPP.

5.2.3.4 Mediators of Inflammatory Response and Injury

Ozone reacts with lipids in the ELF or epithelial cell membranes, creating ozonation products that then stimulate airway epithelial cells, AMs, and PMNs to release a host of pro-inflammatory mediators which include cytokines, chemokines, reactive oxygen species, eicosanoids, and platelet activating factors. At O_3 exposures of $\geq$1 ppm, these mediators recruit PMN and increase expression of MIP-2 mRNA or BALF levels of MIP-2 (Driscoll et al., 1993; Haddad et al., 1995; Bhalla and Gupta, 2000). The increased mRNA expression was associated with an increased neutrophilia in the lung. Zhao et al. (1998) showed that O_3 exposure in mice and rats causes an increase in monocyte chemotactic protein-1 (MCP-1).

Fibronectin, an extracellular matrix glycoprotein, is thought to have a role in lung inflammation and inflammatory disorders, and has shown to be increased with exposures of 1 ppm for 14 days. Gupta et al. (1998) observed an increase in both fibronectin protein and mRNA expression in the lung of rats exposed to 0.8 ppm O_3. A mechanistic role of fibronectin in O_3-induced inflammation and injury was suggested on the basis of comparability of temporal changes in BALF protein, fibronectin and alkaline phosphatase activity (Bhalla et al., 1999). Studies have reported an effect of O_3 on other cytokines and inflammatory mediators. An increase occurred for cytokine-induced neutrophil chemoattractant (CINC) and NF-κB expression *in vivo* (Haddad et al., 1996; Koto et al., 1997), for IL-8 *in vivo* and *in vitro* (Chang et al., 1998), TNFα, fibronectin, IL-1 and CINC release by macrophages *ex vivo* (Pendino et al., 1994; Ishii et al., 1997), and NF-κB and TNFα (Nichols et al., 2001; see 6.9.2). An increase in lung CINC mRNA occurred within 2 hrs after the end of a 3 hr exposure of rats to 1 ppm O_3.

The CINC mRNA expression was associated with neutrophilia at 24 hrs PE. Exposure of guinea pig AMs recovered in BALF and exposed *in vitro* to 0.4 ppm O_3 for 1- h produced a significant increase in IL-6 and TNFα (Arsalane et al., 1995). An exposure of human AMs to an identical O_3 concentration increased TNFα, IL-1b, IL-6 and IL-8 and their mRNAs. Ozone exposure of mice caused an increase in IL-6, MIP-1a, MIP-2, eotaxin and Mt abundance (Johnston et al., 1999a). The IL-6 and MT increase was enhanced in mice deficient in CCSP, suggesting a protective role of Clara cells and their secretions (Mango et al., 1998). CCSP deficiency also increased sensitivity of mice to O_3, as determined by an increase in abundance of MIP-1a and MIP-2 following a 4 hr exposure (Johnston et al., 1999b).

Mast cells, which are located below the epithelium, release proinflammatory mediators and have been shown to contribute to O_3-induced epithelial damage. Mast cell-deficient mice exposed to 2 ppm O_3 showed no inflammation or epithelial injury which was observed in WT mice (Longphre et al., 1996). Greater increases in lavageable macrophages, epithelial cells and PMNs were observed in mast cell-sufficient mice than in mast cell-deficient mice exposed to 0.26 ppm (Kleeberger et., 2001a). Increases in inflammatory cells were also observed in mast cell-deficient mice repleted of mast cells, but O_3-induced permeability increase was not different in genotypic groups exposed to 0.26 ppm. When a mast cell line was exposed to varying O_3 concentrations, spontaneous release of serotonin and modest generation of PGD2 occurred only under conditions that caused cytotoxicity (Peden and Dailey, 1995). Additionally, O_3 inhibited IgE- and A23187- induced degranulation. Mast cells recovered from O_3-exposed peripheral airways of ascaris sensitive dogs released significantly less histamine and PGD2 following *in vitro* challenge with ascaris antigen or calcium ionophore (Spannhake, 1996). Ozone exposure also promoted eosinophil recruitment in the nose and airways in response to instillation of OVA or OVA-pulsed dendritic cells and aggravated allergy like symptoms in guinea pigs (Iijima et al., 2001).

The role of PMNs and cellular mediators in lung injury and epithelial permeability has been investigated using antibodies and inhibitors of known specificity to block inflammatory cell functions and cytokine activity. Treatment of rats with cyclophosphamide prior to O_3 exposure resulted in a decreased recovery of PMNs in the BALF and attenuated permeability induced by O_3 (Bassett et al., 2001). Pretreatment of animals with antiserum against rat neutrophils abrogated PMN accumulation in the lung, but did not alter permeability increase produced by

O_3. DeLorme et al. (2002) showed a relationship between neutrophilic inflammation and AHR. Treatment of rats with anti-neutrophil serum protected the animals from O_3-induced AHR. Studies utilizing antibodies to selected pro- or anti-inflammatory cytokines suggest a role of TNFa, IL-10, and IL-1b in O_3-induced changes in permeability, inflammation and cytokine release (Ishii et al., 1997; Reinhart et al., 1999; Bhalla et al., 2002). An attenuation of O_3-induced increase in permeability and inflammation was also observed in mice treated, either before or after exposure, with UK-74505, a platelet-activating factor (PAF) receptor antagonist (Longphre et al., 1999). These results were interpreted to indicate that O_3-induced epithelial and inflammatory changes are mediated in part by activation of PAF receptors.

Ozone exposure stimulates macrophage motility towards a chemotactic gradient, and macrophages from rats exposed to 0.8 ppm O_3 adhered to epithelial cells (ARL-14) in culture to a greater extent than macrophages from air-exposed controls (Bhalla, 1996). Both macrophage motility and chemotaxis were attenuated by antibodies to cell adhesion molecules CD-11b and ICAM-1, suggesting a role for cell adhesion molecules in O_3-induced cellular interactions. This may also explain the increased tissue localization and reduced recovery of macrophages in BALF (Pearson and Bhalla, 1997) following O_3 exposure. Studies investigating the mechanisms of PMN recruitment in the lung have explored the role of cell adhesion molecules that mediate PMN-endothelial interactions. An exposure of female rats to O_3 had an attenuating effect on CD-18 expression on AMs and vascular PMNs, but the expression of CD62L, a member of selectin family, on vascular PMNs was not affected (Hoffer et al., 1999). In monkeys, O_3-induced inflammation was blocked by treatment with a monoclonal antibody to CD18, suggesting dependence of PMN recruitment on this adhesion molecule (Hyde et al., 1999). Treatment of monkeys with CD18 antibody also reduced tracheal expression of the β6 integrin (Miller et al., 2001). A single 3 hr exposure of rats to O_3 caused an elevation in concentration of ICAM-1, but not CD-18, in the BALF (Bhalla and Gupta, 2000). Takahashi et al. (1995a) found an increase in tissue expression of ICAM-1 in mice exposed to 2 ppm O_3, noting a temporal correlation of inflammatory activity and ICAM-1 expression which varied in different regions of the lung. A comparable pattern of time-related changes in total protein, fibronectin and alkaline phosphatase activity in the BALF of rats exposed to 0.8 ppm O_3 was also noted by Bhalla et al. (1999). Together, these studies support the role of extracellular matrix protein and cell adhesion molecules in the induction of lung inflammation and injury.

5.2.3.5 The Role of Nitric Oxide Synthase and Reactive Nitrogen in Inflammation

Nitric oxide (NO) is a messenger molecule involved in many biological processes, including inflammation. Cells in the respiratory tract (including mast cells, neutrophils, epithelial cells, neurons, and macrophages) produce three differing forms of nitric oxide synthase (NOS), the enzyme that catalyzes the formation of NO. NOS-1(neuronal) and NOS-3 (endothelial) are constitutively expressed, whereas NOS-2 (also referred to as iNOS) is inducible, commonly by pro-inflammatory cytokines. An acute exposure of rats to 2 ppm O_3 caused an increase in BALF macrophage number and total protein, in iNOS expression, in fibronectin, and in TNFα production by AMs (Pendino et al., 1995). All of these effects of O_3 were reduced by pretreatment with gadolinium chloride, a macrophage inhibitor. Macrophages isolated from O_3-exposed mice produced increased amounts of NO, superoxide anion, and PGE2, but production of these mediators by macrophages from NOS knockout mice was not elevated (Fakhrzadeh et al., 2002). Additionally, mice deficient in NOS or mice treated with N^G-monomethyl-L-arginine, an inhibitor of total NOS, were protected from O_3-induced permeability, inflammation, and injury, suggesting a role of NO in the production of O_3 effects (Kleeberger et al., 2001b; Fakhrzadeh et al., 2002). These results contrast with a study showing that O_3 exposure produced greater injury, as determined by measurement of MIP-2, matrix metalloproteinases, total protein, cell content and tyrosine nitration of whole lung protein, in iNOS knockout mice than in wild type mice (Kenyon et al., 2002). This group suggests that protein nitration is related to inflammation and is not dependent on iNOS-derived NO. They point out the possible experimental differences, such as O_3 concentration, for inconsistency between their results and those of Kleeberger et al. (2001b).

Rats pretreated with ebselen, a potent anti-inflammatory, immunomodulator, and NO/peroxynitrite scavenger, and then exposed to 2 ppm O_3 for 4 -h had decreased numbers of neutrophils, lowered albumin levels, and inhibited nitration of tyrosine residues in BALF 18 h PE, though macrophage iNOS expression was not changed (Ishii et al., 2000a). These results suggest an iNOS-independent mechanism for O_3-induced inflammation. Inoue et al. (2000) demonstrated in human transformed bronchial epithelial cells that NO-generating compounds (TNFα, IL-1β, and INF-γ) induce IL-8 production and that NOS inhibitors inhibit IL-8 production. In vivo experiments in the same study using male Hartley-strain guinea pigs exposed to 3 ppm O_3 for 2-h showed that NOS inhibitor pretreatment attenuated-O_3 induced

neutrophil recruitment and AHR at 5 hours after exposure. The NOS inhibitors also blunted the increase in nitrate/nitrite levels and in IL-8 mRNA, at the 5 hours PE. The authors hypothesize that NO, or its derivatives, facilitate AHR and inflammation after O_3 exposure, possibly mediated by IL-8. Jang et al. (2002) showed a dose-dependent increases in nitrate (indicative of in vivo NO generation) with O_3 exposure (0.12, 0.5, 1, or 2 ppm for 3 h). Functional studies of enhanced pause (P_{enh}) demonstrated increases with O_3 which were also dose-dependent. Western blot analysis of lung tissue showed increases in NOS-1, but not in NOS -3 or iNOS isoforms. These results suggest that in mice NOS-1 may induce airway responsiveness by a neutrophilic airway inflammation. The literature regarding the effects of O_3 exposure on NOS activity is complex and conflicting. Similarly, the issue of protein nitration as it relates to cell injury due to O_3 exposure is somewhat controversial.

5.2.3.6 Summary and Conclusions - Inflammation and Permeability Changes

Airway mucosa in the normal lung serves as an effective barrier that controls bidirectional flow of fluids and cells between the air and blood compartments. Ozone disrupts this function, resulting in an increase in serum proteins, bioactive mediators, and PMNs in the interstitium and air spaces of the lung. Generally, the initiation of inflammation is an important component of the defense process; however, its persistence and/or repeated occurrence can result in adverse health effects.

The relative influence of concentration and duration of exposure (i.e., C × T) has been investigated extensively in rats, using BALF protein as an endpoint. Although, the interaction between C and T is complex, C generally dominated the response. The impact of T was C-dependent (at higher Cs, the impact of T was greater); at the lowest C and T values, this dependence appeared to be lost.

In rats, a single 3hr exposure to 0.5 ppm O_3 produced a significant increase in both permeability and inflammation, but a comparable exposure to 0.3 or 0.15 ppm did not produce an effect. In a study comparing the responses of five species exposed to several concentrations of O_3, ranging from 0.2 to 2.0 ppm for 4 h, BAL was performed 18 h PE. Guinea pigs were the most responsive (increased BALF protein at $\geq$ 0.2 ppm); rabbits were the least responsive (effect at 2.0 ppm only); and rats, hamsters, and mice were intermediate (effects at $\geq$ 1.0 ppm). Among rat strains, an acute exposure to O_3 resulted in a greater injury, inflammation BALF levels of IL-

6 in Wistar than in SD or F344 rats. As exposures continue for 3 to 7 days, the increases in BALF protein and PMNs typically peak after a few days (depending upon species tested and exposures) and return towards control even with continuing exposure.

Other factors that have been studied for potential impact on the effects of O_3 include age, gender, nutritional status, genetic variability, exercise and exposure to co-pollutants. The effects of age on lung inflammation are not well known. After an acute exposure to 0.8 or 1 ppm, young mice, rats, and rabbits had greater changes in prostaglandins in BALF, but there were no age-dependent effects on BALF protein or cell number. Comparisons of male and female animals, and vitamin C or ascorbate deficiency did not reveal significant differences in the effects of O_3, but exercise during exposure increased susceptibility.

Ozone also increases the permeability from the air to the blood compartment. Ozone (0.8 ppm; 2 h) caused a 2-fold increase of the transport of labeled DTPA from the rat tracheal lumen to the blood. This coincided with a 2-fold increase in the number of endocytic vesicles in epithelial cells that contained intraluminally instilled HRP as a tracer. These studies also suggest an uneven disruption of tight junctions and alternate transport through endocytotic mechanisms. In studies aimed at detecting the effects of O_3 exposure on regional permeability, O_3 increased the transmucosal transport of DTPA and BSA more in the trachea and bronchoalveolar zone than in the nose. These changes in barrier integrity may allow increased entry of antigens and other bioactive compounds (e.g., bronchoconstrictors) into lung tissue. Data from analyses at regular intervals PR indicate that maximal increases in BALF protein, albumin and number of PMNs occur 8 to 18 h (depending on the study) after an acute exposure ceases.

Recent studies have placed a major focus on mediators released from inflammatory cells to understand the mechanisms of O_3-induced inflammation and injury. Cytokines and chemokines have been shown to be released as a result of stimulation or injury of macrophages, epithelial cells and PMNs. Exposure of guinea pig AMs recovered in BALF and exposed *in vitro* to 0.4 ppm O_3 produced a significant increase in IL-6 and TNFα. An exposure of human AMs to an identical O_3 concentration increased TNFα, IL-1β, IL-6 and IL-8. The expression of MIP-2 mRNA or BALF levels of MIP-2 increased in mice and rats exposed to O_3 concentrations $\geq$ 1 ppm. An increase after O_3 exposure has also been reported for other cytokines and inflammatory mediators, including CINC and fibronectin. The CINC mRNA expression was associated with neutrophilia at 24 hrs PE. Ozone exposure of mice also caused an increase in

IL-6, MIP-1α and eotaxin in mice. Further understanding of the role of mediators has come from studies utilizing antibodies and inhibitors of known specificity. In these studies treatment of rats with an anti IL-6 receptor antibody prior to a nighttime exposure to O_3 abolished O_3-induced cellular adaptive response following a subsequent exposure. Studies utilizing antibodies to selected pro- or anti-inflammatory cytokines suggest a role of TNFα, interleukin-10 (IL-10) and IL-1β in O_3-induced changes in permeability, inflammation and cytokine release.

Studies investigating the mechanisms of PMN recruitment in the lung have explored the role of cell adhesion molecules that mediate PMN-endothelial cell interactions. An increase in tissue expression of ICAM-1 occurred in mice exposed to 2 ppm O_3. A comparable pattern of time-related changes in total protein, fibronectin and alkaline phosphatase activity in the BALF was observed in rats exposed to 1 ppm O_3. In monkeys, the O_3-induced inflammation was blocked by treatment with a monocolonal antibody to CD18, suggesting dependence of PMN recruitment on this adhesion molecule. Together, these studies support the role of extracellular matrix protein and cell adhesion molecules in lung inflammation and injury.

Ozone exposure also affects macrophage functions, and consequently their role in lung inflammation. Macrophages isolated from O_3-exposed mice produced increased amounts of NO, superoxide anion and PGE_2, but production of these mediators by macrophages from NOS knockout mice was not elevated. Additionally, mice deficient in NOS or mice treated with N^G-monomethyl-L-arginine, an inhibitor of total NOS, were protected from O_3-induced permeability, inflammation and injury, suggesting a role of NO in the production of O_3 effects.

5.2.4 Morphological Effects

Most mammalian species show generally similar morphological responses to < 1 ppm O_3, which differ only by region, cell type, exposure parameters, and length of time between exposure and examination. Constant low exposures to O_3 create an early bronchoalveolar exudation, which declines with continued exposure and drops in the PE period. Epithelial hyperplasia also starts early, increases in magnitude for several weeks, plateaus with continuing exposure, and declines slowly during PE. Interstitial fibrosis has a later onset, continues to increase throughout the exposure, and can continue to increase after the exposure ends. Nonhuman primates respond more than rats at this concentration, due to differences in antioxidants, the CAR (predicted to receive the highest dose of O_3), the presence of respiratory bronchioles, acinar volume, and

differences in the nasal cavity's ability to "scrub" the O_3. Ciliated epithelial cells of the airway, Type 1 epithelial cells of the gas-exchange region, and ciliated cells in the nasal cavity are the cells most affected by O_3. Ciliated cells are replaced by nonciliated cells (which are unable to provide clearance function) and Type 1 cells are replaced by Type 2 cells, which are thicker and produce more lipids. Inflammation also occurs, especially in the CAR, wherein the tissue is thickened as collagen accumulates. At exposures of 0.25 ppm O_3 (8 h/day, 18 mo) in monkeys, the distal airway is remodeled as bronchiolar epithelium replaces the cells present in alveolar ducts. In both rodents and monkeys, it appears that the natural seasonal patterns of O_3 exposure alters morphology more than continuous exposures, thus long-term animal studies with uninterrupted exposures may underestimate morphological effects.

5.2.4.1 Short Term Exposure Effects

Morphological effects of key exposure studies generally lasting less than 1 week are summarized in Table AX5-5. Harkema et al. (1997a) reviewed toxicological studies of the nasal epithelial response to short-term O_3. New information regarding the effects of O_3 in this region include demonstrations that the topical anti-inflammatory corticosteriod fluticasone propionate prevents inflammation and mucous cell metaplasia in rats after cumulative O_3 exposure (0.5 ppm O_3, 8h/day, for 3 or 5 days) (Hotchkiss et al., 1998). Exposure to bacterial endotoxin, a common ambient air toxicant, can potentiate mucous cell metaplasia in the nasal transitional epithelium of rats caused by a previous O_3 exposure (Fanucchi et al., 1998). Male F344/N Hsd rats were intranasally instilled with endotoxin after exposure to filtered air (FA) or 0.5 ppm O_3, (8 h/d for 3 d). Mucous cell metaplasia was not found in the air/endotoxin group, but was found in the O_3/saline group and was most severe in the O_3/endotoxin group. A similar synergistic effect was demonstrated by Wagner et al. (2001a,b) with exposure of Fischer rats to O_3 and endotoxin. Ozone alone created epithelial lesions in the nasal transitional epithelium, while endotoxin alone caused lesions in the respiratory epithelium of the nose and conducting airways. The enhanced O_3-induced mucous cell metaplasia was related to neutrophilic inflammation.

Pre-metaplastic responses, such as mucin mRNA upregulation, neutrophilic inflammation, and epithelial proliferation, were shown to be responsible for O_3-induced mucous cell metaplasia in the transitional epithelium of rats (Cho et al., 1999a, 2000). Male F344/N rats exposed to O_3, (0.5 ppm,8 h/d for 1, 2, or 3 d) demonstrated a rapid increase in an airway-specific mucin gene

mRNA rapidly after exposure to O_3, both before and during the onset of mucous cell metaplasia. Neutrophilic inflammation coincided with epithelial DNA synthesis and upregulation of rMuc-5AC, but was resolved before the development of epithelial metaplasia. The mucous cell metaplasia was neutrophil-dependent, whereas O_3-induced epithelial cell proliferation and mucin gene upregulation were neutrophil-independent.

Dormans et al. (1999) compared the extent and time course of fibrotic changes in mice, rats, and guinea pigs exposed to 0.2 and 0.4 ppm O_3 for 3, 7, 28, and 56 days. They found a concentration-related centriacinar inflammation in all three species, with a maximum after 3 days of exposure and total recovery within 3 days after exposure. Repair of O_3 damage by removal of injured epithelial cells is enhanced by the influx of neutrophils (Hyde et al., 1999; Veseley et al., 1999b; Miller et al., 2001; see Section 5.2.3). Labeling indices for rat nasal transitional epithelial cell DNA were greatest 20 to 24 h after O_3 (0.5 ppm for 8h) exposure, but still greater than control by 36 h PE (Hotchkiss et al., 1997).

Very few published studies have explicitly explored susceptibility factors such as species, gender, age, antioxidant defense, acute and chronic airway disease, and exercise. Most typical laboratory species studied have qualitatively similar effects associated with O_3 exposure. Dormans et al. (1999) compared morphological, histological, and biochemical effects in the rat, mouse, and guinea pig after O_3 exposure and after recovery in clean air. Wistar RIV:Tox male rats, NIH male mice, and Hartley Crl:(HA)BR male guinea pigs were continuously exposed to FA, 0.2, or 0.4 ppm for 3, 7, 28, and 56 days. Recovery from 28 days of exposure was studied at intervals of 3, 7, and 28 days PE. The mouse was the most sensitive as shown by a concentration and exposure-time dependent persistence of bronchiolar epithelial hypertrophy, elevated lung enzymes, and slow recovery from exposure. Exposure to the high dose for 56 d in both rats and guinea pigs caused increased amounts of collagen in ductal septa and large lamellar bodies in Type II cells. The inflammatory response was greater in the guinea pig. Overall, the authors rated mice as most susceptible, followed by guinea pigs and rats.

Ferrets, monkeys and rats were exposed to O_3 (1.0 ppm, 8 h) to compare airway effects Sterner-Kock et al. (2000). The ferrets and monkeys had similar epithelial necrosis and inflammation that was more severe than that found in rats. Because ferrets have a similar pulmonary structure as humans (e.g., well-developed respiratory bronchioles and submucosal glands), the authors concluded that the ferret would be a better model than rodents for O_3-

induced airway effects. Age susceptibility is dependent on the endpoint examined. One new study (Dormans et al., 1996) demonstrated that O_3- induced centriacinar lesions are larger in younger rats than in older rats.

Some new studies have examined O_3-induced morphological effects in compromised laboratory animals. Rats with endotoxin-induced rhinitis were more susceptible to mucous cell metaplasia in the nasal transitional epithelium caused by a 3-day exposure to 0.5 ppm O_3 (Cho et al.,1999b). Wagner et al. (2002) reported a similar O_3-induced enhancement of inflammatory and epithelial responses associated with allergic rhinitis. Brown Norway rats were exposed to 0.5 ppm O_3, 8h/day for 1 day or 3 consecutive days and then immediately challenged intranasally with either saline or ovalbumin (OVA). Multiple exposures to O_3 caused greater increases in mucosubstances produced in the nose by allergen challenge.

Recent research has focused on the concept of O_3 susceptible and non-susceptible sites within the respiratory tract, including in situ antioxidant status and metabolic activity. Plopper et al. (1998) examined whether the variability of acute epithelial injury to short-term O_3 exposure within the tracheobronchial tree is related to local tissue doses of O_3 or to local concentrations of reduced glutathione (GSH). Adult male rhesus monkeys exposed to O_3 (0.4 or 1.0 ppm for 2 h) demonstrated significant cellular injury at all sites, but the most damage, along with increased inflammatory cells, occurred in the proximal respiratory bronchiole. A significant reduction in GSH was found in the proximal bronchus at 0.4 ppm O_3 and in the respiratory bronchiole at 1.0 ppm O_3. A significant decrease in the percent of macrophages, along with significant increases in the percent of neutrophils and eosinophils, and a doubling of total lavage protein, were found after exposure to 1.0 ppm O_3 only. The authors concluded that the variability of local O_3 dose in the respiratory tract was related to inhaled O_3 concentration and was closely associated with local GSH depletion and with the degree of epithelial injury.

Plopper and colleagues (e.g., Watt et al.,1998; Paige et al., 2000) explored the site-specific relationship between epithelial effects of O_3 exposure and the metabolism of bioactivated compounds within the respiratory tract of rats. The distribution of CYP2E1-dependent activity, measured with a selective substrate (p-nitrocatechol), was found to be highest in the distal bronchioles and minor daughter airways, and lower in the lobar bronchi and major daughter airways. Short-term O_3 exposure (1 ppm for 8 h) increased CYP2E1 activity in the lobar bronchi/major daughter airways only; however, long-term O_3 exposure (1 ppm for 90 days)

decreased CYP2E1 activity in the major and minor airways, further complicating the interpretation of O_3 effects based on concentration and duration of exposure and recovery. Rats treated i.p. with 1-nitronaphthalene, a pulmonary toxicant requiring metabolic activation, and exposed to 0.8 ppm O_3, 8h/day for 90 days showed greater histopathologic and morphometric effects in the CAR of the lung (Paige et al., 2000). Despite reported tolerance to oxidant stress after long-term O_3 exposure, there was increased severity of ciliated cell toxicity.

5.2.4.2 Summary of Short-Term Morphological Effects

Short-term exposures to O_3 cause similar alterations in lung structure in a variety of laboratory animal species at concentrations of 0.15 ppm in rats and lower concentrations in primates. Cells in the CAR are the primary targets of O_3, but ciliated epithelial cells in the nasal cavity and airways and Type 1 epithelial cells in the gas exchange region are also targets. New work has shown that a topical anti-inflammatory corticosteroid can prevent these effects in nasal epithelia, while exposure to bacterial endotoxin can potentiate the effects. Ozone-induced fibrotic changes in the CAR are maximal at 3 d of exposure and recover 3 d PE with exposures of 0.2 ppm in rodents. New studies of susceptibility factors demonstrated that ferrets and monkeys have similar inflammatory and necrotic responses to 1 ppm O_3, which differs from lesser injury seen in rats. Rats with induced allergic rhinitis are more susceptible to 0.5 ppm than are controls. Important new work has demonstrated variability of local O_3 dose and subsequent injury in the RT due to depletion of GSH. The proximal respiratory bronchiole receives the most acute epithelial injury from exposures $\leq$ 1 ppm, while metabolic effects were greatest in the distal bronchioles and minor daughter airways.

5.2.4.3 Long Term Exposure Effects

Summaries of new studies of morphological effects of exposure lasting longer than 1 week are listed in Table AX5-6 in Annex AX5. In general, as the duration of exposure lengthens, there is not a concomitant linear increase in the intensity of effect of a given endpoint. Rather, as exposure proceeds past 1 week to 1 year, Type 1 cell necrosis and inflammatory responses generally decrease to near control values, and hyperpalstic and fibrotic changes remain elevated. After long-term exposure ended, some indicies of fibrosis persisted and in some cases became more severe during PE periods in clean air.

Effects of O_3 on the upper respiratory tract of F344 rats exposed to O_3 (0.12, 0.5, or 1.0 ppm for 20 months) included marked mucous cell metaplasia in the rats exposed to 0.5 and 1.0 pm O_3, but not at 0.12 ppm O_3 (Harkema et al., 1997a) . In a follow-up study, hyperplasia was found in the nasal epithelium of rats exposed to 0.25 and 0.5 ppm, 8h/day, 7 days/week, for 13 weeks (Harkema et al., 1999). The mucous cell metaplasia, and associated intraepithelial mucosubstances, induced by 0.5 ppm O_3 persisted for 13 weeks after exposure. An acute (8-h) exposure to 0.5 ppm O_3 13 weeks after the chronic exposure induced an additional increase of mucosubstances in the nasal epithelium of rats but not in rats chronically exposed to 0 or 0.25 ppm O_3. The persistent nature of the O_3-induced mucous cell metaplasia in rats reported in this study suggests that O_3 exposure may have the potential to induce similar long-lasting alterations in the airways of humans.

No significant changes in nasal tissue were seen in rats continuously exposed for 49 days to the ambient air of Mexico City, Mexico (Moss et al., 2001), which is in contrast to two rat studies which did demonstrate development of secretory hyperplasia in rats exposed to ambient air of Sao Paulo (Saldiva et al., 1992; Lemos et al., 1994). Because of the persistent nature of these changes in the controlled studies with rats, and the fact that the upper airways of humans are probably more sensitive, like the monkey, the authors suggested that long-term exposure to ambient levels of O_3 could induce significant nasal epithelial lesions that may compromise the upper respiratory tract defense mechanisms of exposed human populations.

Rats exposed to 0.5 ppm O_3 for 1 month exhibited Bcl-2 in protein extracts of nasal epithelium (Tesfaigzi et al., 1998). Further, after 3 and 6 months of exposure, the number of metaplastic mucous cells in the transitional epithelium was indirectly related to the percentage of cells that were Bcl-2 positive . Cells from rats exposed to FA did not express any Bcl-2. This study suggests that apoptosis regulators like Bcl-2 may play a role in the development and resolution of mucous cell metaplasia in the nasal airway

A spectrum of lesions was reported (Herbert et al., 1996) in the nasal cavity and centriacinar lung of male and female mice exposed to 0.5 or 1.0 ppm of O_3 for 2 years, which persisted with continued exposure for 30 months. These lesions included bone loss in the maxilloturbinates, mucosal inflammation, mucous cell metaplasia in the nasal transitional epithelium and increased interstitial and epithelial thickening in the proximal alveolar region. In the CAR, there were increased numbers of nonciliated cells. However, changes in other

endpoints including lung function and lung biochemistry were not evident. The investigators' interpretation of the entire study is that rodents exposed to the two higher O_3 concentrations had some structural hallmarks of chronic airway disease in humans. This interpretation is strengthened further by a comparative pathology study of young (11 to 30 years old) accident victims from Los Angeles, CA, and Miami, FL, (Sherwin et al., 2000) showing increased scores for extent and severity of chronic inflammation in the CAR of Los Angeles residents. (*See Chapter 7 for a more detailed discussion of the population-based studies on O_3.*)

A long-term study using a simulated, seasonal O_3-exposure pattern in infant monkeys was reported by Plopper and colleagues (Evans et al., 2003; Schelegle et al., 2003a; Chen et al., 2003 Plopper and Fanucchi, 2000; Fanucchi et al., 2000) using. Infant rhesus monkeys (30 days old) were exposed to FA, house dust mite allergen aerosol (HDMA), or O_3 + HDMA. The 0.5 ppm O_3 exposures were 8 h/day for 5 days, every 14 days for a total of 11 O_3 episodes. Half of the monkeys were sensitized to house dust mite allergen (*Dermatophagoides farinae*) at 14 and 28 days of age. The sensitized monkeys were exposed to HDMA for 2h/day on Days 3-5 of the FA or O_3 exposures. The lungs were removed during the last FA exposure and the right and left cranial and right middle lobes were separately inflation fixed. Microdisection and morphometric analyses were performed on the conducting airways to the level of the most proximal respiratory bronchiole. Repeated exposures to O_3 or O_3 + HDMA over a 6-month period resulted in an atypical development of the basement membrane zone of airways in nonsensitized developing monkeys. Remodeling in the distal conducting airways was found in the sensitized monkeys as a result of the damage and repair processes occurring with repeated exposure (Evans et al., 2003; Schelegle et al., 2003a; Fanucchi et al., 2000). Lung function changes in these monkeys (Schelegle et al., 2003b), and associated adaptation of the respiratory motor responses (Chen et al., 2003), are described in Section 5.2.5.2. Collectively, these findings provide a pathophysiologic basis for changes in airway function described in children growing up in polluted metropolitan areas (e.g., Tager, 1999) (*See Chapter 7*).

Necropsy of the left caudal lobe of these infant monkeys showed accumulation of eosinophils and mucous cells within the combined epithelium and interstitium compartments in the conducting airways and in the terminal/respiratory bronchioles (Schelegle et al., 2003a) . House dust mite sensitization and HDMA challenge alone, or combined with O_3 exposure, resulted in significantly greater eosinophil accumulation in the conducting airways when

compared to FA and O_3 only exposures. A significant accumulation of eosinophils was found in the terminal/respiratory bronchioles of the sensitized monkeys challenged with HDMA when compared to monkeys exposed to FA, O_3, and HDMA + O_3. The mean mass of mucous cells increased in the fifth generation conducting airways of sensitized animals challenged with HDMA alone and when combined with O_3 exposure, and in the terminal bronchioles of sensitized animals exposed to HDMA + O_3. The tracheal basement membrane of HDMA-sensitized monkeys exposed to HDMA or to HDMA + O_3 was significantly increased over controls; however, there were no significant changes in the airway diameter of proximal and mid-level airways. The authors interpreted these findings to indicate that the combination of cyclic O_3 exposure and HDMA challenge in HDMA-sensitized infant monkeys act synergistically to produce an allergic-reactive airway phenotype characterized by significant eosinophilia of midlevel conducting airways, transmigration of eosinophils into the lumen, and an altered structural development of conducting airways that is associated with increased airway resistance and nonspecific airway reactivity (see Section 5.2.5). Exposures of sensitized young monkeys to HDMA alone, or to O_3 alone, resulted in eosinophilia of the mid-level conducting airways and the terminal/respiratory bronchioles, but without alterations in airway structure or function.

Examination of development of the tracheal basement membrane zone (BMZ) in these monkeys (Evans et al., 2003) showed that with exposures to either O_3 or HDMA + O_3, BMZ development was affected. Abnormalities in the BMZ included: (1) irregular and thin collagen throughout the BMZ; (2) perclecan depeleted or severely reduced; (3) FGFR-1 immunoreactivity was reduced; (4) FGF-2 immunoreactivity was absent in perlecan-deficient BMZ, but was present in the lateral intercelluar space (LIS), in basal cells, and in attenuated fibroblasts; (5) syndecan-4 immumoreactivity was increased in basal cells. The authors interpret these data to suggest that O_3 targets cells associated with synthesis of epithelial BMZ perlecan. The absence of FGF-2, normally stored in the BMZ, could affect downstream signaling in airway epithelium and could be responsible for the abnormal development of the airway seen in this study, and thus be an important mechanism modulating O_3-induced injury. Midlevel bronchi and bronchioles from these monkeys (Larson et al., 2004) demonstrated decrements in the density of epithelial nerves in the axial path between the sixth and seventh airway generations in exposures to O_3. Combined O_3+HDMA exposures exacerbated this reduction. They attribute

this loss of nerve plexuses to neural regression or stunted nerve development, the latter corroborated by the Evans et al. (2003) finding of decreased growth factors following O_3 exposure. Additionally, they found streaks or clusters of cells immunoreactive for protein gene product 9.5 (PGP 9.5, a pan-neuronal marker) and negative for calcitonin gene-related peptide. The functional significance of this is unknown but suggests to the authors a possible injury-repair process induced by O_3.

Remodeling of the distal airways and CAR is one of the most disturbing aspects of the morphological changes occurring after long-term exposure to O_3. Recently, bronchiolization was reported in rats exposed to 0.4 ppm O_3 for only 56 days (van Bree et al., 2001). They also found collagen formation progressively increased with increasing O_3 exposure and remained increased into PE recovery. In addition to centriacinar remodeling, Pinkerton et al. (1998) reported thickening of tracheal, bronchial, and bronchiolar epithelium after 3 or 20 months exposure to 1 ppm O_3, but not to 0.12 ppm. Although some older literature had reported that chronic exposures to $\leq$ 1.0 ppm O_3 cause emphysema, no current literature supports this hypothesis.

5.2.4.4 Summary and Conclusions - Long-Term Morphological Effects

The progression of effects during and after a chronic exposure at a range of 0.5 to 1.0 ppm is complex, with inflammation peaking over the first few days of exposure, then dropping, then plateauing, and finally, largely disappearing. Epithelial hyperplasia follows a somewhat similar pattern. In contrast, fibrotic changes in the tissue increase very slowly over months of exposure, and, after exposure ceases, the changes sometimes persist or increase. Pattern of exposure in this same concentration range determines effects, with 18 mo of daily exposure causing less morphologic damage than exposures on alternating months. This is important as environmental O_3 exposure is typically seasonal. Plopper and colleagues' long term study of infant rhesus monkeys exposed to simulated, seasonal O_3 (0.5 ppm 8h/day for 5 days, every 14 days for 11 episodes) demonstrated : 1) remodeling in the distal airways; 2) abnormalities in tracheal basement membrane; 3) eosinophil accumulation in conducting airways; 4) decrements in airway innervation. These findings advance earlier information regarding possible injury-repair processes occurring with seasonal O_3 exposures.

5.2.5 Effects on Pulmonary Function

5.2.5.1 Acute and Short-Term Exposure Effects on Pulmonary Function

Numerous pulmonary function studies of the effects of short-term O_3 exposure (defined here as ≤ 1 week of exposure) in several animal species have been conducted and generally show responses similar to those of humans (e.g., increased breathing frequency, decreased tidal volume, increased resistance, decreased forced vital capacity [FVC] and changes in the expiratory flow-volume curve). These effects are seen at 0.25 to 0.4 ppm O_3 for several h in a number of species. At concentrations of ≥1 ppm, breathing mechanics (compliance and resistance) are affected. The breathing pattern returns to normal after O_3 exposure. In rats exposed to 0.35 to 1 ppm O_3 for 2 h/day for 5 days, there is a pattern of attenuation of pulmonary function responses similar to that observed in humans. Concurrently, there was no attenuation of biochemical indicators of lung injury or of morphological changes.

New work demonstrating attenuation of pulmonary functions was completed by Wiester et al. (1996) who exposed male Fischer 344 rats to 0.5 ppm O_3 for either 6 or 23 h/day over 5 days. Ozone-induced changes in lung volume were attenuated during the 5 exposure days and returned to control levels after 7 days recovery. The responses to repeated O_3 exposure in rats were exacerbated by reduced ambient temperature, presumably as a result of increased metabolic activity.

Researchers have utilized inbred mouse strains with varying ventilatory responses to O_3 to attempt to model susceptible populations. As differences were seen in inflammatory responses to acute O_3 exposures in C57BL/6J and C3H/HeJ mice, comparisons were made of their ventilatory responses also (Tankersley et al., 1993). Following an exposure of 2 ppm O_3 for 3 h, breathing frequency (f), tidal volume (V_T), and minute ventilation were measured 1 and 24 h in both normocapnia (or air at ~0% CO_2) and hypercapnia (5 or 8% CO_2). They demonstrated that acute O_3 exposures caused altered hypercapnic ventilatory control, which varied between strains. This suggested to the authors that O_3-induced alterations in ventilation are determined, at least in part, by genetic factors.

Paquette et al. (1994) measured ventilatory responses in C57BL/6J and C3H/HeJ mice given repeated subacute exposures. The two strains had differing responses to both normocapnia and hypercapnia. Normocapnic V_E was greater following subacute O_3 exposure in C57BL/6J mice than in C3H/HeJ mice, due to increased and reduced V_T, respectively. This suggests that

the increased V_T in C57BL/6J mice may contribute to the increased susceptibility to lung injury due to a greater dose of O_3 reaching the lower lung. Hypercapnic ventilatory responses following subacute O_3 exposures demonstrated reduced V_E (due to decreased V_T) in C57BL/6J only. Evaluations of O_3 dosimetry were performed in these two strains using $^{18}O_3$-labeled ozone (Slade et al., 1997). Immediately after exposures of 2 ppm $^{18}O_3$ for 2-3 h, C3H/HeJ mice had 46% less ^{18}O in lungs and 61% less in trachea, than C57BL/6J. Additionally, C3H/HeJ mice had a greater body temperature decrease following O_3 exposure than C57BL/6J mice, suggesting that the differences in susceptibility to O_3 are due to differences the ability to decrease body temperature and, consequently decrease the dose of O_3 to the lung.

Tracheal transepithelial potential (V_T) has also been shown to differ in eight mouse strains 6 h after exposure to 2 ppm O_3 for 3 h (Takahashi et al., 1995b). AKR/J, C3H/HeJ, and CBA/J were identified as resistant strains and 129/J, A/J, C57BL/6J, C3HeB/FeJ and SJL/J were identified as susceptible strains. The authors noted that strains' responses to this parameter did not show concordance with inflammatory responses, suggesting to the authors that the two phenotypes are not controlled by the same genetic factors.

Savov et al. (2004) characterized ventilatory responses in nine mouse strains exposed to O_3 (2.0 ppm O_3 for 3 h). Table AX5-4 in Annex AX5 lists the baseline P_{enh}, the P_{enh} following O_3, and the P_{enh} response to methacholine (MCh) following O_3. C57BL/6J was hyporeactive to MCh prior to O_3, but was very responsive to MCh following O_3. Conversely, C3H/HeJ had an intermediate baseline P_{enh} and a small response to MCh following O_3 exposure. This study corroborates the evidence of no consistent relationship between respiratory P_{enh} and inflammation.

5.2.5.2 Summary and Conclusions - Short- and Long-Term Effects on Pulmonary Function

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). Exposures of ~1.0 ppm O_3 affect breathing mechanics (compliance and resistance). Additionally, decreased lung volumes are observed in rats with acute exposures at levels of 0.5 ppm. New work utilizing inbred mouse strains with varying ventilatory responses to O_3 has suggested that: (1) control of the ventilatory response is

determined, at least in part, by genetic factors; (2) increased V_T in some strains may contribute to lung injury due to a greater dose of O_3 reaching the lower lung; (3) some strains' ability to reduce body temperature may account for their decreased O_3-induce lung injury; (4) tracheal transepithelial potential is determined, in part, by genetic factors. Importantly, the genetic loci that appear to be modulating various aspects of pulmonary responses to O_3 differ from each other and from loci controlling inflammatory responses.

Exposures of 2 h/day for 5 days create a pattern of attenuation of pulmonary function 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. Long-term O_3 exposure studies evaluating pulmonary function are not available. Earlier work has demonstrated that repeated daily exposure of rats to an episodic profile of O_3 caused small, but significant decrements in lung function that were consistent with early indicators of focal fibrogenesis in the proximal alveolar region, without overt fibrosis.

5.2.5.3 Ozone Effects on Airway Responsiveness

Effects of O_3 on airway reactivity have been observed in a variety of species at an exposure range of 0.5 to 1 ppm. Many of the new studies on pulmonary function in laboratory animals allow a better prediction of the effects of O_3 exposure on the exacerbation of asthma symptoms and the risk of developing asthma in humans. However, it is necessary to understand the factors that determine airway responsiveness across different mammalian species as discussed in Chapter 4.

The physiological characteristics of asthma include intermittent airway obstruction and increased airway responsiveness to various chemical and physical stimuli. Methods used to assess airway responsiveness in humans include airway challenge with nonspecific bronchoconstrictors (e.g., inhaled methacholine or histamine) and with indirect (e.g., inhalation of adenosine monophosphate, hypertonic saline, mannitol) stimuli to bronchoconstriction (Anderson, 1996). Laboratory animal studies employ intravenous agonist challenges as well as inhalation challenges, though inhaled agonist challenges are preferred in humans. Sommer et al. (2001) reported some differences in the two routes for bronchoconstrictor administration.

Traditional studies of airway responsiveness require sedation in both infants and laboratory animals. Exercise testing is not possible with sedation unless exercise is "simulated" by

increasing ventilation using elevated F_iCO_2 and the need for artificial ventilation in laboratory animal studies may cause breathing patterns that affect O_3 deposition. Joad et al. (2000) reported that when 1 ppm O_3 for 90 min is administered to isolated rat lung at either 2.4 ml/40 bpm or 1.2 m/80 bpm, the more rapid breathing pattern elicits less epithelial cell injury than the slower breathing pattern. Though this study design does not really model rapid shallow breathing elicited in the intact animal, it shows greater reduction in injury in the proximal axial airway compared to its adjacent airway branch and terminal bronchiole. The rapid, shallow breathing pattern protects the large conducting airways of rats, but causes a more even distribution of epithelial cell injury to the terminal bronchioles (Schelegle et al., 2001). Postlethwait et al. (2000) demonstrated that the conducting airways are the primary site of acute cytotoxicity from O_3 exposure. Three-dimensional mapping of the airway tree in SD rat isolated lung exposed to 0, 0.25, 0.5, or 1.0 ppm O_3 showed a concentration-dependent increase in injured cells. Injury was evident in proximal and distal conduction airways, lowest in terminal bronchioles, and highest in the small side branches downstream of bifurcations. These exposure levels did not concurrently elicit changes in LDH activity or total protein in BALF, suggesting that the mapping technique is a more sensitive measure of injury and is useful in dosimetry studies.

Whole-body plethysmography of unanesthetized, unrestrained rodents has been used to indirectly measure pulmonary resistance (Shore et al., 2001, 2002; Goldsmith et al., 2002; Jang et al., 2002). However, these indices of inspiratory/expiratory pressure differences, including enhanced pause (P_{enh}) may be less sensitive than direct measurements of lung airflow resistance (Murphy, 2002). Sommer et al. (1998) demonstrated that unrestrained guinea pigs have a daily variability in pulmonary resistance that is similar to that occurring in humans, indicating that circadian rhythms of airway caliber must be considered when performing airway challenge tests in any species. Changes in airway structure caused by viral infections also must be considered when evaluating laboratory animal studies. Animals with acute viral illness have morphological evidence of inflammatory cell infiltration, bronchiolar wall edema, epithelial hyperplasia, and increased airway mucous plugs that can cause airway narrowing, air trapping, and serious functional changes in the lung (Folkerts et al., 1998).

Exercise-induced bronchoconstriction in humans appears to be mediated by changes in the tonicity of the airway lining fluid (Anderson and Daviskas, 2000). Brannan et al. (1998) suggest that a test in laboratory animals based on the inhalation of mannitol aerosol (hyperosmolar)

might be feasible and provide information similar to that from exercise challenges in cooperative children and adults. Unfortunately, there have been few reports of mannitol or adenosine monophosphate challenges in laboratory animals; most studies have utilized histamine, methacholine, acetylcholine, or carbachol to determine outcome. In active humans with asthma, adenosine monophosphate challenges appear to better reflect ongoing airway inflammation than histamine or methacholine challenges (Polosa and Holgate, 1997; Avital et al, 1995a,b), and might be useful in identifying mechanisms of asthma in laboratory animals and their responsiveness to environmental pollutants.

Airway Responsiveness in Asthma

The increased responsiveness to bronchoconstrictor challenge in asthma is thought to result from a combination of structural and physiological factors that include increased inner-wall thickness, increased smooth-muscle responsiveness, and mucus secretion. These factors also are likely to determine a level of innate airway responsiveness that is genetically influenced. This baseline responsiveness is thought to be modulated in asthma by chronic inflammation and airway remodeling (Stick, 2002). For example, about 90% of children with asthma symptoms in the previous year will exhibit increased airway responsiveness to one or more challenge tests (Sears et al., 1986); however, 10% of healthy children also will respond to one or other of the challenge tests. Longitudinal studies in adults have shown that the development of airway responsiveness is associated with persistence of symptoms (O'Conner et al., 1995) suggesting airway remodeling. This hypothesis is in agreement with the inconsistent relationship reported in the literature between airway responsiveness and markers of inflammation.

Airway Responsiveness in Infants

The age at which nonspecific AHR is first seen in humans is unknown, but it is known that infants show increased responsiveness compared to older children, probably due to differences in dose received. When correction is made for this dose effect, infants and older children appear to have a similar response to inhaled histamine (Stick et al., 1990; Stick, 2002). The importance of this observation is that absolute values of airway responsiveness cannot be used to compare airway responsiveness at different ages, and they certainly cannot be used to compare

responsiveness across different species. However, airway responsiveness can be tracked over time within given populations, or alternatively, by using non-parametric analyses based on ranking subjects at each time point. Such analyses have been used in birth-cohort studies to investigate the role of airway responsiveness in the early genesis of asthma. One unique birth-cohort study has shown that airway responsiveness at one month is a predictor of lung function at six years (Palmer et al., 2001). Data from this study also show that the genetic determinants of atopy and airway responsiveness are independent (Palmer et al., 2000). In another study of infants with wheeze, persistence of AHR was associated with persistence of symptoms, although airway responsiveness at one month of age was neither a sensitive nor a specific predictor of outcome (Delacourt et al., 2001).

The human studies imply that airway responsiveness is a key factor in asthma, but it is not clear if the factors that are important for airway responsiveness in early life are related to inflammation, structure or physiology of the airways, or the combination of all three. Furthermore, it is not clear how viruses, allergens and irritants in the environment modify innate airway responses (Holt et al., 1999), but they are known to be important. Laboratory animal studies have tried to answer some of these key questions.

Airway Responsiveness in Laboratory Animals

A large data base of laboratory animal research has been collected on the role of O_3 in producing an increase in AHR. Exposure levels ($\geq$ 1 ppm for $\geq$ 30 min) in many of these studies are not environmentally relevant, but information can be obtained regarding the mechanisms of action of O_3 concerning: O_3 concentration and peak response time, inhaled versus intravenous challenge with nonspecific bronchoconstrictors, neurogenic mediation, neutrophilic inflammation, and interactions with specific biological agents (e.g., antigens and viruses).

Many species of laboratory animals have been used to study the effects of O_3 on airway bronchoconstriction. Ozone-induced AHR in guinea pigs has been used to model human bronchospasm (Kudo et al., 1996; van Hoof et al., 1996; 1997a; Matsubara et al., 1997a,b; Sun and Chung, 1997; Aizawa et al., 1999a,b; Tsai et al., 1998; Nakano et al., 2000). Because these studies were done at 2 to 3 ppm O_3, these results are not directly relevant for extrapolation to potential airway responses in humans exposed to ambient levels of O_3. Humans with reactive airway disease (e.g., asthma) appear to be sensitive to ambient levels of O_3 (*see Chapters 6*

and 7) and the current understanding is that O_3 exacerbates airway responsiveness to specific allergens, presumably by nonspecifically increasing AHR.

Shore et al. (2000, 2003) have shown that O_3-induced AHR is reduced in immature rats and mice. SD rats exposed to 2 ppm O_3 at ages 2, 4, 6, .8, or 12 weeks and A/J mice exposed to 0.3 to 3 ppm for 3 h at age 2, 4, 8, or 12 weeks had similar concentration-related decreases in V_E except at the youngest ages. This smaller decrement in V_E suggested a delivered dose that was much greater in the younger animals. This group (Shore et al., 2003) has also recently shown that obese mice have greater ventilatory responses to O_3. Exposures of 2.0 ppm O_3 for 3 h to lean, WT C57BL/6J and *ob/ob* mice (mice with a genetic defect in the coding for leptin, the satiety hormone) showed that the *ob/ob* mice had enhanced AHR and inflammation compared to the WT mice. These data correlate with epidemiological data showing increased incidence of asthma in overweight children.

Increased AHR to various nonspecific bronchoconstrictive agents (e.g., ACh, methacholine, histamine, carbachol) given by inhalation or intravenous routes has been previously shown in laboratory animals exposed to O_3 concentrations $\leq$ 1.0 ppm (Table 5-7). Recently, Dye et al. (1999) showed hyperresponsiveness to methacholine in rats 2 h after exposure to 2 ppm O_3 for 2 h. AHR can be induced by specific antigens as well as O_3. The most commonly used laboratory animal model is the OVA sensitized guinea pig. Animals sensitized with OVA have been shown to have similar responses to nonspecific bronchoconstrictors as control animals; however, OVA-sensitized guinea pigs exposed to O_3 showed increased AHR to histamine (Vargas et al., 1994), which was further enhanced by an antigen challenge. When exposed to O_3 before sensitization, repeated exposures to very high levels (5.0 ppm) decreased the OVA sensitization threshold; however, in already sensitized animals, a 2-h exposure to $\geq$ 1.0 ppm enhanced airway responsiveness to OVA, suggesting that O_3 exposure does not modify the development of antigen-induced AHR and, in fact, may enhance AHR at high levels of exposure.

OVA-sensitized guinea pigs (Sun et al., 1997) and mice (Yamauchi et al., 2002) were used to determine the enhancement of antigen-induced bronchoconstriction by acute, high-level O_3 (1.0 ppm O_3 for 1 h). Male Dunkin-Hartley guinea pigs were sensitized by i.p. injection of OVA and exposed to O_3 alone, OVA aerosol, or O_3 + OVA. Ozone exposure alone increased bronchial responsiveness to ACh at 3 h, but not 24 h, while OVA alone had no effect. Combined exposure to O_3 and OVA (1 ppm for 1 h, then 3 min OVA) increased bronchial responsiveness to

ACh 3 h after O_3 exposure. At 24 h following O_3 exposure, AHR increased when OVA challenge was performed at 21 h, suggesting that O_3 pre-exposure can potentiate OVA-induced AHR. Neutrophil counts in the BALF increased at 3 and 24 h after O_3 exposure alone but were not further increased when O_3 exposure was combined with OVA airway challenge; however protein content of the BALF did increase at 3 and 24 h in the O_3 and OVA groups. Thus, this study also indicates that high-ambient O_3 exposure can augment antigen (OVA)-induced AHR in guinea pigs.

Yamauchi et al. (2002) sensitized male C57BL/6 mice by i.p. injection of OVA and then exposed them to O_3. The sensitized mice had AHR to methacholine. Ozone exposure caused significant decreases in dynamic lung compliance, minute ventilation, and P_aO_2 in OVA-sensitized mice, but not in controls. A marker of inflammation (soluble intercellular adhesion molecule-1 [sICAM-1]) was elevated in the BAL fluid of OVA-sensitized mice, but sICAM-1 levels were not significantly changed by O_3 exposure, indicating that the O_3-induced AHR to methacholine was not caused by O_3-induced inflammation.

Ozone-induced AHR may be temporally associated with neutrophils (DeLorme et al., 2002) and other inflammatory cells stimulated by leukotrienes (Stevens et al., 1995a), cytokines (Koto et al., 1997), mast cells (Igarashi et al., 1998; Noviski et al., 1999), or by oxygen radicals (Takahashi et al., 1993; Stevens et al., 1995b; Tsukagoshi et al., 1995; Kudo et al., 1996). Two new studies, however, have shown that inflammation is not a prerequisite of AHR (Stevens et al., 1994; Koto et al., 1997), and some investigators have suggested that O_3-induced AHR may be epithelium dependent (Takata et al., 1995; Matsubara et al., 1995; McGraw et al., 2000). For example, neonatal rats pretreated with capsaicin, which will permanently destroy C-fibers and prevent O_3-induced release of neuropeptides (Vesely et al., 1999a), and then exposed to O_3 when adults, showed a marked increase in airway responsiveness to inhaled aerosolized methacholine (Jimba et al., 1995). Some investigators (Matsumoto et al., 1999; DeLorme et al., 2002) have shown that respective intravenous pretreatment with neutrophil elastase inhibitor or PMN antiserum can block O_3-induced AHR; other investigators (Koto et al., 1995; Aizawa et al., 1997; Takebayashi et al., 1998) have shown that depletion of tachykinins by capsaicin treatment, or by a specific tachykinin receptor antagonist, can block the induction of AHR by O_3. The seemingly disparate responses in laboratory animals may be due to species- or strain-specific differences in inherent reactivity to bronchoconstrictors, or to inherent differences in

susceptibility to O_3-induced inflammation (Zhang et al., 1995; Depuydt et al., 1999; Dye et al., 1999).

Studies that may be potentially relevant to ambient levels of O_3 were conducted in vivo, in an isolated perfused lung model, and in ex vivo lung segments using multihour and repeated multihour exposures with ambient levels of O_3. A study on the relationship between O_3-induced AHR and tracheal epithelial function was conducted in New Zealand white rabbits by Freed et al. (1996). Rabbits exposed to O_3 (0.2 ppm for 7 h) demonstrated significantly decreased PD but no changes in lung resistance. Changes in the compartmentalized lung resistance, measured in response to ACh challenge before and after bilateral vagotomy, were not significantly different in air-exposed rabbits; however, bilateral vagotomy did enhance peripheral lung reactivity in O_3-exposed rabbits. The ACh-induced increase in lung resistance with O_3 exposure (140%) was two times higher than with air exposure, indicating that ambient-level O_3 exposure affects tracheal epithelial function in rabbits and increases central airway reactivity, possibly through vagally-mediated mechanisms.

Pulmonary mechanics and hemodynamics were studied in the New Zealand white rabbit isolated perfused lung model that allowed partitioning of the total pressure gradient into arterial, pre- and post-capillary, and venous components (Delaunois et al., 1998). Exposures to O_3 (0.4 ppm for 4 h) were followed by evaluation of airway responsiveness to ACh, substance P (SP), or histamine immediately or 48 h later. Ozone inhibited pulmonary mechanical reactivity to all three bronchoconstrictors that persisted for 48 h and modified vasoreactivity of the vascular bed, but only at 48 h PE. Arterial segmental pressure, normally insensitive to ACh and SP, was significantly elevated by O_3; precapillary segmental pressure decreased in response to Ach, suggesting that O_3 can induce direct vascular constriction, but the vascular responses are variable and depend on the agonist used and on the species studied.

Airway responsiveness to the same three compounds was evaluated by Segura et al. (1997) in guinea pigs exposed to O_3 (0.15, 0.3, 0.6, or 1.2 ppm for 4 h). Ozone did not cause AHR to ACh or histamine, except at the highest concentration (1.2 ppm O_3) for histamine. However, O_3 did cause AHR to SP at $\geq$ 0.3 ppm, suggesting that O_3 destroys neutral endopeptidases (responsible for SP inactivation) in airway epithelial cells. Vargas et al. (1998), in a follow-up study, demonstrated that guinea pigs chronically exposed to 0.3 ppm O_3 for 4 h/day became adapted to SP-induced AHR. Ozone caused increased sensitivity to SP after 1, 3, 6, 12, and

24 days of exposure that was associated with airway inflammation; however, after 48 days of exposure, the increased sensitivity to SP was lost.

This study is in accordance with Szarek et al. (1995) who demonstrated that AHR associated with acute O_3 exposures does not persist during long-term exposure to ambient-levels of O_3 ($\leq$ 1 ppm). Fischer 344 rats, exposed to 0.0, 0.12, 0.5, or 1.0 ppm O_3, 6h/day, 5 days/week for 20 months, demonstrated significantly reduced responses to bethanechol, ACh, and electrical field stimulation in eighth generation airway segments. This suggests that some adaption had taken place during long-term exposure, possibly increased inner wall thickness.

It is well known that the changes in breathing pattern and lung function caused by O_3 are attenuated with repeated daily exposures for at least 3 to 5 days. But guinea pigs exposed to 0.5 ppm O_3, 8 h/day for 7 days showed enhancement of responsiveness of rapidly adapting airway receptors (Joad et al., 1998). Repeated exposure increased receptor activity to SP, methacholine, and hyperinflation; there were no significant effects on baseline or SP- and methacholine-induced changes in lung compliance and resistance, suggesting that the responsiveness of rapidly adapting receptors was enhanced.

Male and female Hartley guinea pigs exposed to O_3 (0.1 and 0.3 ppm, 4 h/day, 4 days/week for 24 weeks) were evaluated for airway responsiveness following ACh or OVA inhalation challenges (Schlesinger et al., 2002a,b). Ozone exposure did not cause AHR in nonsensitized animals but did exacerbate AHR to both ACh and OVA in sensitized animals that persisted for 4 weeks after exposure. The effects of O_3 on airway responsiveness were gender independent and were concentration-related for the ACh challenges.

Schelegle et al. (2003a) evaluated airway responsiveness in infant rhesus monkeys exposed to a 5 day O_3 episode repeated every 14 days over a 6-month period. Half of the monkeys were sensitized to house dust mite allergen (HDMA; *Dermatophagoides farinae*) at 14 and 28 days of age before exposure to a total of 11 episodes of O_3 (0.5 ppm, 8 h/day for 5 days followed by 9 days of FA), HDMA, or O_3 + HDMA. Baseline R_{aw} was significantly elevated after 10 exposure episodes in the HDMA + O_3 group compared to the FA, HDMA, and O_3 exposure groups. Aerosol challenge with HDMA at the end of the 10^{th} episode did not significantly affect R_{aw}, V_T, f_B, or S_aO_2. Aerosol challenge with histamine was not significantly different after 6 episodes; however, the EC150 R_{aw} for the HDMA + O_3 group was significantly reduced after 10 episodes when compared to the FA, HDMA, and O_3 exposure groups, indicating the

development of AHR in this group sometime between episodes 6 and 10. The results are consistent with altered structural development of the conducting airways.

During repeated episodic exposures to O_3, respiratory responses are first altered to a rapid, shallow breathing pattern, which has long been considered protective, especially to the deep lung. This dogma has been discounted recently as discussed above (Schelegle et al., 2001). Alfaro et al. (2004) examined the site-specific deposition of ^{18}O (1 ppm 2 h) at breathing frequencies of 80, 120, 160, or 200 breaths/minute. At all frequencies, parenchymal areas had a lower content of ^{18}O than trachea and bronchi. As breathing frequency increased from 80 to 160 bpm, the deposition showed a reduction in midlevel trachea and an increase in both mainstream bronchi. At this frequency there was also an increase in deposition in parenchyma supplied by short (cranial) airway paths, consistent with results seen by Schelegle et al., (2001). At 200 bpm ^{18}O deposition in trachea increased, concurrent with increases in right cranial and caudal bronchi regions. Right cranial parenchymal content decreased at 200 bpm, whereas right caudal parenchymal levels did not change at any breathing frequency. These two studies provide evidence that O_3-induced rapid, shallow breathing creates a more evenly distributed injury pattern, with possibly greater protection from focal injury to the large conducting airways including the trachea and the left mainstem bronchus.

Another study of the adaptive phenomena in SD rats used an exposure paradigm consisting of 5 days of daily 8 h 1 ppm O_3 exposures followed by 9 days of recovery in FA (Schelegle et al., 2003b). This O_3/FA pattern was repeated for 4 cycles and demonstrated that the O_3-induced rapid shallow breathing pattern was followed by adaptation occurred with each cycle. But the release of SP from the trachea , the neutrophil content, and cell proliferation became attenuated after the first cycle, suggesting a disconnect from the rapid shallow breathing response. Hypercellularity of the CAR epithelium and thickening of the CAR interstitium, not linked to changes in cell proliferation, were also found. The authors suggest mechanism(s) of injury from repeated O_3 exposures consisting of diminished neutrophilic inflammation/and or release of mitogenic neuropeptides, depressed cell proliferative response, and cumulative distal airway lesion.

Following the initial response of a rapid, shallow breathing pattern, animals eventually adapt with continued episodic exposure despite the continued presence of epithelial damage, altered structural development, and inflammation of the airways. Chen et al. (2003) used a

subset of the monkeys from the Schlegele et al. (2003a) study to demonstrate that attenuation of O_3-induced rapid shallow breathing and lung function changes typically seen with repeated O_3 exposure may be caused by the adaptation of the respiratory motor responses. This episodic O_3 exposure appeared to create neuroplasticity of the nucleus tractus solitarius (NTS; a region of the brainstem which controls respiration), including increased nonspecific excitability of the NTS neurons, an increased input resistance, and an increased spiking response to intracellular injections of depolarizing current.

5.2.5.4 Summary and Conclusions - Effects on Airway Responsiveness

Ozone-induced AHR has been reported in a number of laboratory species at an exposure range of 0.5 to 1.0 ppm and in human asthmatics at ambient levels. In asthmatics, O_3 is thought to exacerbate AHR to specific allergens by nonspecifically increasing AHR. New studies have demonstrated that AHR in asthmatics is due in part to chronic inflammation and airway remodeling. Animal studies have shown that O_3 exposure can augment OVA-induced AHR. Importantly, there is a temporal relationship between inflammatory cell influx and O_3-induced AHR, but inflammation is not a prerequisite of AHR. Repeated O_3 exposures enhance AHR, possibly by modulating rapidly adapting airway receptors or by altering the structure of conducting airways.

Currently reported investigations on AHR with repeated O_3 exposure to nonsensitized laboratory animals have shown equivocal results, especially at the most relevant ambient O_3 concentrations of $\leq$ 0.3 ppm. The few available studies in sensitized laboratory animals are consistent with the O_3-induced exacerbation AHR reported in atopic humans with asthma (*see Chapter 6)* but the results are difficult to extrapolate because of interindividual and interspecies differences in responsiveness to bronchoprovocation and possible adaptation of airway responsiveness with long-term, repeated O_3 exposures. Therefore, further studies in laboratory animals are needed to investigate responses to the different challenges in relation to measurements of airway inflammation and the other physiological and structural factors known to contribute to airway responsiveness in human subjects.

Important new information indicates that rapid shallow breathing in response to O_3 causes a more evenly distributed injury pattern rather than protects from injury. New insights into the mechanisms of O_3-induced AHR suggest that: (1) exercise-induced bronchoconstriction may be

mediated by changes in tonicity of the bronchial smooth muscles; (2) vagally-mediated mechanisms may affect trachela epithelial function and increase central airway reactivity; (3) O_3 may induce direct vascular constriction; (4) O_3 may destroy neural endopeptidases in airway epithelial cells, thus preventing the inactivation of SP; and (5) repeated O_3 exposures may diminish neutrophilic inflammation, depress cell proliferation, and cause cumulative distal airway lesions.

5.2.6 Genotoxicity Potential of Ozone

There has been an historical interest in the ability of ground-level pollution to cause cancer, especially lung cancer. This interest has been amplified in recent years by a report of increased risks of incident lung cancer that were associated with elevated long-term ambient concentrations of O_3, PM_{10}, and SO_2 in nonsmoking California males (Beeson et al., 1998; Abbey et al., 1999). The nationwide American Cancer Society study (Pope et al., 2002) showed no significant effect of O_3 on mortality risk, but a positive association of July-September O_3 concentrations and cardiopulmonary mortality. Studies on children and young adults of Southwest metropolitan Mexico City, repeatedly exposed to high levels of O_3, PM, NO_x, aldehydes, metals, and other components in a complex ambient mixture, also report DNA damage in blood leukocytes and nasal epithelial cells (Valverde et al., 1997; Calderon-Garciduenas et al., 1999), and abnormal nasal biopsies (Calderon-Garciduenas et al., 2001). (*See Chapter 6 for a discussion of the human studies*.)

Many experimental studies have been conducted to explore the mutagenic and carcinogenic potential of O_3. In vitro studies are difficult to interpret due to the high exposure levels and culture systems that allowed the potential formation of artifacts. More recently published 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, again, only on exposures to high doses of O_3 (1 ppm for 72 h and 1 or 2 ppm for 90 min, respectively). Exposing the A/J mouse strain (known to have a high spontaneous incidence pulmonary adenomas) to 0.12, 0.50, and 1.0 ppm O_3 for 6 h/day, 5 days/week for up to 9 months, Witschi et al. (1999) did not find O_3 exposure-related differences in lung tumor multiplicity or incidence.

Similarly, in a sub-chronic exposure study ($B6C3F_1$ mice to 0.5 ppm O_3 for 6 h/day, 5 days/week for 12 weeks) Kim et al. (2001) did not find statistically significant increases in the

incidence of lung tumors. Significant differences in mean body weight as well as mean absolute and relative weights of several organs (e.g., liver, spleen, kidney, testes, and ovary) were observed between O_3-exposed and air-exposed mice. Histopathologic examination of major organs revealed oviductal carcinomas in 3/10 O_3-exposed female mice.

5.2.6.1 Summary and Conclusions - Genotoxicity Potential of Ozone

The weight of evidence from new experimental studies does not appear to support ambient O_3 as a pulmonary carcinogen in laboratory animal models. These new data are in agreement with a definitive study of carcinogenicity of O_3 from the NTP study (National Toxicology Program, 1994; Boorman et al., 1994), which was negative in male and female rats, ambiguous in male mice, and positive only in female mice at high concentrations of O_3 (i.e., 1.0 ppm). However, the new animal studies are not in agreement with epidemiologic studies discussed in Chapter 7, which may suggest significant species differences in this health endpoint.

5.3 SYSTEMIC EFFECTS OF OZONE EXPOSURE

Ozone indirectly affects organs beyond the respiratory system due to O_3 reaction products entering the bloodstream and being transported to target sites. Extra-pulmonary effects could also be due to the exposure-related production of mediators, metabolic products and cell trafficking. Although systemic effects are of interest and indicate a very broad array of O_3 effects, they are of limited influence and difficult to interpret. By protecting from respiratory tract effects, these systemic effects will likely be protected against also. Systemic effects are only summarized briefly here and in Table AX5-8.

5.3.1 Neurobehavioral Effects

Animal behavior, both motor activity and operant behavior, has been shown to be suppressed by acute $_3$ exposures of 0.12 ppm. There is a dose dependent decrease in activity with increasing exposure levels. Additionally, these lowered activity levels tend to attenuate with longer exposure periods. New studies in adult laboratory animals confirm that environmentally- relevant O_3 concentrations from 0.2 to 1.0 ppm can decrease motor activity and affect short- and long-term memory, as tested by passive avoidance conditioning in rats (Rivas-

Arancibia et al., 1998; Avila-Costa et al., 1999; Dorado-Martinez et al., 2001), or water-maze learning tasks in mice (Sorace et al., 2001). The effects have been attributed to reactive oxygen/nitrogen species and/or ozonation products. The memory deficits could be blocked by administration of vitamin E (Guerrero et al, 1999) or taurine (Rivas-Arancibia et al., 2000). Increased freezing and decreased exploratory behaviors were accompanied by decreased serotonin levels and increased levels of NO, glutamate, dopamine and striatal lipoperoxidation in rats exposed to 1 ppm of O_3 for 4 h (Rivas-Arancibia et al., 2003). The O_3-exposed animals also demonstrated neuronal cytoplasm and dendrite vacuolation and dilation of RER cisterns, which the authors interpret as a neurodegenerative process resulting from the oxidative stress of acute O_3 exposure. Nino-Cabrera et al. (2002) demonstrated that a 0.7 ppm O_3 exposure for 4 h can induce ultrastructural alterations in the hippocampus and prefrontal cortex in aged rats. These are areas of the brain where degenerative age-related changes in learning and memory functions have been reported (Bimonte et al., 2003).

Paz (1997) reviewed a series of studies that demonstrated significant alterations of electroencephalographic (EEG) patterns during sleep in animals acutely exposed to O_3 (0.35 to 1.0 ppm). Rats and cats both showed loss of paradoxical sleep time after 2 to 8 h of O_3 exposure (Paz and Bazan-Perkins, 1992; Paz and Huitrón-Reséndiz, 1996). Increased total wakefulness, alterations in circadian rhythm, and a permanent 50% loss of paradoxical sleep time were shown in rat pups born to dams exposed to 1.0 ppm O_3 during gestation (Haro and Paz, 1993). Effects on sleep patterns were associated with alterations in brain neurotransmitter levels (Huitrón-Reséndiz et al., 1994; Gonzalez-Pina and Paz, 1997) thought to be caused by O_3 reaction products or prostaglandins (Koyama and Hayaishi, 1994). The permanent effects in pups caused by high O_3 exposure during gestation were attributed to the diminished antioxidant capability of fetal tissue (Günther et al., 1993).

5.3.2 Neuroendocrine Effects

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 high concentrations of O_3. Concentrations of 0.7 to 1.0 ppm O_3 caused morphological changes in the parathyroid; thymic atrophy; decreased serum levels of thyroid stimulating hormone, triiodothyronine (T_3), thyroxine (T_4), free T_4, and protein binding; and increased prolactin.

In more recent studies, increased toxicity to O_3 was reported in hyperthyroid rats by Huffman et al. (2001) and T_3 supplementation was shown to increase metabolic rate and pulmonary injury in the lungs of O_3-treated animals (Sen et al., 1993).

The mechanisms by which O_3 affects neuroendocrine function are not well understood. Cottet-Emard et al. (1997) examined catecholamine activity in rat sympathetic efferents and brain areas of prime importance to adaptation to environmental stressors. Exposures of 0.5 ppm O_3 for 5 days caused inhibition of norepinephrine turnover in heart (−48% of the control level) but not in lungs and failed to modify the tyrosine hydroxylase activity in superior cervical ganglia, and the catecholamine content in the adrenal glands. In the CNS, O_3 inhibited tyrosine hydroxylase activity in noradrenergic brainstem cell groups and decreased catecholamine turnover was in the cortex (−49%) and striatum (−18%) but not in the hypothalamus. This 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.

High, non-ambient levels of O_3 (e.g., > 1.0 ppm) affect visual and olfactory neural pathways in the rat. For example, Custodio-Ramierez and Paz (1997) reported a significant delay in visual evoked potentials recorded in the visual cortex and the lateral geniculate nucleus of male Wistar rats acutely exposed to high levels of O_3 (1.5, and 3.0 ppm for 4 h). Colin-Barenque et al. (1999), using the same strain, reported cytological and ultrastructural changes in the granule layer of the olfactory bulb after a 4-h exposure to 1 to 1.5 ppm O_3. Although these neural effects are thought to be caused by O_3 reaction products, especially free radicals, the studies do not add much to an understanding of the underlying mechanisms.

5.3.3 Cardiovascular Effects

Studies of the effects on hematological parameters and blood chemistry have shown that erythrocytes are a target of O_3. Exposures to 1.0 ppm O_3 for 3 h have been found to decrease HR, MAP, and core temperature and to induce arrhythmias in some exposures. These effects are more pronounced in adult and awake rats than in younger or sleeping animals. Exposures of 0.2 ppm for 48 h have been shown to cause bradycardia, while exposures of 0.1 ppm for 24 have been shown to cause bradyarrhythmia in rats only.

A more recent study of rats exposed to FA for 6 h, followed 2 days later by a 5-hr exposure to 0.1 ppm O_3, 5 days later by a 5-hr exposure to 0.3 ppm O_3, and 10 days later by a 5-hr exposure to 0.5 ppm O_3 used the the head-out plethysmograph for continuous measurements (Arito et al., 1997). Each of the O_3 exposures was preceded by a 1-hr exposure to FA. Transient rapid shallow breathing with slightly increased HR appeared 1-2 min after the start of O_3 exposures and was attributed to an olfactory response. Persistent rapid shallow breathing with a progressive decrease in HR occurred with a latent period of 1-2 hr. During the last 90-min of exposure, averaged values for relative minute ventilation tended to decrease with the increase in O_3 concentration for young (4-6 mo) but not old (20-22 mo) rats.

New studies utilizing radiotelemetry transmitters in unanesthetized and unrestrained rats, Watkinson et al. (1995; 2001) and Highfill and Watkinson (1996) demonstrated that when HR was reduced during O_3 exposure, the T_{co} and activity levels also decreased. The decreases in T_{co} and blood pressure reported by in these studies and by Arito et al., (1997) suggest that the changes in ventilation and HR are mediated through physiological and behavioral defense mechanisms in an attempt to minimize the irritant effects of O_3 inhalation. Decreased activity was previously reported in laboratory animals during exposure to O_3 (see above).

Similar cardiovascular and thermoregulatory responses in rats to O_3 were reported by Iwasaki et al. (1998). Repeated exposure to 0.1, 0.3, and 0.5 ppm O_3 8 hrs/day for 4 consecutive days caused disruption of circadian rhythms of HR and T_{co} on the first and second exposure days that was concentration-dependent. The decreased HR and T_{co} recovered to control values on the third and fourth days of O_3 exposure.

The thermoregulatory response to O_3 was further characterized by Watkinson et al. (2003). Male Fischer-344 rats were exposed to 0.0 ppm× 24 h/day (air), 0.5 ppm × 6h/day (intermittent) or 0.5 ppm × 23 h/day (continuous) at 3 temperatures, 10° C (cold), 22° C (room), or 34° C (warm). Another protocol examined the effects of O_3 exposure (0.5 ppm) and exercise described as rest, moderate, heavy or CO_2-stimulated ventilation. Both intermittent and continuous O_3 exposure caused decreases in HR and T_{co} and increases in BALF inflammatory markers. Exercise in FA caused increases in HR and T_{co} while exercise in O_3 caused decreases in those parameters. Carbon dioxide and O_3 induced the greatest deficits in HR and T_{co}. Several factors were suggested that may modulate the hypothermic response, including dose, animal mass, and environmental stress).

Laboratory animals exposed to relatively high ambient O_3 concentrations (≥ 0.5 ppm) demonstrate tissue edema in the heart and lungs. This may be due to increased circulating levels of atrial natriuretic factor (ANF), which is known to mediate capillary permeability, vasodilation, and blood pressure (Daly et al., 2002). Increased levels of ANF were reported in the heart, lungs, and circulation of rats exposed to 0.5 ppm O_3 for 8 h (Vesely et al., 1994a,b,c)

5.3.4 Reproductive and Developmental Effects

Early studies of pre- and postnatal exposure to O_3 were performed at relatively high concentrations. Teratogenic effects were not observed with intermittent exposures of 0.44 to 1.97 ppm O_3 during any part of gestation. Continuous exposure during mid-gestation increased the resorption of embryos while exposures during late gestation delayed some behavioral developments (e.g., righting, eye opening). There were no effects on neonatal mortality up to 1.5 ppm O_3, whereas some transient effects on weight gain were observed at exposures of 0.6 ppm O_3.

Recent studies tend to confirm previous conclusions that prenatal exposures to O_3 concentrations < 1.0 ppm do not cause major or widespread somatic or neurobehavioral effects in the offspring of laboratory animals. These studies generally add some weight toward a negative interpretation of the importance of contributions of low, ambient O_3 to lower birth weights and gross development defects reported in neonates born to women exposed to typical ambient pollution (e.g., Renner, 2002; Chen et al., 2002; Ritz and Yu, 1999). Some postnatal O_3 exposure studies continue to find a few, subtle or borderline somatic and behavioral deficits that will require further research to better assess potential risk to developing humans.

Recent studies of somatic and neurobehavioral development in female CD-1 mice exposed during pregnancy (days 7 to 17) to O_3 (0, 0.4, 0.8, or 1.2 ppm) failed to show any O_3 effects on reproductive or behavioral performance (Bignami et al., 1994). The study did find significant decreases in body weight gain and delayed eye opening in pups in the 1.2 ppm exposure group. The lack of effect on behavioral performance contrasts with earlier findings, which may be due to the use of different species, differing exposure durations, cross-fostering used in the latter study different species and exposure durations during pregnancy. A second study using CD-1 mice exposed in utero from conception through day 17 of pregnancy to 0, 0.2, 0.4, and 0.6 ppm O_3 found no significant deficits in reproductive performance, postnatal somatic and

neurobehavioral development, or adult motor activity (Petruzzi et al., 1995). A third study by the same group (Petruzzi et al., 1999), using O_3 exposures (0.3, 0.6, or 0.9 ppm) which continued postnatally until weaning, showed subtle changes in handedness and morphine reactivity. Exposures to 0.6 ppm O_3 caused a reduced preference for the right paw in adulthood. Exposures to 0.9 ppm O_3 altered hot plate avoidance after IP treatment with morphine in adulthood.

CD-1 mice exposed to 0.6 ppm O_3 from birth through weaning demonstrated no impairment of navigational performance during acquisition and only subtle changes during reversal (Dell'Omo et al., 1995a). Additionally, there were no O_3-induced effects on reproductive performance, but offspring showed a significant reduction in body weight. Effects on neurobehavioral development with this exposure were minor, with some attenuation of activity responses and impairment of passive avoidance acquisition (Dell'Omo et al. (1995b). The offspring of CD-1 mice continuously exposed from 30 days prior to the formation of breeding pairs until PND 17 to 0.0, 0.3, or 0.6 ppm O_3 showed only small and selective effects on somatic and sensorimotor development (Sorace et al., 2001).

Morphological changes were found in the anterior cerebellar lobe of rat pups born to dams exposed during the entire gestation period to very high (1.0 ppm) O_3 concentrations for 12 h/day. (Rivas-Manzano and Paz, 1999). Additionally, the dams displayed significantly fewer implantations, increased rate of reabsorptions, a high incidence of spontaneous abortion, and offspring with low birth weight, as noted by previous investigators.

5.3.5 Effects on the Liver, Spleen, and Thymus

Early investigations of the effects of O_3 on liver centered on xenobiotic metabolism, and the prolongation of sleeping time, which was observed at 0.1 ppm O_3. In some species, only adults and especially females were affected. In rats, high (1.0 to 2.0 ppm) acute O_3 exposures caused increased production of NO by hepatocytes and enhanced protein synthesis (Laskin et al., 1994; 1996). 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 in the lungs (Vincent et al., 1996; Laskin et al., 1998; Laskin and Laskin, 2001). Except for the earlier work on xenobiotic metabolism, the responses occurred only after very high acute O_3 exposures.

Examinations of the effects of O_3 on spleen and thymus have shown that O_3 primarily affects T-cell mediated systemic immunity. As with the O_3-associated effects shown in the liver,

most of the statistically significant changes occurred after acute exposures to very high O_3 concentrations and relate to systemic oxidative stress. Using more relevant ambient urban O_3 exposure patterns, effects were not found on systemic immune function of rats.

5.3.6 Effects on Cutaneous and Ocular Tissues

Ozone exposure not only affects various organ systems, when inhaled, but also has direct effects on the exposed skin and eyes. The outermost layer of the skin (stratum corneum; SC) may be oxidized, which can lead to compromise of the skin barrier and an epidermal proinflammatory response (Weber et al., 2001; Cotovio et al., 2001; Thiele, 2001). These effects are found only at very high concentrations (>1-5 ppm) and have not been shown at more relevant ambient levels of exposure. The skin possesses a well-developed defense system against oxidative stress, utilizing nonenzymatic (e.g., vitamin C and E, glutathione, uric acid, α-tocopherol) and enzymatic (e.g., superoxide dismutase, catalase, glutathione reductase and peroxidase) antioxidants (Cross et al., 1998). Ocular tissues have similar antioxidant protective function as the skin but are not as well studied (Mucke, 1996; Rose et al., 1998). Effects of ground-level smog on the eyes have been reported but generally are attributed to related photochemical oxidants like peroxyacetyl nitrate (Vyskocil et al., 1998) or possibly to atmospheric O_3 precursors or reaction products like aldehydes.

Hairless mice (SKH-1) were used to evaluate the cutaneous effects of O_3 (1, 5, and 10 ppm for 2 h or 1 ppm for 2 h on six consecutive days) by Thiele et al. (1997a,b,c,d). In the upper epidermis decreased antioxidant levels were observed at > 1.0 ppm O_3, while in both upper and lower epidermal layers increased malondialdehyde (MDA) was found with exposures > 5 ppm. Dermal application of vitamin E prevented MDA accumulation.

The same mouse model exposed to O_3 (0.8 to 10 ppm for 2 h) was used to demonstrate that O_3 depletes the low molecular weight antioxidants (e.g., α-tocopherol, vitamin C, glutathione, uric acid) in the SC at ≥1.0 ppm and causes increased MDA at ≥ 5 ppm (Weber et al, 1999, 2000,2001). Valacchi et al. (2000) demonstrated that preexposure to O_3 followed by low-dose ultraviolet (UV) radiation (0.33 MED) caused depletion of α-tocopherol at an exposure level of 0.5 ppm O_3. This suggests that combined low doses of UV radiation and near-ambient levels of O_3 may cause oxidative stress on the SC. Additional studies demonstrated that stress-inducible proteins (e.g., heme oxygenase-1) and other heat shock proteins (e.g., HSP27 and HSP70) were

induced in deeper cellular layers of the epidermis following O_3 exposure (8.0 ppm for 2 h) (Valacchi et al., 2002). Prolonged exposure to lower concentrations of O_3 (0.8 ppm) for 6 h also induces cellular stress responses that included the formation of HNE protein adducts, HSP27, and heme-oxygenase-1 in the deeper cellular layers of the skin that continued for up to 18 h after O_3 exposure, followed by repair processes (Valacchi et al., 2003).

The importance of O_3 and UV-induced cellular protein oxidation found in murine skin models to possibly similar environmentally-induced changes in human SC keratins was identified by Thiele et al. (1998, 1999) and Thiele (2001). Using the presence of carbonyl groups in proteins as a marker of reactive oxygen mediated protein oxidation, they reported higher carbonyl levels in the upper SC from the tanned skin of humans and in the skin of healthy human volunteers exposed to model chemical oxidants (e.g., hypochlorite, benzoyl peroxide) that were inversely correlated with vitamin E levels. The environmentally-induced oxidative damage identified in human SC represents an early pathophysiological stage in the development of barrier disruption and inflammation, and possibly has implications for the process of desquamation. The relevance of potentiation of environmental oxidative stress by O_3 exposure of human skin needs further study.

5.3.7 Summary and Conclusions - Systemic Effects of Ozone

Neurobehavioral effects of O_3 at concentrations of 0.2 to 1.0 ppm include decreased motor activity, short- and long-term memory deficits, increased freezing behavior, decreased exploratory behaviors. These effects have been associated with reactive oxygen/nitrogen species, ozonation products, altered neurotransmitter levels, morphological changes in several brain regions, and altered EEG patterns during sleep. Neuroendocrine effects of O_3 include morphological and hormonal changes in the pituitary-thyroid-adrenal axis at concentrations of ~0.75 ppm and alterations of visual and olfactory neural pathways at concentrations > 1 ppm. Mechanisms underlying these effects are not understood at this time. Cardiovascular effects of O_3 at concentrations of 0.3 to 0.5 ppm include decreased HR, T_{CO}, and BP, which have been termed a hypothermic response. Concentrations of O_3 $\geq$0.5 ppm cause tissue edema (possibly mediated by ANF). These data are in accordance with O_3-associated cardiac defects found in neonates and fetuses delivered in southern California during 1987 to 1993, suggesting that high

urban exposures of O_3 and its related co-pollutants can adversely affect the cardiovascular system.

Prenatal exposures to O_3 concentrations < 1.0 ppm did not cause noticeable somatic or neurobehavioral effects in offspring, while concentrations of 1.0 to 1.5 ppm caused varying effects on neonatal mortality. Some studies have shown an effect of O_3 on liver xenobiotic enzymes at concentrations as low as 0.1 ppm, while other studies have shown no alterations in metabolic enzymes at even 1 ppm, with the effects appearing to be highly-species specific. Effects on spleen and thymus appear to only occur at high O_3 concentrations (> 1.0 ppm), while relevant ambient, urban exposures have no effect on systemic immune function in rats. Effects of O_3 on cutaneous and ocular tissue are only seen at high, non-relevant concentrations.

5.4 INTERACTIONS OF OZONE WITH OTHER CO-OCCURRING POLLUTANTS

Animal studies of the effects of O_3 in combination with other air pollutants show that antagonism, addititivity, and synergism can result, depending on the animal species, exposure regimen, and health endpoint. These studies are of three types, ambient air mixtures, laboratory-generated complex mixtures, and binary mixtures. Tables AX5-9 and AX5-10 list binary studies of coexposures to nitrogen dioxide and PM, respectively.

5.4.1 Ozone and Nitrogen Oxides

The most commonly studied copollutant in binary mixtures with O_3 is NO_2. Both early studies and more recent ones indicate that, although interaction may occur between these two pollutants, in general, O_3 often masked the effects of the NO_2 or accounted for most of the response, due to the greater toxicity of O_3. Very generally, additivity occurred after acute exposure and synergism occurred with prolonged exposure. Interpreting the mixture studies is challenging because laboratory exposure patterns rarely simulate real-world exposure patterns. In the case of NO_2 and O_3, NO_2 typically peaks before O_3, with a mixture occurring between the peaks, but most laboratory exposures used mixtures only. Also, most studies of O_3 and NO_2 mixtures used ambient levels of O_3 and levels of NO_2 high above ambient.

Recent work has shown that chronic exposures of rats to O_3 (0.8 ppm) and NO_2 (14.4 ppm) for 6 h/day caused development of respiratory insufficiency and severe weight loss. Half of these animals died after 55 to 78 days of exposure due to severe fibrosis (Farman et al., 1997). Increased total lung collagen and elastin was observed, with loss of mature collagen, suggesting breakdown and remodeling of the lung parenchyma. Morphological examination of these coexposures demonstrate a sequence of events starting with increasing inflammatory and mild fibrotic changes for the first 3 weeks of exposure, and stabilized or even reduced changes after 4 to 6 weeks, and severe increases over 7 to 9 weeks of exposure (Farman et al., 1999). This suggests a repair processes occurring during the middle 4 to 6 weeks of exposure become overwhelmed, leading to progressive fibrosis after 7 to 8 weeks of exposure. When the coexposure was extended for 90 days, lesions were shown to extended far into the acinus, but the extent of tissue involvement was the same after 7, 78, and 90 days of exposure. At the end of exposure, high levels of procollagen types I and III mRNA were observed within central acini in the lungs from the combined exposure group but not in lungs from the rats exposed to O_3 or NO_2 alone.

Sprague-Dawley rats exposed to 0.3 ppm O_3 and the combined exposure of O_3 and 1.2 ppm NO_2 for 3 d demonstrated significant DNA single-strand breaks in AMs (Bermudez et al., 1999). No changes were caused by NO_2-only exposure. The same exposures stimulated the activity of polyADPR synthetase, suggesting a response to lung cellular DNA repair caused by oxidant-induced lung injury (Bermudez, 2001). The laboratory animal model of progressive pulmonary fibrosis, utilizing long-term, combined O_3 (0.4 to 0.8 ppm) and high-level NO_2 (7 to 14 ppm) exposure, causes an initial acute pulmonary inflammation, followed by adaptation and repair, and eventually causing pulmonary fibrosis after 6 to 13 weeks of exposure (Ishii et al., 2000b; Weller et al., 2000). Unfortunately, this model is not very useful for understanding potential interactive effects of ambient concentrations of O_3 and NO_2.

5.4.2 Ozone and Other Copollutants

Ozone and Formaldehyde

Early studies with combined exposures to O_3 and formaldehyde (HCHO) found evidence of both synergistic and non-interactive effects. New work includes studies of biochemical and histopathological endpoints in rats exposed to 0.4 ppm O_3 and 3.6 ppm HCHO, alone and

combined, for 8 h/day for 3 days (Cassee and Feron, 1994). They demonstrated no interactive effects in the nasal respiratory epithelium, despite the high levels of HCHO when compared to typical ambient levels of 1 to 10 ppb (e.g., Rehle et al., 2001). Mautz (2003) studied changes in breathing pattern and epithelial cell proliferation using exposures of 0.6 ppm O_3 and 10 ppm HCHO alone and in combination for 3 h with exercise at two times resting ventilation. Even with exercise, HCHO does not substantially penetrate to the lower respiratory tract to interact with O_3, and does not alter breathing patterns to modify local O_3 dose. Parenchymal injury was, therefore, due to O_3 alone. In the nasal transitional epithelium and in the trachea, however, combined exposure produced additive effects due to the increased volume of toxicants during exercise. No other combined pollutant studies have been published in the peer-reviewed literature, although two studies compared the respiratory effects of O_3 to HCHO. Nielson et al., (1999) compared upper airway sensory irritation caused by HCHO concentrations up to 4 ppm to the lower airway irritation caused by O_3. Using BALB/c mice, they continuously measured f_B, V_t, expiratory flow, T_i, T_e, and respiratory patterns during acute, 30-min exposures. The NOEL for HCHO was 0.3 ppm, compared to 1.0 ppm for O_3.

Thus, O_3 and HCHO do not appear to have additive effects, except during exercise, and that is due to increased volume of gas reaching the tissue. Any possible synergism occurs in the nasal epithelium. HCHO exerts its effects primarily in the upper respiratory tract, whereas the primary site of acute cell injury from O_3 occurs in the conducting airways. EPA is currently completing a toxicological and epidemiological review and risk characterization for formaldehyde.

Ozone and Tobacco Smoke

Early studies of combined exposures of O_3 (1ppm) and tobacco smoke demonstrated altered airway responsiveness to inhaled bronchoconstrictor challenge and tracheal vascular permeability in guinea pigs. A more recent study (Wu et al., 1997) reported that inhalation of cigarette smoke evokes a transient bronchoconstrictive effect in anesthetized guinea pigs. Total pulmonary resistance (R_L) and dynamic lung compliance (C_{dyn}) were compared before and after acute exposure to 1.5 ppm O_3 for 1 h. Cigarette smoke alone (7 ml) at a low concentration (33%) induced a mild and reproducible bronchoconstriction that slowly developed and reached its peak after a delay of > 1 min. After O_3, the same cigarette smoke inhalation challenge evoked an

intense bronchoconstriction that occurred more rapidly, reaching its peak within 20 s, and was sustained for > 2 min. Pretreatment with selective antagonists of neurokinin type 1 and 2 receptors completely blocked the enhanced airway responsiveness suggesting that O_3 exposure induced AHR to inhaled cigarette smoke, which resulted primarily from the bronchoconstrictive effect of endogenous tachykinins.

The above studies were conducted with undiluted tobacco smoke and high O_3 concentrations. To determine the effects of aged and diluted sidestream cigarette smoke (ADSS) as a surrogate of environmental tobacco smoke (ETS) on O_3-induced lung injury, Yu et al. (2002) exposed male B6C3F1 mice to (1) FA, (2) ADSS, (3) O_3, or (4) ADSS followed by O_3 (ADSS/O_3). Exposure to 30 mg/m^3 ADSS, 6 h/day for 3 days, followed by exposure to 0.5 ppm O_3 for 24 h was associated with a significant increase in the number of cells recovered by BAL compared with exposure to ADSS alone or O_3 alone. Neutrophils, lymphocytes, and total protein levels in BAL were increased following the combined exposure when compared with all other groups. Within the CAR, the percentage of proliferating cells was unchanged from control following exposure to ADSS alone but was significantly elevated following exposure to O_3 and further augmented in a statistically significant manner in mice exposed to ADSS/O_3. Following exposure to O_3 alone or ADSS/O_3, the ability of AMs to release IL-6 under LPS stimulation was significantly decreased, while exposure to ADSS alone or ADSS/O_3 caused a significantly increased release of TNFα from AMs under LPS stimulation. These data suggest that ADSS exposure enhances the sensitivity of animals to O_3-induced lung injury.

Acute exposure to ETS also may make a healthy person more susceptible to sequential O_3 exposure by affecting lung barrier function or the underlying epithelium. Toxicological studies with components of ETS (e.g., nicotine receptor agonists, acrolein, and oxidants) have shown that the vagal bronchopulmonary C-fibers are stimulated by acute exposures that initiate both central and local responses (Bonham et al., 2001; Mutoh et al., 2000). The central responses (e.g., tachypnea, cough, bronchoconstriction, increased mucous secretion) are more protective of the lungs; however, local responses may include increased sensitization of the C-fibers to other irritants, including O_3. Active tobacco smokers should not be similarly affected because they already have significant chronic airway inflammation and increased mucus production. In fact, chronic smokers appear to have diminished lung function responses to O_3 (*see Chapter 6*).

5.4.3 Complex (Multicomponent) Mixtures Containing Ozone

Studies using complex (multicomponent) mixtures containing O_3 are difficult to interpret because of chemical interactions between the components, as well as the resultant production of variable amounts of numerous secondary reaction products, and a lack of precise control over the ultimate composition of the exposure environment. Irradiated automobile exhaust mixtures containing total oxdant concentrations (expressed as O_3) in the range of 0.2 to 1.0 ppm have been shown to cause pulmonary function changes in several species.

A more recent attempt has been made to examine multicomponent mixtures resulting from the reaction of O_3 with unsaturated hydrocarbons [e.g., isoprene (C_5H_8) and terpene ($C_{10}H_{16}$)], producing HCHO, formic acid, acetone, acrolein, acetic acid, and other oxidation products, many of which are strong airway irritants. Wilkins et al. (2001) evaluated sensory irritation by measuring mean f_B in the mouse bioassay and found a 50% reduction after 30 min of exposure to reaction products of O_3 and isoprene. The mixture at this time period contained < 0.2 ppm O_3, so the authors attributed the observed effects to the oxidation products. Clausen et al. (2001), using the same mouse model, evaluated the reaction products of O_3 and limonene. A 33% reduction in mean f_B was produced after 30 min of exposure to the mixture containing < 0.3 ppm O_3, again implicating the effects of strong irritant products. Further work needs to be done with these complex reaction mixtures because of their potential impact on the respiratory tract. The results would be particularly important, however, to the reaction of O_3 indoors (*see Chapter 3*).

Pollutant mixtures containing acid aerosols comprise another type of commonly examined exposure atmosphere (studies summarized in Table AX5-10). Earlier studies that employed simultaneous single, repeated, or continuous exposures of various animal species to mixtures of acid sulfates and O_3 found responses for several endpoints, including tracheobronchial mucociliary clearance, alveolar clearance, pulmonary mechanics, and lung morphology, to be due solely to O_3. Some synergism was noted for bacterial infectivity, response to antigen, and effects on lung protein content and the rate of collagen synthesis.

More recent studies found some differences in airway responses to inhaled acid particle-O_3 mixtures that may have been partly due to airway dosimetry. Various physical and chemical mechanisms may be responsible (*see* Schlesinger, 1995). For example, physical adsorption or absorption of O_3 or its reaction products on a particle could result in transport to more sensitive sites, or to sites where O_3, by itself, would not normally be reactive. Chemical reactions on the

surface of particles can form secondary products that are more toxicologically active, or chemical characteristics of the particle may change the residence time or reactivity of oxidation products at the site of deposition. The hypothesis that synergism between O_3 and sulfates is due to decreased pH changing the residence time or reactivity of reactants, such as free radicals, was tested by Chen et al. (1995) and El-Fawal et al. (1995). Male New Zealand white rabbits were exposed for 3 h to 125 $\mu g/m^3$ H_2SO_4, 0.1, 0.3, or 0.6 ppm O_3, and to combinations. Chen et al. (1995) demonstrated that decreased pH following exposure to acid aerosol was correlated with phagocytic activity and capacity of harvested macrophages and that exposure to O_3/ H_2SO_4 the removed this relationship. El-Fawal et al. (1995) showed that responsiveness of rabbit harvested bronchial rings to ACh was increased following O_3 exposure, but that O_3/ H_2SO_4 combinations resulted in antagonism.

Using rat tracheal explant cultures, Churg et al. (1996) demonstrated increased uptake of asbestos or TiO_2 in response to 10 min O_3 (up to 1.0 ppm) pre-exposure suggesting that low concentrations of O_3 may increase the penetration of some types of PM into epithelial cells. Using human epithelial cell cultures, Madden et al. (2000) demonstrated a greater potency for ozonized diesel PM to induce prostaglandin E_2 production. This suggests that O_3 can modify the biological activity of PM derived from diesel exhaust.

Effects of combined exposures of O_3 and resuspended urban particles on cell proliferation in epithelial cells of the terminal bronchioles and the alveolar ducts were examined by Vincent et al. (1997) and Adamson et al. (1999). Rats exposed to 0.8 ppm O_3 in combination with 5 or 50 mg/m^3 particles for 4 h demonstrated greatly potentiated proliferative effects compared to O_3 exposure alone. These findings using resuspended dusts, although at high concentrations, are consistent with the studies demonstrating interaction between H_2SO_4 aerosols and O_3. Effects of acute coexposure to O_3 and fine or ultrafine H_2SO_4 aerosols on lung morphology were examined by Kimmel et al. (1997). They demonstrated that alveolar septal volume was increased in animals co-exposed to O_3 and ultrafine, but not fine, H_2SO_4. Interestingly, cell proliferation was increased only in animals co-exposed to fine H_2SO_4 and O_3, as compared to animals exposed to O_3 alone. Subchronic exposure to acid aerosols (20 to 150 $\mu g/m^3$ H_2SO_4) had no interactive effect on the biochemical and morphometric changes produced by either intermittent or continuous exposure to 0.12 to 0.2 ppm O_3, which suggests that the interactive effects of O_3 and acid aerosol coexposure in the lung disappeared during the long-term exposure (Last and

Pinkerton, 1997). Sindhu et al. (1998) observed an increase in rat lung putrescine levels after repeated, combined exposures to O_3 and a nitric acid vapor.

Other studies have examined interactions between carbon particles and O_3. The interactions of intratracheally instilled carbon particles by followed by either a 7-day or 60-day exposure to 0.5 ppm O_3 in rats was evaulated by Creutzenberg et al. (1995). The carbon particles caused diminished phagocytotic capacity and chemotactic migration capability of AMs that was stimulated by the subsequent O_3 exposure. Inflammatory responses following coexposure of O_3 plus fine, H_2SO_4-coated, carbon particles (MMAD = 0.26 μm) for 1 or 5 days was examined by Kleinman et al. (1999). The response with the O_3-particle mixture was greater after 5 days (4 h/day) than after Day 1. This contrasted with O_3 exposure alone (0.4 ppm), which caused marked inflammation on acute exposure, but no inflammation after 5 consecutive days of exposure.

The effects of a mixture of elemental carbon particles, O_3, and ammonium bisulfate on rat lung collagen content and macrophage activity was examined by Kleinman et al. (2000). Decreases in lung collagen, and increases in macrophage respiratory burst and phagocytosis were observed relative to other pollutant combinations. Mautz et al. (2001) used a similar mixture (i.e., elemental carbon particles, O_3, ammonium bisulfate, but with NO_2 also) and exposure regimen as Kleinman et al. (2000). Also observed were were decreases in pulmonary macrophage Fc-receptor binding and phagocytosis and increases in acid phosphatase staining. Bronchoalveolar epithelial permeability and cell proliferation were increased. Altered breathing-patterns also were observed, with some adaptations occurring.

Bolarin et al. (1997) exposed rats to 50 or 100 $\mu g/m^3$ carbon particles in combination with ammonium bisulfate and O_3. Despite 4 weeks of exposure, they observed no changes in protein concentration in lavage fluid or blood prolyl 4-hydroxylase, an enzyme involved in collagen metabolism. Slight decreases in plasma fibronectin were present in animals exposed to the combined pollutants versus O_3 alone. Thus, the potential for adverse effects in the lungs of animals challenged with a combined exposure to particles and gaseous pollutants is dependent on numerous factors, including the gaseous co-pollutant, concentration, and time.

In a complex series of studies, Oberdörster and colleagues examined the interaction of several pulmonary oxidative stress pollutants. Elder et al. (2000a,b) reported the results of combined exposure to ultrafine carbon particles (100 $\mu g/m^3$) and O_3 (1 ppm) in young and old

Fischer 344 rats that were pretreated with aerosolized endotoxin. In old rats, exposure to carbon and O_3 produced an interaction that resulted in a greater influx in neutrophils than that produced by either agent alone. This interaction was not seen in young rats. Oxidant release from lavage fluid cells also was assessed and the combination of endotoxin, carbon particles, and O_3 produced an increase in oxidant release in old rats. This mixture produced the opposite response in the cells recovered from the lungs of the young rats, indicating that the lungs of the aged animals underwent greater oxidative stress in response to a complex pollutant mix of particles, O_3, and a biogenic agent. Johnston et al. (2000; 2002) reported the results of combined exposure to O_3 (1.0 and 2.5 ppm for 4, 20, or 24 h) and low-dose endotoxin, or to O_3 and endotoxin separately, in newborn and adult C57BL/6J mice. In the first study, adult (8 wk old) mice showed greater sensitivity to O_3 than newborn (36 h old) mice on the basis of mRNAs encoding for various chemokines and cytokines. In contrast, adult and newborn mice responded similarly 2 h after endotoxin exposure (10 ng for 10 min), suggesting that age differences in O_3-generated inflammation is secondary to epithelial cell injury. In the second study, 8 wk old mice exposed to O_3 (1 ppm for 24 h) followed by endotoxin (37.5 EU for 10 min) showed increased responsiveness over either exposure alone, on the basis of increased expression of chemokine and cytokine messages and increased BAL fluid levels of protein and PMNs.

Fanucchi et al. (1998) and Wagner et al. (2001a,b) examined the synergistic effect of coexposure to O_3 and endotoxin on the nasal transitional epithelium of rats that also was mediated, in part, by neutrophils. Fisher 344 rats intranasally instilled with endotoxin and exposed to 0.5 ppm O_3, 8 h per day, for 3 days developed mucous cell metaplasia in the nasal transitional epithelium, an area normally devoid of mucous cells; whereas, intratracheal instillation of endotoxin (20 μg) caused mucous cell metaplasia rapidly in the respiratory epithelium of the conducting airways. A synergistic increase of intraepithelial mucosubstances and morphological evidence of mucous cell metaplasia were found in rat maxilloturbinates upon exposure to both O_3 and endotoxin, compared to each pollutant alone. A similar response was reported in OVA-sensitized Brown Norway rats exposed to 0.5 ppm O_3, 8 h/day for 3 days (Wagner et al., 2002), indicating that coexposure to O_3 and inflammatory biogenic substances like allergens (e.g., OVA) or bacterial endotoxin can augment epithelial and inflammatory responses in rat nasal passages.

In follow-up studies, Wagner et al. (2003) reported that coexposure of rats to O_3 and endotoxin also enhanced epithelial and neutrophilic inflammatory responses in the pulmonary airways. Fisher 344 rats were intranasally instilled with endotoxin and exposed to 1.0 ppm O_3 for 8 h, which was repeated 24 h later. Three days after the last exposure, BALF was analyzed for inflammatory cells and secreted mucosubstances (mucin 5AC), and lung tissue was processed for morphometric analysis. Endotoxin instillation alone caused a dose-dependent increase in BALF neutrophils that was further increased 2-fold in O_3-exposed rats given 20 μg endotoxin, the highest dose. Mucin glycoprotein 5AC also was increased in the BALF at this dose but not at lower endotoxin doses. Ozone exposure alone did not cause mucus hypersecretion, but it did potentiate mucus secretion in rats given both 2 and 20 μg endotoxin and increased intraepithelial mucosubstances 2-fold, which was further substantiated by significant increases in mucin gene (rMuc5AC) mRNA levels in the conducting airways.

The effect of O_3 modifying the biological potency of PM (diesel PM and carbon black) was examined by Madden et al. (2000) in rats. Reaction of NIST Standard Reference Material # 2975 diesel PM with 0.1 ppm O_3 for 48 hr increased the potency (compared to unexposed or air-exposed diesel PM) to induce neutrophil influx, total protein, and LDH in lung lavage fluid in response to intratracheal instillation. Exposure of the diesel PM to high, non-ambient O_3 concentration (1.0 ppm) attenuated the increased potency, suggesting destruction of the bioactive reaction products. Unlike the diesel particles, carbon black particles exposed to 0.1 ppm O_3 did not exhibit an increase in biological potency, which suggested that the reaction of organic components of the diesel PM with O_3 were responsible for the increased potency.

Ulrich et al. (2002) investigated the effect of ambient PM from Ottawa Canada (EHC-93) on O_3-induced inflammation. Male Wistar rats were exposed to 0.8 ppm O_3 for 8 h and allowed to recover before intratracheal instillation of 0.5, 1.5, and 5 mg of EHC-93 in 0.3 ml of saline. The high concentrations of PM used were sufficient to induce pulmonary inflammation, which was not exacerbated by pre-exposure to O_3. Rats from the combined exposure group did have higher and more persistent lung lavage protein and albumin levels, as well as increased plasma fibrinogen levels when compared to PM exposure alone.

The interaction of PM and O_3 was further examined in a murine model of OVA-induced asthma. Kobzik et al. (2001) investigated whether coexposure to inhaled, concentrated ambient particles (CAPs) from Boston, MA and to O_3 could exacerbate asthma-like symptoms. On days

7 and 14 of life, half of the BALB/c mice used in this study were sensitized by intraperitoneal (ip) injection of OVA and then exposed to OVA aerosol on three successive days to create the asthma phenotype. The other half received the ip OVA but were exposed to a phosphate-buffered saline aerosol (controls). The mice were further subdivided ($n \geq 61$/group) and exposed for 5 h to CAPs, ranging from 63 to 1,569 $\mu g/m^3$, 0.3 ppm O_3, CAPs + O_3, or to FA. Pulmonary resistance and airway responsiveness to an aerosolized MCh challenge were measured after exposures. A small, statistically significant increase in pulmonary resistance and airway responsiveness, respectively, was found in both normal and asthmatic mice immediately after exposure to CAPs alone and to CAPs + O_3 but not to O_3 alone or to FA. By 24 h after exposure, the responses returned to baseline levels. There were no significant increases in airway inflammation after any of the pollutant exposures. In this well-designed study of a small-animal model of asthma, O_3 and CAPs did not appear to be synergistic. In further analysis of the data using specific elemental groupings of the CAPs, the acutely increased pulmonary resistance was found to be associated with the AlSi fraction of PM. Thus, some components of concentrated $PM_{2.5}$ may affect airway caliber in sensitized animals, but the results are difficult to extrapolate to people with asthma.

Animal studies have examined the adverse cardiopulmonary effects of complex mixtures in urban and rural environments of Italy (Gulisano et al., 1997), Spain (Lorz and Lopez, 1997), and Mexico (Vanda et al., 1998; Moss et al., 2001). Some of these studies have taken advantage of the differences in pollutant mixtures of urban and rural environments to report primarily morphological changes in the nasopharynx and lower respiratory tract (Gulisano et al., 1997; Lorz and Lopez, 1997) of lambs and pigeons, respectively, after natural, continuous exposures to ambient pollution. Each study has provided evidence that animals living in urban air pollutants have greater pulmonary changes than would occur in a rural and presumably cleaner, environment. The study by Moss et al. (2001) examined the nasal and lung tissue of rats exposed (23 h/day) to Mexico City air for up to 7 weeks and compared them to controls similarly exposed to FA. No inflammatory or epithelial lesions were found using quantitative morphological techniques; however, the concentrations of pollutants were low. Extrapolation of these results to humans is restricted, however, by uncontrolled exposure conditions, small sample sizes, and other unknown exposure and nutritional factors in the studies in mammals and birds, and the negative studies in rodents. They also bring up the issue of which species of

"sentinel" animals is more useful for predicting urban pollutant effects in humans. Thus, in these field studies, it is difficult to assign a specific role to any specific component of the mixture for the significant cardiopulmonary effects reported.

Similar morphological changes (Calderón-Garcidueñas et al., 2000a; 2001) and chest X-ray evidence of mild lung hyperinflation (Calderón-Garcidueñas et al., 2000b) have been reported in children residing in urban and rural areas of Mexico City. (*See Chapter 7 for details of these studies*.) The ambient air in urban areas, particularly in Southwestern Mexico City, is a complex mixture of particles and gases, including high concentrations of O_3 and aldehydes that previously have been shown to cause airway inflammation and epithelial lesions in humans (e.g., Calderón-Garcidueñas et al., 1992, 1994, 1996) and laboratory animals (Morgan et al., 1986; Heck et al., 1990; Harkema et al., 1994, 1997a,b). The described effects demonstrate a persistent, ongoing upper and lower airway inflammatory process and chest X-ray abnormalities in children residing predominantly in highly polluted areas. Again, extrapolation of these results to urban populations of the United States is difficult because of the unique complex of urban air in Mexico City, uncontrolled exposure conditions, and other unknown exposure and nutritional factors.

5.4.4 Summary and Conclusions - Interactions of Ozone with other Co-occurring Pollutants

It is difficult to summarize the role that O_3 plays in exposure responses to binary mixtures, and even harder to determine its role in responses to multicomponent, complex atmospheres. Though the specific mechanisms of action of the individual pollutants within a mixture may be known, the exact bases for toxic interactions have not been elucidated clearly. Certain generic mechanisms that may underlie pollutant interactions: (1) physical, involving adsorption of one pollutant onto another and subsequent transport to more or less sensitive sites or to sites where one of the components of the mixture normally would not deposit in concentrated amounts (probably not involved in O_3-related interactions; (2) production of secondary products that may be more toxicologically active than the primary materials, demonstrated or suggested in a number of studies as a basis for interaction between O_3 and NO_2 and between O_3 and PM; (3) biological or chemical alterations at target sites that affect response to O_3 or the copollutant, which which has been suggested to underlie interactions with mixtures of O_3 and acid sulfates; 4)

O_3- or copollutant-induced physiological change, such as alteration in ventilation pattern, resulting in changes in the penetration or deposition of one pollutant when another is present. This has been implicated in enhanced responses to various O_3-containing mixtures with exercise.

Evaluation of interactions between O_3 and copollutants is a complex procedure. Responses are dependent on a number of host and environmental factors, such that different studies using the same copollutants may show different types or magnitudes of interactions. The occurrence and nature of any interaction is dependent on the endpoint being examined and is also highly related to the specific conditions of each study, such as animal species, health status, exposure method, dose, exposure sequence, and the physicochemical characteristics of the copollutants. Because of this, it is difficult to compare studies, even those examining similar endpoints, that were performed under different exposure conditions. Thus, any description of interactions is really valid only for the specific conditions of the study in question and cannot be generalized to all conditions of exposure to a particular chemical mixture. Furthermore, it is generally not possible to extrapolate the effect of pollutant mixtures from studies on the effects of each component when given separately. In any case, what can be concluded from the database is that interactions of O_3-containing mixtures are generally synergistic (antagonism has been noted in a few studies), depending on the various factors noted above, and that O_3 may produce more significant biological responses as a component of a mixture than when inhaled alone. Furthermore, although most studies have shown that interaction occurs only at higher than ambient concentrations with acute exposure, some have demonstrated interaction at more environmentally relevant levels (e.g., 0.05 to 0.1 ppm O_3 with NO_2) and with repeated exposures.

5.5 EFFECTS OF OTHER PHOTOCHEMICAL OXIDANTS

Peroxyacetyl nitrate (PAN) and peroxypropionyl nitrate (PPN) are the most abundant non-O_3 oxidants in ambient air of industrialized areas, other than the inorganic nitrogenous oxidants such as NO_2, and possibly HNO_3. Ambient levels of PAN and PPN were reported to be decreasing over the 1990's and available air quality data (Grosjean et al., 2001; Grosjean, 2003; Jakobi and Fabian, 1997) indicate that present peak concentrations of PAN and PPN in ambient air from urban areas are in the low ppb range (e.g., < 1 to 10 ppb). The levels found in nonurban areas are considerably lower (Gaffney et al., 1993).

Reactions occur in the troposphere between O_3 and hydrocarbons (e.g., d-limonene) to produce epoxides, hydroperoxides, and peroxides. The majority of the measured ambient hydroperoxides produced is hydrogen peroxide (H_2O_2), although a small amount of organic hydroperoxides (ROOH) also may be formed. Friedlander and Yeh (1998) have estimated that atmospheric aerosols can carry as high as 1 mM of H_2O_2 and organic hydroperoxides (e.g., hydroxymethylhydroperoxide) in the associated water. In vitro cell and tissue damage are induced by high concentrations of liquid phase H_2O_2 (50 μM to 1 mM). Morio et al. (2001) demonstrated that 10 and 20 ppb of inhaled H_2O_2 vapor can penetrate the lower lung where it causes inflammation. It is likely that hygroscopic components of PM transport ambient H_2O_2 into the lower lung and induce tissue injury as well. Exposure of rats to a H_2O_2-fine particle mixture (215 or 429 $\mu g/m^3$ ammonium sulfate) resulted in increased neutrophil influx, and production of inflammatory mediators by AMs (Morio et al., 2001). Hygroscopic secondary organic aerosols generated by the O_3/hydrocarbon reactions and their co-occurrence with H_2O_2 also provides another possible mechanism, yet to be validated, whereby H_2O_2 can be transported into the lower respiratory tract (e.g., Friedlander and Yeh, 1998). Interaction of inhaled O_3 with unsaturated fatty acids on cell membranes and mucus in the airways generates epoxides, hydroperoxides, and secondary ozonation products such as 4-hydroxynonenal (see Section 5.2.1)

Inhalation toxicological information on the effects of the non-O_3 oxidants has been limited to a few studies on PAN, but at concentrations much higher (approximately 100- to 1,000 fold) than levels typically found in ambient air. Such high acute levels cause changes in lung morphology, behavioral modifications, weight loss, and susceptibility to pulmonary infections. Therefore, acute toxicity of PAN is much lower than O_3, and it is unlikely that present ambient PAN levels would affect pulmonary function responses to O_3 (Vyskocil et al., 1998). Cytogenetic studies indicate that PAN is not a potent mutagen, clastogen, or DNA damaging agent in mammalian cells in vivo or in vitro at concentrations several orders of magnitude higher than the generally encountered ambient air levels in most cities (Vyskocil et al., 1998; Kligerman et al., 1995; Heddle et al., 1993). Some studies suggest that PAN may be a weak bacterial mutagen at concentrations much higher than exist in present urban atmospheres (DeMarini et al., 2000; Kleindienst et al., 1990).

5.5.1 Summary and Conclusions - Effects of Other Photochemical Oxidants

Concentrations of PAN and PPN (<1 to 10 ppb) in ambient air are unlikely to affect pulmonary function or cause DNA damage. Levels of 10-20 ppm H_2O_2 can penetrate to the lower lung directly or be transported there by PM, where inflammation can result; however, ambient levels of are typically < ~5 ppb. As toxicology studies of other photochemical oxidants are rare, quantitative scientific evaluations of possible health effects of environmental exposures cannot be completed at this time.

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AX5. ANNEX TO CHAPTER 5 OF OZONE AQCD

AX5.1 INTRODUCTION

This annex serves to provide supporting material for Chapter 5, Toxicological Effects of Ozone and Related Photochemical Oxidants in Laboratory Animals and In Vitro Test Systems. It includes tables that summarize new toxicological literature published since the last O_3 criteria document (U.S. Environmental Protection Agency, 1996). In addition, it provides descriptions of those new finding, in many cases, with more detail than is provided in the chapter.

AX5.2 RESPIRATORY TRACT EFFECTS OF OZONE

AX5.2.1 Biochemical Effects

Biochemically detected effects of O_3 are integrally involved in effects on both structure and function (respiratory and nonrespiratory) of the respiratory tract. However, even the few relatively clear associations (e.g., increases in collagen metabolism/collagen and lung fibrosis) are not fully understood, leading to difficulty in interpretation. For presentation, this section summarizes studies designed to identify biochemical targets of O_3, as well as biochemical measurements of O_3-induced changes in xenobiotic metabolism, antioxidant metabolism and oxygen consumption, lipids and arachidonic acid metabolism, and collagen metabolism. Only descriptions of new studies, published since the previous O_3 CD are included. Detailed discussions of older O_3 literature are found in U.S. Environmental Protection Agency (1986, 1996).

AX5.2.1.1 Cellular Targets of Ozone Interaction

New studies characterizing the cellular targets of O_3 interaction include the following. Figure AX5-1 details the major secondary products of ozone interaction with lung cells.

Frampton et al. (1999) demonstrated the ozonation of PUFA to form nonanal and hexanal in rat BAL after exposures to 0.22 ppm O_3 for 4 h with exercise. Increases in nonanal were not accompanied by significant changes in lung function, in epithelial permeability, or in airway

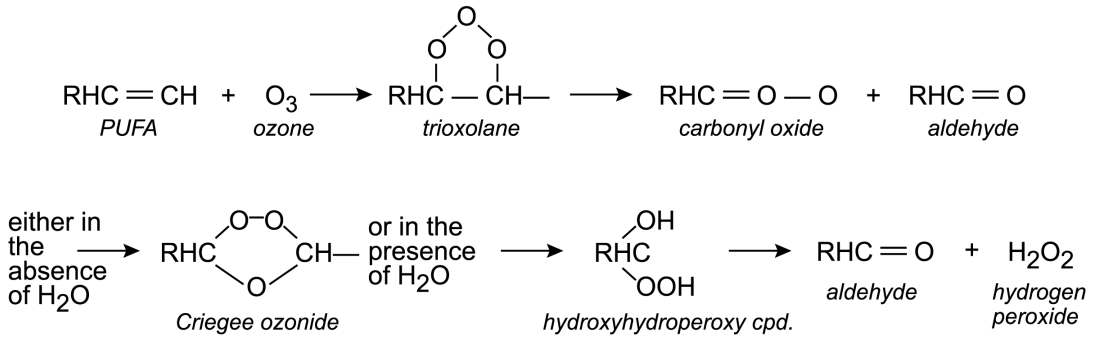

Figure AX5-1. Major secondary products of ozone interaction with lung cells.

inflammation. Hexanal levels did not increase significantly and levels of both aldehydes returned to baseline by 18 h PE. Pryor et al. (1996) exposed rats to 0.5 to 10 ppm O_3 both with and without 5% CO_2 to measure the amount of aldehyde generated in BAL, and also the rate of disappearance of aldehydes from the ELF following the O_3 exposure. Ozone exposure with CO_2 increased the tidal volume and the yield of aldehydes with a maximal aldehyde yield at 2.5 ppm for 1 h. Absolute yields were impossible to ascertain in this system because deposition of O_3 is unknown and aldehyde recovery is not complete due to loss of aldehyde by volatization and by diffusion into underlying tissue. The data showed that at 0.5 ppm O_3 with 5% CO_2, levels of hexanal and nonanal increased at 30 minutes, decreased slightly from that level at 60 minutes, was maximal at 90 minutes and then dropped to 60 minutes levels at 120 minutes. Levels of heptanal did not change appreciably during this time course. Levels of these aldehydes were dependent on a dynamic relationship between their production and the disappearance from the ELF. The authors stated that O_3 is the limiting reagent in this process because the amount of PUFA far exceed the amount of O_3 on a molar basis. Because of the limitations of measuring aldehydes in this study paradigm, it is not useful for quantitative dosimetry; however, the authors suggest the study does serve to demonstrate the use of aldehydes as biomarkers of O_3 exposure since nonanol is produced in an O_3-specific pathway.

Postlethwait et al. (1998) utilized three biologically relevant models (isolated epithelial lining fluid, intact lung, and liposome suspensions) to determine the O_3-induced production of heptanal, nonanal and hexanal in an attempt to estimate formation of lipid-derived bioactive

compounds. Data suggest that PUFAs directly react with O_3 and the amount of bioactive lipids produced is inversely related to ascorbic acid availability. The authors caution that there are limitations to the use of measurements of these reactions products in determining O_3 dose-response relationships due to the heterogenous nature of O_3 reactions in the epithelial lining fluid. Connor et al, (2004) have recently examined the reactive absorption of O_3 within ELF using interfacial films composed of dipalmitoylglycero-3-phosphocholine (DPPC) and rat lung lavage fluid. The films reduced O_3 reactive absorption by antioxidants. Further experiments using a human lung fibroblast cell line exposed to O_3 demonstrated that ascorbic acid (AA) produced cell injury, that high levels of O_3 and AA were needed to induce cell injury, and the DPPC films reduced the amount of cell injury. From these data the authors suggest that O_3 reactions with ELF substrates cause cell injury, that films of active, saturated phospholipids reduce the local dose of O_3-derived reaction products, and that these interfacial phospholipids modulate the distribution of inhaled O_3 and the extent of site-specific cell injury.

Recent studies have examined the formation of ozonation products such as 4-hydroxynonenal (HNE), a toxic aldehyde that reacts with cysteine, histamine, and lysine amino acid residues and creates protein adducts. Hamilton et al. (1998) demonstrated (see Chapter 6) using human AM exposed to 0.4 ppm O_3 for 1 h that exposure caused apoptosis, an increase in a 32-kDa protein adduct, and an increase in ferritin and a 72-kDa heat shock protein. By exposing AM to HNE in vitro, all of these effects are replicated, which the authors interpret to mean that creation of protein adducts and apoptotic cell death are cellular toxic effect of acute O_3 exposure and that it is mediated, at least in part by HNE.

AX5.2.1.2 Monooxygenases

Monooxygenases constitute a class of enzymes, including the cytochrome P-450 (C-P450) or CYP system, that metabolize both endogenous and exogenous substances. Such metabolism can result in detoxification (e.g., drugs like pentobarbital) or activation to more potent metabolites (e.g., carcinogenic metabolites of benzo[*a*]pyrene, B[*a*]P). Although the liver has the greatest capacity for xenobiotic metabolism, the lung also has its complement. The effects of O_3 on this metabolic system are summarized in Table AX5-1.

Lee et al. (1998) characterized the activities of various isoforms of CYPs in both rat and rhesus monkey lung using microdissection techniques and found regiospecific and species-

Table AX5-1. Effects of Ozone on Lung Monooxygenases

Concentration					
ppm	µg/m^3	Duration	Species	Effects[a]	Reference
1.0	1,960	8 h	Rat male, SD 350-600 g	Increases CYP2E1 activity in lobar-bronchi and major daughter airway with 8 h exposure. Decreased CYP2E1 activities in both major and minor daughter airways with 90 day exposure. O_3 does not result in consistent dramatic alterations in CYP2E1 activities.	Watt et al. (1998)
1.0	1,960	90 days			
0.8	1,600	8 h / day for 90 days	Rat, male SD 275-300 g	CYP2B activity increased. Linked to Clara cells in distal lung only — not in trachea or intrapulmonary airway.	Paige et al. (2000a)
1	1,960	2 h	Mice, Clara cell secretory protein deficient, WT strain 129	CCSP-1-mice had increases in IL-6 and MT mRNA that preceded decreases in Clara cell CYP2F2 mRNA. WT mice had levels change but to a lesser degree.	Mango et al. (1998)

[a] CYP = Cytochrome P-450
WT = wild-type
MT = metallothionein
CCSP = Clara Cell Secretary Protein

specific differences. In rat parenchyma both CYP 1A1 and CYP 2B were highest, whereas, in rat airways, CYP 2E1 was highest. In rat airways and parenchyma P450 reductase activities were high, and conversely, low in trachea. Monkeys did not exhibit such site-selective differences in CYP 2B1, CYP 1A1, and P450 reductase; however, they had high CYP2E1 activity in parenchyma and distal bronchioles.

Watt et al. (1998) found that 1 ppm O_3 in both short and long-term exposures in rat increased CYP 2E1 in a region-specific manner (a more detailed discussion is in the Morphology section of this chapter). This group (Paige et al., 2000a) further characterized CYP 2B expression and activity in a long-term O_3 exposure (0.8 ppm 8 h/day for 90 days). Activity of CYP 2B increased 3-fold following this exposure, while CYP 2B like-immunoreactivity increased 2-fold in microsomes prepared from distal lung. Changes in immunodetectable CYP 2B protein and activity were limited to Clara cells in distal lung and not present in trachea or intrapulmonary airways.

Studies have focused on P450 gene expression to examine possible genetic mechanisms that may explain differential O_3-sensitivity (Mango et al., 1998). Mice (129 strain) deficient in Clara cell secretory protein (CCSP-/-), which are oxidant-sensitive, were exposed to 1 ppm O_3 for 2 h. The CCSP null mice demonstrated increases in IL-6 and metallothionein (MT) mRNA that preceded decreases in Clara cell CYP2F2 mRNA (normally expressed at high levels in mouse lung) levels. In 129 strain wild-type mice, RNA levels changed similarly, to a lesser degree.

AX5.2.1.3 Antioxidants, Antioxidant Metabolism, and Mitochondrial Oxygen Consumption

Ozone is an oxidant that produces reactive oxygen species (ROS) involved in the molecular mechanism(s) of toxicity. Antioxidant chemical defenses are important in modulating O_3 toxicity.

Weller et al. (1997) studied the changes in distribution and abundance of copper-zinc (Cu-Zn) and manganese (Mn) SOD in Fischer 344 rats exposed to 1.0 ppm O_3 for up to 3 months. Using immunohistochemistry and immunogold labeling, they identified Cu-Zn SOD positive and Mn SOD positive cells in exposed animals and compared them to controls. In epithelial cells in airways and parenchyma they found reduced Cu-Zn SOD labeling in

O_3-exposed rats. In the CAR regions in both AMs and type II epithelial cells they found significantly increased levels of Mn SOD. Mn SOD levels were not increased in type I epithelial cells, fibroblasts, or Clara cells. The authors suggest that the increased levels of Mn SOD in type II cells in the proximal alveolar duct confer tolerance and protection from further O_3-induced injury.

To look at the effect of antioxidants on modulating O_3-induced oxidant stress, Freed et al. (1999) inhibited antioxidant transport using probenecid (an anion-transport inhibitor) in dogs exposed to 0.2 ppm in a 6h exposure. Blocking antioxidant transport caused heterogeneously distributed increases in peripheral airway resistance and reactivity, supporting the hypothesis that in the lung periphery, endogenous antioxidants moderate the effects of O_3 and that this exposure is a subthreshold stimulus for producing effects on peripheral airway resistance and reactivity in dogs. Treatment with probenecid also inhibited O_3-induced neutrophilic inflammation, which was present in untreated animals exposed to O_3. This finding provides evidence of a dissociation between airway function and inflammation, suggests that O_3-induced inflammation and AHR are independent phenomena, and further, that O_3-induced neutrophilic influx is dependent on a probenecid-sensitive transport process. The authors postulated that probenecid has either a direct or indirect effect on either cytokine or leukotriene transport. Probenecid treatment also caused a 50-60% decrease in plasma urate, a decrease in ascorbate, and a decrease in BALF protein.

Mudway and Kelly (1998) modeled the interactions of O_3 with three ELF antioxidants, AA, uric acid and GSH. They used a continually mixed, interfacial exposure set up in perpex chambers with O_3 concentrations of 0, 0.1, 0.25, 0.5, 1.0 or 1.5 ppm. Exposures were carried out with each oxidant individually, with the antioxidants as a mixture, and with and without human albumin. In all three exposure conditions the ranking of reactivity with O_3 was uric acid > AA > GSH. The reactions did not cause changes in sample pH and no protein carbonyl formations was observed with the antioxidants. They also observed consumption of the antioxidants occurring in a linear fashion with time and a positive relationship to O_3 concentration. They concluded that GSH is not an important substrate for O_3, while uric acid appeared to be the most important substrate which confers protection from O_3 by removing it from inhaled air and limiting the amount that reaches the distal lung. The authors acknowledge

limitations in extrapolating these data to in vivo O_3 exposures due to the absence of surfactant lipids and airway mucus in the model system.

AX5.2.1.4 Lipid Metabolism and Content of the Lung

One of the major postulated molecular mechanisms of action of O_3 is peroxidation of unsaturated fatty acids in the lung, prompting interest in measurement of lipids and lipid metabolism. Several new studies have examined the effects of O3 exposure on phospholipid in lung tissue.

A new mechanism for the toxicity of O_3 was proposed by Pryor et al. (1995). This mechanism suggests that the biochemical changes due to O_3 exposure are relayed to deeper tissue in the lung by a cascade of ozonation products. They consider lipid oxidation products as the most likely molecules to do this because lipids are present in high concentrations in the ELF and they react with O_3 to form stable molecules. These lipid oxidation products cause activation of specific lipases, which then trigger the activation of second messenger pathways (e.g., phospholipase A_2 or phospholipase C). Experiments were completed by this group (Kafoury et al., 1999) using exposures of cultured human bronchial epithelial (NHBE) cells to the lipid ozonation product 1 -palmitoyl-2-(9-oxononanoyl)-sn-glycero-3-phosphocholine (PC-ALF) and 1-hydroxy-1-hydroperoxynonane (HHP-C9). Measurements of PAF, PGE2, IL-6 and IL-8 were completed . PC-ALF elicited release of platelet-activating factor (PAF) and prostaglandin E2, but not IL-6. HHP-C9 caused release of PAF and IL-6 in these cells, but not prostaglandin E2. These results suggest to the authors that O_3-induced production of lipid ozonation products causes release of proinflammatory mediators that then generate an early inflammatory response.

Long et al. (2001) exposed hamsters to 0.12, 1.0 or 3.0 ppm O_3 to evaluate lipid peroxidation and antioxidant depletion. After 6 h exposures to the two higher levels resulted in increased BALF neutrophil numbers and F_2-isoprostanes. The highest exposure only caused increased levels of BALF urate and decreased plasma levels of ascorbate. Exposures to the 0.12 ppm had no effect on BALF neutrophils of F_2-isoprostanes or on plasma antioxidants. Exposures to 1.0 ppm O_3 with 1 h of exercise caused increased levels of F_2-isoprostanes.

Uhlson et al. (2002) evaluated the formation of oxidized phosphlipids by reacting O_3 with calf lung surfactant. Low levels of ozone (0.06, 0.125, and 0.25 ppm) for exposures of 2 to 48 h created a dose- and time-dependent increase in the formation of 1-palmitoyl-2-(9'-oxo-

nonanoyl)-glycerophosphocholine (16:0a/9-al-GPCho), an oxidized phospholipid, which possessed biological activity in three assays. The 16:0a/9-al-GPCho: 1) decreased macrophage viability by necrosis at 6 μM, 2) induced apoptosis in pulmonary epithelial-like A549 cells at 100 to 200 μM , and 3) elicited release of IL-8 from A549 cells at 50 - 100 μM.

AX5.2.1.5 Protein Synthesis

One new study of the effects of O_3 on protein synthesis involved an examination of the time course of lung injury and changes in collagen content in rats exposed acutely or subchronically to 0.4 ppm O_3 (van Bree et al., 2001). They observed centriacinar thickening of septa after 7 days of exposure. This progressed at 28 and 56 days of exposure. After 28 days of O_3, the increase in collagen content in ductular was apparent and it increased progressively until the 56 daytime point. While collagen content decreased with PE recovery, the structural fibrotic changes in ductular septa did not return to control levels. Additionally, they observed the presence of respiratory bronchioles after O_3 exposure, which persisted after an 80-day recovery period. These data suggest that subchronic O_3 exposures in rats creates a progression of structural lung injury that can evolve to a more chronic form, which included fibrosis.

AX5.2.1.6 Gene Expression

Gohil et al. (2003) have used genomic technology to examine differential gene expression in C57BL/6 mice exposed to 1 ppm O_3 for three consecutive nights for 8 h. Utilizing the Affymetrix GeneChip, found O_3-induced changes in the expression of 260 genes, of which 80% were repressed and 20% induced. A number of genes involved in progression of the cell cycle were increased, including ribonucleotide reductase and *S*-adenosyl methionine decarboxylase3. Several NF-κB-activated genes were induced including inhibitor of apoptosis, platelet-derived growth factor receptor α, monocyte chemoattractant protein 1, topoisomerase (DNA) II-α, and serum amyloid 3. These genes are causatively linked with inflammation and in concert with the induced cell cycle genes, may account for increased proliferation of Clara cells and Type II pneumocytes. Ozone caused suppression in the expression of several genes involved in xenobiotic metabolism and in genes coding for major histocompatibility complex. These data suggest O_3 exposure suppresses immune function and xenobiotic metabolism and enhances cellular proliferation.

AX5.2.2 Lung Host Defenses

A number of defense mechanisms operate in the respiratory tract to protect the host from infectious and neoplastic disease. In humans and in animals, the conducting airways of the lungs are primarily protected by the mucociliary escalator. The mucus layer acts to entrap many gaseous and particulate agents and they are cleared from the tract before they have the opportunity to reach underlying tissues. Defects in mucociliary transport can be caused by changing the chemical nature of the mucous secretions, by paralyzing the cilia, or by producing focal lesions in the ciliated epithelium, making it more susceptible to toxic inhalants. Within the gaseous exchange region of the lung, the first line of cellular defense against microbes and nonviable particles is the alveolar macrophage (AM). Impairment of AM function would alter the lung's capabilities to maintain sterility, to clear the lungs of inhaled particles phagocytosized by these cells, to mount an immune response, and to release immunologically-active soluble mediators. Such effects would reduce the host's ability to resist infection and may be involved in the pathogenesis of other chronic diseases. In addition to AM, other local humoral and cell-mediated immune responses are active in protecting the host against such infectious insults, as well as tumor cells. Animal studies have shown that each one of the above defense systems can be altered following exposure to O_3. New studies are summarized in Table AX5-2 are discussed below.

AX5.2.2.1 Clearance

Mucociliary Clearance

To ascertain the mechanism(s) by which O_3 modulates uptake of particles, Churg et al. (1996) prepared 2 mm SD rat tracheal explants and exposed them to either room air or 0.01, 0.05, 0.10, or 1.0 ppm O_3 for 10 minutes. After the O_3 exposure, explants were then submerged in either 5 mg/ml amosite asbestos or 4 mg/ml titanium dioxide for 1 h. Uptake of particles assayed 7 days later indicated a dose-dependent uptake of TiO_2 starting at 0.01 ppm and uptake of asbestos at the two highest doses. To understand the potential role of oxidative stress in this uptake, in some experiments at doses of 0.1 ppm O_3, the reactive oxygen species scavengers catalase or superoxide dismutase, or the iron chelator deferoxamine were added. Uptake of both particles was inhibited by deferoxamine or catalase, but not by superoxide dismutase. Based on these results, the authors concluded that: (1) uptake of particles in the trachea is a direct effect of

Table AX5-2. Effects of Ozone on Lung Host Defenses

Concentration[a]	Duration	Species	Effects[b]	Reference
Microbiologic Endpoints				
0.1, 0.3 ppm	4 h/day, 5 days/week, 1 or 3 weeks	Rat (F344)	No effect on cumulative mortality from subsequent lung infection with 4-8 × 10^6 *Listeria monocytogenes*, but concentration-related effects on morbidity onset and persistence. One-week exposed rats: listeric burdens trended higher than in controls; 0.3 ppm rats displayed continual burden increases and no onset of resolution; in situ IL-1α, TNF-α, and IFNγ levels 48 and 96 h post-infection (4 × 10^6 level) higher than controls. Three-week exposed rats: no O_3-related change in bacterial clearance; IL-1a, TNFa, and IFNg levels higher than control only at 48 h post-infection (4 × 10^6) and only with 0.3 ppm rats	Cohen et al. (2001, 2002)
0.8 ppm	3 h	Rat (F344)	Single exposure to *S. zooepidemicus* led to differential clearance patterns in exposed rats maintained on *ad libitum* or O_3-mitigating calorie-restricted diets	Dong et al. (1998)
0.128 to 1.0	24 h/day for 1 week	Rat	Rats infected with 3.8 x 10^8 *Listeria monocytogenes* (IT). Decreased clearance of *Listeria* at highest exposure (1.0 ppm) and impaired cellular immune response: decreased T/B cell ratios in lymph nodes; delayed-type hypersensitivity response to Listeria-antigen; and depressed lympho-proliferation response in spleen and lymph nodes. Increased formation of lung granulomas.	Steerenberg et al. (1996)
Clearance Endpoints (Non-Microbial)				
0.01 -1.0 ppm	10 min	Rat (SD)	Single 10 min exposure of tracheal explants, followed by 1 h incubation with particles, led to dose-related increases in uptake of amosite asbestos and titanium dioxide particles. Effect inhibited by added catalase or desferoxamine, but not by superoxide dismutase.	Churg et al. (1996)
0.4 ppm	6 h	Dog	Increased tracheal permeability to ^{99m}Tc-DTPA after direct sublobar exposure to O_3. Clearance halftimes remained significantly lower for 1-7 d PE, but recovered by 14 days PE.	Foster and Freed (1999)

Table AX5-2 (cont'd). Effects of Ozone on Lung Host Defenses

Concentration[a]	Duration	Species	Effects[b]	Reference
Alveolar Macrophage Endpoints (General)				
0.8 ppm	3 h	Rat (SD)	Increased ex vivo AM adherence to epithelial cultures mitigated by cell pretreatment with anti-CD11b or anti-ICAM-1 antibodies.	Bhalla (1996)
0.8 ppm	3 h	Rat (SD)	Increased ex vivo AM adherence to epithelial cell cultures mitigated by cell pretreatment with anti-TNF-α/IL-1α antibodies.	Pearson and Bhalla (1997)
1.0 ppm	4 h	Mouse cell line	Increased intracellular calcium resting levels in WEHI-3 cells. Decreased rates of calcium influx due to digitonin.	Cohen et al. (1996)
2.0 ppm	3 h	Rat (SD)	Decreased AM reduced glutathione content. Effect blocked by pretreatment with bacterial endotoxin.	Pendino et al. (1996)
Alveolar Macrophage Endpoints (Functional)				
0.1, 0.3 ppm	4 h/day, 5 days/week, 1 or 3 weeks	Rat (F344)	Superoxide anion: increased AM production (1 week; 0.1, 0.3 ppm); no intergroup differences noted after IFNγ stimulation. H_2O_2: reduced production (1 week; 0.1, 0.3 ppm); further reduced production after treatment with IFNγ (0.1, 0.3 ppm, 1 and 3 weeks).	Cohen et al. (2001)
0.1, 0.3 ppm	4 h/day, 5 days/week, 1 or 3 weeks	Rat (F344)	Increased AM superoxide anion production (1 week; 0.1, 0.3 ppm), Lower H_2O_2 production (1 week; 0.1, 0.3 ppm). Reduced production after treatment with IFNγ - superoxide (0.3 ppm, 1 week) and H_2O_2 (0.1 ppm, 1 week) - relative to cells without IFNγ treatment. No effects from 3-week exposures.	Cohen et al. (2002)
0.3 ppm	5 h/day, 5 days/week, 4 weeks	Rat (F344)	No effect on AM endotoxin-stimulated IL-1α, IL-6, or TNF-α production. Decrease in stimulated, but not spontaneous, superoxide formation; variable effects on H_2O_2 formation. No effect on AM spontaneous, endotoxin-, or IFNγ-stimulated, NO formation.	Cohen et al. (1998)
0.8 ppm	3 h	Rat (SD)	Increased AM motility in response to chemotaxin; effect mitigated by cell pretreatment with anti-CD11b or anti-ICAM-1 antibodies.	Bhalla (1996)
0.8 ppm	3 h	Rat (F344)	Decrease in AM phagocytic activity	Dong et al. (1998)

Table AX5-2 (cont'd). Effects of Ozone on Lung Host Defenses

Concentration[a]	Duration	Species	Effects[b]	Reference
Alveolar Macrophage Endpoints (Functional) (cont'd)				
0.8 ppm	3 h	Mouse (B6J129SV) (C57/BL6X 129 $NOS^{-/-}$)	Increased AM spontaneous and IFNγ+LPS-induced NOS expression and NO production and PGE_2 release. Initial decrease in ROI production, with eventual rebound. Knockout ($NOS^{-/-}$) mice AM incapable of similar response to O3 - no inducible NO or PGE_2 above control levels and consistent decreased ROI production	Fakhrzadeh et al. (2002)
1.0 ppm	24 h/day, 3 days	Rat (Wistar)	Lavage fluid from exposed rats subsequently inhibited IFNγ-induced AM NO production.	Koike et al. (1998, 1999)
Cytokines, Chemokines: Production, Binding, and Inducible Endpoints				
0.1, 0.3 ppm	4 h/day, 5 days/week, 1 or 3 weeks	Rat (F344)	Superoxide anion: no intergroup differences noted after IFNγ stimulation. H_2O_2: reduced production after treatment with IFNγ.	Cohen et al. (2001)
0.1, 0.3 ppm	4 h/day, 5 days/week, 1 or 3 weeks	Rat (F344)	Decreased expression of CD3 among lung lymphocytes (0.1 ppm only; 3 weeks); effect exacerbated by stimulation with IFNα (but not with IL-1α). Decreased expression of CD25 (IL-2R) on $CD3^+$ lymphocytes (0.3 ppm only; 3 weeks); effect worsened by treatment with IL-1α (0.1, 0.3 ppm; 3 weeks). No effects on IL-2-inducible lympho-proliferation. Reduced AM production of ROIs after treatment with IFNγ; superoxide (0.3 ppm, 1 week) and H_2O_2 (0.1 ppm, 1 week) - relative to untreated cells	Cohen et al. (2002)
0.3 ppm	5 h/day, 5 days/week, 4 weeks	Rat (F344)	No effect on AM endotoxin-stimulated IL-1α, IL-6, or TNF-α production.	Cohen et al. (1998)
0.3 ppm 1.0 ppm 2.5 ppm	24 or 96 h 1, 2, or 4 h, 2, 4, or 24 h	Mouse (C57Bl/6J)	0.3 ppm: Increased lung: MIP-2, MCP-1, and eotaxin mRNA expression. 1.0 ppm: After 4 h, increased lung: MIP-2, MCP-1, eotaxin, and IL-6 mRNA expression. 2.5 ppm: After 2 h, increased lung: MIP-2, MCP-1, eotaxin, and IL-6 mRNA expression. No exposure-related increases in lung IL-1α, IL-1β, IL-1Rα, IL-10, IL-12, or IFNγ mRNA expression.	Johnston et al. (1999a)

Table AX5-2 (cont'd). Effects of Ozone on Lung Host Defenses

Concentration[a]	Duration	Species	Effects[b]	Reference
Cytokines, Chemokines: Production, Binding, and Inducible Endpoints (cont'd)				
1.0 ppm	4 h	Mouse cell line	Decreased binding of IFNγ by WEHI-3 cells. Decreased superoxide production by IFNγ-treated cells; no similar effect on H_2O_2 production. Decreased IFNγ-stimulatable phagocytic activity. No effect on IFNγ-inducible Ia (MHC Class II) antigen expression.	Cohen et al. (1996)
1.0 ppm	6 h	Rat (SD)	Increased AM MIP-1α, CINC, TNF-α, and IL-1β mRNA expression. Induced increase in MIP-1α and CINC mRNA temporally inhibited by cell treatment with anti-TNF-α/IL-1β antibodies.	Ishii et al. (1997)
1.0 ppm	24 h/day, 3 days	Rat (Wistar)	Lavage fluid from exposed rats subsequently inhibited: ConA-stimulated lymphocyte IFNγ production, but had no effect on IL-2 production; IL-2-induced lymphopro-liferation; and, IFNγ-induced AM NO production.	Koike et al. (1998, 1999)
1.0 ppm	24 h	Mouse (C57Bl/6J)	Increased lung: MIP-2 (4 h PE) and MCP-1 (4 and 24 h PE) mRNA expression.	Johnston et al. (2001)
1.0 ppm	24 h	Mouse (C57Bl/6J)	Increased lung MIP-2 and MCP-1 mRNA expression (4 and 24 h PE); no effects on mRNA levels of IL-1α, IL-1β, IL-1Rα, IL-6, MIF, MIP-1α, MIP-1β, eotaxin, or RANTES at either timepoint in recovery period. Enhanced expressions of some cytokines/chemokines were maintained longer than normal by coexposure to endotoxin.	Johnston et al. (2002)
1.0 ppm	8 h/day, 3 days	Mouse (C57Bl/6) (C57Bl/6Ai$^-$ NOS$^{-/-}$)	Knockout (NOS$^{-/-}$) mice have more lavageable MIP-2 after exposure than wild-type; both greater than control.	Kenyon et al. (2002)
1.0, 2.5 ppm	4 or 24 h	Mouse (C57Bl/6J)	Dose-related increases in cytokine/chemokine induction. Increased lung MIP-1α, MIP-2, eotaxin (4 and 24 h), IL-6 (4 h only), and iNOS mRNA expression.	Johnston et al. (2000)

Table AX5-2 (cont'd). Effects of Ozone on Lung Host Defenses

Concentration[a]	Duration	Species	Effects[b]	Reference
Cytokines, Chemokines: Production, Binding, and Inducible Endpoints (cont'd)				
0.6, 2.0 ppm	3 h	Mouse (C57BL/6) and Rat	Increased lung MIP-2 (4 h PE) and MCP-1 mRNA expression (24 h PE); PMN and monocyte increased accumulation in lungs consistent with sequential expression of the chemokines. NF-kB activation also increased 20-24 h PE.	Zhao et al. (1998)
0.8 ppm	3 h	Rat (SD)	Increased ex vivo AM adherence to epithelial cells mitigated by cell treatment with anti-TNF-α or IL-1α antibodies.	Pearson and Bhalla (1997)
0.8 ppm	3 h	Mouse (B6J129SV) (C57Bl/6X 129 $NOS^{-/-}$)	Increased AM IFNγ+LPS-induced NOS expression and NO production, as well as induced PGE_2 release. Knockout ($NOS^{-/-}$) mice AM incapable of similar response to O_3 - no inducible NO or PGE_2 above control levels.	Fakhrzadeh et al. (2002)
0.8 ppm	3 h	Mouse (B6J129SV) (C57Bl/6X 129 $NOS^{-/-}$)	Increased AM IFNγ+LPS-induced NOS expression and NO production.	Laskin et al. (2002)
2.0 ppm	3 h	Rat (SD)	Increased AM spontaneous and IFNγ+LPS-induced NOS expression and NO production. AM from exposed rats showed rapid onset/prolonged activation of NF-κB.	Laskin et al. (1998a)
180-500 μg/m^3 and 1% OVA		BALB/C C57BL/6	O_3 - dose-dependent increases in IgE, IL-4, IL-5; recruitment of eosinophils and lymphocytes in BALB/c; O_3 + OVA - increased IgG, antibody titers, leukotrienes, airway responsiveness, immediate cutaneous hypersensitivity reactions in BALB/c. In C58BL/6 only O_3 + OVA caused cutaneous hypersensitivity and altered IgG responses.	Neuhaus-Steinmetz et al. (2000)
Alveolar Macrophage/Lung NO- and iNOS-Related Endpoints				
0.3 ppm	5 h/day, 5d/week, 4 weeks	Rat (F344)	No effect on AM spontaneous, endotoxin-, or IFNγ-stimulated, NO formation.	Cohen et al. (1998)
0.8 ppm	3 h	Mouse (B6J129SV) (C57Bl/6X 129 $NOS^{-/-}$)	Increased AM IFNγ+LPS-induced NOS expression and NO production and PGE_2 release. Knockout ($NOS^{-/-}$) mice AM incapable of similar response to O_3 - no inducible NO or PGE_2 above control levels	Fakhrzadeh et al. (2002)

Table AX5-2 (cont'd). Effects of Ozone on Lung Host Defenses

Concentration[a]	Duration	Species	Effects[b]	Reference
Alveolar Macrophage/Lung NO- and iNOS-Related Endpoints (cont'd)				
0.8 ppm	3 h	Mouse (B6J129SV) (C57Bl/6X 129 $NOS^{-/-}$)	Increased AM spontaneous and IFNγ+LPS-induced NOS expression and NO production. AM from exposed mice showed rapid and prolonged activation of NF-κB, STAT-1 (expression, activity), phosphoinositide 3-kinase, and protein kinase B.	Laskin et al. (2002)
1.0 ppm	8 h/day, 3 days	Mouse (C57Bl/6) ($C57Bl/6Ai^{-}$ $NOS^{-/-}$)	Knockout ($NOS^{-/-}$) mice have more lavageable PMN, MIP-2, and protein in lungs after exposure than wild-type.	Kenyon et al. (2002)
1.0 ppm	24 h/day, 3 days	Rat (Wistar)	Lavage fluid from exposed rats subsequently inhibited IFNγ-induced AM NO production.	Koike et al. (1998, 1999)
1.0, 2.5 ppm	4 or 24 h	Mouse (C57Bl/6J)	Dose-related increase in lung iNOS mRNA expression	Johnston et al. (2000)
2.0 ppm	3 h	Rat (SD)	Increased AM spontaneous, IFNγ, and LPS-induced NO production, as well as spontaneous and LPS-induced NOS expression. Effect somewhat ameliorated by pretreatment with bacterial endotoxin.	Pendino et al. (1996)
2.0 ppm	3 h	Rat (SD)	Increased AM spontaneous and IFNγ+LPS-induced NOS expression and NO production. AM from exposed rats showed rapid onset/prolonged activation of NF-κB.	Laskin et al. (1998b)
3.0 ppm	6 h	Rat (Brown Norway)	Increased lung iNOS mRNA expression. Effect blocked by pretreatment with dexamethasone.	Haddad et al. (1995)
0.12, 0.5, or 2 ppm	3 h	Mice BALB/c	Dose-dependent increases in nitrate and P_{enh}; increases in nNOS but not iNOS or eNOS.	Jang et al. (2002)

Table AX5-2 (cont'd). Effects of Ozone on Lung Host Defenses

Concentration[a]	Duration	Species	Effects[b]	Reference
Surface Marker-Related Endpoints				
0.8 ppm	3 h	Rat (SD)	Increased expression of AM CD11b, but no effect on ICAM-1	Bhalla (1996)
1.0 ppm	4 h	Mouse cell line	No effect on IFNγ-inducible Ia (MHC Class II) antigen expression on WEHI-3 cells.	Cohen et al. (1996)
1.0 ppm	2 h	Rat (SD)	Decreased expression of integrins CD18 on AM and CD11b on PMN. No effect on PMN CD62L selectin.	Hoffer et al. (1999)
1.0 ppm	3 days	Rat (Wistar)	Increased expression of surface markers associated with antigen presentation: Ia (MHC Class II) antigen, B7.1, B7.2, and CD11b/c on BAL cells. Effect attributed to influx of monocytes.	Koike et al. (2001)
0.1, 0.3 ppm	4 h/day, 5 days/week, 1 or 3 weeks	Rat (F344)	Decreased expression of CD3 among lung lymphocytes (0.1 ppm only; 3 weeks); effect exacerbated by stimulation of cells with IFNα (but not with IL-1α). Decreased expression of CD25 (IL-2R) on $CD3^+$ lymphocytes (0.3 ppm only; 3 weeks); effect worsened by treatment of cells with IL-1α (0.1 and 0.3 ppm; 3 weeks).	Cohen et al. (2002)
NK- and Lymphocyte-Related Endpoints				
0.1, 0.3 ppm	4 h/day, 5 days/week, 1 or 3 weeks	Rat (F344)	Decreased expression of CD3 among lung lymphocytes (0.1 ppm only; 3 weeks); effect exacerbated by stimulation of cells with IFNα (but not with IL-1α). Decreased expression of CD25 (IL-2R) on $CD3^+$ lymphocytes (0.3 ppm only; 3 weeks); effect worsened by treatment of cells with IL-1α (0.1 and 0.3 ppm; 3 weeks). Lymphoproliferation: no effect on spontaneous or IL-2-inducible forms; 0.1 ppm increased response to ConA mitogen (1 week only); 0.3 ppm - decreased response to ConA (1 week only).	Cohen et al. (2002)
0.4, 0.8, 1.6 ppm	12 h	Mouse (Balb/c)	Decreased pulmonary delayed-type hypersensitivity reactions to low MW agents, likely via activation of T_H2-dependent pathways.	Garssen et al. (1997)
1.0 ppm	24 h/day, 3 days	Rat (Wistar)	Lavage fluid from exposed rats subsequently inhibited ConA-stimulated lymphocyte IFNγ production, but had no effect on IL-2 production; material also inhibited IL-2-induced lymphoproliferation.	Koike et al. (1999)

Table AX5-2 (cont'd). Effects of Ozone on Lung Host Defenses

Concentration[a]	Duration	Species	Effects[b]	Reference
Susceptibility Factors				
0.3 ppm	24 to 72 h	Mice C57BL/6J C3H/HeJ C3H/HeOuJ	Lavageable protein concentration lowered by inhibition of iNOS and by targeted disruption of *Nos2*; reduced *Nos2* and *Tlr4* mRNA levels in the O_3-resistant C3H/HeJ mice.	Kleeberget et al. (2001b)
1 ppm	4 h	CHO-K1 cell line SP-A	Differences exist biochemically and functionally in SP-A variants. O_3 exposure affects the ability of variants to stimulate TNF-α and IL-8.	Wang et al. (2002)
10 ppm	until death	Mice A/J (O_3 sensitive) and C57BL/6J (O_3-resistant)	No differences in histology or wet-to-dry lung weights; two loci- acute lung injury -1 or -2 confer susceptibility.	Prows et al. (1999) and Prows et al. (1997)

[a]Conversion to $\mu g/m^3$ ≈ ppm value × 1960

[b]Common abbreviations used:
AM = Alveolar macrophage; PE = Postexposure (i.e., time after O_3 exposure ceased); MIP = macrophage inflammatory protein; PMN = Polymorphonuclear leukocyte; MLN = Mediastinal lymph node; CINC = cytokine-induce neutrophil chemoattractant; BAL = Bronchoalveolar lavage; DTPA = diethylenetriaminepentaacetic acid; ROI = reactive oxygen intermediate/superoxide anion; IFN = Interferon; BALT = bronchus-associated lymphoid tissue; MCP = monocyte chemoattractant protein; CON A = Concanavalin A

O_3, as inflammatory cells are not present in the explants, (2) reactive oxygen species are important mediators of particle uptake, with hydrogen peroxide having a primary role in the process, and (3) due to the protective effect of deferoxamine, hydroxyl radical is probably involved in the uptake also.

Pearson and Bhalla (1997) have utilized the radiolabled chelate ^{99m}Tc diethylenetriamine pentaacetic acid (Tc-DTPA) to assess the effect of O_3 exposure on clearance across epithelial surfaces. ^{99m}Tc-DTPA clearance has been found to be significantly increased following a 3-h exposure to 0.8 ppm O_3 in SD rats. Pretreatment with anti-IL-1α and anti-TNF-α did not affect the permeability, suggesting that these soluble mediators are not involved in this process. Foster and Freed (1999) also used ^{99m}Tc-DTPA to examine regional clearance in dogs following a 6-h isolated sublobar exposure to 0.4 ppm O_3 or air. Ozone decreased the clearance halftime of ^{99m}Tc-DTPA by 50% at 1 day following exposure. Seven days PE the halftime was still reduced by 29% and by 14 days PE, clearance had recovered to normal levels. These data provide evidence that a single local exposure to O_3 increases transepithelial clearance, but without any influence on contralateral segements, i.e., only for epithelia directly exposed to O_3, and the altered permeability changes recover to normal levels in 2 weeks.

Alveolar Clearance

New evaluations of the effects of O_3 on alveolar clearance have not been performed.

AX5.2.2.2 Alveolar Macrophages

Effects of O_3 on other AM functions are summarized on Table AX5-2 and new studies are discussed here.

Dong et al. (1998) reported that caloric restriction enhanced phagocytic function in O_3-exposed rats. Whereas ad-libitum fed rats had a prolonged infection and pulmonary inflammation from a *Streptococcus* challenge, calorie restricted rats had no infection and no inflammation.

Ozone exposure has been implicated in altered chemotaxis and cell adhesion properties of AM. Bhalla (1996) reported that macrophages isolated from O_3-exposed SD rats (0.8 ppm O_3 or air, nose-only, for 3 h and then examined AM from BALF at 12 h PE) showed greater mobility and greater adhesion than AM isolated from air-exposed rats. This increased mobility and

adhesion were attenuated when AMs were incubated with monoclonal antibodies to CD11b (leucocyte adhesion molecules) or ICAM-1 (epithelial cell adhesion molecules). The authors suggest that these observed changes in basic cell surface-associated macrophage properties are relevant to subsequent O_3-induced lung inflammatory responses. Using the same O_3-exposure parameters, Pearson and Bhalla (1997) also observed that the increased AM adherence to epithelial cell cultures induced by O_3 exposure was found to be mitigated by pretreatment with antibodies to TNF-α and IL-lα, suggesting that the early inflammatory response to O_3, in part, may be mediated by IL-lα and/or TNF-α.

Additional studies have been carried out to characterize the mechanisms by which O_3 induces decreased lung resistance against microbial pathogens. Cohen et al. (1996) have exposed the WEHI-3 cell line, a BALB/c myelomonocytic AM-like cell, to 1 ppm O_3 for 4 h to determine the effects of O_3 on AM activation by interferon-γ (INF γ). Ozone at this concentration reduced binding of INF γ to AM and affected the AM functional parameters of phagocytic activity, production of reactive oxygen intermediates, and elevation of intracellular calcium. Further,O_3 increased intracellular calcium resting levels and decreased the rates of calcium influx due to digitonin. The authors concluded that this O_3-induced modulation of AM function could be responsible for the increased microbial pathogen survival following O_3 exposure. Pendino et al. (1996) studied the role of glutathione content in AM functions. When BALF recovered from female SD rats exposed to 2 ppm or air for 3 h was assayed for intracellular glutathione (with the fluorescent indicator dye monochlorobimane [MCB]), indicated that AM from O_3-exposed rats had reduced levels of intracellular glutathione compared to air-exposed rats. This reduction in glutathione levels may be due to its interaction with ozonation products from O_3-induced lipid peroxidation.

Bactericidal activity of AM is mediated by hydrogen peroxide production. To study the effect of O_3 on this response function of AM, Cohen et al. (2001,2002) exposed male F-344 rats to either 0.1 or 0.3 ppm O_3 for 4 h/day, 5 days/week or either 1 or 3 weeks and assessed superoxide anion and hydrogen peroxide production in AM recovered from BAL 24 h PE. They found increased superoxide anion production at 1 week 0.1 and 0.3 ppm exposure and did not observe any intergroup differences when stimulated by INFγ. Conversely, hydrogen peroxide production was reduced at both exposure concentrations and durations and was further reduced

with INFγ stimulation. The authors suggest that the compromised killing of bacteria by AM in O_3-exposed rats may be due to the reduction in hydrogen peroxide production.

Laskin et al. (1998a) have examined the activation of AM and type II epithelial cells in female SD rats exposed to 2 ppm O_3 for 3 h. Ozone treatment caused a time-dependent increase in NO levels in both cell types that was correlated with increased expression of iNOS mRNA and protein. Laskin et al (1998b) hypothesized that the inflammatory mediators such as TNF-a and IL-1b may mediate the increase in NO release by activating the expression of iNOS through NF-κB signaling. They demonstrated it by treating the cells with pyrrolidine dithiocarbamate, an inhibitor of NF-κB that caused a dose dependent inhibition of NO production and iNOS expression. This group (Laskin et al., 2002) further investigated the mechanisms by which O_3 activates AM using C57Bl6x129 mice with a targeted disruption of the gene for iNOS. These mice exposed to 0.8 ppm O_3 for 3 h showed no toxicity as measured by BALF protein levels and nitrotyrosine staining of the lung. Additionally, mice overexpressing human Cu, Zn superoxide dismutase (SOD) and mice with a targeted disruption of p50 NF-κB were also resistant to O_3 toxicity. Wild-type mice exposed to O_3 showed an increase in expression of STAT-1, a protein that binds to the regulatory region of iNOS. Taken together, these results suggest to the authors that a number of proteins including NF-κB, phosphoinoside 3-kinase, and STAT-1 that bind to and regulate expression of iNOS are modulated by O_3 exposure. Another study by this group (Fakhrzadeh et al., 2002) used the same iNOS knockout mice strain to further characterize O_3 toxicity. In wild-type mice O_3 exposure causes an increase in AM superoxide anion and prostaglandin (PG)E_2, but in the knockouts, the reactive nitrogen intermediates were not produced and (PG)E_2 was at control levels. Further discussions of the role of nitric oxide synthase and reactive nitrogen in O_3-induced inflammation are contained in Section 5.2.3.5. Additionally, cytokines and chemokines are very important components of the AM response to O_3 and are discussed in detail in Section 5.2.3.4.

AX5.2.2.3 Immune System

Other than by natural protection (e.g., opsonizing antibody, nonspecific phagocytosis by AM), the immune system defends the lung by mounting three major waves of response: natural killer (NK) cells (nonspecific lymphocytes that kill viruses, bacteria, and tumor cells), followed by cytotoxic T-lymphocytes (T_{CTL}- lymphocytes that lyse specifically recognized microbial and

tumor-cell targets), followed by antigen-specific antibodies. These T-cell types are involved with other immunologically active cells (e.g., B-cells and AM), which in a complex manner, interact in immunological defense. To date, only a few of these mechanisms have been investigated in the context of their role in O_3 susceptibility. Effects on the systemic immune system can be different from those in the lung (see Section 5.3). New studies reporting O_3-induced effects on the immune system in the lung are described here.

Garssen et al. (1997) have studied the effects of O_3 on non-IgE-mediated pulmonary hyper-immune reactions induced by picryl chloride (PCI). BALB/c mice sensitized with PCI, both actively and passively (by adoptive transfer of lymphoid cells from pre-sensitized mice), were then challenged with picryl sulfonic acid (PSA). The mice were exposed to12 h of 0.4, 0.8, or 1.6 mg/m^3 O_3 during one night, at 4 days or 7 days after skin sensitization (which was either just before or just after PSA challenge, i.e., during the induction or effector phase). Non-sensitized mice showed no changes in tracheal reactivity to carbacol with O_3 exposure. Sensitized mice were hyperreactive to carbachol 48 h after PSA challenge, whereas sensitized mice exposed to all concentrations of O_3 showed no significant tracheal hyperreactivity to carbachol. The sensitized mice also demonstrated a suppressed inflammatory reaction (PMN) with 1.6 mg O_3 exposure. O_3 exposure following PSA challenge also caused a suppression of tracheal hyperresponsiveness. In a separate experiment wherein mice were exposed to O_3 before sensitization and then lymphoid cells from these mice were injected into non-exposed mice, the recipients also demonstrated an inhibition of the induction of hyperreactivity. These results are opposite to the effect on type I (IgE-mediated) allergic reactions, which the authors suggest is due to activation of Th-2 cell-dependent reactions that are possibly potentiated by O_3 or to a direct effect by O_3 on Th-1 cells or other cells that are crucial for the tracheal hyperreactivity and inflammation seen in this mouse model.

Recent evidence also point towards the potential interaction between the innate and acquired immune system with O_3 exposure. Kleeberger et al. (2000) performed a genome screen on O_3-susceptible (C57BL/6J) and O_3-resistant (C3H/HeJ) mice and identified a candidate gene on chromosome 4, Toll-like receptor 4 (*Tlr4*), a gene implicated in endotoxin susceptibility and innate immunity. When O_3-resistant strain C3H/HeJ and C3H/HeOuJ (differing from the O_3-resistant strain by a polymorphism in the coding region of *Tlr4*) were exposed to 0.3 ppm for 24 to 72 h, greater protein concentrations were demonstrated in the OuJ strain. The two strains

exhibited differential expression of *Tlr4* mRNA with O_3 exposure. These data point to a quantitative trait locus on chromosome 4 as being responsible for a significant portion of the genetic variance in O_3 -induced lung hyperpermeability. Further investigation by this laboratory (Kleeberger et al., 2001a) using these mouse strains showed lavageable protein concentration was lowered by inhibition of inducible nitric oxide synthase (iNOS) and by targeted disruption of *Nos2*. Further comparisons on O_3 exposure in these two strains (C3H/HeJ and C3H/HeOuJ) demonstrated reduced *Nos2* and *Tlr4* mRNA levels in the O_3-resistant C3H/HeJ mice. These data are consistent with the hypothesis that O_3 -induced lung hyperpermeability is mediated by iNOS . These studies also suggested a role for toll-like receptor 4 (TLR4) in the host response to O_3 similar to the role it has demonstrated in LPS sensitivity (Schwartz, 2002; Wells et al., 2003). TLR4 signaling is thought to be critical to linking the innate and acquired immune system through antigen presenting cells and Th1/Th2 differentiation.

Neuhaus-Steinmetz et al. (2000) compared the response to repeated O_3 (180-500 $\mu g/m^3$) and OVA (1%) exposure in "IgE-high responder" (BALB/c) and "IgE-low responder " (C57BL/6) mice. In BALB/c mice exposed to O_3, a T-helper (Th)2-like response consisting of dose-dependent increases in IgE, IL-4, IL-5, and recruitment of eosinophils and lymphocytes into airways was generated. Concurrent O_3/OVA exposures in BALB/c mice increased IgG, antibody titers, leukotrienes, airway responsiveness, and immediate cutaneous hypersensitivity reactions. In C57BL/6 mice only the combined O_3/OVA exposure caused immediate cutaneous hypersensitivity and altered IgG responses, thus demonstrating that O_3 has the potential for shifting the immune response toward a Th2-like pattern in two mouse strains with differing potentials for developing allergic reactions. Becker et al. (1991) have demonstrated changes in IgG production with O_3 exposures of 1.0, 0.5, and 0.1 ppm for 2 h in vitro with human lymphocytes. Subsequent to O_3 exposure, when lymphocytes were stimulated with pokeweed mitogen (PWM, a T-cell-dependent stimulus) or *Staphylococcus aureus* Cowan 1 strain (SAC, a T-cell-independent stimulus), both B and T cells were found to be affected by O_3 preexposure. T cells also demonstrated an increase in IL-6 and a decrease in IL-2, suggesting that O_3 may have direct effects on IgG-producing cells and concurrently an effect that is mediated by altered production of T cell immunoregulatory molecules.

Surfactant protein A and D (SP-A and SP-D) are members of the collectin family, named for their composition of both collagens and lectins. The surfactant proteins are secreted by airway epithelial cells and are a part of the innate immune response with important immunomodulatory function. Perturbations in lung immune defenses lead to a feedback loop between inflammation and SP-A and SP-D levels (reviewed in Hawgood and Poulain, 2001). Oosting et al. (1991) investigated whether surfactant protein A (SP-A) is a target of O_3 toxicity by exposing human and canine SP-A to 0.75 ppm for 4 h. Functionally the in vitro exposure inhibited SP-A self-association and SP-A-mediated lipid vesicle aggregation. Additionally SP-A decreased binding of SP-A to mannose. Structurally, O_3 oxidized tryptophan and methionine residues on the protein. Additional work by Oosting et al. (1992) further examined the effect of O_3 on the function of SP–A from dog and human. In vitro O_3 exposure at 0.4 or 0.75 ppm for 4 h reduced the ability of SP-A to inhibit phospholipid secretion by alveolar type II cells and reduced the capacity of SP-A to induce superoxide anion production and enhance phagocytosis of herpes simplex virus (HSV). In vivo exposures of rats (0.4 ppm for 12 h) generated SP-A less capable of stimulating superoxide anion production by AM. These data suggested that inhibition of interactions between SP-A and alveolar cells may be one mode of toxicity of O_3. Bridges et al. (2000) reported that both SP-A and SP-D directly protect surfactant phospholipids and macrophages from oxidative damage. Both proteins were found to block accumulation of TBARS and conjugated dienes generated during oxidation of surfactant lipids or low density lipoprotein particles by a mechanism that does not involve metal chelation or oxidative modification of the proteins. Wang et al. (2002) expressed human variants of PS-A in CHO cells and then exposed the expressed protein to O_3 (1ppm for 4 h). All of the eight SP-A variants studied showed decreased ability to stimulate cytokine (TNF- and IL-8) production in THP-1 cells, a macrophage-like cell line. Each variant exhibited a unique time- and dose-dependent pattern of stimulation of cytokine production with O_3 exposure, suggesting their potential role in underlying susceptibility to O_3 toxicity. Targeted disruption of mouse SP- A and SP-D (Hawgood et al, 2002) caused increases in bronchoalveolar lavage phospholipid, macrophage, and protein through 24 weeks of age. Further, the deficient mice developed patchy lung inflammation and air space enlargement consistent with emphysema.

AX5.2.2.4 Interaction with Infectious Microorganisms

Numerous investigations have sought to understand the effects of O_3 on overall functioning of host defense systems by challenging animals with infectious agents before, during, or after exposure and observing the outcome, typically mortality. This work (summarized in Table AX5-2) shows that the results are dependent on microbial species, animal species, and O_3 exposure and its temporal relationship to infectious challenge.

Recent studies of O_3-induced modulation of cell-mediated immune responses showed effects on the onset and persistence of infection. Cohen et al. (2001,2002) exposed male F-344 rats to either 0.1 or 0.3 ppm O_3 for 4 h/day, 5 days/week or either 1 or 3 weeks. One day later the rats were instilled with viable Listeria monocytogenes (4×10^6 for 8×10^6) and then tested at 1, 58, 72, or 96 h postinfection. There was no observed effect on cumulative mortality, but there was a concentration-related effect on morbidity onset and persistence. In the one week-exposed rats the listeric burdens trended higher than in controls and the high dose rats showed continual burden increased and without resolution. Levels of IL-1α, TNF-α, and IFNγ were higher than controls at the 48 and 96 h time period. In the 3-week-exposed rats there were no changes in bacterial clearance. Levels of IL-1α, TNF-α, and IFNγ were higher than controls only at the 48 h time period only in the 0.3 ppm-exposed rats. These observations suggest that exposure to O_3 may be causing a possible imbalance between Th-1 and Th-2 cells, which can subsequently lead to suppression of the resistance to intracellular pathogens. In this case, the host defense to Listeria monocytogenes, which is predominantly a Th-1-type response, is adversely affected.

AX5.2.3 Inflammation and Lung Permeability Changes

Ozone has long been recognized to cause lung inflammation and increased permeability, which are distinct events under control by independent mechanisms. The normal lung has an effective barrier function that controls bidirectional flow of fluids and cells between the air and blood compartments. Ozone disrupts this function, resulting in an increase in serum proteins, bioactive mediators and polymorphonuclear leukocytes (PMNs) in the air spaces of the lung. These inflammatory changes have been detected microscopically in tissue preparations and by analyses of bronchoalveolar lavage fluid (BALF). Generally, the initiation of inflammation is part of a defense process; however, its persistence and/or repeated occurrence can result in health

effects. For example, at the early stage, the increase in PMNs may enable more phagocytosis of microbes, and increased edema fluids or protein associated with the PMN influx could provide an enhanced medium for microbial growth, however, these are untested hypotheses. Mediators in the influxing fluid and secreted from the increased number of PMNs can recruit other cells (e.g., fibroblasts and AMs). If released, the large stores of proteolytic enzymes in these phagocytic cells could damage lung tissue over the longer term. Cheek et al. (1994) suggest that PMNs may also play a role in removal of O_3-injured cells. The exact role of inflammation in causation of lung disease is not known, nor is the relationship between inflammation and changes in lung function. However, it is associated with acute changes in pulmonary function and chronic diseases such as asthma, chronic bronchitis and emphysema. Table AX5-3 summarizes key new studies on the potential for O_3 to increase lung permeability and to cause inflammation.

AX5.2.3.1 Time Course of Inflammation and Lung Permeability Changes

Studies on the time course of the inflammatory response (e.g., Cheng et al., 1995) indicated that the maximal increases in BALF protein, albumin and number of PMNs occur 8 to 18 h (depending on the study) post acute exposure. In rats, a single 3-h exposure to 0.5 ppm O_3 produced a significant increase in both permeability and inflammation, but a comparable exposure to 0.3 or 0.15 ppm did not produce an effect (Bhalla and Hoffman, 1997). Dye et al. (1999) reported that an acute exposure to 0.5 ppm O_3 results in a significantly greater lung injury, inflammation and BALF levels of IL-6 in Wistar rats than in Sprague Dawley or F344 rats. BALF cell count, PGE and IL-6 levels were consistently lower in F344 rats compared to other strains. Sun et al. (1997) exposed OVA-sensitized male Dunkin-Hartley guinea pigs to 1 ppm O_3 for 3 h and examined protein levels and PMN levels at 3 h PE. PMN levels were significantly increased, without any change in BAL protein levels, suggesting a lack of correlation between the two endpoints. When guinea pigs exposed to 1 ppm O_3 for 1 h were evaluated 24 h PE, they exhibited the same trend, increase in PMN without a concordant increase in BAL protein levels. Increased AHR observed in the first group but not in the second group suggest a dissociation between PMN levels and AHR.

Depending upon species tested and exposure regimens, continuous exposure for 3 to 7 days resulted in an increase in BALF protein and PMNs that typically peak after a few days and return

Table AX5-3. Effects of Ozone on Lung Permeability and Inflammation

Concentration					
ppm	**µg/m^3**	**Duration**	**Species**	**Effects[a]**	**Reference**
0.1 0.2 0.5	196 392 980	0.5 h in vitro	Primary rat alveolar type II cells	Decreased resistance (R_t) after 0.5 ppm from 2 to 24 h PE and at 48 h in monolayers subjected to PMNs. Significantly lower R_t after PMN treatment at 0.2 and 0.5 ppm.	Cheek et al. (1995)
0.1 0.2 0.4 1.0	196 322 784 1,960	1 h, in vitro	Guinea pig (Hartley) and human alveolar macrophages	Exposure of guinea pig alveolar macrophages to 0.4 ppm for 60 minutes produced a significant increase in IL-6 and TNF-α, and an exposure of human alveolar macrophages to identical O_3 concentration increased TNF-α, IL-1b, IL-6 and IL-8 protein and mRNA expression.	Arsalane et al. (1995)
0.2 0.4 0.8	392 784 1,568	23 h/day for 1 week	Guinea pigs, F (Hartley), 260-330 g	Increase in BALF protein and albumin immediately after 0.8 ppm exposure, with no effect of ascorbate deficiency in diet. O_3-induced increase in BALF PMN number was only slightly augmented by ascorbate deficiency.	Kodavanti et al. (1995)
0.26	510	8 h/day, 5 days/week for 1-90 days	Mice, M (mast cell-deficient and -sufficient), 6-8 weeks old	Greater increases in lavageable macrophages, epithelial cells and PMNs in mast cell -sufficient and mast cell-deficient mice repleted of mast cells than in mast cell-deficient mice. O_3-induced permeability increase was not different in genotypic groups.	Kleeberger et al. (2001b)
0.3	588	48 h and 72 h. Exposures repeated after 14 days	Mice, M (C57BL/6J and C3H/HeJ) 6-8 weeks old	Greater BALF protein, inflammatory cell and LDH response in C57BL/6J than in C3H/HeJ after initial exposure. Repeated exposure caused a smaller increase in BALF protein and number of macrophages, lymphocytes and epithelial cells in both strains, but PMN number was greater in both strains of mice compared to initial exposure.	Paquette et al. (1994)
0.1 0.3 1.0	196 588 1,960	60 min	Rat basophilic leukemia cell line (RBL-2H3)	O_3 inhibited IgE- and A23187 - indued degranulation. Spontaneous release of serotonin and modest generation of PGD2 occurred only under conditions that caused cytotoxicity.	Peden and Dailey (1995)
0.3 2.0	588 3,920	72 h 3 h	Mice (C57BL/6J and C3H/HeJ)	Greater PMN response in C57BL/6J than in C3H/HeJ after acute and subacute exposures. Responses of recombinant mice were discordant and suggested two distinct genes controlling acute and subacute responses.	Tankersley and Kleeberger, (1994)

Table AX5-3 (cont'd). Effects of Ozone on Lung Permeability and Inflammation

Concentration		Duration	Species	Effects[a]	Reference
ppm	µg/m^3				
0.3	588	48 h	Mice (C57BL/6J and C3H/HeJ)	Susceptibility to O_3 is linked to a quantitative trait locus, and TNF-α is identified as a candidate gene.	Kleeberger et al. (1997)
0.3 1.0 2.5	588 1,960 4,900	24 or 48 h 1, 2 or 4 h 2, 4 or 24 h	Mice M (C57BL/6J) 8 weeks old	0.3 ppm for 24 h caused increase in mRNA for eotaxin, MIP-1a and MIP-2 1 ppm for 4 h caused increase in mRNA for eotaxin, MIP-1a, MIP-2 and IL-6 2.5 ppm for 2 and 4 h caused increase in mRNA for MIP-1a, MIP-2 and IL-6 and metallothionein. Greater increases and lethality after 24 h.	Johnston et al. (1999a)
0.3	588	72 h	Mice, M {HeJ, OuJ, Nos2 (+/+) [C57BL/6J-Nos2 (+/+)], and Nos2 (-/-) [C57BL/6J-Nos2 (-/-)]}, 6-8 weeks old	O_3 induced permeability was decreased by pretreatment with a nitric oxide synthase inhibitor and in animals with iNOS gene knocked out.	Kleeberger et al. (2001a)
0.4	784	5 weeks	Guinea pigs, M (Hartley), 5 weeks old (350-450g)	Ovalbumin instillation in the nose caused an increase in O_3-induced infiltration of eosinophils in nasal epithelium.	Iijima et al. (2001)
0.15, 0.3 or 0.5	294 588 980	3 h	Rat, M (SD) 6-8 weeks old	Time-related increase in permeability and inflammation, with a peak at 8 h PE, after 0.5 ppm. No change following exposure to 0.15 or 3 ppm.	Bhalla and Hoffman (1997)
0.5	980	4 h, 12-4 PM for daytime and 7-11 PM for nighttime exposures. Exposures repeated 16 h later.	Rat, M (Wistar), 60-90 days old	Significantly greater increase in IL-6, but not inflammation, following a nighttime exposure compared to daytime exposure. An initial nighttime exposure resulted in lesser inflammation following a subsequent exposure. Pretreatment with IL-6 receptor antibody abrolished cellular adaptive response without affecting inflammatory response induced by initial nighttime exposure.	McKinney et al. (1998)
0.5 1.0 2.0	980 1,960 3,920		Rat, M (Fisher) 90 days old	Increase in BALF protein and albumin occurred immediately after 2 ppm exposure, and at 18 h after 1 ppm. No increase after 0.5 ppm. The movement of water and protein into airspace were not coupled.	Cheng et al. (1995)

Table AX5-3 (cont'd). Effects of Ozone on Lung Permeability and Inflammation

Concentration		Duration	Species	Effects[a]	Reference
ppm	µg/m^3				
1.0-2.0	0-3,920	3 h	Mice (C57BL/6), 6-8 weeks old and rats (Wistar), 14-16 weeks old	Steady state MCP-1 mRNA increase after 0.6 ppm, with maximal increase after 2 ppm. After 2 ppm, MIP-2 mRNA peaked at 4 h PE and MCP-1 mRNA peaked at 24 h PE. BALF neutrophils and monocytes peaked at 24 and 72 h PE, respectively. BALF MCP-1 activity induced by O_3 was inhibited by an anti-MCP-1 antibody.	Zhao et al. (1998)
0.5	980	24 h following a 3-day (6 h/day) exposure to cigarette smoke	Mice, M (B6C3F1) 25 ± 2 g	BALF protein, neutrophils and lymphocytes were increased in animals exposed to smoke and then to O_3. Macrophages from this group also responded with greater release of TNF-α upon LPS stimulation	Yu et al. (2002)
0.5	980	8 h during nighttime	Rat, M (Wistar, SD and F344) 90 days old	Exposure resulted in a significantly greater injury, inflammation and BALF levels of IL-6 in Wistar than in SD or F344 rats.	Dye et al. (1999)
0.8	1,568	2h and 6 h	Rats, M (Fisher), Juvenile (2 months; 180-250 g), Adult (9 months; 370-420 g), Old (18 months; 375-425 g), Senescent (24 months; 400-450 g)	Comparable effect on the leakage of alveolar protein in rats of different age groups, but a greater increase occurred in interleukin-6 and N-acetyl-beta-D-glucosaminidase in senescent animals than in juvenile and adult rats.	Vincent et al. (1996).
0.8	1,568	3 h	Rat, M (SD) 6-8 weeks old	Increased adhesion of macrophages from exposed animals to rat alveolar type II epithelial cells in culture. Treatment with anti-TNF-α + anti-IL-1a antibody decreased adhesion in vitro, but not permeability in vivo	Pearson and Bhalla (1997)
0.8	1,568	3 h	Rat, M (SD) 6-8 weeks old	Increase in fibronectin protein in BALF and lung tissue, and fibronectin mRNA in lung tissue. The increase produced by O_3 was amplified in animals pre-treated intra-tracheally with rabbit serum to induce inflammation.	Gupta et al. (1998)
0.8	1,568	3 h	Rat, M (SD) 200-225 g	Treatment of animals with IL-10 prior to O_3 exposure caused a reduction in O_3 induced BALF protein, albumin and fibronectin and tissue fibronectin mRNA	Reinhart et al. (1999)

Table AX5-3 (cont'd). Effects of Ozone on Lung Permeability and Inflammation

Concentration		Duration	Species	Effects[a]	Reference
ppm	µg/m^3				
0.8	1,568	8 h	Monkey (Rhesus), 3 years 8 months-3 years 10 month old	Pretreatment of monkeys with a monoclonal anti-CD18 antibody resulted in a significant inhibition of O_3-induced neutrophil emigration and accumulation of necrotic airway epithelial cells.	Hyde et al. (1999)
0.8	1,568	48 h	Rat, M (SD) 6-8 weeks old	Cyclophosphamide treatment ameliorated O_3-induced BALF neutrophils and albumin after short term and 1-day exposure. Anti-neutrophil serum reduced lavageable neutrophils but did not affect permeability.	Bassett et al. (2001)
1.0-2.0	1,960-3,920	3 h			
0.8	1,568	8 h	Monkeys, M (Rhesus), 3 years 8 months-3 years 10 months old (5.1-7.6 kg)	Tracheal epithelium of exposed animals expressed b6 integrin. The integrin expression was reduced or undetectable in animals treated with CD-18 antibody.	Hyde et al. (1999)
0.8	1,568	3 h	Mice, F (C57BL6X129NOSII knockout and wild-type B6J129SV F2)	Alveolar macrophages from O_3 exposed wild-type mice produced increased amounts of NO, peroxynitrite, superoxide anion, and PGE2. Nitrogen intermediates were not produced and PGE2 was at control level in exposed NOSII knockout mice. These mice were also protected from O_3-induced inflammation and injury.	Fakhrzadeh et al. (2002)
1.0	1,960	5 min exposure of airway segments following bronchoscopy	Dogs, M (Mongrel) Adult	Mast cells from O_3-exposed airways of ascaris sensitive dogs released significantly less histamine and PGD2 following in vitro challenge with ascaris antigen or calcium ionophore.	Spannhake (1996)
1.0	1,968	8 h	Monkeys (Rhesus)	Increase in steady state IL-8 mRNA in airway epithelium. Increase in IL-8 protein staining declined at 24 h after exposure.	Chang et al. (1998)
0.2 0.5 1.0	392 980 1,960	In vitro at liquid/air interface	Primary TBE, BEAS-2b S and HBE1	Dose related increase in IL-8 release in the conditioned media. Ozone produced greater toxicity in cell lines than in primary cultures.	

Table AX5-3 (cont'd). Effects of Ozone on Lung Permeability and Inflammation

Concentration		Duration	Species	Effects[a]	Reference
ppm	µg/m^3				
1.0	1,960	3 h	Rats, M (Brown Norway) 200-250 g	Increase in lung CINC mRNA within 2hr after the end of exposure and increase in BALF neutrophils at 24 h. Treatment with anti- CINC antibody reduced neutrophil influx, but not bronchial hyperreactivity.	Koto et al. (1997)
1.0	1,960	3 h	Rat, M (SD) 6-8 weeks old	Time-related increase in BALF protein, fibronectin (Fn), and alkaline phosphatase (AP) activity. Fn mRNA detected in macrophages , and AP in type II cells and in BALF PMNs from exposed animals only.	Bhalla et al. (1999)
1	1,960	2 h	Rats, F (SD)	The expression of CD18 on alveolar macrophages and CD11b on blood PMNs was lowered by exposure, but CD62L expression on blood PMNs was not affected.	Hoffer et al. (1999)
1	1,960	3 h	Rat, M (SD) 6-8 weeks old	Time-related increase in BALF albumin, PMNs, MIP-2 and ICAM-1, and increase in MIP-2 mRNA only at early time point in BALF macrophages. MIP-2 mRNA not detected in lung tissue.	Bhalla and Gupta (2000)
1	1,960	3 h	Rat, M (SD) 250-275 g	Ozone induced increase in BALF albumin, fibronectin and PMN number was associated with an increase in expression of TNF-α, IL-1a, IL-6 and IL-10 mRNA. Pretreatment with anti-TNF-α antibody caused downregulation of gene expression and in reduction of BALF albumin and PMN number, but not fibronectin.	Bhalla et al. (2002)
1	1,960	6 h	Rat, M (S-D), 200-250 g	Increase in number of macrophages with mRNA transcripts and immunocytochemical staining of IL-1, TNF-α, MIP-2 and cytokine-induced neutrophil chemoattractact (CINC). Chemokine activities were reduced by treatment of macrophages with anti-IL-1b and anti-TNF-α antibodies.	Ishii et al. (1997)
0.5 1.0 2.5	980 1,960 4,900	4 h	Mice, M (129 wild-type or clara cell seceretory protein -/-), 2-5 months old	Increases in IL-6 and metallothionein mRNA by 2 h after exposure to 1 ppm. mRNA increases were further enhanced in ccsp -/- mice.	Mango et al. (1998)

Table AX5-3 (cont'd). Effects of Ozone on Lung Permeability and Inflammation

Concentration		Duration	Species	Effects[a]	Reference
ppm	µg/m^3				
1.0	1,960	8 h/night for three nights	Mice, (C57Bl/6 wild-type and iNOS knockout)	O_3 exposure produced greater injury, as determined by measurement of MIP-2, matrix metalloproteinases, total protein, cell content and tyrosine nitration of whole lung protein, in iNOS knockout mice than in wild-type mice.	Kenyon et al. (2002)
1.0	1,960	4 h	Mice, M (129 strain, wild-type and clara cell secretory protein-deficient), 2-3 mo old	Increases in abundance of mRNAs encoding eotaxin, MIP-1a and MIP-2 in CCSP-/-, but no change in wild-type mice.	(Johnston et al. (1999b)
1.2	2,352	6 h	Rat, M (BN), 200-250 g	Eotaxin mRNA expression in the lungs increased 1.6-fold immediately after and 4-fold at 20 h. Number of lavageable eosinophils increased 3- and 15-fold respectively at these time points. Alveolar macrophages and bronchial epithelial cells stained positively for eotaxin.	Ishii et al. (1998)
2.0	3,920	3 h	Mice, M (C57BL/6J)	O_3-induced increase in protein and PMNs in BALF, and pulmonary epithelial cell proliferation were significantly reduced in animals pre-treated with UK-74505, a platelet activating factor-receptor antagonist.	Longphre et al. (1999)
2.0	3,920	3 h	Rat, F (SD) 6-8 weeks old	BALF cells from exposed animals released 2 to 3 times greater IL-1 and TNF-α, and greater fibronectin. Immunocytochemistry showed greater staining of these mediators in lung tissue from exposed rats.	Pendino et al. (1994)
2.0	3,920	3 h	Rat, F (SD) 6-8 weeks old	Increase in BALF macrophage number and total protein. Increase in iNOS expression, and increase in Fibronectin and TNF-α production by alveolar macrophages. O_3 effects were reduced by pretreatment with gadolinium chloride, a macrophage inhibitor.	Pendino et al. (1995)
2.0	3,920	4 h	Rat, M (Wistar), 200-225 g	A transient increase in tissue neutrophils correlated with an elevation and subsequent decline in airway hyperresponsiveness. Pretreatment of rats with anti-neutrophil serum protected the animals from O_3-induced airway hyperresponsiveness	DeLorme et al. (2002)

Table AX5-3 (cont'd). Effects of Ozone on Lung Permeability and Inflammation

Concentration		Duration	Species	Effects[a]	Reference
ppm	µg/m^3				
2.0	3,920	3 h	Mice, M (mast cell-sufficient and -deficient, and deficient mice repleted with mast cells) 6-8 weeks old	Significant increases in BALF PMNs and total protein in mast cell -sufficient mice than in mast cell-deficient mice. Mast cell-deficient mice that were repleted with mast cells showed increases compared to mast cell-deficient mice that were not repleted with mast cells.	Longphre et al. (1996)
1.1	1,960	8 h	Rat-depleted of neutrophils	Epithelial necrosis in the nasal cavity, bronchi, and distal airways. Proliferation of terminal bronchiolar epithelial cells also decreased by O_3 exposure.	Vesely et al. (1999a)
0.32	627	48 h (subcutaneous) 3 h (acute)	Mice C57BL/6J WT TNRF1KO TNRF2KO	TNFR1 and TNFR2 KOs less sensitive to subacute O_3 exposure than WT. With acute exposures, airway hyperreactivity was diminished in KO mice compared to WT mice, but lung inflammation and permeability were increased.	Cho et al. (2001)
0.3	588	24 to 72 h	Mice C57BL/6J C3H/HeJ C3H/HeOuJ	Differential expression of *Tlr4* mRNA.	Kleeberger et al. (2000)
2.0	3,920	3 h	Mouse C3H/HeJ, A/J, C57BL/6J, 129/SvIm,CAST/Ei,BTBR,DBA/2J, FVB/NJ,BALB/cJ.	Two strains consistently O_3-resistant: C3H/HeJ and A/J. Two strains consistently O_3-vulnerable: C57BL/6J and 129/SvIm Five strains with inconsistent phenotypes with intermediate responses: CAST/Ei, BTBR, DBA/2J, FVB/NJ, and BALB/cJ.	Savov et al. (2004)

[a] PMN = Polymorphonuclear leukocyte.
PE = Postexposure (time after O_3 exposure ceased).
BAL = Bronchoalveolar lavage.
BALF = Bronchoalveolar lavage fluid.

towards control even with continuing exposure. Van Bree et al. (2002) reported adaptation of rats to O_3 following 5 days of exposure. Animals exposed for 5 days had lower BALF proteins, fibronectin, IL-6, and inflammatory cells than animals exposed for 1 day. Postexposure challenge with single O_3 exposures at different time points showed that a recovery of susceptibility to O_3 (as measured by BALF levels of albumin, IL-6, and the number of macrophages and neutrophils) occurred at ~15-20 days, but total protein and fibronectin levels remained attenuated even at 20 days post-5-day exposure. The recovery with regards to BrdU labeling occurred in 5-10 days after the 5 day exposure. McKinney et al. (1998) investigated the role of IL-6 in the adaptive response induced by repeated O_3 exposures and observed a significant increases in IL-6 levels following a nighttime exposure of rats to 0.5ppm O_3 as compared to a daytime exposure. The kinetics of inflammation were similar following these exposures, but a second exposure subsequent to the nighttime exposure resulted in lesser inflammation than an exposure subsequent to a daytime exposure. Pretreatment of rats with an anti-IL-6 receptor antibody prior to the nighttime exposure abolished O_3-induced adaptation with regards to IL-6.

The time course of the influx of PMNs into the lung and the BALF fluid levels of macrophage inflammatory protein-2 were found to be roughly similar to that for proteins (Bhalla and Gupta, 2000). Adherence of neutrophils to pulmonary vascular endothelium is maximal within 2 h after exposure and returns to control levels by 12 h PE (Lavnikova et al., 1998). In an in vitro system utilizing rat alveolar type II cell monolayers, O_3 produced a dose-dependent increase in permeability (Cheek et al., 1995). At higher O_3 levels, neutrophils exacerbated the injury, but their presence after the exposure expedited restoration of epithelial barrier.

Vesely et al. (1999a) have demonstrated that neutrophils contribute to the repair process in O_3 -injured airway epithelium. When rats were depleted of neutrophils by rabbit anti-rat neutrophil serum, and exposed to 1 ppm O_3 for 8 h, epithelial necrosis in the nasal cavity, bronchi, and distal airways were observed, suggesting a role for neutrophils in repair processes. Proliferation of terminal bronchiolar epithelial cells, as assessed by BrdU-incorporation, was also decreased by O_3 exposure, suggesting a role for neutrophils in this process.

AX5.2.3.2 Concentration and Time of Exposure

The relative influence of concentration and duration of exposure (i.e., C × T) has been investigated extensively in rats, using BALF protein as an endpoint. Though the interaction between C and T is complex, concentration generally dominated the response. The impact of T was C-dependent (at higher Cs, the impact of T was greater); at the lowest C and T values, this dependence appeared to be lost.

New studies evaluating C × T relationships have not been found.

AX5.3.3.3 Susceptibility Factors

Factors that have been studied for potential impact on the effects of O_3 include age, gender, nutritional status, genetic variability, exercise and exposure to co-pollutants.

The effects of age on lung inflammation are not well known. Vincent et al. (1996) found O_3 did not differentially affect the leakage of alveolar protein in rats of different age groups, but an O_3-induced increase in IL-6 and N-acetyl-beta-D-glucosaminidase (NAG) was observed in senescent animals compared to juvenile and adult rats. Johnston et al. (2000b) compared gene expression of chemokines and cytokine in newborn and 8-week-old C57Bl/6J mice exposed to 1.0 or 2.5 ppm O_3 for 4, 20, or 24 h. The animals were killed immediately after exposure, total RNA was isolated from lung tissue, ribonuclease protection assays were completed for a number of cytokines/chemokines including IL-12, IL-10, IL-1α, IL-1β, IL-1Ra, MIF, IFN-γ, MIP-1α, MIP-2,IL-6, and Mt. The newborn mice displayed increased levels of Mt mRNA only, while the 8-week-old mice had increases in MIP-1α, MIP-2, IL-6, and Mt mRNA. Comparisons were made with mice of the same age groups with exposures to endotoxin (10 min). Both age groups displayed similar cytokine/chemokine profiles with endotoxin exposure. This suggested to the authors that the responses to endotoxin, which does not cause epithelial injury, and the responses to O_3, which does, demonstrate that differences in inflammatory control between newborn and adult mice is secondary to epithelial injury.

Ascorbate deficiency had been found to have only minimal effect on injury and inflammation in guinea pigs exposed to O_3 (Kodavanti et al., 1995). Elsayed (2001) demonstrated that a general dietary restriction to 20% of the freely-fed diet for 60 days caused an extreme reduction in body weight in 1-month-old SD rats. These rats, exposed to 0.8 ppm continuously for 3 days, had levels of antioxidants and detoxifying enzymes that were increased

less than in freely fed animals. Lung injury, as detected by cell proliferation in CAR and BALF levels of protein, number of neutrophils, was increased in sidestream cigarette smoke exposed mice that were subsequently exposed to O_3 (Yu et al., 2002). Macrophages from smoke + O_3 exposed animals also responded by a greater release of TNF-α following LPS stimulation when compared to macrophages exposed to air, smoke or O_3 alone.

Recent lines of evidence illustrate the importance of genetic susceptibility in O_3 health effects. The effects of acute and subacute exposures were studied by Tankersley and Kleeberger (1994) in inflammation-prone (susceptible) C57BL/6J(B6) and inflammation-resistant C3H/HeJ(C3) strains of mice. Based on the neutrophilic response to O_3 in these two strains and in recombinant mice, the authors concluded that the acute and subacute exposures are controlled by two distinct genes, referred to as *Inf*-1and *Inf*-2 respectively. Exposures, when repeated fourteen days after the initial exposures, caused a smaller increase in BALF protein and number of macrophages, lymphocytes and epithelial cells in both strains, but PMN number was greater in both strains compared to initial exposure (Paquette et al., 1994).

Further studies by Kleeberger et al. (1997) identified another potential susceptibility gene, tumor necrosis factor (*Tnf*), on a qualitative trait locus on mouse chromosome 17. *Tnf* codes for the pro-inflammatory cytokine TNF-α. By neutralizing the function of TNF-α with a specific antibody, they were able to confer protection against O_3 injury in susceptible mice. Cho et al. (2001) demonstrated a role for tumor necrosis factor receptor 1 and 2 (TNFR1 and TNFR2, respectively) signaling in subacute (0.3 ppm for 48 h) O_3-induced pulmonary epithelial injury and inflammation. TNFR1 and TNFR2 knockouts were less sensitive to subacute O_3 exposure than wild-type C57BL/6J mice. With acute exposures to O_3 (2 pm for 3 h), airway hyperreactivity was diminished in knockout mice compared to wild-type mice, but lung inflammation and permeability were increased. Based on these studies, it has been hyposthesized that in subacute O_3 exposures, TNF-α is a susceptibility gene in mice, and further, that independent mechanisms control lung inflammation and permeability. Further evidence for the mechanistic separation of hyperresponsiveness and PMN infiltration was provided by Shore et al. (2001) from studies using wild-type and TNFR knockout mice exposed to 2 ppm O_3 for 3 h. Numbers of PMN in BAL collected 21 h PE were not changed in air exposures, but were increased to the same extent in both wild-type and TNFR knockout mice, whereas hyperresponsiveness was increased only in wild-type mice, but not in the knockouts.

Prows et al (1999) characterized the differences between A/J (O_3-sensitive) and C57BL/6J (O_3-resistant) mice exposed to 10 ppm O_3, which continued until the animals died. Sensitive animals survived ≤13 h and resistant mice survived > 13 h. Histological examination of the lungs and wet-to-dry lung weight ratios did not differ between the two strains. Though the dose given was not environmentally relevant, the genome wide scans confirmed earlier findings (Prows et al. 1997) that two loci (acute lung injury-1 and -3, Ali1 and Ali3, respectively) on chromosome 11 control susceptibility to death after O_3 exposure.

A recent comprehensive characterization of lung injury in nine inbred mouse strains (Savov et al., 2004) has been summarized in Table AX5-4. The exposure consisted of 3 h of 2.0 ppm O_3, followed by room air, for 6 h or 24 h PE and analyzed for plethysmography, MCh challenge, BALF, histology, and single nucleotide polymorphisms (SNPs). This group identified two strains (C3H/HeJ and A/J) as consistently O_3-resistant, and two strains (C57BL/6J and 129/SvIm) as consistently O_3-vulnerable. Five strains were characterized as having inconsistent phenotypes with intermediate responses to O_3 (CAST/Ei, BTBR, DBA/2J, FVB/NJ, and BALB/cJ). Their in silico genome scan identified on chromosome 1, a 170- to 189-Mb region associated with the 6-h airway hyperreactivity response and the 24-h inflammatory response; on chromosome 7, a 30- to 40-Mb region associated with the 6-h inflammatory response and the 6-h protein increase; and on chromosome 17, a 30- to 40-Mb region associated with the 24-h airway hyperreactivity response. They found no consistent correlation between the concentration of total protein in BALF and influx of inflammatory cells (PMN), which they attribute to be regulated by different genes. They did find a correlation between O_3-induced increases in IL-6 and PMN concentration.

Ozone-induced changes in CCSP (called CC16 by this group) expression were evaluated in five inbred mouse strains: C57BL/6J and CBA both considered sensitive to acute O_3-induced inflammation, C3H/HeJ and AKR/J both considered resistant, and SJL/J considered intermediate (Broeckaert et al., 2003). Two exposures paradigms were used, 1.8 ppm O_3 for 3 h or 0.11 ppm O_3, 24/h day for up to 3 days, and BALF and serum were assayed immediately after exposure or at 6 h PE. Both exposure levels caused a transient increase in CC16 in serum that correlated with BALF changes in protein, LDH, and inflammatory cells. There was an inverse relationship between preexposure levels of CC16 in BALF and epithelial damage based on serum CC16 levels and BALF markers of inflammation. There was also an inverse relationship between

Table AX5-4. Inbred Mouse Strain Susceptibility

	P_{enh}			PMN		Protein		IL-6		% PCNA	Overall Response to O_3
Mouse Strain	**Baseline**	**O_3 only**	**O_3 then MCh**	**6h**	**24h**	**6h**	**24h**	**6h**	**24h**	**24h**	
C57BL/6J	hyporeactive	susceptible	much more responsive	↑	↑↑↑	ns	ns	↑↑	↑	> 4	highly sensitive
129/SvIm	hyperreactive	susceptible	more responsive	↑↑	↑↑	↑	ns	↑↑	↑	> 2	highly sensitive
BTBR	hyperreactive	susceptible	more responsive	↑↑	↑↑	↑↑	↑↑	↑↑↑	↑	< 1	intermediate
BALB/cJ	intermediate	susceptible	more responsive	↑ ns	↑ ns	ns	↑	↑	ns	< 1	intermediate
DBA/2J	intermediate	resistant	less responsive	↑↑	↑	ns	↑	↑	↑	> 1	intermediate
A/J	very hyperreactive	resistant	much less responsive	↑↑	↑↑	ns	ns	↑↑	ns	> 4	highly resistant
FVB/NJ	intermediate	resistant	less responsive	↑↑	↑↑	↑	↑	↑	ns	> 1	intermediate
CAST/Ei	intermediate	resistant	less responsive	↑↑↑	↑↑↑	↑	↑↑	↑↑↑	↑	< 1	intermediate
C3H/HeJ	intermediate	resistant	less responsive	↑	↑	ns	↑	↑↑	ns	< 1	highly resistant

preexposure levels of albumin in BALF and lung epithelium damage. Based on these results, the authors conclude that a major determinant of susceptibility to O_3 is basal lung epithelial permeability. As all of the mouse strains had similar levels of preexposure CC16 mRNA, they explored the possible role of CC16 isozymes in differences among strains. The CC16 monomer a 7kD protein exist in two isoforms with differing p*I* values, CC16a (4.9) and CC16b (5.2). To evaluate the role of CC16 isoform profiles in permeability differences between C57BL/6J and C3H/HeJ, this group evaluated the CC16 protein profiles in BALF of the strains before and after O_3 exposure following two-dimensional protein electrophoresis analysis. C57BL/6J mice had lower levels of CC16a (the more acidic form) than C3H/HeJ. But both the strains had similar levels of CC16b. Based on these observations Broeckaert et al (2003) conclude that greater epithelial permeability observed in C57BL/6J may be due to difference in the expression of CC16a and possibly other antioxidant/inflammatory proteins.

Wattiez et al. (2003) examined BALF protein from C57BL/6J (O_3-sensitive) and C3H/HeJ (O_3-resistant) mice exposed to filtered air using a two-dimensional polyacrylamide gel approach to analyze the protein profiles. C3H/HeJ mice expressed 1.3 times more Clara cell protein16 (CC16) than C57BL/6J mice, and further, expressed more of the acidic isoform of CC16. Strain-specific differential expression of isoforms of the antioxidant protein 2 (AOP2), the isoelectric point 5.7 isoform in C3H/HeJ and isoelectric point 6.0 isoform in C57BL/6J were observed. These studies suggested a potential role for the strain-specific differential expression in their protein toward differential susceptibility to oxidative stress.

AX5.2.3.4 Mediators of Inflammatory Response and Injury

While neutrophils in the lung characterize an inflammatory response to O_3, the release of chemotactic mediators by inflammatory cells indicates their state of activation and their role in continued inflammation and injury. Studies in recent years have placed a greater focus on these mediators to understand the mechanisms implicated in O_3-induced inflammation and injury. Cytokines and chemokines have been shown to be released as a result of stimulation or injury of macrophages, epithelial cells and PMNs. Many of these mediators have been implicated in PMN recruitment in the lung following O_3 exposure. The expression of macrophage inflammatory protein 2 (MIP-2) mRNA or BALF levels of MIP-2 increased in mice and rats exposed to O_3 concentrations equal to or greater than 1 ppm (Driscoll et al. 1993; Haddad et al., 1995; Bhalla

and Gupta, 2000). The increased mRNA expression was associated with an increased neutrophilia in the lung. Ozone exposure also caused an increase in monocyte chemotactic protein-1 (MCP-1) mRNA in mice and rats (Zhao et al., 1998). These studies implicate MCP-1 in O_3-induced monocyte accumulation in the lung and suggest a role of NFkB in MCP-1 gene expression. Fibronectin, an extracellular matrix glycoprotein, has been studied for its role in lung inflammation and inflammatory disorders. Gupta et al. (1998) observed an increase in both fibronectin protein and mRNA expression in the lung of rats exposed to 0.8 ppm O_3. A mechanistic role of fibronectin in O_3-induced inflammation and injury was suggested on the basis of comparability of temporal changes in BALF protein, fibronectin and alkaline phosphatase activity (Bhalla et al., 1999). Numerous studies have reported O_3-induced differential expression of various cytokines and inflammatory mediators both in vivo and ex vivo: increased expression of cytokine-induced neutrophil chemoattractant (CINC) and NF-κB expression in vivo (Haddad et al., 1996; Koto et al., 1997)*;* IL-8 both in vivo and in vitro (Chang et al., 1998); tumor necrosis factor (TNF-α), fibronectin, interleukin-1 (IL-1), and CINC release by macrophages ex vivo (Pendino et al., 1994; Ishii et al., 1997), and NF-αB and TNF-α (Nichols et al., 2001). An increase in lung CINC mRNA occurred within 2 h after the end of a 3 h exposure of rats to 1 ppm O_3. The CINC mRNA expression was associated with neutrophilia at 24 h post-O_3 exposure. Exposure of guinea pig alveolar macrophages recovered in BALF and exposed in vitro to 0.4 ppm O_3 for 60 minutes produced a significant increase in IL-6 and TNF-α (Arsalane et al., 1995). An exposure of human AMs to an identical O_3 concentration increased TNF-α, IL-1b, IL-6 and IL-8. This exposure also caused an increase in mRNA expression for TNF-α, IL-1b, IL-6 and IL-8 in human cells. Ozone exposure caused an increase in IL-6, MIP-1a, MIP-2, eotaxin and metallothionein (MT) expression in mice (Johnston et al., 1999a). The IL-6 and MT increase was enhanced in mice deficient in Clara cell secretory protein (CCSP), suggesting a protective role of Clara cells and their secretions (Mango et al., 1998). CCSP deficiency also increased sensitivity of mice to O_3, as determined by an increase in abundance of MIP-1a and MIP-2 following a 4 h exposure (Johnston et al., 1999b).

A role for mast cells in airway responses is proposed on the basis of chronic exposure studies demonstrating greater increases in lavageable macrophages, epithelial cells and PMNs in mast cell-sufficient mice compared to mast cell-deficient mice exposed to 0.26 ppm O_3 (Kleeberger et al., 2001b). Similar results were earlier reported by the same group using a higher

O_3 concentration (Longphre et al., 1996). Increases in inflammatory cells were also observed in mast cell-deficient mice repleted with mast cells, but O_3-induced permeability increase was not different in genotypic groups exposed to 0.26 ppm. When a mast cell line was exposed to varying O_3 concentrations, spontaneous release of serotonin and modest generation of PGD2 occurred only under conditions that caused cytotoxicity (Peden and Dailey, 1995). Additionally, O_3 inhibited IgE- and A23187-induced degranulation. Mast cells recovered from O_3-exposed peripheral airways of ascaris sensitive dogs released significantly less histamine and PGD2 following in vitro challenge with ascaris antigen or calcium ionophore (Spannhake, 1996). Ozone exposure also promoted eosinophil recruitment in the nose and airways in response to instillation of ovalbumin or ovalbumin-pulsed dendritic cells and aggravated allergy like symptoms in guinea pigs (Iijima et al., 2001).

Treatment of rats with cyclophosphamide prior to O_3 exposure resulted in a decreased recovery of PMNs in the BALF and attenuated permeability induced by O_3 (Bassett et al., 2001). Additionally, they found that pretreatment of animals with antiserum against rat neutrophils abrogated PMN accumulation in the lung, but did not alter permeability changes produced by O_3. DeLorme et al. (2002) showed a relationship between neutrophilic inflammation and airway hyperresponsiveness. Treatment of rats with anti-neutrophil serum protected the animals from O_3-induced airway hyperresponsiveness. Studies utilizing antibodies to selected pro- or anti-inflammatory cytokines suggest a role of TNF-α, interleukin-10 (IL-10) and IL-1b in O_3-induced changes in permeability, inflammation and cytokine release (Ishii et al., 1997; Reinhart et al., 1999; Bhalla et al., 2002). An attenuation of O_3-induced increase in permeability and inflammation was also observed in mice treated, either before or after exposure, with UK-74505, a platelet-activating factor receptor antagonist (Longphre et al., 1999), suggesting that O_3-induced epithelial and inflammatory changes are mediated in part by activation of PAF receptors.

Ozone exposure stimulates macrophage motility towards a chemotactic gradient, and macrophages from rats exposed to 0.8 ppm O_3 adhered to epithelial cells (ARL-14) in culture to a greater extent than macrophages from air-exposed controls (Bhalla, 1996). Both macrophage motility and chemotaxis were attenuated by antibodies to cell adhesion molecules CD-11b and ICAM-1. An exposure of female rats to O_3 had an attenuating effect on CD-18 expression on alveolar macrophages and vascular PMNs, but the expression of CD62L, a member of selectin family, on vascular PMNs was not affected (Hoffer et al., 1999). In monkeys, the O_3-induced

inflammation was blocked by treatment with a monocolonal antibody to CD18, suggesting dependence of PMN recruitment on this adhesion molecule (Hyde et al., 1999). Treatment of monkeys with CD 18 antibody also reduced tracheal expression of the beta6 integrin (Miller et al., 2001). A single 3 h exposure of rats to O_3 resulted in an increase in neutrophil adhesion to epithelial cells in culture (Bhalla and Young, 1992) and caused an elevation in concentration of ICAM-1, but not CD-18, in the BALF (Bhalla and Gupta, 2000). Takahashi et al. (1995a) found an increase in tissue expression of ICAM-1 in mice exposed to 2 ppm O_3. They noted a temporal correlation of inflammatory activity and ICAM-1 expression which varied in different regions of the lung. A comparable pattern of time-related changes in total protein, fibronectin and alkaline phosphatase activity in the BALF of rats exposed to 0.8 ppm O_3 was also observed by Bhalla et al. (1999).

AX5.2.3.5 Role of Nitric Oxide Synthase and Reactive Nitrogen in Inflammation

An acute exposure of rats to 2 ppm O_3 caused an increase in the expression of iNOS activity with an increase in BALF macrophage number and total protein and increase in fibronectin and TNF-α production by AMs (Pendino et al., 1995). All of these effects of O_3 were reduced by pretreatment with gadolinium chloride, a macrophage inhibitor. Macrophages isolated from O_3-exposed mice produced increased amounts of nitric oxide, superoxide anion and PGE2, but production of these mediators by macrophages from NOS knockout mice was not elevated (Fakhrzadeh et al., 2002). Additionally, mice deficient in NOS or mice treated with N^G-monomethyl-L-arginine, an inhibitor of total NOS, were protected from O_3-induced permeability, inflammation and injury, suggesting a role of nitric oxide in the production of O_3 effects (Kleeberger et al., 2001b; Fakhrzadeh et al., 2002). Another study demonstrated greater injury (as determined by measurement of MIP-2, matrix metalloproteinases, total protein, cell content and tyrosine nitration of whole lung protein) in iNOS knockout mice than in wild-type mice on O_3 exposure (Kenyon et al., 2002). They proposed that protein nitration differences are related to inflammation and may not be dependent on iNOS-derived NO.

Ishii et al. (2000a) performed studies using pretreated rats with ebselen (a potent anti-inflammatory, immunomodulator and NO/peroxynitrite scavenger) and then exposed to 2 ppm O_3 for 4 h. The pretreated rats had decreased numbers of neutrophils, lowered albumin levels, and inhibited nitration of tyrosine residues in BALF 18 h PE, without changes in macrophage iNOS

expression. These results suggest that an iNOS-independent mechanism may be involved in O_3-induced inflammation. Inoue et al. (2000) demonstrated in human transformed bronchial epithelial cells that NO-generating compounds (TNF-α, IL-1β, and INF-γ) induce IL-8 production and that NOS inhibitors inhibit IL-8 production. In vivo experiments in the same study using male Hartley-strain guinea pigs exposed to 3 ppm O_3 for 2 h showed that NOS inhibitor pretreatment attenuated-O_3 induced neutrophil recruitment and airway hyperresponsiveness at 5 h after exposure. The NOS inhibitors also blunted the increase in nitrate/nitrite levels and in IL-8 mRNA, at the 5 h PE. The authors hypothesize that NO, or its derivatives, facilitate airway hyperresponsiveness and inflammation after O_3 exposure, possibly mediated by IL-8. Jang et al. (2002) have attempted to characterize the mechanism by which short-term O_3 exposures (0.12, 0.5, 1, or 2 ppm for 3 h) cause airway inflammation and responsiveness in BALB/c mice. Using a modified Griess reaction, measurement of nitrate and nitrite in BAL fluid after O_3 exposure showed dose-dependent increases in nitrate, which is indicative of in vivo NO generation. Functional studies of enhanced pause (P_{enh}) demonstrated increases with O_3 which were also dose-dependent. Western blot analysis of lung tissue showed increases in NOS-1, but not in NOS -3 or iNOS isoforms. The authors conclude that in mice NOS-1 may induce airway responsiveness by a neutrophilic airway inflammation.

AX5.2.4 Morphological Effects

AX5.2.4.1 Introduction

All laboratory animal species studied to date show generally similar morphological responses to < 1 ppm O_3. The precise characteristics of the structural changes due to O_3 are dependent on the exposure regimen, time of examination, distribution of sensitive cells, and the type of centriacinar region (i.e., junction between the end of the terminal bronchioles and the first few generations of either respiratory bronchioles or alveolar ducts, depending on the species).

The presentation of this morphology section will begin with effects of short-term exposure (≤ 1 week). The subsequent discussion of long-term exposure effects (>1 week) is an artificial separation, but consistent with the division of studies in other effects sections. The long-term studies that included evaluation of various durations of exposures are presented totally in this subsection to illustrate the effects of exposure duration and to provide a better understanding of possible chronic effects of multi-seasonal, ambient O_3 exposures in the population (Chapter 7).

AX5.2.4.2 Short-Term Exposure Effects on Morphology

Morphological effects of key new exposure studies generally lasting less than 1 week are summarized in Table AX5-5. The following discussion is by region of the respiratory tract first, followed by exposure and susceptibility factors.

Hotchkiss et al. (1998) explored the efficacy of a topical anti-inflammatory corticosteriod, fluticasone propionate (FP), in preventing the inflammation and mucous cell metaplasia in rats after cumulative O_3 exposure. Male F-344 rats were exposed to filtered air or 0.5 ppm O_3, 8 h/day, for 3 or 5 days. Immediately before and after exposure, the rats were given FP (25 μg) by intranasal instillation (50 μL/nasal passage) or an equivalent amount of vehicle only. Nasal tissues were processed for light microscopy 2 h and 3 days after the 3- and 5-day exposures, respectively. Rats treated with FP had 30 to 60 % less nasal inflammation after 3 and 5 days of O_3 exposure and 85% less mucous cell metaplasia after the 5-day exposure compared with vehicle-instilled, O_3-exposed controls.

Fanucchi et al. (1998) reported that exposure to bacterial endotoxin, a common ambient air toxin, can potentiate mucous cell metaplasia in the nasal transitional epithelium of rats caused by a previous O_3 exposure. Male F344/N Hsd rats were intranasally instilled with saline or 100 μg/ml endotoxin after exposure to filtered air or 0.5 ppm O_3, 8 h/day for 3 days, and evaluated 6 h and 3 days PE. Mucous cell metaplasia was not found in the air/endotoxin group, but was found in the O_3/saline group and was most severe in the O_3/endotoxin group. A similar synergistic effect of O_3 and endotoxin on the nasal epithelium was reported in Fischer rats by Wagner et al. (2001a,b). When exposed to O_3 alone (0.5 ppm, 8 h/day for 3 days), rats developed epithelial lesions in the nasal transitional epithelium. Exposure to endotoxin alone (20 μg) caused lesions in the respiratory epithelium of the nose and conducting airways. Endotoxin enhancement of the O_3-induced mucous cell metaplasia was related to neutrophilic inflammation.

Cho et al. (1999a, 2000) reported that O_3-induced mucous cell metaplasia in the transitional epithelium of rats may be dependent on pre-metaplastic responses, such as mucin mRNA upregulation, neutrophilic inflammation, and epithelial proliferation. Male F344/N rats were exposed to 0.5 ppm O_3, 8 h/day for 1, 2, or 3 days and 2 h, or 1, 2, or 4 days PE were assayed for the parameters listed above. A rapid increase in an airway-specific mucin gene (rMuc-5AC mRNA) occurred rapidly after exposure to O_3, both before and during the onset of mucous cell metaplasia. Neutrophilic inflammation coincided with epithelial DNA synthesis and

Table AX5-5. Effects of Ozone on Lung Structure: Short-Term Exposures

Concentration					
ppm	µg/m^3	Duration	Species	Effects[a]	Reference
0.1 0.5 1.0	196 980 1,960	8 h/ day x 1 day 8 h/day x 1 day 8 h/day x 1, 10, 75, and 90 days	Rat; male; Sprague-Dawley	No dose-related response on CYP2E1, one of six P450 enzymes identified in respiratory tissue. CYP2E1 activity was elevated (250% and 280%) in the lobar bronchi / major daughters airways immediately after 1.0 ppm O_3 exposure for 1day and 10 days, respectively, but not in the trachea or distal bronchioles; CYPE1 activity was unchanged and decreased after 1.0 ppm O_3 exposure for 75 and 90 days, respectively.	Watt et al. (1998)
0.2 0.4	392 784	3, 7, 28, and 56 days; 3-, 7-, and 28-day recovery from 28 days of exposure	Mouse; male; NIH; Rat; male; Wistar RIV:Tox Guinea pig; male; Hartley Crl:(HA)BR	Concentration-related centriacinar inflammation, with a maximum after 3 days of exposure; number of alveolar macrophages and pulmonary cell density increased progressively until 56 days of exposure, with the guinea pig the most sensitive species. Concentration and exposure-time dependent hypertrophy of bronchiolar epithelium in mouse only. Exposure to 0.2 ppm for 3 and 7 days caused significant histological and morphometric changes in all 3 species; exposure for 56 days caused alveolar duct fibrosis in rat and guinea pigs. Total recovery in rats after 28-day exposure, but not in guinea pigs or mice .	Dormans et al. (1999)
0.2 0.4 0.8	392 784 1,568	23 h/day for 7 days	Guinea pig; female; Hartley; ±AH_2 diet	Treatment-related lesions were observed after exposure to 0.4 and 0.8 ppm O_3; lesions were primarily seen in the terminal bronchioles and consisted of mononuclear cell and neutrophilic infiltrate and thickening of the peribronchiolar interstitium. Effects were only marginally exacerbated by the AH_2 (ascorbic acid) deficient diet and lesions were resolved after 1 week in FA.	Kodavanti et al. (1995)
0.4	784	12 h/day; 1- or 7-day exposure	Rat; (Wistar RiV:TOX; M & F; 1,3,9, & 18 months of age	Centriacinar inflammation (increased alveolar macrophages and PMNs; increased proximal and ductular septal density) was greatest in young rats (1 month and 3 months for 1-and 7-day exposures, respectively) and decreased with age. No major gender differences were noted.	Dormans et al. (1996)
0.4 1.0	784 1,960	2 h	Monkey; adult male rhesus	Reduced glutathione (GSH) increased in the proximal intrapulmonary bronchus after 0.4 ppm O_3 and in the respiratory bronchiole after 1.0 ppm O_3. Local O_3 dose (measured as excess ^{18}O) varied by as much as a factor of three in different airways of monkeys exposed to 1.0 ppm, with respiratory bronchioles having the highest concentration and the parenchyma the lowest concentration. After exposure to 0.4 ppm, the O_3 dose was 60% to 70% less and epithelial injury was minimal, except in the respiratory bronchiole, where cell loss and necrosis occurred, but was 50% less than found at 1.0 ppm.	Plopper et al. (1998)

Table AX5-5 (cont'd). Effects of Ozone on Lung Structure: Short-Term Exposures

Concentration					
ppm	µg/m^3	Duration	Species	Effects[a]	Reference
0.5	980	8 h + BrdU to label epithelial cells	Rat; male; F344	O_3 exposure induced a transient influx of neutrophils and a significant (17%) loss of NTE cells 2-4 h after exposure. Increased epithelial DNA synthesis was first detected 12 h PE. LI and ULLI indices of epithelial cell DNA synthesis were greatest 20-24 h and still elevated 36 h PE; numeric density of NTE cells returned to control levels 20-24 h PE.	Hotchkiss et al. (1997)
0.5	980	8 h/day, 3 or 5 days; + fluticasone propionate (FP) intranasally	Rat; male; F344	No significant difference of FP on morphometry of the maxilloturbinates; O_3 exposure caused neutrophilic rhinitis with 3.3- and 1.6-fold more intraepithelial neutrophils (3-day and 5-day exposure, respectively) and marked mucous cell metaplasia (5-day exposure only) with numerous mucous cells and approximately 60 times more IM in the nasal transitional epithelium; FP-treated rats exposed to O_3 had minimal nasal inflammation and mucous cell metaplasia.	Hotchkiss et al. (1998)
0.5	980	8 h/day, 3 days + endotoxin (100 µg/mL) intranasally	Rat; male; F344/N Hsd	Endotoxin-induced neutrophilia in nasal mucosa with NTE; mucous cell metaplasia was not detected in air/endotoxin-exposed rats, was observed in O_3/saline-exposed rats, and was most severe in O_3/endotoxin-exposed rats.	Fanucchi et al. (1998)
0.5	980	8 h/day, 1, 2, or 3 days + BrdU to label epithelial cells + antirat neutrophil antiserum	Rat; male; F344/N	Acute O_3 exposure induced a rapid increase in rMuc-5AC mRNA levels prior to the onset of mucous cell metaplasia; neutrophilic inflammation coincided with epithelial DNA synthesis and upregulation, but was resolved when mucous cell metaplasis first appeared in the NTE. Maxilloturbinates lined with NTE determined the epithelial labeling index, numeric densities of neutrophils, total epithelial and mucous secretory cells, amount of stored intraepithelial mucosubstances, and steady-state ratMUC-5AC (mucin) mRNA levels. Four days after a 3-d exposure, antiserum-treated, O_3-exposed rats had 66% less stored intraepithelial mucosubstances and 58% fewer mucous cells in their NTE than did controls. Antiserum treatment had no effects on O_3-induced epithelial cell proliferation or mucin mRNA upregulation.	Cho et al. (1999a, 2000)

Table AX5-5 (cont'd). Effects of Ozone on Lung Structure: Short-Term Exposures

Concentration					
ppm	**µg/m³**	**Duration**	**Species**	**Effects[a]**	**Reference**
0.5	980	8 h/day, 3 days + endotoxin	Rat; F-344	Enhanced epithelial lesions in the NTE and respiratory epithelium of the nose and conducting airways by endotoxin and O_3 exposures, respectively; synergistic effects of coexposure mediated by neutrophils. Endotoxin increased rMuc-5AC mRNA levels in the NTE of O_3-exposed rats; neutrophil depletion, however, had no effect on endotoxin-induced upregulation of mucin gene mRNA levels. Endotoxin enhanced the O_3-induced increase in stored mucosubstances (4-fold increase), but only in neutrophil-sufficient rats	Wagner et al. (2001a,b)
0.5	980	8 h/day, 1 and 3 days + OVA (1%, 50 µL/nasal passage)	Rat; Brown Norway	O_3 enhanced the appearance of eosinophils in the maxilloturbinates of OVA-challenged rats but did not increase inflammation in other nasal tissues; O_3/OVA coexposures for 3 days increased the number of epithelial cells as well as the appearance of mucus-containing cells in the NTE lining the maxilloturbinates.	Wagner et al. (2002)
1	1,960	8 h	Rat; Sprague-Dawley Ferret; young male Monkey; young male rhesus	Severe, acute infiltration of neutrophils along with necrotic bronchiolar epithelium in all lung regions, especially in the centriacinar region; necrosis and inflammation was more severe in ferrets and monkeys than in rats.	Sterner-Kock et al. (2000)

[a]AM = Alveolar macrophage.
PE = Postexposure (i.e., time after O_3 exposure ceased).
LM = Light microscopy.
EM = Electron microscopy.
RB = Respiratory bronchiole.
TB = Terminal bronchiole.
IAS = Interalveolar septum.
PMN = Polymorphonuclear leukocyte.

upregulation of rMuc-5AC, but was resolved before the development of epithelial metaplasia. In the follow-up study, the investigators found that only the mucous cell metaplasia was neutrophil-dependent, whereas O_3-induced epithelial cell proliferation and mucin gene upregulation were neutrophil-independent.

In the ***centriacinar region***, different species have similar responses to low levels of O_3 ($\geq$ 0.2 ppm for 1 week; Dormans et al., 1999). Dormans et al. (1999) compared the extent and time course of fibrotic changes in mice, rats, and guinea pigs exposed to 0.2 and 0.4 ppm O_3 for 3, 7, 28, and 56 days. They found a concentration-related centriacinar inflammation in all three species, with a maximum occurring after 3 days of exposure and total recovery within 3 days.

The effects of ***exposure duration*** are complex and are likely responsible for the similar patterns of biochemical responses (see Section 5.2.1). Repair of the damage by removal of injured epithelial cells is enhanced by the influx of neutrophils (Hyde et al., 1999; Veseley et al., 1999a; Miller et al., 2001; see Section 5.2.3).

Hotchkiss et al. (1997) reported that labeling indices for rat nasal transitional epithelial cell DNA were greatest 20 to 24 h after an 8-h exposure to 0.5 ppm O_3, but still greater than control by 36 h PE.

Exploring the role of ***susceptibility factors*** on morphological changes, Dormans et al. (1999) compared morphological, histological, and biochemical effects in the rat, mouse, and guinea pig after O_3 exposure and after recovery in clean air. Wistar RIV:Tox male rats, NIH male mice, and Hartley Crl:(HA)BR male guinea pigs were continuously exposed to filtered air, 0.2, or 0.4 ppm for 3, 7, 28, and 56 days. Recovery from 28 days of exposure was studied at intervals of 3, 7, and 28 days PE. Morphometric analysis was performed only on lung parenchyma with proximal alveoli and smaller alveolar ducts and no distinct species-specific differences were noted. The mouse was the most sensitive as shown by a concentration and exposure-time dependent persistence of bronchiolar epithelial hypertrophy, elevated lung enzymes, and slow recovery from exposure. In both rats and guinea pigs, 56 days of exposure to 0.4 ppm O_3 caused increased amounts of collagen in ductal septa and large lamellar bodies in Type II cells; however, the inflammatory response was greater in the guinea pig. Overall, the authors rated mice as most susceptible, followed by guinea pigs and rats.

In another comparative study of airway effects, Sterner-Kock et al. (2000) exposed ferrets, monkeys and rats to 1.0 ppm O_3 for 8 h. The ferrets developed epithelial necrosis and

inflammation that was similar to the monkey, and more severe than that found in rats. Because ferrets have a similar pulmonary structure as humans (e.g., well-developed respiratory bronchioles and submucosal glands), the authors concluded that the ferret would be a better model than rodents for O_3-induced airway effects.

Younger rats were found to have larger centriacinar lesions than older rats after a 1- or 7-day exposure to 0.4 ppm O_3 (Dormans et al., 1996). Thus, age susceptibility is dependent on the endpoint examined.

Rats with endotoxin-induced rhinitis were more susceptible to mucous cell metaplasia in the nasal transitional epithelium caused by a 3-day exposure to 0.5 ppm O_3 (Cho et al.,1999b). Wagner et al. (2002) reported a similar O_3-induced enhancement of inflammatory and epithelial responses associated with allergic rhinitis. Brown Norway rats were exposed to 0.5 ppm O_3, 8 h/day for 1 day or 3 consecutive days and then immediately challenged intranasally with either saline or ovalbumin.

More recent research has focused on the concept of O_3 susceptible and non-susceptible sites within the respiratory tract, including in situ antioxidant status and metabolic activity. Plopper et al. (1998) examined whether the variability of acute epithelial injury to short-term O_3 exposure within the tracheobronchial tree is related to local tissue doses of O_3 or to local concentrations of reduced glutathione (GSH). Adult male rhesus monkeys were exposed for 2 h to filtered air or O_3 (0.4 or 1.0 ppm). The O_3 was generated by $^{18}O_2$ for determination of local O_3 dose in the trachea, proximal bronchi, distal bronchi, and proximal respiratory bronchioles. Analyses of GSH and extracellular components (BAL) also were performed. Significant cellular injury was found at all sites, but the most damage, along with increased inflammatory cells, occurred in the proximal respiratory bronchiole. A significant reduction in GSH was found in the proximal bronchus at 0.4 ppm O_3, and in the respiratory bronchiole at 1.0 ppm O_3. A significant decrease in the percent of macrophages, along with significant increases in the percent of neutrophils and eosinophils, and a doubling of total lavage protein, were found after exposure to 1.0 ppm O_3 only. The authors concluded that the variability of local O_3 dose in the respiratory tract was related to inhaled O_3 concentration and was closely associated with local GSH depletion and with the degree of epithelial injury.

Plopper et al. (e.g., Watt et al.,1998; Paige et al., 2000) explored the site-specific relationship between epithelial effects of O_3 exposure and the metabolism of bioactivated

compounds within the respiratory tract of rats. The distribution of cytochrome P450 (CYP2E1)-dependent activity, measured with a selective substrate (p-nitrocatechol), was found to be highest in the distal bronchioles and minor daughter airways, and lower in the lobar bronchi and major daughter airways. Short-term O_3 exposure (1 ppm for 8 h) increased CYP2E1 activity in the lobar bronchi/major daughter airways only; however, long-term O_3 exposure (1 ppm for 90 days) decreased CYP2E1 activity in the major and minor airways, further complicating the interpretation of O_3 effects based on concentration and duration of exposure and recovery. Rats treated i.p. with 1-nitronaphthalene, a pulmonary toxicant requiring metabolic activation, and exposed to 0.8 ppm O_3, 8 h/day for 90 days showed greater histopathologic and morphometric effects in the centriacinar region of the lung (Paige et al., 2000b). Despite reported tolerance to oxidant stress after long-term O_3 exposure, there was increased severity of ciliated cell toxicity.

AX5.2.4.3 Long-Term Exposure Effects on Morphology

Key new exposure studies describing the morphological effects of O_3 exposures lasting longer than 1 week are summarized in Table AX5-6.

Marked mucous cell metaplasia was found in F344 rats exposed to 0.5 and 1.0 ppm O_3, but not 0.12 ppm for 20 months (Harkema et al., 1997a). In a follow-up study, hyperplasia was found in the nasal epithelium of rats exposed to 0.25 and 0.5 ppm, 8 h/day, 7 days/week, for 13 weeks (Harkema et al., 1999). The mucous cell metaplasia, and associated intraepithelial mucosubstances, induced by 0.5 ppm O_3 persisted for 13 weeks after exposure. An acute (8-h) exposure to 0.5 ppm O_3 13 weeks after the chronic exposure induced an additional increase of mucosubstances in the nasal epithelium of rats, but not in rats chronically exposed to 0 or 0.25 ppm O_3.

Rats continuously exposed for 6 months to the ambient air of São Paulo, Brazil (11 ppb O_3; 1.25 ppm CO; 35 $\mu g/m^3$ PM; 29 $\mu g/m^3$ SO_2) also developed secretory hyperplasia in the upper airways (Lemos et al., 1994). No significant changes in nasal tissue, however, were seen in rats continuously exposed for 49 days to the ambient air of Mexico City, Mexico (Moss et al., 2001).

Apoptosis regulators like Bcl-2 may play a role in the development and resolution of mucous cell metaplasia in the nasal airway (Tesfaigzi et al., 1998). In rats exposed to 0.5 ppm O_3 for 1 month, Bcl-2 was found in protein extracts of nasal epithelium. After 3 and 6 months of exposure, the number of metaplastic mucous cells in the transitional epithelium was indirectly

Table AX5-6. Effects of Ozone on Lung Structure: Long-Term Exposures

Concentration		Duration	Species	Effects[a]	Reference
ppm	µg/m^3				
Mexico City Ambient: 0.018 (≥ 0.12 for 18 1-h intervals)	35.3	23 h/day for 7 weeks	Rat; male; F344; 8 weeks old	No inflammatory or epithelial lesions in nasal airways or respiratory tract.	Moss et al. (2001)
0.12 0.5 1.0	235 980 1,960	6 h/day, 5 days/week for 20 months	Rat; male; F344; 6-8 weeks old	LM morphometry of CAR remodeling. Thickened tips of alveolar septa lining ADs (alveolar entrance rings) 0.2 mm from TB in rats exposed to 0.12 ppm and to 0.6 mm in rats exposed to 1.0 ppm. At 0.5 and 1.0 ppm, atrophy of nasal turbinates, mucous cell metaplasia in NTE, increased volume of interstitium and epithelium along ADs due to epithelial metaplasia, and bronchiolar epithelial hyperplasia. At 1.0 ppm, increased AMs and mild fibrotic response (increase in interstitial matrix and cellular interstitium; the latter due to increase in volume in interstitial fibroblasts). More effects in PAR than in terminal bronchioles. Effects not influenced by gender or by aging. Effects similar to, or model of, early fibrotic human disease (e.g., idiopathic pulmonary fibrosis).	Catalano et al. (1995a,b); Chang et al. (1995); Harkema et al. (1994, 1997a,b) Pinkerton et al. (1995); Plopper et al. (1994a); Stockstill et al. (1995)
0.12 0.50 1.0	235 980 1.960	6 h/day, 5 days/week for 24 and 30 months	Mouse; male and female; B6C3F1; 6-7 weeks old	Effects in the nose and centriacinar region of the lung at 0.5 and 1.0 ppm. Nasal lesions were mild: hyaline degeneration, hyperplasia, squamous metaplasia, fibrosis, suppurative inflammation of transitional and respiratory epithelium; and atrophy of olfactory epithelium. Lung lesions: alveolar/bronchiolar epithelial metaplasia and histiocytosis in terminal bronchioles, alveolar ducts, and proximal alveoli. Severity was greatest in mice exposed to 1.0 ppm O_3, but there was minimal interstitial fibrosis.	Herbert et al. (1996)
0.12 1.0	235 1,960	6 h/day, 5 days/week, for 2 or 3 months	Rat; F344/N	Morphometric changes (epithelial thickening, bronchiolarization) occurred after 2 or 3 months exposure to 1.0 ppm O_3; effects were similar to those found with 20 months exposure (*see Pinkerton et al., 1995*)	Pinkerton et al. (1998)

Table AX5-6 (cont'd). Effects of Ozone on Lung Structure: Long-Term Exposures

Concentration					
ppm	µg/m^3	Duration	Species	Effects[a]	Reference
0.25 0.5	490 980	8 h/day, 7 days/week for 13 weeks	Rat; male; F344/N HSD; 10-14 weeks old	Mucous cell hyperplasia in nasal 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. Mucous cell metaplasia found only after 0.5 ppm O_3, but still detectable 13 weeks PE.	Harkema et al. (1999)
0.4	784	23.5 h/day for 1, 3, 7, 28, or 56 days	Rat; Wistar 7 weeks old	Acute inflammatory response (increased PMNs and plasma protein in BALF) reached a maximum at day 1 and resolved within 6 days during exposure; AMs in BALF increased progressively up to day 56, and slowly returned to near control levels with PE recovery. Histological examination and morphometry of the lungs revealed CAR inflammatory responses throughout O_3 exposure; thickening of septa was observed at day 7. Ductular septa thickened progressively at days 7, 28, and 56 of exposure; showed increased collagen at day 28, which was further enhanced at day 56. Increased RBs with continuous exposure. Collagen and bronchiolization remained present after a recovery period.	Van Bree et al. (2002)
0.5	980	8 h/day for 1, 3, and 6 months	Rat; male; F344/N	Increased Bcl-2, a regulator of apotosis, after 1 month, decreasing somewhat thereafter, returning to baseline by 13 weeks PE; increased number of metaplastic mucous cells in NTE after 3 and 6 months.	Tesfaigzi et al. (1998)
0.5	980	8 h/day for 5 days, every 5 days for a total of 11 episodes	Monkey; bonnet; 30-day-old infants	Increased density and distribution of goblet cells in RB whole mounts stained with AB/PAS; extensive remodeling of distal airway with O_3 and O_3 + HDMA challenge; increased airways resistance and reactivity, and respiratory motor adaptation also occurred. Authors conclude that periodic cycles of acute injury and repair associated with the episodic nature of environmental patterns of O_3 exposure alters postnatal morphogenesis and epithelial differentiation in the distal lung of infant primates.	Evans et al. (2003); Schelegle et al. (2003a); Chen et al. (2003); Fanucchi et al. (2000); Plopper and Fanucchi (2000)

Table AX5-6 (cont'd). Effects of Ozone on Lung Structure: Long-Term Exposures

Concentration					
ppm	µg/m^3	Duration	Species	Effects[a]	Reference
0.8	1,568	8 h/day for 90 days + 1-NN (100 mg/kg)	Rat; male; Sprague-Dawley	Increased O_3-induced centriacinar toxicity (histopathology, TEM, morphometry) of 1-Nitronaphthalene (1-NN), a pulmonary cytotoxicant requiring metabolic activation, especially to ciliated cells.	Paige et al. (2000b)
0.5	980	11 episodes of 5 days each, 8 h/day followed by 9 days of recovery	Monkey; *Macaca mulatta*; 30 days old	In small conducting airways O_3 caused decrements in density of airway epithelial nerves. Reduction greater with HDMA + O_3. O_3 or HDMA+O_3 caused increase in number of PGP 9.5 (pan-neuronal marker) in airway. CGRP-IR nerves were in close contact with the PGP9.5 positive cells. Appearance of clusters of $PGP9.5^+/CGRP^-$ cells. Suggests episodic O_3 alters developmental pattern of neural innervation of epithelial compartment.	Larson et al. (2004)
0.5	980	11 episodes of 5 days each, 8 h/day followed by 9 days of recovery	Monkey; *Macaca mulatta*; 30 days old	Abnormalities in the BMZ included: (1) irregular and thin collagen throughout the BMZ; (2) perclecan depeleted or severely reduced; (3) FGFR-1 immunoreactivity was reduced; (4) FGF-2 immunoreactivity was absent in perlecan-deficient BMZ, but was present in the lateral intercelluar space (LIS), in basal cells, and in attenuated fibroblasts; (5) syndecan-4 immunoreactivity was increased in basal cells.	Evans et al. (2003)

[a]TB = Terminal bronchiole.
PE = Postexposure (i.e., time after O_3 exposure ceased).
AM = Alveolar macrophage.
LM = Light microscopy.
EM = Electron microscopy.
RB = Respiratory bronchiole.
IAS = Interalveolar septum.
C × T = Product of concentration and time.

related to the percentage of cells that were Bcl-2 positive. Cells from rats exposed to filtered air did not express any Bcl-2.

A similar spectrum of lesions also was reported (Herbert et al., 1996) in the nasal cavity and centriacinar lung of male and female mice exposed to 0.5 or 1.0 ppm of O_3 for 2 years, which persisted with continued exposure for 30 months. Few changes, however, were found in other endpoints (e.g., lung function or lung biochemistry) examined in these rats. The investigators' interpretation of the entire study is that rodents exposed to the two higher O_3 concentrations had some structural hallmarks of chronic airway disease in humans.

A fifth long-term study was reported in infant monkeys by Plopper et al. (Evans et al., 2003; Schelegle et al., 2003a, 2003b; Chen et al., 2003; Plopper and Fanucchi, 2000; Fanucchi et al., 2000) using a shorter simulated, seasonal O_3-exposure pattern, but at a higher O_3 concentration (0.5 ppm) than the protocol used by Tyler et al. (1988, 1991a). Infant rhesus monkeys (30 days old) were exposed to filtered air, house dust mite allergen aerosol (HDMA), or O_3 + HDMA. The O_3 exposures were 8 h/day for 5 days, every 14 days for a total of 11 O_3 episodes. Half of the monkeys were sensitized to house dust mite allergen (*Dermatophagoides farinae*) at 14 and 28 days of age. The sensitized monkeys were exposed to HDMA for 2h/day on days 3-5 of the FA or O_3 exposures. The lungs were removed during the last filtered air exposure and the right and left cranial and right middle lobes were separately inflation fixed. Microdisection and morphometric analyses were performed on the conducting airways to the level of the most proximal respiratory bronchiole. Repeated exposures to O_3 or O_3 + HDMA over a 6-month period resulted in an atypical development of the basement membrane zone of airways in nonsensitized developing monkeys. A profound remodeling in the distal conducting airways was found in the sensitized monkeys as a result of the damage and repair processes occurring with repeated exposure (Evans et al., 2003; Schelegle et al., 2003a; Fanucchi et al., 2000).

Schelegle et al. (2003a) reported the lung histopathology results from the O_3 exposures to infant monkeys. At necropsy, cross sections of the left caudal lobe were prepared from each animal. The accumulation of eosinophils and mucous cells within the combined epithelium and interstitium compartments was determined in the conducting airways and in the terminal/respiratory bronchioles. House dust mite sensitization and HDMA challenge alone, or combined with O_3 exposure, resulted in significantly greater eosinophil accumulation in the conducting airways when compared to FA and O_3 only exposures. A significant accumulation of

eosinophils was found in the terminal/respiratory bronchioles of the sensitized monkeys challenged with HDMA when compared to monkeys exposed to FA, O_3, and HDMA + O_3. The mean mass of mucous cells increased in the fifth generation conducting airways of sensitized animals challenged with HDMA alone and when combined with O_3 exposure, and in the terminal bronchioles of sensitized animals exposed to HDMA + O_3. The tracheal basement membrane of house dust mite-sensitized monkeys exposed to HDMA or to HDMA + O_3 was significantly increased over controls; however, there were no significant changes in the airway diameter of proximal and mid-level airways. The authors interpreted these findings to indicate that the combination of cyclic O_3 exposure and HDMA challenge in house dust mite-sensitized infant monkeys act synergistically to produce an allergic-reactive airway phenotype characterized by significant eosinophilia of midlevel conducting airways, transmigration of eosinophils into the lumen, and an altered structural development of conducting airways. Exposures of sensitized young monkeys to HDMA alone, or to O_3 alone, resulted in eosinophilia of the mid-level conducting airways and the terminal/respiratory bronchioles, but without alterations in airway structure or function. Evans et al. (2003) examined development of the tracheal basement membrane zone (BMZ) in these monkeys and found that with exposures to either O_3 or HDMA + O_3, BMZ development was affected. Abnormalities in the BMZ included: (1) irregular and thin collagen throughout the BMZ; (2) perclecan depeleted or severely reduced; (3) FGFR-1 immunoreactivity was reduced; (4) FGF-2 immunoreactivity was absent in perlecan-deficient BMZ, but was present in the lateral intercelluar space (LIS), in basal cells, and in attenuated fibroblasts; (5) syndecan-4 immunoreactivity was increased in basal cells. The authors interpret these data to suggest that O_3 targets cells associated with synthesis of epithelial BMZ perlecan. The absence of FGF-2, normally stored in the BMZ, could affect downstream signaling in airway epithelium and could be responsible for the abnormal development of the airway seen in this study, and thus be an important mechanism modulating O_3-induced injury.

Mid-level bronchi and bronchioles from these monkeys were examined for alterations in airway innervation (Larson et al., 2004). They found decrements in the density of epithelial nerves in the axial path between the sixth and seventh airway generations in exposures to O_3. Combined O_3+HDMA exposures exacerbated this reduction. They attribute this loss of nerve plexuses to neural regression or stunted nerve development, the latter corroborated by the Evans et al. (2003) finding of decreased growth factors following O_3 exposure. Additionally, they found

streaks or clusters of cells immunoreactive for protein gene product 9.5 (PGP 9.5, a pan-neuronal marker) and negative for calcitonin gene-related peptide. The functional significance of this is unknown, and presumed to be implicated in injury-repair process induced by O_3.

Recently, bronchiolization was reported in rats exposed to 0.4 ppm O_3 for only 56 days (van Bree et al., 2001). Collagen formation progressively increased with increasing O_3 exposure, and remained increased into PE recovery. In addition to centriacinar remodeling, Pinkerton et al. (1998) reported thickening of tracheal, bronchial, and bronchiolar epithelium after 3 or 20 months exposure to 1 ppm. No such responses were observed at either time point for exposures to 0.12 ppm O_3.

AX5.2.5 Effects on Pulmonary Function

AX5.2.5.1 Introduction

Numerous pulmonary function studies of the effects of short-term O_3 exposure (defined here as $\leq$ 1 week of exposure) in several animal species have been conducted and generally show responses similar to those of humans (e.g., increased breathing frequency, decreased tidal volume, increased resistance, decreased forced vital capacity [FVC] and changes in the expiratory flow-volume curve). The breathing pattern returns to normal after O_3 exposure.

This section will provide a brief overview of acute and short-term exposure effects and then focus on functional changes observed after long-term exposure to O_3 (defined here as >1 week of exposure) and on the new studies on O_3-induced airway hyperresponsiveness (AHR).

AX5.2.5.2 Acute and Short-Term Exposure Effects on Pulmonary Function

Wiester et al. (1996) exposed male Fischer 344 rats to 0.5 ppm O_3 for either 6 or 23 h/day over 5 days. Ozone-induced changes in lung volume were attenuated during the 5 exposure days and returned to control levels after 7 days recovery. The responses to repeated O_3 exposure in rats were exacerbated by reduced ambient temperature, presumably as a result of increased metabolic activity.

Recent work has utilized inbred mouse strains with varying ventilatory responses to O_3 to attempt to model susceptible populations. As differences were seen in inflammatory responses to acute O_3 exposures in C57BL/6J and C3H/HeJ mice, comparisons were made of their ventilatory responses also (Tankersley et al., 1993). Following an exposure of 2 ppm O_3 for 3 h, breathing

frequency (f), tidal volume (V_T), and minute ventilation were measured 1 and 24 h in both normocapnia (or air at ~0% CO_2) and hypercapnia (5 or 8% CO_2). They demonstrated that acute O_3 exposures caused altered hypercapnic ventilatory control, which varied between strains. The observations from this study indicate that control of ventilation is at least in part regulated by other genetic factors.

The Paquette et al. (1994) study discussed in 5.3.3.3 also measured ventilatory responses in C57BL/6J and C3H/HeJ mice on repeated subacute exposures to O_3. C57BL/6J and C3H/HeJ had differing responses to both normocapnia and hypercapnia. Normocapnic V_E was greater following subacute O_3 exposure in C57BL/6J mice than in C3H/HeJ mice, due to increased and reduced V_T, respectively. The authors speculated that this increased V_T in C57BL/6J mice may contribute to the increased susceptibility to lung injury due to a greater dose of O_3 reaching the lower lung. Hypercapnic ventilatory responses following subacute O_3 exposures demonstrated reduced V_E (due to decreased V_T) in C57BL/6J only. Evaluations of O_3 dosimetry were performed in these two strains using ^{18}O-labeled O_3 (Slade et al., 1997). Immediately after exposures to 2 ppm $^{18}O_3$ for 2-3 h, C3H/HeJ mice had 46% less ^{18}O in lungs and 61% less in trachea, than C57BL/6J. Additionally, C3H/HeJ mice had a greater body temperature decrease following O_3 exposure than C57BL/6J mice. The authors suggested that the differences in susceptibility to O_3 are due to differences the ability to decrease body temperature and, consequently decrease the dose of O_3 to the lung.

Takahashi et al. (1995b) measured tracheal transepithelial potential (V_T) in eight mouse strains 6 h after exposure to 2 ppm O_3 for 3 h. AKR/J, C3H/HeJ, and CBA/J were identified as resistant strains and 129/J, NJ, C57BL/6J, C3HeB/FeJ and SJL/J were identified as susceptible strains. The pattern of inheritance for this trait suggested an autosomal recessive pattern. The authors noted that strains' responses to this parameter did not show concordance with inflammatory responses, suggesting to the authors that the two phenotypes are not controlled by the same genetic factors.

The Savov et al. (2004) study discussed in 5.2.3.3 (2.0 ppm O_3 for 3 h) characterized ventilatory responses using whole body plethysmography and enhanced pause index (P_{enh}) in the nine mouse strains evaluated. Table AX5-4 lists the baseline P_{enh}, the P_{enh} following O_3, and the P_{enh} response to methacholine (MCh) following O_3. C57BL/6J was hyporeactive to MCh prior to

O_3, but was very responsive to MCh following O_3. Conversely, C3H/HeJ had an intermediate baseline P_{enh} and a small response to MCh following O_3 exposure.

AX5.2.5.3 Long-Term Exposure Effects on Pulmonary Function

New long-term O_3 exposure studies evaluating pulmonary function are not available.

AX5.3.5.4 Acute and Chronic Exposure Effects on Airway Responsiveness

New studies in laboratory animals allow possible ways of predicting, with increased specificity, the effects of O_3 exposure on the exacerbation of asthma symptoms and the risk of developing asthma in humans. A variety of methods have been used to assess airway responsiveness in humans, including airway challenge with nonspecific bronchoconstrictors (e.g., inhaled methacholine or histamine) and with indirect (e.g., inhalation of adenosine monophosphate, hypertonic saline, mannitol) stimuli to bronchoconstriction (Anderson, 1996). Although inhaled agonist challenges are preferred in humans, laboratory animals studies have employed intravenous (i.v.) agonist challenges as well as inhalation challenges. The comparability of these two routes for bronchoconstrictor administration has not been well studied, however differences have been reported (e.g., Sommer et al., 2001). Most challenge tests require an outcome measure that reflects airway function, such as pulmonary resistance, dynamic lung compliance, or deceased forced expiratory flow and volume. In a generalized sense, resistance is a measure of large airway function, and dynamic compliance is a measure of small airway function. In regards to pulmonary mechanics, human infants are much like laboratory rodents because both have very compliant chest walls. Therefore, this discussion will make comparisons from the published literature on airway responsiveness in human infants as recently reviewed, for example, by Stick, 2002.

As with infants, a limitation of the traditional types of studies in laboratory rodents is the requirement for sedation. The need for artificial ventilation in laboratory animal studies may cause breathing patterns that affect O_3 deposition (see Section 5.2). Joad et al. (2000) reported that when 1 ppm O_3 for 90 min is administered to isolated rat lung at either 2.4 ml/40 bpm or 1.2 m/80 bpm, the more rapid breathing pattern elicits less epithelial cell injury than the slower breathing pattern. They further showed greater reduction in injury in the proximal axial airway compared to its adjacent airway branch and terminal bronchiole. For rats, normal respiration is

approximately 100 bpm, so this paradigm does not really model rapid shallow breathing elicited in the intact animal. Schelegle et al. (2001) showed that the large conducting airways of rats are protected by rapid, shallow breathing, but there is a more even distribution of epithelial cell injury to the terminal bronchioles. Recent observations (Postlethwait et al., 2000) demonstrate that the conducting airways are the primary site of acute cytotoxicity from O_3 exposure. By utilizing a new analytic approach of three-dimensional mapping of the airway tree in SD rat isolated lung exposed to 0, 0.25, 0.5, or 1.0 ppm O_3 for 20 to 90 minutes, they showed a concentration-dependent increase in injured cells. Injury was evident in proximal and distal conduction airways, lowest in terminal bronchioles, and highest in the small side branches downstream of bifurcations. These exposure levels did not concurrently elicit changes in LDH activity or total protein in BALF.

More recent methods of studying laboratory animals utilize unanesthetized, unrestrained rodents in a whole-body plethysmograph (e.g., Shore et al., 2001, 2002; Goldsmith et al., 2002; Jang et al., 2002), but pulmonary resistance is measured indirectly using several indices of inspiratory/expiratory pressure differences, including enhanced pause (Penh), that may be less sensitive than direct measurements of lung airflow resistance (Murphy, 2002). Also, in another study by Sommer et al. (1998), unrestrained guinea pigs were shown to have a daily variability in pulmonary resistance that is similar to that occurring in humans. Therefore, circadian rhythms of airway caliber must be considered when performing airway challenge tests in any species. Animals with acute viral illness have morphological evidence of inflammatory cell infiltration, bronchiolar wall edema, epithelial hyperplasia, and increased airway mucous plugs that can cause airway narrowing, air trapping, and serious functional changes in the lung (Folkerts et al., 1998).

Exercise-induced bronchoconstriction in humans appears to be mediated by changes in the tonicity of the airway lining fluid (Anderson and Daviskas, 2000) and, therefore, a test in laboratory animals based on the inhalation of mannitol aerosol (hyperosmolar) might be feasible and provide information similar to that from exercise challenges in cooperative children and adults (Brannan et al., 1998). In active humans with asthma, adenosine monophosphate challenges appear to better reflect ongoing airway inflammation than histamine or methacholine challenges (Polosa and Holgate, 1997; Avital et al, 1995a,b), and might be useful in identifying mechanisms of asthma in laboratory animals and their responsiveness to environmental pollutants.

Airway responsiveness in asthma

The increased responsiveness to bronchoconstrictor challenge in asthma is thought to result from a combination of structural and physiological factors that include increased inner-wall thickness, increased smooth-muscle responsiveness, and mucus secretion. This baseline responsiveness is thought to be modulated in asthma by chronic inflammation and airway remodeling (Stick, 2002). Longitudinal studies in adults have shown that the development of airway responsiveness is associated with persistence of symptoms (O'Conner et al., 1995).

Airway responsiveness in infants

The age at which nonspecific airway hyperresponsiveness first appears in humans is unknown, although both genetic and environmental factors are most likely to play a role. Although underlying physiological or structural factors may determine this relative increase in responsiveness in infants compared with older children, the most likely explanation is that infants receive a relatively larger dose of inhaled challenge agent than older children. Thus, when a correction is made for this dose effect, infants and older children appear to have a similar response to inhaled histamine (Stick et al., 1990; Stick, 2002). Airway responsiveness at one month is a predictor of lung function at six years (Palmer et al., 2001). Data from this study also show that the genetic determinants of atopy and airway responsiveness are independent (Palmer et al., 2000). In another study of infants with wheeze, persistence of airway hyperresponsiveness was associated with persistence of symptoms, although airway responsiveness at one month of age was neither a sensitive nor a specific predictor of outcome (Delacourt et al., 2001).

The human studies imply that airway responsiveness is a key factor in asthma, but it is not clear if the factors that are important for airway responsiveness in early life are related to inflammation, structure or physiology of the airways, or the combination of all three. Furthermore, it is not clear how viruses, allergens and irritants in the environment modify innate airway responses (Holt et al., 1999), but they are known to be important.

Airway responsiveness in laboratory animals

Laboratory animals, including rodents (mice, rats, guinea pigs), rabbits, cats, dogs, and nonhuman primates have been used to study the effects of O_3 exposure on airway bronchoconstriction. New studies examining airway responsiveness in laboratory animals are

listed in Table AX5-7. Ozone-induced AHR in guinea pigs has been used as a model of bronchospasm (e.g., Kudo et al., 1996; van Hoof et al., 1996; 1997a,b; Matsubara et al., 1997a,b; Sun and Chung, 1997; Aizawa et al., 1999a,b; Tsai et al., 1998; Nakano et al., 2000). In this model, the guinea pigs are acutely exposed for 1 or 2 h to high O_3 concentrations (2 to 3 ppm). The model is useful for understanding mechanisms of bronchospasm, but are not directly relevant for extrapolation to potential airway responses in humans exposed to ambient levels of O_3. Dye et al. (1999) showed hyperresponsiveness to methacholine in rats 2 h after exposure to 2 ppm O_3 for 2 h.

Shore et al. (2000) have shown that O_3-induced AHR is reduced in immature SD rats. The animals were exposed to 2 ppm O_3 for 3 h in nose-only-exposure plethysmographs and baseline V_E was normalized for body weight. V_E was reduced, primarily as a result of decreased V_T, with O_3 exposure. Adults rats had 40-50% decreases in V_E, 6-wk-old rats had smaller decreases, and 2- and 4 week-old rats had no significant changes in V_E.. This suggested to the authors that the higher baseline V_E in the young rats, combined with the smaller decreases in V_E with O_3 exposure, created a much larger dose in the immature rats. Shore et al. (2002) completed complementary studies in A/J mice at ages 2, 4, 8, or 12 weeks using exposures of 0.3 to 3 ppm for 3 h. Ozone caused a similar concentration-related decreases in V_E except in the 2- and 4-week old mice. This suggested that the young mice are less sensitive than adult mice to O_3 in terms of AHR. Lean and obese mice were also compared for differences in AHR response to O_3 exposure (2.0 ppm O_3 for 3 h). Shore et al. (2003) exposed lean, WT C57BL/6J mice and mice with a genetic defect in the gene that codes for leptin, the satiety hormone. These *ob/ob* mice had enhanced AHR and inflammation compared to the WT mice.

Airway hyperresponsiveness can be induced by specific antigens as well as O_3. The most commonly used laboratory animal model is the ovalbumin (OVA) sensitized guinea pig. Animals sensitized with OVA have been shown to have similar responses to nonspecific bronchoconstrictors (e.g., carbachol) as control animals; however, OVA-sensitized guinea pigs exposed to O_3 showed increased AHR to histamine (Vargas et al., 1994). Guinea pigs were sensitized by inhalation exposure to ovalbumin and subsequently challenged with histamine; the main endpoint was specific airway resistance. When exposed to O_3 before sensitization, repeated exposures to very high levels (5.0 ppm) decreased the OVA sensitization threshold; however, in already sensitized animals, a 2-h exposure to $\geq$ 1.0 ppm enhanced airway responsiveness to

Table AX5-7. Effects of Ozone on Airway Responsiveness

Ozone Concentration[a]		Exposure Duration	Challenge[b]		Drugs	Species, Sex, Strain, and Age[c]	Observed Effect(s)	Reference
ppm	µg/m^3		Agent	Route				
0.1 0.3	196 588	4 h/day, 4 days/week for 24 weeks	ACh OVA	inh inh	none	Guinea pig, M & F, Hartley	O_3 exposure did not produce airway hyperresponsiveness to ACh in nonsensitized animals; in OVA-sensitized animals, there was increased responsiveness to both nonspecific (ACh) and specific (OVA) airway challenge that persisted for 4 weeks after exposure 0.1 and 0.3 ppm O_3. Effects were not gender specific and were not associated with BALF inflammatory indicators, but were associated with antigen-specific antibodies in blood.	Schlesinger et al. (2002a,b)
0.15 0.30 0.60 1.2	294 588 1,176 2,352	4 h	ACh Hist SP	iv iv iv	Na Pentobarbital	Guinea pig, M Hartley, 500-600g	Increased airway responsiveness to Hist, but not Ach, 16-18 h after 1.2 ppm O_3 exposure only. Increased responsiveness to SP occurred after exposure to $\geq$ 0.3 ppm O_3	Segura et al. (1997)
0.3	588	4 h/day for 1, 3, 6, 12, 24, or 38 days	SP	iv	Na Pentobarbital	Guinea pig, M Hartley, 500-600 g	Increased airway responsiveness to SP occurred 16-18 h after exposure to 0.3 ppm O_3 for 1, 3, 6, 12, and 24 days; but not after 48 days. Highly significant correlation between airway responsiveness and BALF total cells, Ams, neutrophils, and eosinophils, suggesting that airway inflammation is involved.	Vargas et al. (1998
0.5	980	8 h/day for 5 days, repeated every 14 days for 6 months	Hist	inh	Ketamine + Diprivan	Rhesus monkey, M, 30 days old	Increased airway responsiveness to Hist after 10 episodes of exposure to O_3 + HDMA in sensitized infant monkeys.	Schelegle et al. (2003b)
1	1,960	1 h	Ach OVA	inh inh	Urethane	Guinea pig, M, Dunkin-Hartley, 250-300 g	Increased bronchial responsiveness at 3 h, but not 24 h after O_3; OVA had no effect on baseline, but enhanced airway responsiveness 24 h after O_3	Sun et al. (1997)
1	1,960	1 h	Mch OVA	inh inh	Urethane	Mouse, M, C57BL/6, 6 weeks old	Ozone caused increased C_{dyn} and V_E, and decreased P_aO_2 in OVA- sensitized mice	Yamauchi et al. (2002)
2	3,920	2 h	Mch	inh	Ketamine + Zylazine	Rat, M, F344 14 months old	Increased airway responsiveness to MCh 2 h PE	Dye et al. (1999)
3	5,880	1 h	Hist OVA	iv iv		Guinea pig, M, Hartley, 500-700 g	Increased airway responsiveness to histamine after O_3 exposure in OVA sensitized guinea pigs, with enhanced responsiveness after OVA challenge	Vargas et al. (1994)

[a]Table ordered according to ozone concentration.
[b]MCh = methylcholine, ACh = acetylcholine, Hist = histamine, 5-HT = 5-hydroxytryptamine, SP = substance P, FS = field stimulation, CCh = carbachol, TX = thromboxane, KCl = potassium chloride, Pt = platinum; Route: iv = intravenous, inh = inhalation., sc = subcutaneous, ip = intraperitoneal
[c]Age or body weight at start of exposure.

ovalbumin. Thus, O_3 exposure does not modify the development of antigen-induced AHR and, in fact, may enhance AHR at high levels of exposure.

The enhancement of antigen-induced bronchoconstriction by acute, high-level O_3 was further explored in OVA-sensitized guinea pigs (Sun et al., 1997) and mice (Yamauchi et al., 2002). Male Dunkin-Hartley guinea pigs were sensitized by i.p. injection of OVA (1 mL 20 μg) and exposed to filtered air or 1 ppm O_3 for 1 h (Sun et al., 1997). Airway responsiveness to inhaled acetylcholine was measured 3 h and 24 h after air and O_3 exposures and BAL was performed. Four other groups of OVA-sensitized animals were exposed to OVA aerosol or to O_3 alone, or in combination, and airway responsiveness to acetylcholine was measured 3 h and 24 h PE. In the combined exposure groups, OVA aerosol exposure was initiated either immediately after, or 21 h after O_3 exposure. In this study, O_3 exposure increased bronchial responsiveness to acetylcholine at 3 h, but not 24 h, while OVA alone had no effect. Combined exposure to O_3 and OVA (1 ppm for 1 h, then 3 min OVA) increased bronchial responsiveness to acetylcholine 3 h after O_3 exposure. At 24 h following O_3 exposure, AHR increased when OVA challenge was performed at 21 h, suggesting to authors that O_3 pre-exposure can potentiate OVA-induced AHR. Neutrophil counts in the BALF increased at 3 and 24 h after O_3 exposure alone, but were not further increased when O_3 exposure was combined with OVA airway challenge; however protein content of the BALF did increase at 3 and 24 h in the O_3 and OVA groups. Thus, this study also indicates that high-ambient O_3 exposure can augment antigen(OVA)-induced AHR in guinea pigs.

Male C57BL/6 mice were sensitized by i.p. injection of OVA (50 μg) and exposed to filtered air or 1 ppm O_3 for 1 h (Yamauchi et al., 2002). Airway responsiveness to methacholine was measured 24 h after an inhalation challenge to OVA (10 mg/mL) in OVA-sensitized and control groups, and 3 h after the OVA inhalation challenge in animals exposed to O_3 to maximize the susceptible time period for AHR (Yamaguchi et al., 1994). Pulmonary function was measured by plethysmography before and at 10-min intervals during the 1-h air and O_3 exposures. Blood gases and BAL fluid were monitored immediately after exposure. Mice sensitized to OVA had AHR to methacholine. Ozone exposure caused significant decreases in dynamic lung compliance, minute ventilation, and P_aO_2 in OVA-sensitized mice, but not in controls. A marker of inflammation (soluble intercellular adhesion molecule-1 [sICAM-1]) was elevated in the BAL fluid of OVA-sensitized mice, but sICAM-1 levels were not significantly changed by O_3

exposure, indicating that the O_3-induced AHR to methacholine was not caused by O_3-induced inflammation.

Ozone-induced AHR may be temporally associated with neutrophils (DeLorme et al., 2002) and other inflammatory cells stimulated by leukotrienes (Stevens et al., 1995a), cytokines (Koto et al., 1997), mast cells (Igarashi et al., 1998; Noviski et al., 1999), or by oxygen radicals (Takahashi et al., 1993; Stevens et al., 1995b; Tsukagoshi et al., 1995; Kudo et al., 1996). Two new studies have shown that inflammation is not a prerequisite of AHR (Stevens et al., 1994; Koto et al., 1997), and some investigators have suggested that O_3-induced AHR may be epithelium dependent (Takata et al., 1995; Matsubara et al., 1995; McGraw et al., 2000). For example, neonatal rats pretreated with capsaicin, which will permanently destroy C-fibers and prevent O_3-induced release of neuropeptides (Vesely et al., 1999b), and then exposed to O_3 when adults, showed a marked increase in airway responsiveness to inhaled aerosolized methacholine (Jimba et al., 1995). Some investigators (Matsumoto et al., 1999; DeLorme et al., 2002) have shown that respective intravenous pretreatment with neutrophil elastase inhibitor or PMN antiserum can block O_3-induced AHR; other investigators (Koto et al., 1995; Aizawa et al., 1997; Takebayashi et al., 1998) have shown that depletion of tachykinins by capsaicin treatment, or by a specific tachykinin receptor antagonist, can block the induction of AHR by O_3. The seemingly disparate responses in laboratory animals may be due to species- or strain-specific differences in inherent reactivity to bronchoconstrictors, or to inherent differences in susceptibility to O_3-induced inflammation (Zhang et al., 1995; Depuydt et al., 1999; Dye et al., 1999).

The studies referenced above are useful for gaining an understanding of how acute O_3 exposure modulates specific airway responsiveness to allergen challenge, but the O_3 concentrations used in these studies are not typical of ambient exposures in the population. More recently published studies that may be potentially relevant to ambient levels of O_3 were conducted in vivo, in an isolated perfused lung model, and in ex vivo lung segments using multihour and repeated multihour exposures with ambient levels of O_3. A study on the relationship between O_3-induced AHR and tracheal epithelial function was conducted in New Zealand white rabbits by Freed et al. (1996). Rabbits were exposed to filtered air or to 0.2 ppm O_3 for 7 h. Tracheal transepithelial potential difference (PD) was measured 3 h after exposure and lung resistance and reactivity were partitioned into central and peripheral components using forced oscillation. Exposure to O_3 significantly decreased PD, but did not change lung resistance.

Changes in the compartmentalized lung resistance, measured in response to bronchoconstrictor aerosol challenge (acetylcholine) before and after bilateral vagotomy, were not significantly different in air-exposed rabbits; however, bilateral vagotomy did enhance peripheral lung reactivity in O_3-exposed rabbits. The acetylcholine-induced increase in lung resistance with O_3 exposure (140%) was two times higher than with air exposure, indicating that ambient-level O_3 exposure affects tracheal epithelial function in rabbits and increases central airway reactivity, possibly through vagally-mediated mechanisms.

Delaunois et al. (1998) studied pulmonary mechanics and hemodynamics in the isolated perfused lung model that allowed partitioning of the total pressure gradient into arterial, pre- and post-capillary, and venous components. New Zealand white rabbits were exposed to filtered air or to 0.4 ppm O_3 for 4 h and evaluated for airway responsiveness to acetylcholine, substance P, or histamine immediately or 48 h later. Ozone exposure did not significantly change baseline values of pulmonary resistance and dynamic compliance, but inhibited pulmonary mechanical reactivity to all three bronchoconstrictors that persisted for 48 h. Ozone also modified vasoreactivity of the vascular bed, but only at 48 h PE. Arterial segmental pressure, normally insensitive to acetylcholine and substance P, was significantly elevated by O_3; precapillary segmental pressure decreased in response to acetylcholine. The authors concluded that O_3 can induce direct vascular constriction, but the vascular responses are variable and depend on the agonist used and on the species studied.

Guinea pigs were exposed to filtered air, 0.15, 0.3, 0.6, or 1.2 ppm O_3 for 4 h and evaluated for airway responsiveness to acetylcholine, substance P, or histamine 16 to 18 h later (Segura et al., 1997). Ozone did not cause airway hyperresponsiveness to acetylcholine or histamine, except at the highest concentration (1.2 ppm O_3) for histamine. However, O_3 did cause hyperresponsiveness to substance P at $\geq$ 0.3 ppm. The authors speculated that O_3 destroys neutral endopeptidases, responsible for substance P inactivation, that are located in airway epithelial cells. In a follow-up study at the same laboratory, Vargas et al. (1998) reported that guinea pigs chronically exposed to 0.3 ppm O_3 for 4 h/day became adapted to substance P-induced AHR. Ozone caused increased sensitivity to substance P after 1, 3, 6, 12, and 24 days of exposure that was associated with airway inflammation; however, after 48 days of exposure, the increased sensitivity to substance P was lost.

The effects of repeated short-term exposure or long-term exposure to O_3 on airway responsiveness have been investigated in several laboratory animal studies. Both Vargas et al. (1998), discussed above, and Szarek et al. (1995) reported that the AHR associated with acute O_3 exposures does not persist during long-term exposure to ambient-levels of O_3 ($\leq$ 1 ppm). In the Szarek et al. (1995) study, Fischer 344 rats were exposed to 0.0, 0.12, 0.5, or 1.0 ppm O_3, 6h/day, 5 days/week for 20 months. Eighth generation airway segments were isolated from the exposed rats and circumferential tension development was measured in response to bethanechol, acetylcholine, and electrical field stimulation and normalized to smooth muscle area. Maximum responses of the small bronchi of male rats were significantly reduced after exposure to 0.12 and 0.5 ppm O_3, suggesting some adaption had taken place during long-term exposure, possibly increased inner wall thickness.

Changes in breathing pattern and lung function caused by O_3 are attenuated with repeated daily exposures for at least 3 to 5 days. Joad et al. (1998), however, reported that repeated daily O_3 exposure enhances, rather than diminishes, the responsiveness of rapidly adapting airway receptors. Guinea pigs were exposed to 0.5 ppm O_3, 8 h/day for 7 days and then studied for measurement of impulse activity of the rapidly adapting receptors, dynamic lung compliance, and lung resistance at baseline and in response to substance P, methacholine, hyperinflation, and removal of end-expired airway pressure. Repeated exposure increased receptor activity to substance P, methacholine, and hyperinflation; there were no significant effects on baseline or substance P- and methacholine-induced changes in lung compliance and resistance. Because agonist-induced changes in receptor activity precede lung function changes, the authors concluded that the responsiveness of rapidly adapting receptors was enhanced.

Schlesinger et al. (2002a,b) evaluated airway responsiveness following acetylcholine or OVA inhalation challenges in male and female Hartley guinea pigs exposed to 0.1 and 0.3 ppm O_3, 4 h/day, 4 days/week for 24 weeks. The bronchoprovocation tests were performed at 4 week intervals during exposure and at 4- to 8-week intervals during the PE period in nonsensitized animals and in animals sensitized to allergen (OVA) prior to, or concurrent with, O_3 exposure. Ozone exposure did not cause AHR in nonsensitized animals, but did exacerbate AHR to both acetylcholine and OVA in sensitized animals that persisted for 4 weeks after exposure. The effects of O_3 on airway responsiveness were gender independent and they were concentration-related for the acetylcholine challenges. The study did not show any evidence of adaptation.

Schelegle et al. (2003a) evaluated airway responsiveness in infant rhesus monkeys exposed to a 5 day O_3 episode repeated every 14 days over a 6-month period. Half of the monkeys were sensitized to house dust mite allergen (*Dermatophagoides farinae*) at 14 and 28 days of age before exposure to a total of 11 episodes of O_3 (0.5 ppm, 8 h/day for 5 days followed by 9 days of FA), house dust mite allergen aerosol (HDMA), or O_3 + HDMA. Monkeys were sedated for measurement of airway responsiveness and then anesthetized for measurement of pulmonary mechanics (e.g., R_{aw}, R_{rs}) using a head-out body plethysmograph. A necropsy was performed after all pulmonary function measurement were taken (see the previous Section 5.3.4 for results). The HDMA and histamine aerosol challenges were administered until R_{aw} doubled. Data were expressed as the concentration increasing R_{aw} by 150% (EC150 R_{aw}). Other measurements included V_T, f_B, and S_aO_2 (estimated by pulse oximeter). Baseline R_{aw} was significantly elevated after 10 exposure episodes in the HDMA + O_3 group compared to the FA, HDMA, and O_3 exposure groups. Aerosol challenge with HDMA at the end of the 10th episode did not significantly affect R_{aw}, V_T, f_B, or S_aO_2. Aerosol challenge with histamine was not significantly different after 6 episodes; however, the EC150 R_{aw} for the HDMA + O_3 group was significantly reduced after 10 episodes when compared to the FA, HDMA, and O_3 exposure groups, indicating the development of airway hyperresponsiveness in this group sometime between episodes 6 and 10.

Using ^{18}O exposures at 1 ppm for 2 h and breathing frequencies of 80, 120, 160, or 200 breaths/minute, Alfaro et al., (2004) examined the site-specific deposition of ^{18}O. At all frequencies, parenchymal areas had a lower content of ^{18}O than trachea and bronchi. As breathing frequency increased from 80 to 160 bpm, the deposition showed a reduction in midlevel trachea and an increase in both mainstream bronchi. At this frequency there was also an increase in deposition in parenchyma supplied by short (cranial) airway paths. At 200 bpm ^{18}O deposition in trachea increased, concurrent with increases in right cranial and caudal bronchi regions. Right cranial parenchymal content decreased at 200 bpm, whereas right caudal parenchymal levels did not change at any breathing frequency. The authors list some limitations of this study, such as the possible effect on regional distribution of ventilation by the use of the negative-pressure ventilator, the effect of paralysis on airway geometry, and possible translocation of ^{18}O during the 2 h exposure period. But the evidence provided by these studies strongly suggests that the effect of rapid, shallow breathing is to create a more evenly distributed

injury pattern, with possibly greater protection from focal injury to the large conducting airways including the trachea and the left mainstem bronchus.

Schelegle et al. (2003b) examined adaptive phenomena in SD rats using an exposure paradigm consisting of 5 days of daily 8 h 1 ppm O_3 exposures followed by 9 days of recovery in filtered air. This O_3/FA pattern was repeated for 4 cycles and animals were analyzed on day 1 and day 5 of each exposure and at the end of the filtered air period. The O_3 -induced rapid shallow breathing pattern followed by adaptation occurred with each cycle, however, the release of substance P from the trachea , the neutrophil content, and cell proliferation, as visualized by BrdU labeling, became attenuated after the first cycle, thus displaying a disconnect from the rapid shallow breathing response. The repeated cycles of O_3 also created hypercellularity of the CAR epithelium and thickening of the CAR interstitium, not linked to changes in cell proliferation. The authors hypothesize a mechanism of injury from repeated O_3 exposures that consists of: (1) diminished neutrophilic inflammation/and or release of mitogenic neuropeptides, (2) depressed cell proliferative response, and (3) cumulative distal airway lesion.

Using a subset of monkeys from the same study (Schelegle et al., 2003b) reported above, Chen et al. (2003) reported that attenuation of O_3-induced rapid shallow breathing and lung function changes typically seen with repeated O_3 exposure may be caused by the adaptation of the respiratory motor responses. The monkeys were killed 3 to 5 days after exposure to 11 "episodes" of O_3 and brain stem coronal slices were prepared. Whole cell recordings were performed on neurons from the nucleus tractus solitarius (NTS), the brain stem region that processes lung sensory signals. Episodic O_3 exposure resulted in neuroplasticity of the NTS including increased nonspecific excitability of the NTS neurons, an increased input resistance, and an increased spiking response to intracellular injections of depolarizing current.

AX5.2.6 Genotoxicity Potential of Ozone

Many experimental studies have been conducted to explore the mutagenic and carcinogenic potential of O_3. Recently published 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 after high O_3 exposures (1 ppm for 72 h and 1 or 2 ppm for 90 min, respectively).

Witschi et al. (1999) exposed female strain A/J mice to 0.12, 0.50, and 1.0 ppm O_3 for 6 h/day, 5 days/week for up to 9 months. After 5 months, one-third of the O_3-exposed mice were

compared to one-half of the controls exposed to filtered air. There was no statistically significant difference in lung tumor multiplicity or incidence. The remaining O_3-exposed mice were split into two groups. Ozone exposure continued in one group for an additional 4 months and the mice in the second group were allowed to recover in filtered air. Again, there were no statistically significant differences in lung tumor multiplicity between control mice and mice exposed to any concentration of O_3 for 9 months. The highest, and only statistically significant lung tumor incidence, was found in the mice exposed to 0.5 ppm O_3. In the O_3-exposed mice allowed to recover in filtered air, only the mice exposed to 0.12 ppm O_3 had statistically significant increases in lung tumor incidence and multiplicity. These results were considered by the authors to be spurious and of no significance for data interpretation.

Kim et al. (2001) evaluated the effects of O_3 inhalation exposure in $B6C3F_1$ mice. No increased incidence of lung tumors was found after exposure to 0.5 ppm O_3 for 6 h/day, 5 days/week for 12 weeks. There were statistically significant differences in mean body weight between O_3-exposed mice and air-exposed controls, as well as significant differences in the mean absolute and relative weights of several organs (e.g., liver, spleen, kidney, testes, and ovary). Histopathologic examination of major organs revealed oviductal carcinomas in 3/10 O_3-exposed female mice.

AX5.3 SYSTEMIC EFFECTS OF OZONE EXPOSURE

Mathematical models of O_3 dosimetry predict that essentially no O_3 penetrates to the blood of the alveolar capillaries, and thus is unlikely to enter the bloodstream (*see Section 5.2*). However, numerous studies have indicated that inhalation of O_3 can produce effects in lymphocytes, erythrocytes, and serum, as well as several organ systems. The mechanism by which O_3 causes such systemic changes is unknown but it seems most likely that some reaction product of O_3, which then penetrates to the blood and is transported to some target site, is a probable mechanism. Extra-pulmonary effects could also be due to the exposure-related production of mediators, metabolic products and cell trafficking. Although systemic effects are of interest and indicate a very broad array of O_3 effects, they are of limited influence and difficult to interpret. By protecting from respiratory tract effects, these systemic effects will likely be

protected against also. New studies of systemic effects are discussed here and summarized in Table AX5-8.

AX5.3.1 Neurobehaviorial Effects

Acute exposures to increasing O_3 concentrations affect animal behavior (see Table AX5-8 for details of studies). Recently reported studies in adult laboratory animals confirm that relevant O_3 concentrations from 0.2 to 1.0 ppm can decrease motor activity and affect short- and long-term memory. This has been shown in passive avoidance conditioning studies in rats (Rivas-Arancibia et al., 1998; Avila-Costa et al., 1999; Dorado-Martinez et al., 2001), and in water-maze learning tasks in mice (Sorace et al., 2001). The effects have been attributed to reactive oxygen/nitrogen species and/or ozonation products. The memory deficits could be blocked by administration of vitamin E (Guerrero et al, 1999) or taurine (Rivas-Arancibia et al., 2000). Rivas-Arancibia et al. (2003) demonstrated in rats that 1 ppm of O_3 for 4 h caused increased freezing and decreased exploratory behaviors that were accompanied by decreased serotonin levels and increased levels of NO, glutamate, dopamine and striatal lipoperoxidation. Morphological changes were also observed in O_3-exposed animals, including neuronal cytoplasm and dendrite vacuolation and dilation of rough endoplasmic reticulum cisterns, which the authors interpret as a neurodegenerative process resulting from the oxidative stress of acute O_3 exposure. A recent study by Nino-Cabrera et al. (2002) reports that 0.7 ppm O_3 exposure for 4 h can induce ultrastructural alterations in the hippocampus and prefrontal cortex in aged rats, areas of the brain where degenerative age-related changes in learning and memory functions have been reported (Bimonte et al., 2003).

In a series of studies reviewed by Paz (1997), animals acutely exposed to O_3 concentrations from 0.35 to 1.0 ppm demonstrated significant alterations of electroencephalographic (EEG) patterns during sleep. For example, rats and cats both showed loss of paradoxical sleep time after 2 to 8 h of O_3 exposure (Paz and Bazan-Perkins, 1992; Paz and Huitrón-Reséndiz, 1996). A permanent 50% loss of paradoxical sleep time, as well as increased total wakefulness and alterations in circadian rhythm, were shown in rat pups born to dams exposed to 1.0 ppm O_3 during the entire period of gestation (Haro and Paz, 1993). The sleep pattern effects were associated with alterations in brain neurotransmitter levels (Huitrón-Reséndiz et al., 1994; Gonzalez-Pina and Paz, 1997) and most likely caused by O_3 reaction products or prostaglandins

Table AX5-8. Systemic Effects of Ozone

Ozone Concentration ppm	Ozone Concentration µg/m³	Duration	Species	Effects[a]	Reference
NEUROBEHAVIORAL EFFECTS					
0.1 0.2 0.5 1.0	196 392 980 1,960	4 h	Rat Wistar male	Rats exposed for 4 h to 0.2, 0.5, and 1 ppm O_3 showed long-term memory deterioration and decreased motor activity, which was reversed 24 h later. Brain and pulmonary Cu/Zn SOD levels were increased in animals exposed to 0.1, 0.2, and 0.5 ppm O_3, but decreased in animals exposed to 1 ppm O_3.	Rivas-Arancibia et al. (1998)
0.1 0.4 0.7 1.1 1.5	196 784 1,372 2,156 2,940	4 h	Rat Wistar male	O_3 caused memory impairment at ≥ 0.7 ppm (one trial passive avoidance test), decreased motor activity at ≥ 1.1 ppm, and increased lipid peroxidation at ≥ 0.4 ppm. Lipid perioxidation levels from the frontal cortex, hippocampus, striatum and cerebellum increased with increasing O_3 concentration.	Dorado-Martinez et al. (2001)
0.3 0.6	588 1,176	30 days	Mouse CD-1 M, F	O_3 exposure slightly but selectively affected neurobehavioral performance in male mice assessed with a 5-min open-field test on exposure days 4 and 19 and on day 3 after the end of the exposure. O_3 exposure, however, did not grossly affect neurobehavioral development. Reversal learning in the Morris water maze test was consistently impaired in both prenatally and adult exposed mice. In addition, longer latency to step-through in the first trial of the passive avoidance test and a decrease in wall rearing in the hot-plate test were recorded in O_3 prenatally exposed mice. Except for the first open-field test, altered responses were observed only in animals exposed to 0.3 ppm O_3.	Sorace et al. (2001)
0.35 0.75 1.5	686 1,470 2,940	12 h	Rat Wistar male	O_3 exposure decreased paradoxical sleep after 2 h of exposure, and increased slow wave sleep after 12 h of exposure at all O_3 concentrations; 5-HT concentrations in the pons increased with increasing O_3 concentration.	Paz and Huitrón-Reséndiz (1996)
0.7	1,372	4 h	Rat	Vitamin E administered before or after O_3 exposure blocked memory deterioration (passive avoidance)and increases in lipid peroxidation levels in the striatum, hippocampus and frontal cortex that were associated with oxidative stress.	Guerrero et al. (1999)

Table AX5-8 (cont'd). Systemic Effects of Ozone

Ozone Concentration					
ppm	**µg/m^3**	**Duration**	**Species**	**Effects[a]**	**Reference**
NEUROBEHAVIORAL EFFECTS (cont'd)					
0.7	1,372	4 h	Rat Wistar male 27 months old	O_3 exposure increased ultrastructural alterations in the hippocampus and prefrontal cortex in aged rats compared with controls. These areas are related to learning and memory functions, which are the first degenerative aging changes observed.	Nino-Cabrera et al. (2002)
0.7 0.8	1,372 1,568	4 h	Rat	Taurine (43 mg/kg) given before or after O_3 exposure improved memory deterioration in an age-specific manner. Old rats showed peroxidation in all control groups and an improvement in memory with taurine. When taurine was applied before O_3, peroxidation levels were high in the frontal cortex of old rats and the hippocampus of young rats; in the striatum, peroxidation caused by O_3 was blocked when taurine was applied either before or after exposure.	Rivas-Arancibia et al. (2000)
1	1,960	12 h/day during dark period	Rat	O_3 exposure during pregnancy affects the neural regulation of paradoxical sleep and circadian rhythm of rat pups 30, 60, and 90 days after birth.	Haro and Paz (1993)
1	1,960	4 h	Rat Wistar male	O_3 caused alterations in long-term memory and a significant reduction of dendritic spines. Results provide evidence that deterioration in memory is probably due to the reduction in spine density in the pyramidal neurons of the hippocampus.	Avila-Costa et al. (1999)
1	1,960	3 h	Rat	O_3 or its reaction products affect the metabolism of major neurotransmitter systems as rapidly as after 1 h of exposure. There were significant increases in dopamine (DA), and its metabolites noradrenaline (NA) and 3,4 dihydroxyphenylacetic acid (DOPAC), and 5-hydroxyindolacetic acid (5-HIAA) in the midbrain and the striatum.	Gonzalez-Pina and Paz (1997)
1.5	2,940	24 h	Rat Wistar male	Adult rats exposed to O_3 spend decreased time in wakefulness and paradoxical sleep and a significant increase in time in slow-wave sleep. Neurochemical changes include increased metabolism of serotonin in the medulla oblongata, pons, and midbrain.	Huitrón-Reséndiz et al. (1994)

Table AX5-8 (cont'd). Systemic Effects of Ozone

Ozone Concentration					
ppm	**µg/m^3**	**Duration**	**Species**	**Effects[a]**	**Reference**
NEUROENDOCRINE EFFECTS					
0.5	980	20 h/day for 5 days	Rat	O_3 produced marked neural disturbances in structures involved in the integration of chemosensory inputs, arousal, and motor control. O_3 inhibited tyrosine hydroxylase activity in noradrenergic brainstem cell groups, including the locus ceruleus (-62%) and the caudal A2 subset (−57%). Catecholamine turnover was decreased by O_3 in the cortex (−49%) and striatum (−18%) but not in the hypothalamus.	Cottet-Emard et al. (1997)
0.5 to 3.0	980 to 5,880	3 h	Rat Sprague-Dawley male	Hyperthyroid, T_4-treated rats (0.1 - 1.0 mg/kg/day for 7 days) had increased pulmonary injury (BALF LDH, albumin, PMNs) at 18 h PE compared to control rats.	Huffman et al. (2001)
1.0	1,960	24 h	Rat Sprague-Dawley male	Hyperthyroid, T3-treated rats had increased metabolic activity and O_3-induced pulmonary injury, but lipid peroxidation, as assessed by alkane generation, was not affected.	Sen et al. (1993)
CARDIOVASCULAR EFFECTS					
0.1 0.3 0.5	196 588 980	5 h	Rat Wistar young (4-6 month) and old (22-24 month)	Transient rapid shallow breathing with slightly increased HR appeared 1-2 min after the start of O_3 exposure, possibly due to olfactory sensation; persistent rapid shallow breathing with a progressive decrease in HR occurred with a latent period of 1-2 h. The last 90-min averaged values for relative minute ventilation tended to decrease with the increase in the level of exposure to O_3 and these values for young rats were significantly lower than those for old rats. An exposure of young rats to 0.1 ppm O_3 for shorter than 5 h significantly decreased the tidal volume and HR and increased breathing frequency, but no significant changes were observed in old rats. There were no differences between young and old rats in non-observable-adverse-effect-levels (NOAELs) for the O_3-induced persistent ventilatory and HR responses, when the NOAELs were determined by exposure to 0.3 and 0.5 ppm O_3.	Arito et al. (1997)

Table AX5-8 (cont'd). Systemic Effects of Ozone

Ozone Concentration ppm	Ozone Concentration µg/m³	Duration	Species	Effects[a]	Reference
CARDIOVASCULAR EFFECTS (cont'd)					
0.1 0.3 0.5	196 588 980	8 h/day for 4 days	Rat Wistar male	Circadian rhythms of HR and core body temperature were significantly decreased on the first and second O_3 exposure days in a concentration dependent manner, and returned to control levels on the third and fourth days.	Iwasaki et al. (1998)
0.25 to 2.0	490 to 3920	2 h to 5 days	Rat Mouse Guinea pig	Robust and consistent decreases in HR and core body temperature; smaller decreases in metabolism, minute ventilation, blood pressure, and cardiac output that vary inversely with ambient temperature and body mass.	Watkinson et al. (2001)
0.5	588	6 h/day 23 h/day for 5 days	Rat F-344 male	Minimal extrapulmonary effects were observed at a core body temperature of 34 °C; O_3 exposures at 22 and 10 °C produced significant decreases in heart rate (160 and 210 beats/min, respectively), core body temperature (2.0 and 3.5 °C, respectively), and body weight (15 and 40 g, respectively). Decreases in these functional parameters reached their maxima over the first 2 exposure days and returned to control levels after the 3rd day of exposure.	Watkinson et al. (1995); Highfill and Watkinson (1996)
0.5	588	8 h	Rat F-344 male	O_3 exposure increased atrial natriuretic peptides in the heart, lung, and circulation, suggesting they mediate the decreased BP and pulmonary edema observed with similar O_3 exposures.	Vesely et al. (1994a,b,c)

Table AX5-8 (cont'd). Systemic Effects of Ozone

Ozone Concentration					
ppm	**µg/m^3**	**Duration**	**Species**	**Effects[a]**	**Reference**
REPRODUCTIVE AND DEVELOPMENTAL EFFECTS					
0.2 0.4 0.6	392 784 1,176	Continuous up to day 17 of pregnancy	Mouse CD-1	No significant effects on either reproductive performance, postnatal somatic and neurobehavioral development (as assessed by a Fox test battery) or adult motor activity (including within-session habituation); some subtle or borderline behavioral deficits were noted, however.	Petruzzi et al. (1995)
0.3 0.6 0.9	588 1,176 1,764	Continuous up to postnatal day 26	Mouse CD-1	O_3 caused subtle CNS effects, but did not affect the animals' capability to learn a reflexive response (limb withdrawal); females exposed to 0.6 ppm O_3 showed a reduced preference for the right paw than both their same-sex controls and 0.6 ppm males. The effect was more robust in the case of an organised avoidance response (wall-rearing).	Petruzzi et al. (1999)
0.3 0.6	588 1,176	Continuous until gestational day 17	Mouse CD-1	Exposure to O_3 did not grossly affect neurobehavioral development, as assessed by somatic and sensorimotor development (postnatal day (PND) 2-20), homing performance (PND 12), motor activity (PND 21), passive avoidance (PND 22-23), water maze performances (PND 70-74), and response to a nociceptive stimulus (PND 100).	Sorace et al. (2001)
0.4 0.8 1.2	784 1,568 2,352	Continuous during gestation days 7-17	Mouse CD-1	No effect of O_3 on reproductive performance; no significant somatic developmental effects in O_3-exposed pups except for a delay in eye opening that was not concentration dependent.	Bignami et al. (1994)

Table AX5-8 (cont'd). Systemic Effects of Ozone

Ozone Concentration ppm	Ozone Concentration µg/m^3	Duration	Species	Effects[a]	Reference
REPRODUCTIVE AND DEVELOPMENTAL EFFECTS (cont'd)					
0.6	1,176	Continuous from birth to weaning	Mouse CD-1	Exposure to O_3 did not produce any significant impairment of the acquisition phase during swimming navigation, a sensitive indicator for hippocampal damage; however, O_3 slightly increased the swimming paths during the last day of the reversal phase. Mice exposed to O_3 showed a slightly but significantly higher swimming speed during all the days, which was unrelated to differences in body weight and to navigational performances. Moreover, mice exposed to O_3 (with the exception of one animal) had a strong tendency to make turns to the left while the controls, independent of sex, preferred clockwise turns.	Dell'Omo et al. (1995a,b)
1.0 2.0	1,960 3,920	3 h	Rat Sprague-Dawley female	High O_3 exposure stimulates hepatocytes to produce increased amounts of nitric oxide as well as protein, possibly mediated by cytokines such as TNF-α produced by alveolar macrophages. When macrophage function is blocked, hepatic injury induced by O_3 is prevented.	Laskin et al. (1994, 1996, 1998); Laskin and Laskin (2001)
2.0	3,920	2 h	Rat F-344	Utilizing electron paramagnetic resonance (EPR) spectroscopy of chloroform extracts of liver homogenates, a significant flux of hydrogen peroxide produced from the reaction of O_3 with lipids of the extracellular lining could be a source of biologically relevant amounts of hydroxyl radical. EPR signals for carbon-centred alkoxyl and alkyl adducts were detected with C-phenyl N-tert-butyl nitrone (PBN) in the liver of animals exposed to O_3.	Vincent et al. (1996)

Table AX5-8 (cont'd). Systemic Effects of Ozone

Ozone Concentration					
ppm	**µg/m³**	**Duration**	**Species**	**Effects[a]**	**Reference**
EFFECTS ON CUTANEOUS TISSUE					
0.5	980	2 h	Mouse hairless female	α tocopherol levels in the stratum corneum (SC) were not affected by O_3 exposure (0.5 ppm) alone, but were significantly depleted by combined exposure to UV and O_3.	Valacchi et al. (2000)
0.8	1,568	6 h	Mouse SKH-1 hairless	Increased lipid peroxidation in the skin epidermis and dermis activated stress proteins HSP27 and HO-1, and activated a proteolytic enzyme system (MMP-9) related to matrix injury and repair processes.	Valacchi et al. (2003)
0.8 1.0 10.0	1,568 1,960 19,600	2 h	Mouse SKH-1 hairless	High O_3 depletes hydrophilic antioxidants in the SC: Vit. C decreased to 80%, GSH decreased to 41%, and uric acid decreased to 44% of control levels after exposure to ≥ 1.0 ppm O_3	Weber et al. (2000)
1.0 5.0 10.0	1,960 9,800 19,600	2 h	Mouse SKH-1 hairless	Vit. E levels decreased and malondialdehyde levels increased in the SC with increasing O_3 concentration.	Thiele et al. (1997a)
1.0 5.0 10.0	1,960 9,800 19,600	2 h	Mouse	High O_3 exerts an oxidizing effect on the outermost layer of the skin (SC); depletes low-molecular-weight antioxidants (α tocopherol, vit. C, glutathione, uric acid) in a concentration dependent manner; increases malondialdehyde levels associated with lipid peroxidation	Weber et al. (2000)

[a]RER = Rough endoplasmic reticulum.
PE = Postexposure (i.e., time after O_3 exposure ceased).
TSH = Thyroid stimulating hormone.
T_3 = Triiodothyronine.
T_4 = Thyroxine.
cyt. = Cytochrome.
NADPH = Reduced nucotinamide adenine dinucleotide phosphate.
NADH =
B[*a*]P = Benzo[*a*]pyrene.
NK = Natural killer.
PHA = Phytohemagglutin.
ConA = Concanavalin A.
LPS = Lipopolysaccharide.
SRBC = Sheep red blood cell.
TBA = Thiobarbituric acid.
ONP =
IgE = Immunoglobulin E.

(Koyama and Hayaishi, 1994). The permanent effects in pups caused by high O_3 exposure during gestation were attributed to the diminished antioxidant capability of fetal tissue (Günther et al., 1993).

AX5.3.2 Neuroendocrine Effects

Several new studies have examined the interaction of O_3 with the pituitary-thyroid-adrenal axis. Sen et al. (1993) found that T_3 supplementation increased metabolic rate and pulmonary injury in the lungs of O_3-treated animals. Increased toxicity to O_3 was later reported in hyperthyroid rats by Huffman et al. (2001).

Mechanisms involved in the interaction of O_3 and the neuroendocrine system are still are not well understood. Cottet-Emard et al. (1997) studied the effects of exposure to 0.5 ppm O_3 for 5 days on catecholamine activity in rat sympathetic efferents and brain areas of prime importance to adaptation to environmental stressors. Catecholamine activity was assessed by estimating the turnover rate of catecholamines and in vivo tyrosine hydroxylase activity in peripheral and central structures (i.e., heart, lungs, superior cervical ganglia, cerebral cortex, hypothalamus and striatum), and in A2 cell groups within the nucleus tractus solitarius (NTS) and locus ceruleus (A6). Ozone inhibited norepinephrine turnover in heart (−48% of the control level) but not in lungs and failed to modify the tyrosine hydroxylase activity in superior cervical ganglia, and the catecholamine content in the adrenal glands. In the central nervous system, O_3 inhibited tyrosine hydroxylase activity in noradrenergic brainstem cell groups, including the locus ceruleus (−62%) and the caudal A2 subset (−57%). Catecholamine turnover was decreased by O_3 in the cortex (−49%) and striatum (−18%) but not in the hypothalamus. These data show 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 described in Section 5.2.1.

High, non-ambient levels of O_3 (e.g., > 1.0 ppm) have been shown to affect visual and olfactory neural pathways in the rat. Custodio-Ramierez and Paz (1997) reported a significant delay in visual evoked potentials recorded in the visual cortex and the lateral geniculate nucleus of male Wistar rats acutely exposed for 4 h to high levels of O_3 (1.5, and 3.0 ppm). Also using Wistar rats, Colin-Barenque et al. (1999) reported cytological and ultrastructural changes in the granule layer of the olfactory bulb after a 4-h exposure to 1 to 1.5 ppm O_3.

AX5.3.3 Cardiovascular Effects

New studies evaluating the cardiovascular effects of O_3 have monitored continuous cardiovascular and ventilatory measurements in unanesthetized rats exposed to O_3. Using the head-out plethysmograph for continuous measurements, Arito et al. (1997) exposed rats to filtered air for 6 h, followed 2 days later by a 5-h exposure to 0.1 ppm O_3, 5 days later by a 5-h exposure to 0.3 ppm O_3, and 10 days later by a 5-h exposure to 0.5 ppm O_3. Each of the O_3 exposures was preceded by a 1-h exposure to filtered air. Transient rapid shallow breathing with slightly increased HR appeared 1-2 min after the start of O_3 exposures and was attributed to an olfactory response. Persistent rapid shallow breathing with a progressive decrease in HR occurred with a latent period of 1-2 h. During the last 90-min of exposure, averaged values for relative minute ventilation tended to decrease with the increase in O_3 concentration for young (4-6 months) but not old (20-22 months) rats.

In a series of studies utilizing radiotelemetry transmitters for monitoring ECG, HR, core body temperature (T_{co}), and motor activity in unanesthetized and unrestrained rats, Watkinson et al. (1995; 2001) and Highfill and Watkinson (1996) demonstrated that when HR was reduced during O_3 exposure, the T_{co} decreased in association with reduced activity. The decreases in body temperature and associated decreases in blood pressure reported by Watkinson et al., and also by Arito et al. (1997), suggested that the pattern and magnitude of the ventilation and HR responses were mediated through some physiological and behavioral defense mechanisms acting to minimize the irritant effects of O_3 inhalation.

The adaptation of cardiovascular and thermoregulatory responses to O_3 also was reported by Iwasaki et al. (1998) in ECG electrode- and thermistor sensor implanted rats after repeated exposure to 0.1, 0.3, and 0.5 ppm O_3 8 h/day for 4 consecutive days. Circadian rhythms of HR and T_{co} were disrupted on the first and second O_3 exposure days in a concentration dependent manner. The 8-h and 12-h averaged values of HR and T_{co} decreased significantly on the first and second exposure days and recovered to control values after small but significant rebound increases on the third and fourth days of O_3 exposure.

More recent reports by Watkinson et al. (2003) further examined the thermoregulatory response to O_3 exposures. Male Fischer-344 rats were exposed to one of three possible O_3 levels (0.0 ppm x 24 h/day [air], 0.5 ppm x 6h/day [intermittent] or 0.5 ppm x 23 h/day [continuous]) at one of three temperatures (10° C [cold], 22° C [room], or 34° C [warm]) for a total of 9 treatment

groups. Another protocol examined the effects of O_3 exposure (0.5 ppm) and exercise described as rest, moderate, heavy or CO_2-stimulated ventilation. Both intermittent and continuous O_3 exposure caused decreases in HR and T_{co} and increases in BALF inflammatory markers. Exercise in filtered air caused increases in HR and T_{co} while exercise in O_3 caused decreases in those parameters. Carbon dioxide and O_3 induced the greatest deficits in HR and T_{co}. The authors discuss several factors which may modulate the hypothermic response, including:1) dose, 2) animal mass (i.e., smaller animals show a greater response), and 3) environmental stress (e.g., restraint, exercise). The authors further discuss possible problems with extrapolation to humans that may be caused by this response (discussed in Chapter 4).

The tissue edema reported in the heart and lungs of laboratory animals exposed to relatively high ambient O_3 concentrations ($\geq$ 0.5 ppm) may be caused by increased circulating levels of atrial natriuretic factor (ANF) which is known to be a possible mediator of increased capillary permeability, vasodilation, and decreased blood pressure (Daly et al., 2002; Vesely et al. (1994a,b,c) reported increased levels of ANF in the heart, lungs, and circulation of rats exposed to 0.5 ppm O_3 for 8 h.

AX5.3.4 Reproductive and Developmental Effects

New studies on the developmental effects of O_3 demonstrate that prenatal exposures to O_3 concentrations < 1.0 ppm do not cause major or widespread somatic or neurobehavioral effects in the offspring of laboratory animals. Animal studies evaluating O_3-induced reproductive effects have not been completed.

Developmental Effects

A study of somatic and neurobehavioral development was reported by Bignami et al. (1994) in female CD-1 mice exposed during pregnancy (days 7 to 17) to O_3 concentrations of 0, 0.4, 0.8, or 1.2 ppm. They did not find any O_3 effects on reproductive or behavioral performance, or on neonatal mortality, but found a significant decrease in body weight gain and delayed eye opening in pups in the 1.2-ppm exposure group. A follow-up study by Petruzzi et al. (1995) did not find any significant deficits in reproductive performance, postnatal somatic and neurobehavioral development, or adult motor activity in CD-1 mice exposed in utero from conception through day 17 of pregnancy to 0, 0.2, 0.4, and 0.6 ppm O_3. In a subsequent study by Petruzzi et al. (1999),

subtle changes in handedness and morphine reactivity were found when the O_3 exposures (0.3, 0.6, or 0.9 ppm) continued postnatally until weaning [post natal day (PND) 26]. Female mice exposed to 0.6 ppm O_3 showed a reduced preference for the right paw at PND 70, and 0.9 ppm O_3 altered hot plate avoidance after IP treatment with morphine (10 mg/kg) at PND 100.

Dell'Omo et al. (1995a,b) exposed CD-1 mice to 0.6 ppm O_3 from birth through weaning (PND 22 or 26). Swimming navigation was tested (Dell'Omo et al., 1995a) at 12 to 13 weeks of age using acquisition and reversal trials. Exposure to O_3 did not produce any significant impairment of navigational performance during acquisition and only subtle changes during reversal. As in previous studies, there were no significant effects of O_3 on reproductive performance but O_3 offspring showed a significant reduction in body weight. Ozone effects on neurobehavioral development were not large and very selective, with some attenuation of activity responses and impairment of passive avoidance acquisition (Dell'Omo et al. (1995b). Similarly, only small and selective effects on somatic and sensorimotor development were found in the offspring of CD-1 mice continuously exposed from 30 days prior to the formation of breeding pairs until PND 17 to 0.0, 0.3, or 0.6 ppm O_3 (Sorace et al., 2001).

AX5.3.5 Effects on the Liver, Spleen, and Thymus

Liver

New studies on the effects of O_3 on liver showed that, in rats, high (1 to 2 ppm) acute O_3 exposures caused increased production of NO by hepatocytes and enhanced protein synthesis (Laskin et al., 1994; 1996).

The O_3-associated effects shown in the liver are most likely mediated by inflammatory cytokines (e.g., TNF alpha) or other cytotoxic mediators (e.g., hydroxyl radicals) released by activated macrophages in the lungs (Vincent et al., 1996; Laskin et al., 1998; Laskin and Laskin, 2001). Except for the earlier work on xenobiotic metabolism, the responses occurred after very high acute O_3 exposures.

Spleen and Thymus

Ozone has been shown to primarily affect T-cell mediated systemic immunity. New studies evaluating O_3-induced effects on spleen and thymus have not been completed.

AX5.3.6 Ozone Effects on Cutaneous and Ocular Tissues

Ground-level smog exposure not only affects various organ systems, when inhaled, but may potentially have direct effects on exposed skin and eyes. Several new studies have examined the effects of O_3 on skin. Ozone can have an oxidizing effect on the outermost layer of the skin, called the stratum corneum (SC) where it may compromise skin barrier function and possibly induce an epidermal proinflammatory response (e.g., Weber et al., 2001; Cotovio et al., 2001; Thiele, 2001); however, these cutaneous effects of O_3 are found only at very high concentrations used in experimental studies and have not been shown at more relevant ambient- or near-ambient levels (< 0.5 ppm) of O_3 exposure. The lack of ambient-level O_3 effects on the skin is most likely due to a well-developed defense against oxidative stress, utilizing nonenzymatic (e.g., vitamin C and E, glutathione, uric acid, α-tocopherol) and enzymatic (e.g., superoxide dismutase, catalase, glutathione reductase and peroxidase) antioxidants found in many living organisms (Cross et al., 1998).

Effects of ground-level smog on the eyes have also been reported, but generally are attributed to related photochemical oxidants like peroxyacetyl nitrate (Vyskocil et al., 1998) or possibly to atmospheric O_3 precursors or reaction products like aldehydes. Ocular tissues also have similar antioxidant protective function as in the skin, but are not well studied (Mucke, 1996; Rose et al., 1998).

The cutaneous effects of O_3 exposure were first reported by Thiele et al. (1997a,b,c,d). Hairless mice (SKH-1) were exposed to 1, 5, and 10 ppm O_3 for 2 h or to 1 ppm O_3 for 2 h on six consecutive days and skin layers from the epidermis and dermis were separated for analysis of antioxidants and lipid peroxidation products. Decreased antioxidant levels (α-tocopherol; ascorbic acid) were found in the upper epidermis and increased malondialdehyde (MDA), a lipid peroxidation product, was found in both the upper and lower epidermal layers. Effects were dose dependent and became significant ($p < 0.05$) for α-tocopherol and MDA after single exposures to >1.0 and >5.0 ppm O_3, respectively. Repeated O_3 exposures caused significantly higher MDA concentrations. The effects of MDA accumulation could be prevented by enriching the skin with vitamin E.

In the SKH-1 hairless mouse model, Weber et al. (1999, 2000, 2001) demonstrated that 0.8 to 10 ppm O_3 exposure for 2 h depletes the low molecular weight antioxidants (e.g., α-tocopherol, vitamin C, glutathione, uric acid) in the SC in a dose-response manner and causes

increased MDA. The effects were statistically significant ($p < 0.05$) at ≥ 1 ppm O_3 and ≥ 5 ppm for antioxidant depletion and increased MDA, respectively. Reactive aldehydes were found in the epidermis at the highest O_3 concentration tested, though this level of exposure is of limited relevance. Preexposure to O_3 followed by low-dose ultraviolet (UV) radiation (0.33 MED) decreased the significance level to 0.5 ppm O_3, probably through combined oxidative stress on the SC (Valacchi et al., 2000). Stress-inducible proteins (e.g., heme oxygenase-1) and other heat shock proteins (e.g., HSP27 and HSP70) were found in deeper cellular layers of the epidermis after 2 h of exposure to 8.0 ppm O_3 (Valacchi et al., 2002). Prolonged exposure to lower concentrations of O_3 (0.8 ppm) for 6 h also induces cellular stress responses that included the formation of HNE protein adducts, HSP27, and heme-oxygenase-1 in the deeper cellular layers of the skin that continued for up to 18 h after O_3 exposure, followed by repair processes (Valacchi et al., 2003).

AX5.4 INTERACTIONS OF OZONE WITH OTHER CO-OCCURRING POLLUTANTS

Ozone is part of a complex mixture of air pollutants with a composition and pattern that varies geographically and temporally (by hour of the day, day of the week, and season). Health effects caused by the complex mixture are undoubtedly different (either subtly or significantly) from the additive effects of a few of the hundreds of compounds present. The only disciplinary approach that can evaluate a "real-world" complex mixture is epidemiology (Chapter 7). However, because of the difficulty in evaluation of causative factors and quantitative relationships in epidemiology studies, it is useful to consider animal toxicological studies of mixtures. Such studies can be divided into three categories: (1) ambient air mixtures, (2) laboratory-generated complex mixtures (e.g., gasoline combustion mixtures having ultraviolet-irradiation, other reaction mixtures with O_3 and several other components), and (3) binary mixtures. In most cases, experimental designs in the first two classes did not have an O_3-only group, making it difficult to impossible to discern the influence of O_3. The more recent mixture studies that are discussed here typically have been with NO_2, sulfuric acid (H_2SO_4), or ammonium sulfate ($[NH_4]_2SO_4$).

Interpreting the mixture studies in terms of real-world risk is difficult because laboratory exposure patterns do not always represent real-world exposure patterns. For example, in the real world, NO_2 often peaks before O_3 peaks, with a mixture occurring between the peaks, but most laboratory exposures used mixtures only. Also, most studies of O_3 and NO_2 mixtures used ambient levels of O_3 and levels of NO_2 high above ambient. As shall be seen, all interaction possibilities have occurred, depending upon the composition of the mixture, the endpoint examined, and the exposure regimen. In some cases, no interaction was found. Most often, additivity (the effects of the mixture are equal to the sum of the effects of the individual components) or synergism (the effects of the mixture are greater than the sum of the effects of the individual components) was observed. Antagonism (the effects of the mixture are less than the sum of the individual components) was rarely found.

AX5.4.1 Ozone and Nitrogen Oxides

The most commonly studied copollutant in binary mixtures with O_3 is NO_2. New studies evaluating the effects of combined O_3/NO_2 exposures are listed in Table AX5-9.

Recent work has demonstrated that chronic exposures of rats to 0.8 ppm O_3 and 14.4 ppm NO_2 for 6 h/day caused the rats to develop insufficiency and severe weight loss (Farman et al., 1997). Half of these animals died after 55 to 78 days of exposure due to severe fibrosis. Biochemical analysis of lung tissue demonstrated increased total lung collagen and elastin, with loss of mature collagen, indicating there was breakdown and remodeling of the lung parenchyma. In follow-up rat studies, Farman et al. (1999) reported a sequence of events starting with increasing inflammatory and mild fibrotic changes for the first 3 weeks of exposure to 0.8 ppm O_3 and 14.4 ppm NO_2, stabilized or even reduced changes after 4 to 6 weeks, and severe increases over 7 to 9 weeks of exposure. The authors speculated that repair processes occurring during the middle 4 to 6 weeks of exposure become overwhelmed, leading to progressive fibrosis after 7 to 8 weeks of exposure. When combined exposures to 0.8 ppm O_3 and 14.4 ppm NO_2 for 6 h/day, 7 days/week were extended for 90 days, Farman et al. (1999) found that the lesion extended far into the acinus, but the extent of tissue involvement was the same after 7, 78, and 90 days of exposure. At the end of exposure, in situ hybridization for procollagen types I and III demonstrated high levels of messenger RNA within central acini in the lungs from the combined exposure group, but not in lungs from the rats exposed to O_3 and NO_2 alone.

Table AX5-9. Interactions of Ozone With Nitrogen Dioxide

Concentration								
O_3		NO_2						
ppm	µg/m³	µg/m³	ppm	Duration	Species	Endpoints[a]	Interaction	Reference
MORPHOLOGY								
0.8	1,568	27,072	14.4	6 h/day , 7 days/week for 90 days	Rat, M (S-D) 10-12 weeks old	Morphometry of lung parenchyma; DNA probes for procollagen; in situ mRNA hybridization	syngeristic; more peripheral centriacinar lesion, but same after 7, 78, and 90 days of exposure.	Farman et al. (1999)
0.3	588	2,256	1.2	Continuous for 3 days	Rat, M (S-D) 3 months old	DNA single strand breaks; polyADPR synthetase of AMs; total cells, protein, and LDH in BALF	None; effect due to O_3	Bermudez et al. (1999, Bermudez (2001)
BIOCHEMISTRY (cont'd)								
0.8	1,568	27,072	14.4	6 h/day, 7 days/week for 9 weeks	Rat, M (S-D) 10-12 weeks old	Lung hydroxyproline, hydrooxypyridinium, DNA, and protein content of whole lung; morphology and labeling index	Synergistic; fibrosis after 7-8 weeks of exposure	Farman et al. (1997)

BAL = Bronchoalveolar lavage.
PG = Prostaglandin.
G-6-PD = Glucose-6-phosphate dehydrogenase.
GDT = GSH-disulfide transhydrogenase.
GSHPX = GSH peroxidase.
SOD = Superoxide dismutase.
DR = Disulfide reductase.
NADPH-CR = Reduced nicotinamide adenine dinucleotide phosphate-cytochrome c reductase.
GSH = Glutathione.
6-PG-D = 6-phosphogluconate dehydrogenase.

Bermudez et al. (1999) reported that a 3-day exposure to 0.3 ppm O_3, and the combined exposure of O_3 and 1.2 ppm NO_2, caused significant DNA single-strand breaks in the alveolar macrophages of Sprague-Dawley rats. No changes were caused by NO_2-only exposure. In a follow-up study, Bermudez (2001) showed that the same exposures stimulated the activity of polyADPR synthetase, an indicator of response to lung cellular DNA repair caused by oxidant-induced lung injury.

Other published reports (Ishii et al., 2000b; Weller et al., 2000) indicate that the laboratory animal model of progressive pulmonary fibrosis, utilizing long-term, combined O_3 (0.4 to 0.8 ppm) and high-level NO_2 (7 to 14 ppm) exposure, causes an initial acute pulmonary inflammation, followed by adaptation and repair, and eventually causing pulmonary fibrosis after 6 to 13 weeks of exposure.

AX5.4.2 Ozone and Other Copollutants

Although the bulk of the toxicological database for binary mixtures of O_3 involves NO_2 or acidic sulfate and nitrate aerosols (see Section 5.4.3), a few studies have examined responses to combinations of O_3 with other single pollutants, such as formaldehyde (HCHO); or with surrogates of pollutants treated as a single pollutant, such as tobacco smoke. New studies evaluating coexposures of O_3 with acid aerosols and particle mixtures are listed in Table AX5-10.

Interactive effects of ozone and formaldehyde.

A study by Cassee and Feron (1994) focused on biochemical and histopathological changes in the nasal respiratory epithelium of rats exposed 8 h/day for 3 days to 0.4 ppm O_3 and 3.6 ppm HCHO, alone and combined. No interactive effects were found, however, despite the high levels of HCHO when compared to typical ambient levels of 1 to 10 ppb (e.g., Rehle et al., 2001). In a follow-up to their previous combined exposure study in rats, Mautz (2003) elaborated on changes in breathing pattern and epithelial cell proliferation attributed to O_3 and HCHO. Rats were exposed to 0.6 ppm O_3 and 10 ppm HCHO alone and in combination for 3 h with exercise at two times resting ventilation. Even with exercise, HCHO does not penetrate to the lower respiratory tract to interact with O_3, and does not alter breathing patterns to modify local O_3 dose. Parenchymal injury was, therefore, due to O_3 alone. In the nasal transitional epithelium and in the trachea, however, combined exposure produced additive effects due to the increased volume of

Table AX5-10. Interactions Of Ozone With Particles

Concentration							
O_3		PM					
ppm	µg/m³	mg/m³[a]	Duration[b]	Species	Endpoints[c]	Interaction	Reference
SULFURIC ACID							
0.1 0.2	196 392	0.02 - 0.15 (0.4 - 0.8 µm)	23.5 h/day or intermittent 12 h/day for up to 90 days	Rat S-D male	Morphology Biochemistry	No interaction	Last and Pinkerton (1997)
0.1 0.3 0.6	196 588 1,176	0.50 (0.3 µm) 0.125 (0.3 µm)	3 h	Rabbit NZW male	AM intracellular pH homeostasis and H^+ extrusion	Antagonism	Chen et al. (1995)
0.1 0.3 0.6	196 588 1,176	0.50 (0.3 µm) 0.125 (0.3 µm)	3 h	Rabbit NZW male	Airway responsiveness (in vitro bronchial rings + ACh)	Antagonism	El-Fawal et al. (1995)
0.6	1,176	0.5 (0.06 and 0.3 µm MMD)	4 h/day for 2 days	Rat	Morphology: volume percentage of total parenchyma containing injured alveolar septae; bromode oxyuridine cell labeling index in the periacinar region	Synergism: ultrafine + O_3, but not fine Synergism: fine + O_3	Kimmel et al. (1997)
PARTICLE MIXTURES							
0.1	196	Diesel PM (NIST #2975) reacted with O_3 for 48 h	24 h (IT)	Rat S-D	Inflammation	Synergism	Madden et al. (2000)
0.16 0.30 0.59	314 588 1,156	0.05 - 0.22 mg/m³ ammonium bisulfate 0.03 - 0.10 mg/m³ C 0.11 - 0.39 pm NO_2 0.02 - 0.11 mg/m³ HNO_3; (0.3 µm MMAD)	4 h/day, 3 days/week for 4 weeks	Rat F344N male	breathing pattern, morphology, lavagable protein, and clearance	Complex interactions, but possible loss of typical attenuation seen with O_3 only exposure, reflecting persistence of inflammation	Mautz et al. (2001)

Table AX5-10 (cont'd). Interactions Of Ozone With Particles

Concentration							
O_3		PM					
ppm	µg/m³	mg/m³[a]	Duration[b]	Species	Endpoints[c]	Interaction	Reference
PARTICLE MIXTURES (cont'd)							
0.2	392	0.07and 0.14 mg/m³ ammonium bisulfate (0.45 µm MMMD); 0.05 and 0.10 mg/m³ carbon	4 h/day, 3 days/week for 4 weeks	Rat F344 male 22-24 months old	BAL protein and albumin; plasma hydroxylase and fibronectin	Questionable interaction	Bolarin et al. (1997)
0.2	392	0.50 mg/m³ ammonium bisulfate (0.45 µm MMMD) and elemental carbon)	4 h/day, 3 days/week for 4 weeks; nose only	Rat F344N-NIA 22-24 months old	DNA labeling of dividing lung epithelial and interstitial cells by 5-bromo-2-deoxyuridine	Synergism	Kleinman et al. (2000)
0.3	588	0.063 to 1.57 mg/m³ CAPs (Boston) + ip OVA sensitization	5 h	Mouse BALB/c	Airway function	Interaction: increased R_L and airway responsiveness	Kobzik et al. (2001)
0.4	784	0.20 and 0.50 mg/m³ fine, H_2O_2-coated carbon (0.26 µm MMMD)	4 h/day for 1 or 5 days	Rat S-D 300 g	Inflammation	Synergism for effect on day 5	Kleinman et al. (1999)
0.5	980	Endotoxin (IN) 100 µg 24 h and 48 h after the 3rd O_3 exposure	8 h/day for 3 days	Rat F344 10-12 weeks old	Nasal morphology	Synergism: increased intraepithelial mucosubstances and mucous cell metaplasia	Fanucchi et al. (1998) Wagner et al. (2001a,b)
0.5	980	OVA (IN) 50 µl (1%)	8 h/day for 1 day or 3 consecutive days	Rat Brown Norway 10-12 weeks old	Nasal morphology	Synergism: increased intraepithelial mucosubstances and mucous all metaplasia	Wagner et al. (2002)

Table AX5-10 (cont'd). Interactions Of Ozone With Particles

Concentration							
O_3		PM					
ppm	µg/m³	mg/m³[a]	Duration[b]	Species	Endpoints[c]	Interaction	Reference
PARTICLE MIXTURES (cont'd)							
0.8	1,600	0.5 mg. 1.5 mg or 5 mg of PM from Ottawa Canada (EHC-93)	2, 4, and 7 days after IT instillation	Rat	Inflammation	Interaction: increased TNF-α in BALF	Ulrich et al. (2002)
1	1,960	0.11 mg/m³ ultra fine carbon (25 nm CMD) + endotoxin (IH))	6 h	Rat F344 male, 10 weeks and 22 months old; Mouse TSK male, 14-17 months old	Inflammation	Interaction: increased PMNs and ROS release from BALF cells for old rats and mice primed with endotoxin; depressed in young rats	Elder et al. (2000a,b)
1	1,960	Endotoxin (37.5 EU) for 10 minutes	4, 20, or 24 h	Mouse C57BL/6J 8 weeks old	Inflammation	Synergism: increased BALF protein and PMNs	Johnston et al. (2000, 2002)
1	1,960	Endotoxin (IN) 0, 2, or 20 µg in 120 µL	8 h, repeated after 24 h	Rat F344	Lung morphometric analysis and inflammation	Synergism: increased BALF PMNs and mucin glycoprotein; increased intraepithelial mucosubstances and mucingene mRNA	Wagner et al. (2003)

[a]VMD = Volume median diameter.
σg = Geometric standard deviation.
MMAD = Mass median aerodynamic diameter.
CMD = Count median diameter.
PM = Particulate Matter.
OVA= Ovalbumin.
AED = Aerodynamic diameter.
[b]Unless indicated otherwise, whole-body exposures used.
[c]BAL = Bronchoalveolar lavage.
AM = Alveolar macrophage.
IN = Intranasal
IT = Intratracheal.

toxicants during exercise. No other combined pollutant studies have been published in the peer-reviewed literature, although two studies compared the respiratory effects of O_3 to HCHO. Nielson et al. (1999) compared upper airway sensory irritation caused by HCHO concentrations up to 4 ppm to the lower airway irritation caused by O_3. Using BALB/c mice, they continuously measured f_B, V_t, expiratory flow, T_i, T_e, and respiratory patterns during acute, 30-min exposures. The NOEL for HCHO was 0.3 ppm, compared to 1.0 ppm for O_3.

Interactive effects of ozone and tobacco smoke.

Wu et al. (1997) also reported that inhalation of cigarette smoke evokes a transient bronchoconstrictive effect in anesthetized guinea pigs. To examine whether O_3 increases airway responsiveness to cigarette smoke, effects of smoke inhalation challenge on total pulmonary resistance (R_L) and dynamic lung compliance (C_{dyn}) were compared before and after acute exposure to 1.5 ppm O_3 for 1 h. Before O_3 exposure, inhalation of two breaths of cigarette smoke (7 ml) at a low concentration (33%) induced a mild and reproducible bronchoconstriction that slowly developed and reached its peak ($\Delta R_L = 67 \pm 19\%$, $\Delta C_{dyn} = -29 \pm 6\%$) after a delay of > 1 min. After exposure to O_3, the same cigarette smoke inhalation challenge evoked an intense bronchoconstriction that occurred more rapidly, reaching its peak ($\Delta R_L = 620 \pm 224\%$, $\Delta C_{dyn} = -35 \pm 7\%$) within 20 s, and was sustained for > 2 min. By contrast, sham exposure to room air did not alter the bronchomotor response to cigarette smoke challenge. Pretreatment with selective antagonists of neurokinin type 1 and 2 receptors completely blocked the enhanced airway responsiveness. The authors concluded that O_3 exposure induced airway hyperresponsiveness to inhaled cigarette smoke, which resulted primarily from the bronchoconstrictive effect of endogenous tachykinins.

To determine the effects of aged and diluted sidestream cigarette smoke (ADSS) as a surrogate of environmental tobacco smoke (ETS) on O_3-induced lung injury, Yu et al. (2002) exposed male B6C3F1 mice to (1) filtered air, (2) ADSS, (3) O_3, or (4) ADSS followed by O_3 ($ADSS/O_3$). Exposure to 30 mg/m^3 ADSS, 6 h/day for 3 days, followed by exposure to 0.5 ppm O_3 for 24 h was associated with a significant increase in the number of cells recovered by BAL compared with exposure to ADSS alone or O_3 alone. The proportion of neutrophils and lymphocytes, as well as total protein level in BAL, also was significantly elevated following the combined exposure when compared with all other groups. Within the centriacinar regions of the

lungs, the percentage of proliferating cells identified by bromodeoxyuridine (BrdU) labeling was unchanged from control following exposure to ADSS alone, but was significantly elevated following exposure to O_3 (280% of control), and further augmented in a statistically significant manner in mice exposed to ADSS/O_3 (402% of control). Following exposure to O_3 alone or combined with ADSS, the ability of AMs to release interleukin (IL)-6 under lipopolysaccharide (LPS) stimulation was significantly decreased, while exposure to ADSS alone or ADSS/O_3 caused a significantly increased release of TNF-α from AMs under LPS stimulation. The authors concluded that ADSS exposure enhances the sensitivity of animals to O_3-induced lung injury.

Toxicological studies with components of ETS (e.g., nicotine receptor agonists, acrolein, and oxidants) have shown that the vagal bronchopulmonary C-fibers are stimulated by acute exposures that initiate both central and local responses (Bonham et al., 2001; Mutoh et al., 2000). The central responses (e.g., tachypnea, cough, bronchoconstriction, increased mucous secretion) are more protective of the lung; however, local responses may include increased sensitization of the C-fibers to other irritants, including O_3.

AX5.4.3 Complex (Multicomponent) Mixtures Containing Ozone

Ambient pollution in most areas is a complex mix of more than two chemicals, and a number of new studies have examined the effects of exposure to multicomponent atmospheres containing O_3. Some of these studies attempted to simulate photochemical reaction products occurring under actual atmospheric conditions. However, the results of these studies are often difficult to interpret because of chemical interactions between the components, as well as the resultant production of variable amounts of numerous secondary reaction products, and a lack of precise control over the ultimate composition of the exposure environment. In addition, the role of O_3 in the observed biological responses is often obscure.

A recent attempt has been made to examine multicomponent mixtures resulting from the reaction of O_3 with unsaturated hydrocarbons [e.g., isoprene (C_5H_8) and terpene ($C_{10}H_{16}$)], producing HCHO, formic acid, acetone, acrolein, acetic acid, and other oxidation products, many of which are strong airway irritants. Wilkins et al. (2001) evaluated sensory irritation by measuring mean f_B in the mouse bioassay and found a 50% reduction after 30 min of exposure to reaction products of O_3 and isoprene. The mixture at this time period contained < 0.2 ppm O_3, so the authors attributed the observed effects to the oxidation products. Clausen et al. (2001), using

the same mouse model, evaluated the reaction products of O_3 and limonene. A 33% reduction in mean f_B was produced after 30 min of exposure to the mixture containing < 0.3 ppm O_3, again implicating the effects of strong irritant products.

Pollutant mixtures containing acid aerosols comprise another type of commonly examined exposure atmosphere. Most of these mixtures included acidic sulfate aerosols as the copollutant. Peak (1-h) ambient levels of sulfuric acid (H_2SO_4) are estimated at 75 $\mu g/m^3$, with longer (12-h) averages about one-third of this concentration.

More recent studies found some differences in airway responses to inhaled acid particle-O_3 mixtures that may have been partly due to airway dosimetry. Various physical and chemical mechanisms may be responsible (*see* Schlesinger, 1995). For example, physical adsorption or absorption of O_3 or its reaction products on a particle could result in transport to more sensitive sites, or to sites where O_3, by itself, would not normally be reactive. Chemical reactions on the surface of particles can form secondary products that are more toxicologically active, or chemical characteristics of the particle may change the residence time or reactivity of oxidation products at the site of deposition. Chen et al. (1995) and El-Fawal et al. (1995) tested this hypothesis on rabbit pulmonary macrophages and on airway reactivity, respectively. Male New Zealand white rabbits were exposed for 3 h to 125 $\mu g/m^3$ H_2SO_4, 0.1, 0.3, or 0.6 ppm O_3, and to combinations of O_3 and H_2SO_4. Decreased pH following exposure to acid aerosol was correlated with phagocytic activity and capacity of harvested macrophages; however, exposure to the mixture did not show this relationship (Chen et al., 1995). Responsiveness of harvested bronchial rings to acetylcholine was increased following O_3 exposure, but the combination of O_3 and H_2SO_4 resulted in antagonism (El-Fawal et al., 1995).

Churg et al. (1996) demonstrated increased uptake of asbestos or TiO_2 into rat tracheal explant cultures in response to 10 min O_3 (up to 1.0 ppm) pre-exposure. These data suggest that low concentrations of O_3 may increase the penetration of some types of PM into epithelial cells. More recently, Madden et al. (2000) demonstrated a greater potency for ozonized diesel PM to induce prostaglandin E_2 production from human epithelial cell cultures, suggesting that O_3 can modify the biological activity of PM derived from diesel exhaust (*see details below*).

Vincent et al. (1997) and Adamson et al. (1999) exposed rats to 0.8 ppm O_3 in combination with 5 or 50 mg/m^3 of resuspended urban particles for 4 h. Although PM alone caused no change in cell proliferation (^{3}H-thymidine labeling), co-exposure to either concentration of resuspended

PM with O_3 greatly potentiated the proliferative effects of exposure to O_3 alone. These interactive changes occurred in epithelial cells of the terminal bronchioles and the alveolar ducts. Kimmel et al. (1997) examined the effect of acute coexposure to O_3 and fine or ultrafine H_2SO_4 aerosols on rat lung morphology. They determined morphometrically that alveolar septal volume was increased in animals coexposed to O_3 and ultrafine, but not fine, H_2SO_4. Interestingly, cell labeling, an index of proliferative cell changes, was increased only in animals co-exposed to fine H_2SO_4 and O_3, as compared to animals exposed to O_3 alone. Last and Pinkerton (1997) found that subchronic exposure to acid aerosols (20 to 150 $\mu g/m^3$ H_2SO_4) had no interactive effect on the biochemical and morphometric changes produced by either intermittent or continuous O_3 exposure (0.12 to 0.2 ppm). Thus, the interactive effects of O_3 and acid aerosol coexposure in the lung disappeared during the long-term exposure. Sindhu et al. (1998) observed an increase in rat lung putrescine levels after repeated, combined exposures to O_3 and a nitric acid vapor.

Other studies have examined interactions between carbon particles and O_3. Creutzenberg et al. (1995) treated rats with a high concentration of carbon particles by intratracheal instillation followed by either a 7-day or 60-day exposure to 0.5 ppm O_3. The phagocytotic capacity and chemotactic migration capability of alveolar macrophages was impaired by carbon black, but was stimulated by O_3. Kleinman et al. (1999) examined the effects of O_3 plus fine, H_2SO_4-coated, carbon particles (MMAD = 0.26 μm) for 1 or 5 days. They found that the inflammatory response with the O_3-particle mixture was greater after 5 days (4 h/day) than after day 1. This contrasted with O_3 exposure alone (0.4 ppm), which caused marked inflammation on acute exposure, but no inflammation after 5 consecutive days of exposure.

Kleinman et al. (2000) examined the effects of a mixture of elemental carbon particles, O_3, and ammonium bisulfate on rat lung collagen content and macrophage activity. Decreases in lung collagen, and increases in macrophage respiratory burst and phagocytosis were observed relative to other pollutant combinations. Mautz et al. (2001) used a similar mixture (i.e., elemental carbon particles, O_3, ammonium bisulfate, but with NO_2 also) and exposure regimen as Kleinman et al. (2000). There were decreases in pulmonary macrophage Fc-receptor binding and phagocytosis and increases in acid phosphatase staining. Bronchoalveolar epithelial permeability and cell proliferation were increased. Altered breathing-patterns also were observed, with some adaptations occurring.

Bolarin et al. (1997) exposed rats to 50 or 100 μg/m^3 carbon particles in combination with ammonium bisulfate and O_3. Despite 4 weeks of exposure, they observed no changes in protein concentration in lavage fluid or blood prolyl 4-hydroxylase, an enzyme involved in collagen metabolism. Slight decreases in plasma fibronectin were present in animals exposed to the combined pollutants versus O_3 alone. Thus, the potential for adverse effects in the lungs of animals challenged with a combined exposure to particles and gaseous pollutants is dependent on numerous factors, including the gaseous co-pollutant, concentration, and time.

In a complex series of studies, Oberdörster et al. examined the interaction of several pulmonary oxidative stress pollutants. Elder et al. (2000a,b) reported the results of combined exposure to ultrafine carbon particles (100 μg/m^3) and O_3 (1 ppm) in young and old Fischer 344 rats that were pretreated with aerosolized endotoxin. In old rats, exposure to carbon and O_3 produced an interaction that resulted in a greater influx in neutrophils than that produced by either agent alone. This interaction was not seen in young rats. Oxidant release from lavage fluid cells also was assessed and the combination of endotoxin, carbon particles, and O_3 produced an increase in oxidant release in old rats. This mixture produced the opposite response in the cells recovered from the lungs of the young rats, indicating that the lungs of the aged animals underwent greater oxidative stress in response to a complex pollutant mix of particles, O_3, and a biogenic agent. Johnston et al. (2000; 2002) reported the results of combined exposure to O_3 (1 and 2.5 ppm for 4, 20, or 24 h) and low-dose endotoxin, or to O_3 and endotoxin separately, in newborn and adult C57BL/6J mice. In the first study, adult (8 week old) mice showed greater sensitivity to O_3 than newborn (36 h old) mice on the basis of mRNAs encoding for various chemokines and cytokines. In contrast, adult and newborn mice responded similarly 2 h after endotoxin exposure (10 ng for 10 min), suggesting that age differences in O_3-generated inflammation is secondary to epithelial cell injury. In the second study, 8 week old mice exposed to O_3 (1 ppm for 24 h) followed by endotoxin (37.5 EU for 10 min) showed increased responsiveness over either exposure alone, on the basis of increased expression of chemokine and cytokine messages and increased BAL fluid levels of protein and PMNs.

Fanucchi et al. (1998) and Wagner et al. (2001a,b) examined the synergistic effect of co-exposure to O_3 and endotoxin on the nasal transitional epithelium of rats that also was mediated, in part, by neutrophils. Fisher 344 rats intranasally instilled with endotoxin and exposed to 0.5 ppm O_3, 8 h per day, for 3 days developed mucous cell metaplasia in the nasal

transitional epithelium, an area normally devoid of mucous cells; whereas, intratracheal instillation of endotoxin (20 μg) caused mucous cell metaplasia rapidly in the respiratory epithelium of the conducting airways. A synergistic increase of intraepithelial mucosubstances and morphological evidence of mucous cell metaplasia were found in rat maxilloturbinates upon exposure to both O_3 and endotoxin, compared to each pollutant alone. A similar response was reported in OVA-sensitized Brown Norway rats exposed to 0.5 ppm O_3, 8 h/day for 3 days (Wagner et al., 2002), indicating that coexposure to O_3 and inflammatory biogenic substances like allergens (e.g., OVA) or bacterial endotoxin can augment epithelial and inflammatory responses in rat nasal passages.

In follow-up studies, Wagner et al. (2003) reported that coexposure of rats to O_3 and endotoxin also enhanced epithelial and neutrophilic inflammatory responses in the pulmonary airways. Fisher 344 rats were intranasally instilled with endotoxin and exposed to 1.0 ppm O_3 for 8 h, which was repeated 24 h later. Three days after the last exposure, BALF was analyzed for inflammatory cells and secreted mucosubstances (mucin 5AC), and lung tissue was processed for morphometric analysis. Endotoxin instillation alone caused a dose-dependent increase in BALF neutrophils that was further increased 2-fold in O_3-exposed rats given 20 μg endotoxin, the highest dose. Mucin glycoprotein 5AC also was increased in the BALF at this dose, but not at lower endotoxin doses. Ozone exposure alone did not cause mucus hypersecretion, but it did potentiate mucus secretion in rats given both 2 and 20 μg endotoxin and increased intraepithelial mucosubstances 2-fold, which was further substantiated by significant increases in mucin gene (rMuc5AC) mRNA levels in the conducting airways.

The effect of O_3 modifying the biological potency of PM (diesel PM and carbon black) was examined by Madden et al. (2000) in rats. Reaction of NIST Standard Reference Material # 2975 diesel PM with 0.1 ppm O_3 for 48 h increased the potency (compared to unexposed or air-exposed diesel PM) to induce neutrophil influx, total protein, and LDH in lung lavage fluid in response to intratracheal instillation. Exposure of the diesel PM to high, non-ambient O_3 concentration (1.0 ppm) attenuated the increased potency, suggesting destruction of the bioactive reaction products. Unlike the diesel particles, carbon black particles exposed to 0.1 ppm O_3 did not exhibit an increase in biological potency, which suggested that the reaction of organic components of the diesel PM with O_3 were responsible for the increased potency. Reaction of particle components with O_3 was ascertained by chemical determination of specific classes of organic compounds.

Ulrich et al. (2002) investigated the effect of ambient PM from Ottawa Canada (EHC-93) on O_3-induced inflammation. Male Wistar rats were exposed to 0.8 ppm O_3 for 8 h and allowed to recover before intratracheal instillation of 0.5, 1.5, and 5 mg of EHC-93 in 0.3 ml of saline. The high concentrations of PM used were sufficient to induce pulmonary inflammation, which was not exacerbated by pre-exposure to O_3. Rats from the combined exposure group did have higher and more persistent lung lavage protein and albumin levels, as well as increased plasma fibrinogen levels when compared to PM exposure alone.

The interaction of PM and O_3 was further examined in a murine model of ovalbumin (OVA)-induced asthma. Kobzik et al. (2001) investigated whether coexposure to inhaled, concentrated ambient particles (CAPs) from Boston, MA and to O_3 could exacerbate asthma-like symptoms. On days 7 and 14 of life, half of the BALB/c mice used in this study were sensitized by intraperitoneal (ip) injection of OVA and then exposed to OVA aerosol on three successive days to create the asthma phenotype. The other half received the ip OVA, but were exposed to a phosphate-buffered saline aerosol (controls). The mice were further subdivided ($n \geq 61$/group) and exposed for 5 h to CAPs, ranging from 63 to 1,569 $\mu g/m^3$, 0.3 ppm O_3, CAPs + O_3, or to filtered air. Pulmonary resistance and airway responsiveness to an aerosolized MCh challenge were measured after exposures. A small, statistically significant increase in pulmonary resistance and airway responsiveness, respectively, was found in both normal and asthmatic mice immediately after exposure to CAPs alone and to CAPs + O_3, but not to O_3 alone or to filtered air. By 24 h after exposure, the responses returned to baseline levels. There were no significant increases in airway inflammation after any of the pollutant exposures. In this well-designed study of a small-animal model of asthma, O_3 and CAPs did not appear to be synergistic. In further analysis of the data using specific elemental groupings of the CAPs, the acutely increased pulmonary resistance was found to be associated with the AlSi fraction of PM. Thus, some components of concentrated $PM_{2.5}$ may affect airway caliber in sensitized animals, but the results are difficult to extrapolate to people with asthma.

Six unique animal studies have examined the adverse cardiopulmonary effects of complex mixtures in urban and rural environments of Italy (Gulisano et al., 1997), Spain (Lorz and Lopez, 1997), and Mexico (Vanda et al., 1998; Calderón-Garcidueñas et al., 2001; Moss et al., 2001). Five of these studies have taken advantage of the differences in pollutant mixtures of urban and rural environments to report primarily morphological changes in the nasopharynx and lower

respiratory tract (Gulisano et al., 1997; Lorz and Lopez, 1997; Calderón-Garcidueñas et al., 2001a) of lambs, pigeons, and dogs, respectively, after natural, continuous exposures to ambient pollution. Each study has provided evidence that animals living in urban air pollutants have greater pulmonary changes than that would occur in a rural and presumably cleaner, environment. The study by Moss et al. (2001) examined the nasal and lung tissue of rats exposed (23 h/day) to Mexico City air for up to 7 weeks and compared them to controls similarly exposed to filtered air. No inflammatory or epithelial lesions were found using quantitative morphological techniques; however, the concentrations of pollutants were low.

The ambient air in urban areas, particularly in Southwestern Mexico City, is a complex mixture of particles and gases, including high concentrations of O_3 and aldehydes that previously have been shown to cause airway inflammation and epithelial lesions in laboratory animals (Harkema et al., 1994, 1997a,b).

AX5.6 EFFECTS OF OTHER PHOTOCHEMICAL OXIDANTS

Complex atmospheric physical and chemical processes involving two classes of precursor pollutants, volatile organic compounds and nitrogen oxides (NO_x), lead to the formation of O_3 and other photochemical oxidants found in ambient air, such as peroxyacyl nitrates, nitric acid (HNO_3), and sulfuric acid, and to the formation of other compounds, such as PM, formaldehyde (HCHO), and other carbonyl compounds (*see Chapter 2*). Peroxyacetyl nitrate (PAN) and peroxypropionyl nitrate (PPN) are the most abundant of the non-ozone oxidants in ambient air of industrialized areas, other than the inorganic nitrogenous oxidants such as NO_2, and possibly HNO_3.

Tropospheric reactions between O_3 and hydrocarbons (e.g., d-limonene) produce epoxides, hydroperoxides, and peroxides. Hydrogen peroxide (H_2O_2) presumably constitutes the majority of the measured ambient hydroperoxides (< 5 ppb), although a small amount of organic hydroperoxides (ROOH) also may be formed. On the basis of equilibrium calculations and limited data, Friedlander and Yeh (1998) estimated that atmospheric aerosols can carry as high as 1 mM of H_2O_2 and organic hydroperoxides (e.g., hydroxymethylhydroperoxide) in the associated water. High concentrations of liquid phase H_2O_2 (50 µM to 1 mM) are known to induce in vitro cell and tissue damage, and recent in vivo studies indicate that 10 and 20 ppb of inhaled H_2O_2

vapor can penetrate the lower lung where it causes inflammation (Morio et al., 2001). It is likely that hygroscopic components of PM transport ambient H_2O_2 into the lower lung and induce tissue injury as well. Exposure of rats to a H_2O_2-fine particle mixture (215 or 429 $\mu g/m^3$ ammonium sulfate) resulted in increased neutrophil influx, and production of inflammatory mediators by alveolar macrophages (Morio et al., 2001). Hygroscopic secondary organic aerosols (SOA) generated by the O_3/hydrocarbon reactions and their co-occurrence with H_2O_2 also provides another possible mechanism whereby H_2O_2 can be transported into the lower respiratory tract (e.g., Friedlander and Yeh, 1998).

Therefore, acute toxicity of PAN is much lower than O_3 and it is unlikely that present ambient PAN levels would affect pulmonary function responses to O_3 (Vyskocil et al., 1998). Cytogenetic studies indicate that PAN is not a potent mutagen, clastogen, or DNA damaging agent in mammalian cells in vivo or in vitro at concentrations several orders of magnitude higher than the generally encountered ambient air levels in most cities (Vyskocil et al., 1998; Kligerman et al., 1995; Heddle et al., 1993). Some studies suggest that PAN may be a weak bacterial mutagen at concentrations much higher than exist in present urban atmospheres (DeMarini et al., 2000; Kleindienst et al., 1990).

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6. CONTROLLED HUMAN EXPOSURE STUDIES OF OZONE AND RELATED PHOTOCHEMICAL OXIDANTS

6.1 INTRODUCTION

In the previous chapter, results of ozone (O_3) studies in laboratory animals and in vitro test systems were presented. The extrapolation of results from animal studies is one mechanism by which information on potential adverse human health effects from exposure to O_3 is obtained. More direct evidence of human health effects due to O_3 exposure can be obtained through controlled human exposure studies of volunteers or through field and epidemiologic studies of populations exposed to ambient O_3 (*see Chapter 7*). Controlled human exposure studies typically use fixed concentrations of O_3 under carefully regulated environmental conditions and subject activity levels. This chapter discusses studies in which volunteers were exposed for up to 8 h to between 0.08 to 0.75 ppm O_3 while at rest or during varying intensities of exercise.

The majority of controlled human studies have investigated the effects of exposure to O_3 in young non-smoking healthy adults (18 to 35 years of age) performing continuous exercise (CE) or intermittent exercise (IE). Varied combinations of O_3 concentration, exercise routine, and exposure duration have been used in these studies. The responses to ambient O_3 concentrations include decreased inspiratory capacity; mild bronchoconstriction; rapid, shallow breathing pattern during exercise; and symptoms of cough and pain on deep inspiration. 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 s (FEV_1). In addition to physiological pulmonary responses and respiratory symptoms, O_3 has been shown to result in airway hyperresponsiveness and inflammation.

The most salient observations from studies reviewed in the 1996 EPA Ozone Air Quality Criteria Document or O_3 AQCD (U.S. Environmental Protection Agency, 1996) were that: (1) young healthy adults exposed to O_3 concentrations $\geq$ 0.08 ppm develop significant reversible, transient decrements in pulmonary function if minute ventilation ($\dot{V}_E$) or duration of exposure are increased sufficiently, (2) children experience similar spirometric responses but lesser

symptoms from O_3 exposure relative to young adults, (3) O_3-induced spirometric responses are decreased in the elderly relative to young adults, (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, and (5) subjects exposed repeated to O_3 over several days develop a tolerance to successive exposures, as demonstrated by an attenuation of responses, which is lost after about a week without exposure.

There are several important limitations associated with these clinical studies: (1) the ability to study only short-term, acute effects; (2) difficulties in trying to link short-term effects with long-term consequences; (3) the use of a small number of volunteers that may not be representative of the general population; and (4) the statistical limitations associated with the small sample size. Sample size affects the power of a study, and having a small number of samples causes a risk of Type II error, i.e., the incorrect conclusion that no difference exists between treatments or groups when comparisons are not significantly different. This affects the confidence in estimates of a minimum O_3 concentration at which some degree of pulmonary impairment will occur in both the general population and susceptible subpopulations. As a result, the conclusions drawn from many of the studies cited in this chapter may underestimate the presence of responses at low O_3 concentrations and low activity levels.

Most of the scientific information summarized in this chapter comes from the literature published since the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996). In addition to further study of physiological pulmonary responses and respiratory symptoms, much of this literature has focused on mechanisms of inflammation and cellular responses to injury induced by O_3 inhalation. A more thorough discussion and review of this literature appears in Annex AX6 of this document. In summarizing the literature, effects are described if they are statistically significant at a probability (p-value) of less than 0.05, otherwise trends are noted as such.

As spirometry typically *improves* in healthy young adults with exercise exposures to filter air (FA), the term "O_3-induced" is used herein and in the annex to designate effects that have been corrected for responses during FA exposures. For healthy adults, an O_3-induced change in lung function is the difference between the *decrement* experienced with O_3 exposure and the *improvement* observed with FA exposure. However, the distinction between an O_3-induced change and a post- versus preexposure change is particularly important in individuals with

respiratory disease who may experience exercise-induced *decrements* in pulmonary function during both FA and O_3 exposures. Hence, in subjects with respiratory disease, exercise-induced responses could be mistaken for O_3-induced responses in the absence of a correction for FA responses.

6.2 PULMONARY FUNCTION EFFECTS OF OZONE EXPOSURE IN HEALTHY SUBJECTS

6.2.1 Introduction

As reviewed in the 1986 and 1996 O_3 AQCD's (U.S. Environmental Protection Agency, 1986, 1996), 0.5 ppm is the lowest O_3 concentration at which statistically significant reductions in FVC and FEV_1 have been reported in sedentary subjects. On average, young adults (n = 23; mean age, 22 yrs) exposed at rest for 2 h to 0.5 ppm O_3 had O_3-induced decrements of ~4% in FVC and ~7% in FEV_1 (Folinsbee et al., 1978; Horvath et al., 1979). During exercise, spirometric and symptoms responses are observed at lower O_3 concentrations. For acute exposures of 2 h or less to ≥0.12 ppm O_3 , if $\dot{V}_E$ is sufficiently increased by exercise, healthy human subjects generally experience decreases in TLC, inspiratory capacity (IC), FVC, FEV_1, mean forced expiratory flow from 25% to 75% of FVC (FEF_{25-75}), and tidal volume (V_T) and increases in specific airways resistance (sRaw), breathing frequency (f_B), and airway responsiveness. These exposure also cause symptoms of cough, pain on deep inspiration, shortness of breath, throat irritation, and wheezing. With exposures of 4- to 8-h in duration, statistically significant pulmonary function and symptoms responses are observed at lower O_3 concentrations and lower $\dot{V}_E$ than in shorter duration studies.

6.2.2 Acute Exposure for Up to 2 h

With heavy CE ($\dot{V}_E$ = 89 L/min), an O_3-induced decrement of 9.7% in FEV_1 has been reported for healthy young adults (n = 17; age, 24 ±3 yrs) exposed for only 1 h to 0.12 ppm O_3 (Gong et al., 1986). With moderate-to-heavy IE (15 min intervals of rest and exercise [$\dot{V}_E$ = 68 L/min]), McDonnell et al. (1983) reported a physiologically small, but significant, O_3-induced decrement of 3.4% in FEV_1 for young healthy adults (n = 22, age, 22 ±3 yrs)

exposed for 2 h to 0.12 ppm O_3. Using the same 2 h exposure protocol, Linn et al. (1986) found no statistically significant spirometic responses at O_3 concentrations of 0.16 ppm and lower. However, the subjects in the Linn et al. (1986) study were potentially exposed concurrently in Los Angeles to ambient O_3 levels of between 0.12 and 0.16 ppm and were on average 3 yrs older than the subjects in the McDonnell et al. (1983) study. (*The attenuating effects of increasing age and repeated O_3 exposures are discussed in Sections 6.5.1 and 6.6, respectively.*) The disparities between the Linn et al. (1986) and McDonnell et al. (1983) studies demonstrate the difficulty in determining a no-effect-level for O_3 based on relatively small study populations.

Studies analyzing large data sets (≥300 subjects) provide better predictive ability of acute changes in FEV_1 at low levels of O_3 and $\dot{V}_E$ than possible via comparisons between smaller studies. Such an analysis was performed by McDonnell et al. (1997), who examined FEV_1 responses in 485 healthy white males (18 to 36 years of age; subjects recruited from the area around Chapel Hill, NC) exposed once for 2 h to O_3 concentrations of up to 0.40 ppm at rest or with IE. Decrements in FEV_1 were modeled by sigmoid-shaped curve as a function of subject age, O_3 concentration, $\dot{V}_E$, and duration of exposure. Figure 6-1 illustrates the predicted O_3-induced decrements in FEV_1 for young healthy adults (20 yrs of age) exposed for up to 2 h to O_3 during moderate IE ($\dot{V}_E$ = 30 L/min). The responses illustrated for 0.1 ppm in Figure 6-1 are approximately the same as responses predicted for an exposure to 0.3 ppm at rest. Although not illustrated in the figure, the predicted FEV_1 decrements increase with $\dot{V}_E$. Regarding applicability to the general population, the McDonnell et al. (1997) model has an apparent limitation of considering only data for white males. However, two other large studies (n = 372; 18 to 35 yrs of age; subjects recruited from the area around Chapel Hill, NC) found no significant gender nor race effects on spirometric responses to O_3 exposure (Seal et al., 1993, 1996).

Ultman et al. (2004) recently reported pulmonary responses in 60 young heathy non-smoking adults (32 M, 28 F) exposed to 0.25 ppm O_3 for 1 h with CE at a target $\dot{V}_E$ of 30 L/min. Consistent with findings reported in the 1996 O_3 criteria document, considerable intersubject variability in FEV_1 decrements was reported by Ultman et al. (2004) with responses ranging from −4 to 56%. One-third of the subjects had FEV_1 decrements of > 15% and 7% of the subjects had decrements of > 40%. It should be pointed out that the McDonnell et al. (1997)

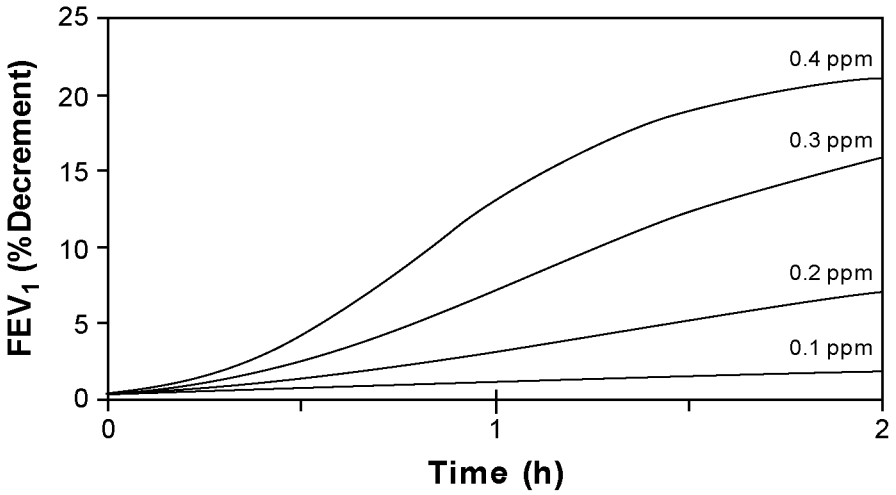

Figure 6-1. Predicted O_3-induced decrements in FEV_1 as a function of exposure duration and O_3 concentration in young healthy adults (20 yrs of age) during moderate IE ($\dot{V}_E$ = 30 L/min). Predictions are for Model 1 coefficients in Table 3 of McDonnell et al. (1997).

model predicts only *average* responses. In a more recent study, McDonnell et al. (1999) also reported a model predicting average symptom responses from O_3 exposure. Unfortunately, neither of these papers (McDonnell et al., 1997, 1999) provide predictions of intersubject variability in response. (*Section 6.4 of this Chapter discusses intersubject variability in response to O_3 exposure*).

6.2.3 Prolonged Ozone Exposures

In the exposure range of 0.08 to 0.16 ppm O_3, a number of studies using moderate quasi continuous exercise (QCE; 50 min exercise and 10 min rest per h) for 4 to 8 h have shown significant responses under the following conditions: 0.16 ppm for 4 h with QCE at $\dot{V}_E \approx$ 40 L/min (Folinsbee et al., 1994), 0.08 to 0.12 ppm for 6.6 h with QCE at $\dot{V}_E \approx$ 35 to 40 L/min (Adams, 2002; Adams, 2003a; Folinsbee et al., 1988; Horstman et al., 1990), and 0.12 ppm for 8 h of IE (30 min per h) at $\dot{V}_E \approx$ 40 L/min (Hazucha et al., 1992). Symptoms and spirometric responses increased with duration of exposure, O_3 concentration, and total $\dot{V}_E$. Airway resistance is only modestly affected with moderate or even heavy exercise combined with O_3 exposure (Folinsbee et al., 1978; McDonnell et al., 1983; Seal et al., 1993).

6.2.3.1 Effect of Exercise Ventilation Rate on FEV_1 Response to 6.6 h Ozone Exposure

It is well established that response to O_3 exposure is a function of $\dot{V}_E$ in studies of 2 h or less in duration (*See Section AX6.2.2*). It is reasonable to expect that response to a prolonged 6.6-h O_3 exposure is also function of $\dot{V}_E$, although quantitative analyzes are lacking. Data from five similar prolonged exposure studies are available for evaluation of FEV_1 responses as a function of exercise $\dot{V}_E$ (Adams, 2000; Adams and Ollison, 1997; Folinsbee et al., 1988, 1994; Horstman et al., 1990). Each of these studies exposed similarly aged subjects (mean ages 22 to 25 yrs) to 0.12 ppm O_3 for 6.6 h. In total, ten sets of mean FEV_1 decrements were available for exercise $\dot{V}_E$ ranging from 20 to 43 L/min, although no data were available for $\dot{V}_E$ between 20 and 30 L/min (*data illustrated in Figure AX6-2*). As in 2 h exposure studies, FEV_1 decrements are a function $\dot{V}_E$ in prolonged 6.6-h exposure studies as demonstrated by a significant correlation between these variables (Pearson, $r = 0.95$, $p < 0.001$; Spearman, $r = 0.84$, $p < 0.01$).

6.2.3.2 Exercise Ventilation Rate as a Function of Body/Lung Size on FEV_1 Response to 6.6 h Ozone Exposure

Based on the assumption that the total inhaled O_3 dose (product of O_3 concentration, exposure duration, and $\dot{V}_E$) is proportional to the lung size, exercise $\dot{V}_E$ are typically selected to be a multiple of body surface area (BSA) or FVC. Data from several recent studies do not support the contention that $\dot{V}_E$ should be normalized. In an analysis of data from 485 young adults, McDonnell et al. (1997) found that any effect of BSA, height, or baseline FVC on percent decrement in FEV_1 was small to nonexistent. This is consistent with Messineo and Adams (1990), who compared pulmonary function responses in young adult women having small ($n = 14$) or large ($n = 14$) lung sizes (mean FVC of 3.74 and 5.11 L, respectively) and found no significant group difference in FEV_1 decrements. For 30 subjects exposed to 0.12 ppm O_3 for 6.6 h, Adams (2000) also reported that FEV_1 responses were more closely related to $\dot{V}_E$ than to $\dot{V}_E$ normalized to BSA. The O_3 dosimetry study of Bush et al. (1996) suggested that normalization of the O_3 dose might more appropriately be a function of anatomic dead space. Ozone penetrates deeper into the lungs of individuals with larger conducting airway volumes, however, FEV_1 responses in subjects exposed for 2 h to 0.25 ppm O_3 do not appear to be associated with O_3 uptake (Ultman et al., 2004).

6.2.3.3 Comparison of 2 h IE to 6.6 h O_3 Exposure Effects on Pulmonary Function

Adams (2003b) examined whether prolonged 6.6-h QCE exposure to a relatively low O_3 concentration (0.08 ppm) and the 2-h IE exposure at a relatively high O_3 concentration (0.30 ppm) elicited consistent individual subject FEV_1 responses. Individual subject O_3 exposure reproducibility was first examined via a regression plot of the postexposure FEV_1 response to the 6.6-h chamber exposure as a function of postexposure FEV_1 response to the 2-h IE chamber exposure. The R^2 of 0.40, although statistically significant, was substantially less than that observed in a comparison of individual FEV_1 response to the two 2-h IE exposures by chamber and face mask, respectively ($R^2 = 0.83$). The Spearman rank order correlation for the chamber 6.6-h and chamber 2-h exposure comparison was also substantially less (0.49) than that obtained for the two 2-h IE exposures (0.85). The primary reason for the greater variability in the chamber 6.6-h exposure FEV_1 response as a function of that observed for the two 2-h IE exposures is very likely related to the increased variability in response upon repeated exposure to O_3 concentrations lower than 0.18 ppm (R = 0.57, compared to a mean R of 0.82 at higher concentrations) reported by McDonnell et al. (1985a). This rationale is supported by the lower R (0.60) observed by Adams (2003b) for the FEV_1 responses found in 6.6 h chamber and face mask exposures to 0.08 ppm O_3, compared to an R of 0.91 observed for responses found at 0.30 ppm O_3.

6.2.4 Triangular Ozone Exposures

To further explore the factors that determine responsiveness to O_3, Hazucha et al. (1992) designed a protocol to examine the effect of varying, rather than constant, O_3 concentrations. Subjects were exposed to an O_3 level that increased linearly from 0 to 0.24 ppm for the first 4 h and then decreased linearly from 0.24 to 0 ppm over the second 4 h of the 8 h exposure (triangular concentration profile) and to a constant level exposure of 0.12 ppm O_3 for 8 h. While total inhaled O_3 doses for the constant and the triangular concentration profile were almost identical, the FEV_1 response was dissimilar. For the constant 0.12 ppm O_3 exposure, FEV_1 declined ~5% by the fifth hour and then remained at that level. With the triangular O_3 concentration profile, there was minimal FEV_1 response over the first 3 h followed by a rapid decrease in FEV_1 (−10.3%) over next 3 h. During the seventh and eighth hours, FEV_1 improved

to −6.3% despite continued exposure to a lower O_3 concentration (0.12 to 0.00 ppm, mean = 0.06 ppm).

More recently, Adams (2003a) used a less abrupt triangular O_3 exposure profile at concentrations assumed to be typical of outdoor ambient conditions (beginning at 0.03 ppm, increasing steadily to 0.15 ppm in the fourth hour and decreasing steadily to 0.05 ppm at 6.6 h (mean = 0.08 ppm). Postexposure values for FEV_1 and symptoms were not significantly different between the 6.6 h triangular and a square-wave 0.08 ppm O_3 exposure. There was no evidence of FEV_1 response recovery with the triangular exposure as observed by Hazucha et al. (1992). Rather, FEV_1 responses observed by Adams (2003a) for the triangular exposure seemed to plateau during the last 2 h, i.e., −5.46% at 4.6 h, −6.27% at 5.6 h, and −5.77% at 6.6 h.

With square-wave O_3 exposures between 0.08 to 0.12 ppm, FEV_1 decrements may increase with time of exposure (and O_3 dose) or reach plateau (Horstman et al., 1990; McDonnell et al., 1991). For the triangular exposures used by Hazucha et al. (1992) and Adams (2003a), maximal FEV_1 responses occurred 1 h to 2 h after peak O_3 concentration and 1 h to 2 h before the maximal O_3 dose occurred (at the end of the O_3 exposure).

6.2.5 Mechanisms of Pulmonary Function Responses

Inhalation of O_3 for several hours while physically active elicits both subjective respiratory tract symptoms and acute pathophysiologic changes. The typical symptomatic response consistently reported in studies is that of tracheobronchial airway irritation. This is accompanied by decrements in lung capacities and volumes, bronchoconstriction, airway hyperresponsiveness, airway inflammation, immune system activation, and epithelial injury. The severity of symptoms and the magnitude of response depend on inhaled dose, O_3 sensitivity of an individual and the extent of tolerance resulting from previous exposures. The development of effects is time- dependent during both exposure and recovery periods with considerable overlap of evolving and receding effects. The time sequence, magnitude and the type of responses of this complex series of events, both in terms of development and recovery, indicate that several mechanisms, activated at different time of exposure must contribute to the overall lung function response (U.S. Environmental Protection Agency, 1996).

Available information on recovery from O_3 exposure indicates that an initial phase of recovery proceeds relatively rapidly, and some 40 to 65% of the acute spirometric and symptom

response appears to occur within about 2 h (Folinsbee and Hazucha, 1989). Following a 2 h exposure to 0.4 ppm O_3 with IE, Nightingale et al. (2000) observed a 13.5% decrement in FEV_1. By 3 h postexposure, however, only a 2.7% FEV_1 decrement persisted as illustrated in Figure 6-2. A similar postexposure recovery in FVC was also observed. Gerrity et al. (1993) suggested that for healthy young adults transient increases in mucus clearance (mediated by cholinergic receptors) due to O_3 exposure may be coincident to pulmonary function responses, i.e., the transient increases in clearance and decrements in lung function return to baseline values within 2 to 3 h postexposure. However, there is some indication that the spirometric responses, especially at higher O_3 concentrations, are not fully recovered within 24 h (Folinsbee and Horvath, 1986; Folinsbee et al., 1998). Small residual lung function effects are almost completely resolved within 24 hours. In hyperresponsive individuals, the recovery takes longer, as much as 48 hours, to return to baseline values. Collectively, these observations suggest that there is a rapid recovery of O_3-induced spirometric responses and symptoms, which may occur during resting exposure to O_3 (Folinsbee et al., 1977) or as O_3 concentration is reduced during exposure (Hazucha et al., 1992), and a slower phase, which may take at least 24 h to complete (Folinsbee and Hazucha, 2000). Repeated exposure studies at higher concentrations typically show that FEV_1 response to O_3 is enhanced on the second of several days of exposure (Table AX6-8). This enhanced response suggests a residual effect of the previous exposure, about 22 h earlier, even though the preexposure spirometry may be the same as on the previous day. The absence of the enhanced response with repeated exposure at lower O_3 concentrations may be the result of a more complete recovery or less damage to pulmonary tissues (Folinsbee et al., 1994).

As the exposure to O_3 progresses, airway inflammation begins to develop and the immune response at both cellular and subcellular level is activated. Airway hyperreactivity develops more slowly than pulmonary function effects, while neutrophilic inflammation of the airways develops even more slowly and reaches the maximum 3 to 6 h postexposure. The cellular responses (e.g., release of immuno-modulatory cytokines) appear to still be active as late as 20 h postexposure (Jörres et al., 2000). Following cessation of exposure, the recovery in terms of breathing pattern, pulmonary function and airway hyperreactivity progresses rapidly and is almost complete within 4 to 6 hours in moderately responsive individuals. More slowly developing inflammatory and cellular changes may persist for up to 48 hours.

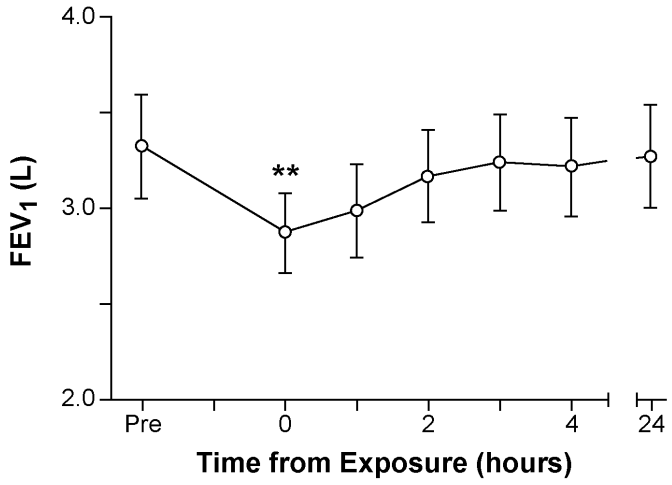

Figure 6-2. Recovery of FEV_1 responses following a 2 h exposure to 0.4 ppm O3 with IE. Immediately postexposure, FEV_1 was significantly (p<0.001) decreased. At 3 h postexposure, FEV_1 was at 97% of the preexposure value.**

Adapted from Nightingale et al. (2000).

6.2.5.1 Pathophysiologic Mechanisms

Breathing pattern changes

Human studies consistently report that inhalation of O_3 alters the breathing pattern without significantly affecting minute ventilation. A progressive decrease in tidal volume and a "compensatory" increase in frequency of breathing to maintain steady minute ventilation during exposure suggests a direct modulation of ventilatory control. These changes parallel a response of many animal species exposed to O_3 and other lower airway irritants (Tepper et al., 1990). Bronchial C-fibers and rapidly adapting receptors appear to be the primary vagal afferents responsible for O_3-induced changes in ventilatory rate and depth in both humans (Folinsbee and Hazucha, 2000) and animals (Coleridge et al., 1993; Hazucha and Sant'Ambrogio, 1993; Schelegle et al., 1993).

The potential modulation of breathing pattern by activation of sensory afferents located in extrathoracic airways by O_3 has not yet been studied in humans. Nasal only O_3 exposure of rats produces changes in breathing pattern that are similar to changes observed in humans (Kleinman et al., 1999).

Symptoms and lung function changes

As discussed, in addition to changes in ventilatory control, O_3 inhalation by humans will also induce a variety of symptoms, reduce vital capacity (VC) and related functional measures, and increase airway resistance.

Schelegle et al. (2001) recently demonstrated that the reduction in VC due to O_3 exposure is a reflex action and not a voluntary termination of inspiration as result of discomfort. They reported that O_3-induced symptom responses (mediated in part by bronchial C-fibers) are substantially reduced by inhaled topical anesthetic. However, the anesthetic had a minor and irregular effect on pulmonary function decrements and tachypnea. Since respiratory symptom response were largely abolished, these findings support reflex inhibition of VC due to stimulation of both bronchial and pulmonary C-fibers.

The involvement of nociceptive bronchial C-fibers modulated by opioid receptors in limiting maximal inspiration and eliciting subjective symptoms in humans was studied by Passannante et al. (1998). Sufentanil (an opioid agonist and analgesic) rapidly reversed O_3-induced symptom responses and reduced spirometric decrements in "strong" responders. The incomplete recovery in FEV_1 following sufentanil administration, however, suggests involvement of non-opioid receptor modulated mechanisms as well. Interestingly, naloxone (opioid receptor antagonist) had no significant effect on FEV_1 decrements in "weak" responders. Plasma levels of β-endorphin (a potent pain suppressor) were not related with O_3 responses.

Airway hyperreactivity

In addition to limitation of maximal inspiration and its effects on other spirometric endpoints, activation of airway sensory afferents also plays a role in receptor-mediated bronchoconstriction and an increase in airway resistance. Despite this common mechanism, post-O_3 pulmonary function changes and either early or late bronchial hyperresponsiveness (BHR) to inhaled aerosolized methacholine or histamine are poorly correlated either in time or magnitude. Fentanyl and indomethacin, the drugs that have been shown to attenuate O_3-induced lung function decrements in humans, did not prevent induction of BHR when administered to guinea pigs prior to O_3 exposure (Yeadon et al., 1992). Neither does post-O_3 BHR seem to be related to airway baseline reactivity. These findings imply that the mechanisms are either not

related or are activated independently in time. Animal studies (with limited support from human studies) have suggested that an early post-O_3 BHR is, at least in part, vagally mediated (Freed, 1996) and that stimulation of C-fibers can lead to increased responsiveness of bronchial smooth muscle independently of systemic and inflammatory changes which may be even absent (Joad et al., 1996). In vitro study of isolated human bronchi have reported that O_3-induced airway sensitization involves changes in smooth muscle excitation-contraction coupling (Marthan, 1996). Characteristic O_3-induced inflammatory airway neutrophilia which at one time was considered a leading BHR mechanism, has been found in a murine model, to be only coincidentally associated with BHR and there was no cause and effect relationship (Zhang et al., 1995). However, this observation does not rule out involvement of other cells such as eosinophils or T-helper cells in BHR modulation. There is some evidence that release of inflammatory mediators by these cells can sustain BHR and bronchoconstriction. In vitro and animal studies have also suggested that airway neutral endopeptidase activity can be a strong modulator of BHR (Marthan et al., 1996; Yeadon et al., 1992). Late BHR observed in some studies is plausibly due to a sustained damage of airway epithelium and continual release of inflammatory mediators (Foster et al., 2000). Thus, O_3-induced BHR appears to be a product of many mechanisms acting at different time periods and levels of the bronchial smooth muscle signaling pathways (*The effects of O_3 on BHR are described in Section 6.8*).

6.2.5.2 Mechanisms at a Cellular and Molecular Level

Stimulation of vagal afferents by O_3 and reactive products, the primary mechanism of lung function impairment is enhanced and sustained by what can be considered in this context to be secondary mechanisms activated at a cellular and molecular level. The complexity of these mechanisms is beyond the scope of this section and the reader is directed to Section 6.9 of this chapter for greater detail. A comprehensive review by Mudway and Kelly (2000) discusses the cellular and molecular mechanisms of O_3-induced pulmonary response in great detail.

Stimulation of bronchial C-fibers by O_3 not only inhibits maximal inspiration but, through local axon reflexes, induces neurogenic inflammation. This pathophysiologic process is characterized by release of tachykinins and other proinflammatory neuropeptides. Ozone exposure has been shown to elevate C-fiber-associated tachykinin substance P in human bronchial lavage fluid (Hazbun et al. 1993) and to deplete neuropeptides synthesized and

released from C-fibers in human airway epithelium rich in substance P-immunoreactive axons. Substance P and other transmitters are known to induce granulocyte adhesion and subsequent transposition into the airways, increase vascular permeability and plasma protein extravasation, cause bronchoconstriction, and promote mucus secretion (Solway and Leff, 1991). Although the initial pathways of neurogenic, antigen-induced, and generally immune-mediated inflammation are not the same, they eventually converge leading to further amplification of airway inflammatory processes by subsequent release of cytokines, eicosanoids, and other mediators. Significantly negative correlations between O_3-induced leukotriene ($LTC_4/D_4/E_4$) production and spirometric decrements (Hazucha et al., 1996), and an increased level of postexposure PGE_2, a mediator known to stimulate bronchial C-fibers, show that these mediators play an important role in attenuation of lung function due to O_3 exposure (Mohammed et al., 1993; Hazucha et al., 1996). Moreover, because the density of bronchial C-fibers is much lower in the small than large airways, the reported post O_3 dysfunction of small airways assessed by decrement in FEF_{25-75} (Weinman et al., 1995; Frank et al., 2001) may be due in part to inflammation. Also, because of the relative slowness of inflammatory responses as compared to reflex effects, O_3-triggered inflammatory mechanisms are unlikely to initially contribute to progressive lung function reduction. It is plausible, however, that when fully activated, they sustain and possibly further aggravate already impaired lung function. Indeed, a prolonged recovery of residual spirometric decrements following the initial rapid improvement after exposure termination could be due to slowly resolving airway inflammation. Bronchial biopsies performed 6 h postexposure have shown that O_3 caused a significant decrease in immunoreactivity to substance P in the submucosa (Krishna et al., 1997). A strong negative correlation with FEV_1 also suggests that the release of substance P may be a contributing mechanism to persistent post-O_3 bronchoconstriction (Krishna et al., 1997). Persistent spirometry changes observed for up to 48 h postexposure could plausibly be sustained by the inflammatory mediators, many of which have bronchoconstrictive properties (Blomberg et al., 1999).

6.3 SUBJECTS WITH PREEXISTING DISEASE

Individuals with respiratory disease are of primary concern in evaluating the health effects of O_3 because even a small change in function is likely to have more impact on a person with reduced reserve, i.e., O_3-induced effects are superimposed on preexisting pulmonary impairment.

6.3.1 Subjects with Chronic Obstructive Pulmonary Disease

For patients with COPD performing light to moderate IE, no decrements in pulmonary function were observed after 1- and 2-h exposures to ≤ 0.30 ppm O_3 (Kehrl et al., 1985; Linn et al., 1982a, 1983a; Solic et al., 1982) and only small decreases in forced expiratory volume were observed for 3-h exposures of chronic bronchitics to 0.41 ppm O_3 (Kulle et al., 1984). More recently, Gong et al. (1997a) found no significant difference in response between age-matched controls and COPD patients to a 4 h exposure to 0.24 ppm O_3 with IE. Although the clinical significance is uncertain, small transient decreases in arterial blood oxygen saturation have also been observed in some of these studies.

6.3.2 Subjects with Asthma

Based on studies reviewed in the 1996 criteria document (U.S. Environmental Protection Agency, 1996), asthmatics appear to be at least as sensitive to acute effects of O_3 as healthy nonasthmatic subjects.

Several recent studies support a tendency for slightly increased spirometric responses in mild asthmatics versus healthy subjects. Alexis et al. (2000) reported reductions in FVC (12%, 10%) and FEV_1 (13%, 11%) for 13 mild asthmatic and 9 healthy subjects, respectively, exposed to 0.4 ppm O_3 for 2 h with IE ($\dot{V}_E$ = 30 L/min). The FVC and FEV_1 responses were attenuated by indomethacin in the healthy subjects but not the asthmatics. As assessed by the magnitude of reductions in mid-flows (viz. FEF_{25}, FEF_{50}, FEF_{60p}, FEF_{75}) following O_3 exposure, the small airways tended to be more affected in asthmatics than healthy subjects. In a larger study, Jörres et al. (1996) exposed 24 asthmatics, 12 allergic rhinitics, and 10 healthy subjects to 0.25 ppm O_3 for 3 h with IE. The O_3-induced FEV_1 decrements tended to be greater in the diseased populations (allergic rhinitis, 14.1%; asthmatics, 12.5%; healthy controls, 10.2%). Scannell et al. (1996) exposed 18 asthmatics to 0.2 ppm O_3 for 4 h with IE ($\dot{V}_E \approx 25$ L/min/m^2

BSA). An O_3-induced increase in sRaw tended to be greater in the asthmatics compared to 81 healthy subjects who underwent similar experimental protocols (Aris et al., 1995; Balmes et al., 1996).

Similar O_3-induced spirometric responses are suggested by some studies. The Scannell et al. (1996) study of 18 asthmatics reported FEV_1 and FVC decrements that were similar to 81 healthy subjects (Aris et al., 1995; Balmes et al., 1996). Similar group decrements in FEV_1 and FVC were reported by Hiltermann et al. (1995), who exposed 6 asthmatics and 6 healthy subjects to 0.4 ppm O_3 for 2 h with light IE. Basha et al. (1994) also reported similar spirometric responses between 5 asthmatic and 5 healthy subjects exposed to 0.2 ppm O_3 for 6 h with IE. The lack of significant differences in the Hilltermann et al. (1995) and Basha et al. (1994) studies is not compelling given the extremely small sample sizes and corresponding lack of statistical power. The Basha et al. (1994) study was also confounded by the asthmatics having an average preexposure FEV_1 that was about 430 mL lower (a 12% difference) on the O_3-day relative to the air-day. Hence, only the Scannell et al. (1996) study supports similar O_3-induced spirometric responses in asthmatics versus healthy subjects.

One study has reported that asthmatics tend to have smaller O_3-induced FEV_1 decrements relative healthy subjects (3% versus 8%, respectively) when exposed to 0.2 ppm O_3 for 2 h with IE (Mudway et al., 2001). However, the asthmatics in the Mudway et al. (2001) study also tended to be older than the healthy subjects, which could partially explain their lesser response.

In a longer exposure duration (7.6 h) study, Horstman et al. (1995) exposed 17 mild-to-moderate asthmatics and 13 healthy controls to 0.16 ppm O_3 or FA with QCE ($\dot{V}_E \approx 30$ L/min). The FEV_1 decrement observed in the asthmatics was significantly greater than in the healthy subjects (19% versus 10%, respectively). There was also tendency for a greater O_3-induced decrease in FEF_{25-75} in asthmatics relative to the healthy subjects (24% versus 15%, respectively). A significant positive correlation in asthmatics was also reported between O_3-induced spirometric responses and baseline lung function, i.e., responses increased with severity of disease.

With repeated O_3 exposures asthmatics, like healthy subjects (*see Section 6.6*) develop tolerance. Gong et al. (1997b) exposed 10 asthmatics to 0.4 ppm O_3, 3 h per day with IE ($\dot{V}_E \approx 32$ L/min), for 5 consecutive days. Symptom and spirometric responses were greatest on the first (-35 % FEV_1) and second (-34 % FEV_1) exposure days, and progressively diminished

toward baseline levels (−6 % FEV_1) by the fifth exposure day. Similar to healthy subjects, asthmatics lost their tolerance 4 and 7 days later.

Other studies have reported that asthmatics have a somewhat exaggerated inflammatory response to acute O_3 exposure relative to healthy controls (e.g., McBride et al., 1994; Basha et al., 1994; Peden et al., 1995, 1997; Peden, 2001a; Scannell et al., 1996; Hiltermann et al., 1997, 1999; Michelson et al., 1999; Vagaggini et al., 1999; Newson et al., 2000; Holz et al., 2002) also (*see Section 6.9*). Inflammatory responses do not appear to be correlated with lung function responses in either asthmatic or healthy subjects (Balmes et al., 1996, 1997; Holz et al., 1999). This lack of correlations between inflammatory and spirometric responses may be due to differences in the time kinetics of these responses (Stenfors et al., 2002). In addition, airway responsiveness to inhaled allergens is increased by O_3 exposure in subjects with allergic asthma for up to 24 h (*see Section 6.8*).

6.3.3 Subjects with Allergic Rhinitis

Allergic rhinitis is a condition defined by inflammation of the nasal membranes. Nayak (2003) recently reviewed the commonalities between asthma and allergic rhinitis. Clinically, greater than 60% of asthmatics have allergic rhinitis and slightly less than 40% of allergic rhinitics have asthma. Leukotrienes and histamine are well-recognized mediators of responses (viz., inflammation, hyperresponsiveness, and bronchoconstriction) in both asthma and allergic rhinitis. Although, rhinitis and asthma are distinguished as affecting the upper and lower airways, respectively, it has been suggested that these diseases are manifestations of the same disease entity.

Given the prevalence of concomitant asthma and rhinitis and their common response mediators, it should be expected that allergic rhinitics might respond more similarly to asthmatics than healthy individuals. Regarding spirometric responses, Jörres et al. (1996) provide the only data demonstrating a trend in support of this supposition.

Studies demonstrating the interaction between air pollutants and allergic processes in the human nasal airways and rhinoconjunctival tissue have been reviewed by Peden (2001b) and Riediker et al. (2001), respectively. Ozone exposure of subjects with allergic rhinitis has been shown to induce nasal inflammation and increase airway responsiveness to nonspecific bronchoconstrictors.

Peden et al. (1995), who studied allergic asthmatics exposed to O_3, found that O_3 causes an increased response to nasal allergen challenge in addition to nasal inflammatory responses. Their data suggested that allergic subjects have an increased immediate response to allergen after O_3 exposure. In a follow-up study, Michelson et al. (1999) reported that 0.4 ppm O_3 did not promote early-phase-response mediator release or enhance the response to allergen challenge in the nasal airways of mild, asymptomatic dust mite-sensitive asthmatic subjects. Ozone did, however, promote an inflammatory cell influx, which helps induce a more significant late-phase response in this population.

Jörres et al. (1996) found that O_3 causes an increased response to bronchial allergen challenge in subjects with allergic rhinitis. This study also measured responses in healthy subjects and mildly allergic asthmatics (*see Sections AX6.3.2 and AX6.8*). All subjects were exposed to 0.25 ppm O_3 for 3 h with IE. Statistically significant O_3-induced decrements in FEV_1 occurred in rhinitics (14.1%), asthmatics (12.5%), and the healthy controls (10.2%), but these responses did not differ statistically between groups. Methacholine responsiveness was significantly increased in asthmatics, but not in subjects with allergic rhinitis. Airway responsiveness to an individual's historical allergen (either grass and birch pollen, house dust mite, or animal dander) was significantly increased after O_3 exposure when compared to FA exposure. The authors concluded that subjects with allergic rhinitis, but without asthma, could be at risk if a high O_3 exposure is followed by a high dose of allergen.

Holz et al. (2002) extended the results of Jörres et al. (1996) by demonstrating that repeated daily exposure to lower concentrations of O_3 (0.125 ppm for 4 days) causes an increased response to bronchial allergen challenge in subjects with preexisting allergic airway disease, with or without asthma. These investigators observed no major difference in the pattern of bronchial allergen response between asthmatics or rhinitics, except for a 10-fold increase in the dose of allergen required to elicit a similar response ($\geq$ 20% decrease in FEV_1) in the asthmatic subjects. Early phase responses were more consistent in subjects with rhinitis and late-phase responses were more pronounced in subjects with asthma. There also was a tendency towards a greater effect of O_3 in subjects with greater baseline response to specific allergens (chosen on the basis of skin prick test and history, viz., grass, rye, birch, or alder pollen, house dust mite, or animal dander). These data suggest that the presence of allergic bronchial sensitization, but not a history of asthma, may be a key determinant of increased airway allergen

responsiveness following exposure to O_3 (*for a more complete discussion of airway responsiveness*) see Section AX6.8.

6.3.4 Subjects with Cardiovascular Disease

Possibly due to the age of subjects studied, O_3 exposure does not appear to result in significant pulmonary function impairment or evidence of cardiovascular strain in patients with cardiovascular disease relative to healthy controls. Gong et al. (1998) exposed 10 hypertensive and 6 healthy adult males, 41 to 78 years of age, to 0.3 ppm O_3 for 3 h with IE at 30 L/min. For all subjects combined (no significant group differences), there was an O_3-induced decrement of 7% in FEV_1 and an 70% increase in the alveolar-arterial oxygen tension gradient. The overall results did not indicate any major acute cardiovascular effects of O_3 in either the hypertensive or normal subjects.

6.4 INTERSUBJECT VARIABILITY AND REPRODUCIBILITY OF RESPONSE

Analysis of factors that contribute to intersubject variability is important for the understanding of individual responses, mechanisms of response, and health risks associated with acute O_3 exposures. A large intersubject variability in response to O_3 has been reported by numerous investigators (Adams et al., 1981; Aris et al., 1995; Folinsbee et al., 1978; Kulle et al., 1985; McDonnell et al., 1983). The magnitude of individual variability in FEV_1 response in 2 h IE exposures increases at higher O_3 concentrations (Kulle et al., 1985: McDonnell et al., 1983). McDonnell (1996) examined the FEV_1 response data from three 6.6-h exposure studies conducted at the EPA Health Effects Research Laboratory, and showed that the FEV_1 responses in FA were small with most tightly grouped around zero. With increasing O_3 concentrations between 0.08 and 0.12 ppm, the mean response became asymmetrical with a few individuals experiencing quite large decrements in FEV_1 (*Intersubject variability observed in O3 dosimetry studies is discussed in Chapter 4.2*).

As an example of the variation in spirometric responses to O_3 exposure, Hazucha et al. (2003) analyzed the distribution of O_3 responsiveness in 240 subjects (18 to 60 years of age) exposed to 0.42 ppm O_3 (on 3 occasions) for 1.5 h with IE at $\dot{V}_E$ = 20 L/min/m^2 BSA. Across all

ages, 18% of subjects were weak responders (≤ 5% FEV_1 decrement), 39% were moderate responders, and 43% were strong responders (≥ 15% FEV_1 decrement). Younger subjects (≤ 35 years of age) were predominately strong responders, whereas, older subjects (> 35 years of age) were mainly weak responders. The influence of age on intersubject variability was also noted by Passannante et al. (1998) who found that subjects under 35 years of age were more like to be strong responders than older individuals. For repeated exposures, Hazucha et al. (2003) reported that the reproducibility of FEV_1 responses was related to the length of time between exposures. The Spearman correlation coefficient of 0.54 was found between responses for exposures separated by 105 days (median), whereas, a correlation coefficient of 0.85 was found between responses for exposures separated by only 7 days (median).

The more reproducible the subject's response, the more precisely it indicates his/her intrinsic responsiveness. In 2 h IE O_3 exposures, McDonnell et al. (1985b) found a relatively poor FEV_1 reproducibility (R = 0.58) at the lowest concentration, 0.12 ppm, due, in part, to a lack of specific O_3 response or a uniformly small response in the majority of subjects. It was concluded that for 2 h IE O_3 exposures equal to or greater than 0.18 ppm, the intersubject differences in magnitude of change in FVC and FEV_1 are quite reproducible over time (21 to 385 days; mean = 33 days) and are due primarily to differences in intrinsic responsiveness of individual subjects to O_3 exposure.

Intersubject variability, mechanisms of response, and health risks associated with acute O_3 exposures are complicated by a poor association between various O_3-induced responses. In a retrospective study of 485 male subjects (ages 18 to 36 yrs) exposed to one of six O_3 concentrations at one of three activity levels for 2 h, McDonnell et al. (1999) observed significant, but low, Spearman rank order correlations between FEV_1 response and symptoms of cough (R = 0.39), shortness of breath (R = 0.41), and pain on deep inspiration (R = 0.30). These authors concluded that these responses are related mechanistically to some degree, but indicates that there is not a single factor which is responsible for the observed individual differences in O_3 responsiveness across the spectrum of symptom and lung function responses.

The effect of large intersubject variability on the ability to predict individual responsiveness to O_3 was demonstrated by McDonnell et al. (1993). These investigators analyzed the data of 290 male subjects (18 to 32 years of age) who underwent repeat 2 h IE exposures to one or more O_3 concentrations ranging from 0.12 to 0.40 ppm. They attempted to

identify personal characteristics (i.e., age, height, baseline pulmonary functions, presence of allergies, and past smoking history) that might predict individual differences in FEV_1 response. Only age contributed significantly to intersubject responsiveness (younger subjects were more responsive), accounting for just 4% of the observed variance. Interestingly, O_3 concentration accounted for only 31% of the variance, strongly suggesting the importance of as yet undefined individual characteristics that determine FEV_1 responsiveness to O_3. The authors concluded that much individual variability in FEV_1 response to O_3 remains unexplained.

6.5 FACTORS MODIFYING RESPONSIVENESS TO OZONE

6.5.1 Influence of Age

Beyond the age of 18 to 20 yrs, spirometric and symptom responses to O_3 exposure begin to decline with increasing age. In healthy individuals, the rate of decline in O_3 responsiveness appears to be greater in younger (18 to 35 yrs) versus middle aged (35 to 55 yrs) individuals (Passannante et al., 1998; Hazucha et al., 2003). Beyond this age (> 55 yrs), acute O_3 exposure elicits minimal spirometric changes. An average FEV_1 decrement of ~3% has been reported by Gong et al. (1997a) for this older population under a "worst case" exposure scenario (0.24 ppm O_3 with 4 h IE). Although Gong et al. (1997a) and others have examined responses to O_3 exposure in subjects of various ages, the exposure conditions differ between most studies so that age effects remain uncertain.

Three recent studies, which analyzed large data sets (≥240 subjects) of similarly exposed subjects, show clearly discernable changes in FEV_1 responses to O_3 as a function of age. Seal et al. (1996) analyzed O_3-induced spirometric responses in 371 young nonsmokers (18 to 35 years of age) exposed for 2.3 h during IE at a $\dot{V}_E$ of 25 L/min/m^2 BSA. On average, for the same O_3 concentration (C), the response of 25, 30, and 35 year old individuals are predicted to be 83, 65, and 48%, respectively, of the response in 20 year olds. For example, a 5.4% decrement in FEV_1 is predicted for 20 year old exposed to 0.12 ppm O_3 for 2.3 h IE ($\dot{V}_E$ = 25 L/min/m^2 BSA), whereas, a similarly exposed 35 yr old is predicted to have only a 2.6% decrement.

McDonnell et al. (1997) examined FEV_1 responses in 485 healthy white males (18 to 36 years of age) exposed once for 2 h to an O_3 concentration of 0.0, 0.12, 0.18, 0.24, 0.30, or

0.40 ppm at rest or one of two levels of IE ($\dot{V}_E$ of 25 and 35 L/min/m^2 BSA). For the same exposure conditions (C, $\dot{V}_E$, and duration), the average responses of 25, 30, and 35 year old individuals are predicted to be 69, 48, and 33%, respectively, of the response in 20 year olds. Hazucha et al. (2003) analyzed the distribution of O_3 responsiveness in 240 subjects (18 to 60 years of age) exposed to 0.42 ppm O_3 for 1.5 h with IE at $\dot{V}_E$ = 20 L/min/m^2 BSA. In males, the FEV_1 responses of 25, 35, and 50 year olds are predicted to be 94, 83, and 50% , respectively, of the average response in 20 year old males. In females, the FEV_1 responses of 25, 35, and 50 year olds are predicted to be 82, 46, and 18%, respectively, of the average response in 20 year old females.

For subjects aged 18 to 36 yrs, McDonnell et al. (1999) recently reported that symptom responses from O_3 exposure also decrease with increasing age. Whether the same age-dependent pattern of O_3 sensitivity decline also holds for airway reactivity or inflammatory endpoints has not been determined.

6.5.2 Gender and Hormonal Influences

Several studies have suggested that physiological differences between the genders may predispose females to a greater susceptibility to O_3. Lower plasma and nasal lavage fluid levels of uric acid (the most prevalent antioxidant) in females relative to males 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 gender when absorption is normalized to anatomical dead space (Bush et al., 1996). More recently, Ultman et al. (2004) reported that the whole lung uptake fraction of O_3 was significantly greater in males (91.4%) than females (87.1%). But, this increase in O_3 uptake in the males was consistent with their larger V_T and smaller f_B relative to the females. Furthermore, O_3 uptake was not correlated with spirometric responses. 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). Although, during the follicular phase of the menstrual cycle, lung function response to O_3 is enhanced (Fox et al., 1993).

6.5.3 Racial, Ethnic and Socioeconomic Status Factors

A few epidemiologic studies have implied that minorities are more responsive to O_3 than then caucasians. However, this may be more of a consequence of the overall quality of health care and SES then an innate sensitivity to oxidants (Gwynn and Thurston, 2001; Seal et al, 1996). The paucity of data has prevented making any definitive conclusions on the influence of race, ethnic or other related factors on the responsiveness to O_3.

6.5.4 Influence of Physical Activity

Any physical activity will increase minute ventilation and therefore the dose of inhaled O_3. Consequently, the intensity of physiological response following an acute exposure will be strongly associated with minute ventilation (see Figure 6-3).

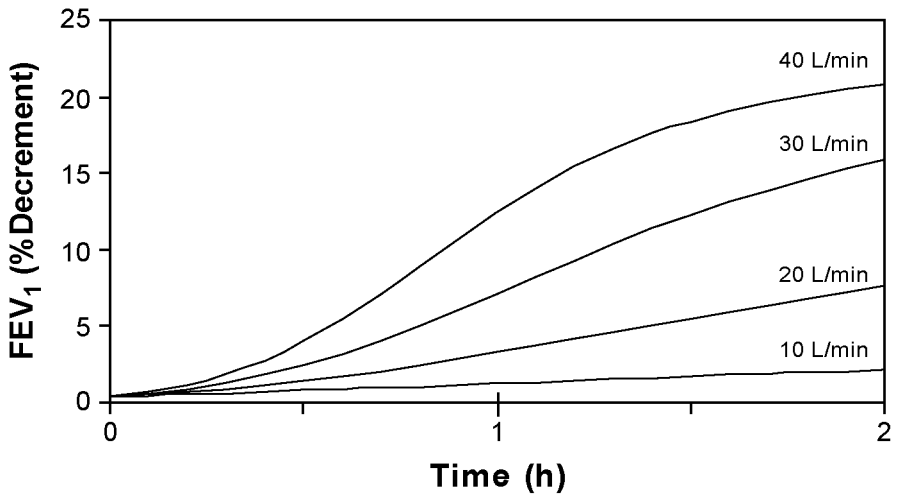

Figure 6-3. Predicted O_3-induced decrements in FEV_1 as a function of exposure duration and level of IE (line labels are $\dot{V}_E$ levels) in young healthy adults (20 yrs of age) exposed to 0.3 ppm O_3. The illustrated activity levels range from rest ($\dot{V}_E$ = 10 L/min) to moderate exercise ($\dot{V}_E$ = 40 L/min). Predictions are for Model 1 coefficients in Table 3 of McDonnell et al. (1997).

Source: Based on McDonnell et al. (1997).

6.5.5 Environmental Factors

Since the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) few human laboratory studies have examined the potential influence of environmental factors such as rural versus urban environment, passive cigarette smoke exposure, and bioactive admixtures such as endotoxin on healthy individual's pulmonary function changes due to O_3.

New controlled human exposure studies have confirmed that smokers are less responsive to O_3 than nonsmokers. Spirometric and plethysmographic pulmonary function decline, nonspecific airway hyperreactivity, and inflammatory response of smokers to O_3 were all weaker than those reported for nonsmokers. Although all these responses are intrinsically related, the functional association between them, as in nonsmokers, has been weak. Similarly, the time course of development and recovery of these effects, as well their reproducibility, was not different from nonsmokers. 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., 1997; Torres et al., 1997).

The effect on environment tobacco smoke (ETS) on O_3 responses has received very little attention. In one study, preexposure of mice to sidestream cigarette smoke (ETS surrogate), elicited no immediate effects, but potentiated subsequent O_3-induced inflammatory responses (Yu et al., 2002) (*See Chapter 5.4.2 for additional ETS details*).

The influence of ambient temperature on pulmonary effects induced by O_3 exposure in humans has been studied infrequently under controlled laboratory conditions. Several experimental human studies have reported additive effects of heat and O_3 exposure (see U.S. Environmental Protection Agency, 1986, 1996). Foster et al. (2000) exposed 9 young healthy subjects for 130 min (IE 10 min at 36 to 39 l/min) to filtered air and to ramp profile O_3 at 22° and 30 °C, 45-55% RH. The O_3 exposure started at 0.12 ppm, reached the peak of 0.24 ppm mid-way through and subsequently declined to 0.12 ppm at the end of exposure. At the end of exposure FEV_1 decreased significantly ($p < 0.5$) by ~8% at 22°C and ~6.5% at 30 °C. One day (19 h) later, the decline of 2.3% from baseline was still significant ($p < 0.05$). FVC decrements were smaller and significant only for the 22 °C condition immediately postexposure. There was a decline in specific airway conductance (sGaw; $p < 0.05$) at 30°C but not at 22 °C. The nonspecific bronchial responsiveness to methacloline assessed as PC_{50} sGaw was significantly

($p < 0.05$) higher one day following O_3 exposure at both temperatures but more so at 30 °C. Thus, these findings suggest that elevated temperature may partially attenuate spirometric responses but enhance airway reactivity.

6.5.6 Oxidant-Antioxidant Balance

The first line of defense against oxidative stress is antioxidant present in epithelial 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., 1999), however, the concentration and the activity of antioxidant enzymes either in ELF or plasma do not appear to be related to O_3 responsiveness (Avissar et al., 2000; Blomberg et al., 1999; Samet et al., 2001). 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 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 post-exposure bronchial hyperresponsiveness (Trenga et al., 2001). Animal studies (*described in Chapter 5.2.1.3*) have also demonstrated the protective effects of ELF antioxidants during O_3 exposures.

6.5.7 Genetic and Other Factors

Several recent studies (Bergamaschi et al., 2001) have reported that genetic polymorphism of antioxidant enzymes may modulate pulmonary function and inflammatory response to O_3 challenge. It appears that healthy carriers of NQO1 wild type in combination with GSTM1 null genotype are more responsive to O_3. Adults with GSTM1 null only genotype did not show O_3 hyperresponsiveness. In contrast, asthmatic children with GSTM1 null genotype (Romieu et al, 2004) were reported to be more responsive to O_3. In general, the findings between studies are inconsistent and additional, better controlled studies, are needed to clarify influence of genetic polymorphism on O_3 responsiveness.

6.6 REPEATED O_3 EXPOSURE EFFECTS

Based on studies reviewed here and in the previous O_3 criteria documents (U.S. Environmental Protection Agency, 1986, 1996), several conclusions can be drawn about repeated 1 to 2-h O_3 exposures. Repeated exposures to O_3 can cause an enhanced (i.e., greater) pulmonary function response on the second day of exposure (*see Tables AX6-8 and AX6-9 for added detail*). This enhancement appears to be dependent on the interval between the exposures (24 h is associated with the greatest increase) and is absent with intervals > 3 days (Bedi et al., 1985; Folinsbee and Horvath, 1986; Schonfeld et al., 1989). An enhanced response also 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 an attenuation of pulmonary function responses, typically after 3 to 5 days of repeated exposure. This attenuated response persists for less than 1 week (Kulle et al., 1982; Linn et al., 1982b) or as long as 2 weeks (Horvath et al., 1981). In temporal conjunction with pulmonary function changes, symptoms induced by O_3, such as cough, PDI, and chest discomfort, are increased on the second exposure day and attenuated with repeated exposure thereafter (Folinsbee et al., 1980, 1998; Foxcroft and Adams, 1986; Linn et al., 1982b). O_3-induced changes in airway responsiveness persist longer and attenuate more slowly than pulmonary function and symptoms responses (Dimeo et al., 1981; Kulle et al., 1982), although this has been studied only on a limited basis (Folinsbee et al., 1994). In longer-duration (4 h to 6.6 h), 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) [*Effects of repeated exposures on inflammatory responses are discussed in Section 6.9.4*).

6.7 EFFECTS ON EXERCISE PERFORMANCE

The effects of acute O_3 inhalation on endurance exercise performance have been examined in numerous controlled laboratory studies. These studies were discussed in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) and can be divided into two categories: (1) those that examined the effects of acute O_3 inhalation on maximal oxygen uptake ($\dot{V}O_{2max}$) and

(2) those that examined the effects of acute O_3 inhalation on the ability to complete strenuous continuous exercise protocols of up to 1 h in duration.

In brief, endurance exercise performance and $\dot{V}O_{2max}$ may be limited by acute exposure to O_3 (Adams and Schelegle, 1983; Schelegle and Adams, 1986; Gong et al., 1986; Foxcroft and Adams, 1986; Folinsbee et al., 1977; Linder et al., 1988). Gong et al. (1986) and Schelegle and Adams (1986) found that significant reductions in maximal endurance exercise performance may occur in well-conditioned athletes while they perform CE ($\dot{V}_E$ > 80 L/min) for 1 h at O_3 concentrations ≥ 0.18 ppm. Reports from studies of exposure to O_3 during high-intensity exercise indicate that breathing discomfort associated with maximal ventilation may be an important factor in limiting exercise performance in some, but not all, subjects.

6.8 EFFECTS ON AIRWAY RESPONSIVENESS

Airway or bronchial hyperresponsiveness (BHR) refers to a condition in which the propensity for the airways to bronchoconstrict due to a variety of stimuli becomes augmented. Airway responsiveness is typically quantified by measuring the decrement in pulmonary function (i.e., spirometry or plethysmography) following the inhalation of small amounts of an aerosolized bronchoconstrictor agent (specific [antigen, allergen] or nonspecific [methacholine, histamine]) or a measured stimulus (e.g., exercise, cold air).

Ozone exposure causes an increase in nonspecific airway responsiveness as indicated by a reduction in the concentration of methacholine or histamine required to produce a given reduction in FEV_1 or increase in SRaw. Increased airway responsiveness is an important consequence of exposure to O_3 because its presence means that the airways are predisposed to narrowing on inhalation of a variety of stimuli (e.g., specific allergens, SO_2, cold air).

Ozone exposure of asthmatic subjects, who characteristically have increased airway responsiveness at baseline, can cause further increases in responsiveness (Kreit et al., 1989). Similar relative changes in airway responsiveness are seen in asthmatics exposed to O_3 despite their markedly different baseline airway responsiveness. Several studies (Jörres et al., 1996; Kehrl et al., 1999; Molfino et al., 1991) have been published suggesting an increase in specific (i.e., allergen-induced) airway reactivity. An important aspect of increased airway

responsiveness after O_3 exposure is that this represents a plausible link between ambient O_3 exposure and increased hospital admissions for asthma.

Changes in airway responsiveness after O_3 exposure appear to be resolved more slowly than changes in FEV_1 or respiratory symptoms (Folinsbee and Hazucha, 2000). Furthermore, in studies of repeated exposure to O_3, changes in airway responsiveness tend to be somewhat less susceptible to attenuation with consecutive exposures than changes in FEV_1 (Dimeo et al., 1981; Folinsbee et al., 1994; Gong et al., 1997b; Kulle et al., 1982). Increases in airway responsiveness do not appear to be strongly associated with decrements in lung function or increases in symptoms.

The mechanism of O_3-induced increases in airway responsiveness is only partially understood, but it appears to be associated with a number of cellular and biochemical changes in airway tissue. Although inflammation could play a role in the increase in airway responsiveness, cyclooxygenase inhibitors have not been effective at blocking the O_3-induced influx of PMNs into BAL fluid (Hazucha et al., 1996; Ying et al., 1990). Therefore, O_3-induced airway responsiveness may not be due to the presence of PMNs in the airway or to the release of arachidonic acid metabolites. Rather, it seems likely that the mechanism for this response is multifactorial, possibly involving the presence of cytokines, prostanoids, or neuropeptides; activation of macrophages, eosinophils, or mast cells; and epithelial damage that increases direct access of mediators to the smooth muscle or receptors in the airways that are responsible for reflex bronchoconstriction.

6.9 INFLAMMATION AND HOST DEFENSE EFFECTS

6.9.1 Introduction

Short-term exposure of humans to O_3 can cause acute inflammation and that long-term exposure of laboratory animals results in a chronic inflammatory state (*see Chapter 5*). The relationship between repetitive bouts of acute inflammation in humans caused by O_3 and the development of chronic respiratory disease is unknown.

The presence of neutrophils (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. It is apparent, however, that inflammation within airway tissues may persist beyond the point that

inflammatory cells are found in BAL fluid. Soluble mediators of inflammation such as the cytokines (IL-6, IL-8) and arachidonic acid metabolites (e.g., PGE_2, $PGF_{2\alpha}$, thromboxane, and leukotrienes [LTs] such as LTB_4) have been measured in the BAL fluid of humans exposed to O_3. In addition to their role in inflammation, many of these compounds have bronchoconstrictive properties and may be involved in increased airway responsiveness following O_3 exposure.

Some recent evidence suggests that changes in small airways function may provide a sensitive indicator of O_3 exposure and effect, despite the fact that inherent variability in their measurement by standard spirometric approaches make their assessment difficult. Observations of increased functional responsiveness of these areas relative to the more central airways, and of persistent effects following repeated exposure, may indicate that further investigation of inflammatory processes in these regions is warranted.

6.9.2 Inflammatory Response in the Upper Respiratory Tract

The nasal passages constitute the primary portal for inspired air at rest and, therefore, the first region of the respiratory tract to come in contact with airborne pollutants. Nikasinovic et al. (2003) recently reviewed the literature of laboratory-based nasal inflammatory studies published since 1985. Nasal lavage (NL) has provided a useful tool for assessing O_3-induced inflammation in the nasopharynx. Increased levels of PMNs in the NL fluid of humans exposed to 0.5 ppm O_3 at rest for 4 h has been reported (Graham et al., 1988; Bascom et al., 1990).

Graham and Koren (1990) compared inflammatory mediators present in both the NL and BAL fluids of humans exposed to 0.4 ppm O_3 for 2 h. Similar increases in PMN were observed in NL and BAL, suggesting a qualitative correlation between inflammatory changes in the lower airways (BAL) and the upper respiratory tract (NL). Torres et al. (1997) compared NL and BAL in smokers and nonsmokers exposed to 0.22 ppm O_3 for 4 h. In contrast to Graham and Koren (1990), they did not find a relationship between numbers or percentages of PMNs in the nose and the lung, perhaps in part due to the variability observed in their NL recoveries. Albumin, a marker of epithelial cell permeability, was increased 18 h later, but not immediately after exposure, as seen by Bascom et al. (1990).

McBride et al. (1994) reported that asthmatic subjects were more sensitive than non-asthmatics to upper airway inflammation at an O_3 concentration (0.24 ppm (1.5 h)) that did not

affect lung or nasal function or biochemical mediators. A significant increase in the number of PMNs in NL fluid was detected in the asthmatic subjects both immediately and 24 h after exposure. Peden et al. (1995) also found that O_3 at a concentration of 0.4 ppm had a direct nasal inflammatory effect, and reported a priming effect on the response to nasal allergen challenge, as well. A subsequent study in dust mite-sensitive asthmatic subjects indicated that O_3 at this concentration enhanced eosinophil influx in response to allergen, but did not promote early mediator release or enhance the nasal response to allergen (Michelson et al., 1999). Similar to observations made in the lower airways, the presence of O_3 molecular "targets" in nasal lining fluid is likely to provide some level of local protection against exposure. In a study of healthy subjects exposed to 0.2 ppm O_3 for 2 h, Mudway and colleagues (1999) observed a significant depletion of uric acid in NL fluid at 1.5 h following exposure.

6.9.3 Inflammatory Response in the Lower Respiratory Tract

Seltzer et al. (1986) were the first to demonstrate that exposure of humans to O_3 resulted in inflammation in the lung. Bronchoalveolar lavage fluid (3 h post-exposure) from subjects exposed to O_3 contained increased PMNs as well as increased levels of PGE_2, $PGF_{2\alpha}$, and TXB_2 compared to fluid from air-exposed subjects. Koren et al. (1989a,b) described inflammatory changes 18 h after O_3 exposure. In addition to an eightfold increase in PMNs, Koren et al. reported a two-fold increase in BAL fluid protein, albumin, and immunoglobulin G (IgG) levels, suggestive of increased epithelial cell permeability. There was a 12-fold increase in IL-6 levels, a two-fold increase in PGE_2, and a two-fold increase in the complement component, C3a. Evidence for stimulation of fibrogenic processes in the lung was shown by significant increases in coagulation components, Tissue Factor and Factor VII (McGee et al., 1990), urokinase plasminogen activator and fibronectin (Koren et al., 1989a). Subsequent studies by Lang et al., (1998), using co-cultures of cells of the BEAS-2B bronchial epithelial line and of the HFL-1 lung fibroblast line, provided additional information about O_3-induced fibrogenic processes. They demonstrated that steady-state mRNA levels of both alpha 1 and procollagens type I and III in the fibroblasts were increased following O_3 exposure and that this effect was mediated by the O_3-exposed epithelial cells. This group of studies demonstrated that exposure to O_3 results in an inflammatory reaction in the lung, as evidenced by increases in PMNs and proinflammatory compounds. Furthermore, they demonstrated that cells and mediators capable of damaging

pulmonary tissue are increased after O_3 exposure, and provided early suggestion of the potential importance of the epithelial cell-myofibroblast "axis" in modulating fibrotic and fibrinolytic processes in the airways.

Isolated lavage of the mainstream bronchus using balloon catheters or BAL using small volumes of saline have been used to assess O_3-induced changes in the large airways. Studies collecting lavage fluid from isolated airway segments after O_3 exposure indicate increased neutrophils in the airways (Aris et al., 1993; Balmes et al., 1996; Scannell et al., 1996). Other evidence of airway neutrophil increase comes from studies in which the initial lavage fraction ("bronchial fraction") showed increased levels of neutrophils (Schelegle et al., 1991; Peden et al., 1997; Balmes et al., 1996; Torres et al., 1997). Bronchial biopsies show increased PMNs in airway tissue (Aris et al.,1993) and, in sputum collected after O_3 exposure, neutrophil numbers are elevated (Fahy et al., 1995).

Increased BAL protein, suggesting O_3-induced changes in epithelial permeability (Koren et al., 1989a, 1991 and Devlin et al., 1991) supports earlier work in which increased epithelial permeability, as measured by increased clearance of radiolabled diethylene triamine pentaacetic acid (^{99m}Tc-DTPA) from the lungs of humans exposed to O_3, was demonstrated (Kehrl et al., 1987). In addition, Foster and Stetkiewicz (1996) have shown that increased permeability persists for at least 18-20 h and the effect is greater at the lung apices than at the base. In a study of mild atopic asthmatics exposed to 0.2 ppm O_3 for 2 h, Newson, et al. (2000) observed a 2-fold increase in the percentage of PMNs present at 6 hours post exposure, with no change in markers of increased permeability as assessed by sputum induction. By 24 h, the neutrophilia was seen to subside while levels of albumin, total protein, myeloperoxidase, and eosinophil cationic protein increased significantly. It was concluded that the transient PMN influx induced by acute exposure of these asthmatic subjects was followed by plasma extravasation and the activation of both PMNs and eosinophils within the airway tissues. Such changes in permeability associated with acute inflammation may provide better access of inhaled antigens, particulates, and other substances to the submucosal region.

Devlin et al. (1991) reported an inflammatory response in subjects exposed to 0.08 and 0.10 ppm O_3 for 6.6 h. Increased numbers of PMNs and levels of IL-6 were found at both O_3 concentrations, suggesting that lung inflammation from O_3 can occur as a consequence of prolonged exposure to ambient levels while exercising. Interestingly, those individuals who had

the largest increases in inflammatory mediators in this study did not necessarily have the largest decrements in pulmonary function, suggesting that separate mechanisms underlie these two responses. The absence of a relationship between spirometric responses and inflammatory cells and markers has been reported in several studies (Balmes et al., 1996; Schelegle et al., 1991; Torres et al., 1997; Hazucha et al., 1996; Blomberg et al., 1999). These observations relate largely to disparities in the times of onset and duration following single exposures.

As indicated above, a variety of potent proinflammatory mediators have been reported to be released into the airway lumen following O_3 exposure. Studies of human alveolar macrophages (AM) and airway epithelial cells exposed to O_3 *in vitro* suggest that most mediators found in the BAL fluid of O_3-exposed humans are produced by epithelial cells. Macrophages exposed to O_3 *in vitro* showed only small increases in PGE_2 (Becker et al., 1991). In contrast, airway epithelial cells exposed *in vitro* to O_3 showed large concentration-dependent increases in PGE_2, TXB_2, LTB_4, LTC_4, and LTD_4 (McKinnon et al., 1993) and increases in IL-6, IL-8, and fibronectin at O_3 concentrations as low as 0.1 ppm (Devlin et al., 1994). Macrophages lavaged from subjects exposed to 0.4 ppm (Koren et al., 1989a) showed changes in the rate of synthesis of 123 different proteins, whereas AMs exposed to O_3 *in vitro* showed changes in only six proteins, suggesting that macrophage function was altered by mediators released from other cells. Furthermore, recent evidence suggests that the release of mediators from AMs may be modulated by the products of O_3-induced oxidation of airway lining fluid components, such as human surfactant protein A (Wang et al., 2002).

Although the release of mediators has been demonstrated to occur at exposure concentrations and times that are minimally cytotoxic to airway cells, potentially detrimental latent effects have been demonstrated in the absence of cytotoxicity. These include the generation of DNA single strand breaks (Kozumbo et al., 1996) and the loss of cellular replicative activity (Gabrielson et al., 1994) in bronchial epithelial cells exposed *in vitro*, and the formation of protein and DNA adducts. A highly toxic aldehyde formed during O_3-induced lipid peroxidation is 4-hydroxynonenal (HNE). Healthy human subjects exposed to 0.4 ppm O_3 for 1 h underwent BAL 6 h later. Analysis of lavaged alveolar macrophages by Western blot indicated increased levels of a 32-kDa HNE-protein adduct, as well as 72-kDa heat shock protein and ferritin, in O_3- versus air-exposed subjects (Hamilton et al., 1998). In a recent study of healthy subjects exposed to 0.1 ppm O_3 for 2 h (Corradi et al., 2002), formation of 8-hydroxy-2'-

deoxyguanosine (8-OHdG), a biomarker of reactive oxidant species (ROS)-DNA interaction, was measured in peripheral blood lymphocytes. At 18 h post exposure, 8-OHdG was significantly increased in cells compared to pre-exposure levels, presumably linked to concurrent increases in chemical markers of ROS. Of interest, the increase in 8-OHdG was only significant in a subgroup of subjects with the wild genotype for NAD(P)H:quinone oxidoreductase and the null genotype for glutathione-S-transferase M1, suggesting that polymorphisms in redox enzymes may confer "susceptibility' to O_3 in some individuals. The generation of ROS following exposure to O_3 has been shown to be associated with a wide range of responses. In a recent study, ROS production by alveolar macrophages lavaged from subjects exposed to 0.22 ppm for 4 h was assessed by flow cytometry (Voter et al., 2001). Levels were found to be significantly elevated 18 h post exposure and associated with several markers of increased permeability. An *in vitro* study of human tracheal epithelial cells exposed to O_3 indicated that generation of ROS resulted in decrease in synthesis of the bronchodilatory prostaglandin, PGE_2, as a result of inactivation of prostaglandin endoperoxide G/H synthase 2 (Alpert et al., 1997). These and similar studies indicate that the responses to products of O_3 exposure in the airways encompass a broad range of both stimulatory and inhibitory activities, many of which may be modulated by susceptibility factors upstream in the exposure process, at the level of compensating for the imposed oxidant stress.

The inflammatory responses to O_3 exposure also have been studied in asthmatic subjects (Basha et al., 1994; Scannell et al., 1996; Peden et al., 1997). In these studies, asthmatics showed significantly more neutrophils in the BAL (18 h post-exposure) than similarly exposed healthy individuals. In one of these studies (Peden et al., 1997), which included only allergic asthmatics who tested positive for Dematophagoides farinae antigen, there was an eosinophilic inflammation (2-fold increase), as well as neutrophilic inflammation (3-fold increase). In a study of subjects with intermittent asthma that utilized a 2-fold higher concentration of O_3 (0.4 ppm) for 2 h, increases in eosinophil cationic protein, neutrophil elastase and IL-8 were found to be significantly increased 16 h post-exposure and comparable in induced sputum and BAL fluid (Hiltermann et al, 1999). In two studies (Basha et al., 1994; Scannell et al., 1996), IL-8 was significantly higher in post-O_3 exposure BAL in asthmatics compared to non-asthmatics, suggesting a possible mediator for the increased neutrophilic inflammation in those subjects. In a recent study comparing the neutrophil response to O_3 at a concentration and exposure time

similar to those of the latter three studies, Stenfors and colleagues (2002) were unable to detect a difference in the increased neutrophil numbers between 15 mild asthmatic and 15 healthy subjects by bronchial wash at the 6 h post-exposure time point. These results suggest that, at least with regard to neutrophil influx, differences between healthy and asthmatic individuals develop gradually following exposure and may not become evident until later in the process. In another study, mild asthmatics who exhibited a late phase underwent allergen challenge 24 hrs before a 2 h exposure to 0.27 ppm O_3 or filtered air in a cross-over design (Vagaggini et al., 2002). At 6 h post-exposure, eosinophil numbers in induced sputum were found to be significantly greater after O_3 than after air. Studies such as these suggest that the time course of eosinophil and neutrophil influx following O_3 exposure can occur to levels detectable within the airway lumen by as early as 6 h. They also suggest that the previous or concurrent activation of proinflammatory pathways within the airway epithelium may enhance the inflammatory effects of O_3. For example, in an *in vitro* study of epithelial cells from the upper and lower respiratory tract, cytokine production induced by rhinovirus infection was enhanced synergistically by concurrent exposure to O_3 at 0.2 ppm for 3 h (Spannhake et al, 2002). The use of bronchial mucosal biopsies has also provided important insight into the modulation by O_3 of existing inflammatory processes within asthmatics. In a study of healthy and allergic asthmatic subjects exposed to 0.2 ppm O_3 or filtered air for 2 h, biopsies were performed 6 hr following exposure (Bosson et al., 2003). Monoclonal antibodies were used to assess epithelial expression of a variety of cytokines and chemokines. At baseline (air exposure), asthmatic subjects showed significantly higher expression of interleukins (IL)-4 and -5. Following O_3 exposure, the epithelial expression of IL-5, IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF) and epithelial cell-derived neutrophil-activating peptide 78 (ENA-78) was significantly greater in asthmatic subjects, as compared to healthy subjects. *In vitro* studies of bronchial epithelial cells derived by biopsy from nonatopic, nonasthmatic subjects and asthmatic subjects also demonstrated the preferential release of GM-CSF and also of regulated on activation, normal T cell-expressed and -secreted (RANTES) from asthmatic cells following O_3 exposure.

The time course of the inflammatory response to O_3 in humans has not been explored fully. Nevertheless, studies in which BAL was performed 1-3 h (Devlin et al., 1990; Koren et al., 1991; Seltzer et al., 1986) after exposure to 0.4 ppm O_3 demonstrated that the inflammatory response is quickly initiated, and other studies (Koren et al., 1989a,b; Torres et al., 1997;

Scannell et al., 1996; Balmes et al., 1996) indicated that, even 18 h after exposure, inflammatory mediators such as IL-6 and PMNs were still elevated. However, different markers show peak responses at different times. Ozone-induced increases in IL-8, IL-6, and PGE_2 are greater immediately after O_3 exposure, whereas BAL levels of fibronectin and plasminogen activator are greater after 18 h. PMNs and some products (protein, Tissue Factor) are similarly elevated both 1 and 18 h after O_3 exposure (Devlin et al., 1996; Torres et al., 1997). Schelegle et al. (1991) found increased PMNs in the "proximal airway" lavage at 1, 6, and 24 h after O_3 exposure, with a peak response at 6 h. In a typical BAL sample, PMNs were elevated only at the later time points. This is consistent with the greater increase 18 h after exposure seen by Torres et al. (1997). In addition to the influx of PMNs and (in allergic asthmatics) eosinophils, lymphocyte numbers in BAL were also seen to be elevated significantly at 6 h following exposure of healthy subjects to 0.2 ppm O_3 for 2 h (Blomberg et al., 1997). Analysis of these cells by flow cytometry indicated the increased presence of CD3+, CD4+ and CD8+ T cell subsets. This same laboratory later demonstrated that within 1.5 h following exposure of healthy subjects to the same O_3 regimen, expression of human leukocyte antigen (HLA)-DR on lavaged macrophages underwent a significant, 2.5-fold increase (Blomberg et al., 1999). The significance of these alterations in immune system components and those in IL-4 and IL-5 expression described above in the studies of Bosson et al. (2003) has not been fully explored and may suggest a role for O_3 in the modulation of immune inflammatory processes.

6.9.4 Effects of Repeated Exposures and Adaptation of Responses

Physiologic and symptomatic responses in humans following repeated exposure to O_3 were discussed in Section 6.6. Inflammatory responses upon repeated O_3 exposures are discussed in this section. Animal studies suggest that while inflammation may be diminished with repeated exposure, underlying damage to lung epithelial cells continues (Tepper et al., 1989). Markers from BALF following both 2-h (Devlin et al., 1997) and 4-h (Christian et al., 1998; Jörres et al., 2000) 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) examined the inflammatory responces of humans repeatedly exposed to 0.4 ppm O_3 for 5 consecutive days. Several indicators of inflammation (e.g., PMN influx, IL-

6, PGE_2, BAL protein, fibronectin) were attenuated after 5 days of exposure (i.e., values were not different from FA). Several markers (LDH, IL-8, total protein, epithelial cells) did not show attenuation, indicating that tissue damage probably continues to occur during repeated exposure. The recovery of the inflammatory response occurred for some markers after 10 days, but some responses were not normalized even after 20 days. The continued presence of markers of cellular injury indicates a persistent but not necessarily recognized due to attention of spirometric and symptom responses to O_3.

Christian et al. (1998) randomly subjected heathy subjects to a single exposure and to 4 consecutive days of exposure to 0.2 ppm O_3 for 4 h. Both "bronchial" and "alveolar" fractions of the BAL showed decreased numbers of PMNs and fibronectin concentration at day 4 versus the single exposure, and a decrease in IL-6 levels in the alveolar fraction.

Following a similar study design and exposure parameters, Jörres et al. (2000) found both functional and BAL cellular responses to O_3 were abolished at 24 h 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 for bronchitis, erythema and the numbers of neutrophils in the mucosal biopsies were increased. Their results indicate that, despite reduction of some markers of inflammation in BAL and measures of large airway function, inflammation within the airways persists following repeated exposure to O_3.

Holz, et al. (2002) made a comparison of early and late responses to allergen challenge following O_3 in subjects with allergic rhinitis or allergic asthma. With some variation, both early and late FEV_1 and cellular responses in the two subject groups were significantly enhanced by 4 consecutive days of exposure to 0.125 ppm O_3 for 3 h.

In another study, Frank and colleagues (2001) exposed healthy subjects to FA and to O_3 (0.25 ppm, 2 h) on 4 consecutive days each, with pulmonary function measurements being made prior to and following each exposure. BAL was performed on day 5, 24 h following the last exposure. On day 5, PMN numbers remained significantly higher following O_3 compared to FA. Of particular note in this study was the observation that small airway function, assessed by grouping values for isovolumetric FEF_{25-75}, Vmax50 and Vmax75 into a single value, showed persistent reduction from day 2 through day 5. Following exposure of and asthmatic and healthy subjects for one day to 0.4 ppm O_3 for 2 h, Alexis et al. (2000) have also reported that variables representing small airways function (viz., FEF_{25}, FEF_{50}, FEF_{60P}, FEF_{75}) demonstrated the

greatest O_3-induced decline in the asthmatic subjects. These data suggest that techniques monitoring the function in the small peripheral airway regions, the primary sites of O_3 uptake in the lung, may provide important information regarding both acute and cumulative effects of O_3 exposure.

6.9.5 Effect of Anti-Inflammatory and other Mitigating Agents

Pretreatment of healthy subjects with non-steroidal anti-inflammatory drugs (ibuprofen, etc.) has been found to partially suppress development of airway inflammation and pulmonary function changes (U.S. Environmental Protection Agency, 1996). Although atropine blocked the increase in Raw in response to O_3 exposure, it did not alter the spirometric or symptom responses (Beckett et al., 1985). Similarly, albuterol and salbutamol, which had no effect on O_3-induced changes in spirometry, also had no effect of symptom responses (McKenzie et al., 1987; Gong et al., 1988). The anti-inflammatory medications indomethacin and ibuprofen, which partially inhibit the spirometric responses to O_3 exposure, also cause a reduction in respiratory symptoms (Schelegle et al., 1987; Hazucha et al., 1994). Indomethacin attenuates decrements in FEV_1 and FVC in healthy subjects, but not asthmatics (Alexis et al., 2000). In contrast, inhalation of the corticosteroid budesonide does not prevent or even attenuate O_3-induced responses in healthy subjects as assessed by measurements of lung function, bronchial reactivity and airway inflammation (Nightingale et al., 2000). In asthmatic subjects, budesonide decreases airway neutrophil influx following O_3 exposure (Vagaggini et al., 2001). This suggests that corticosteroids may be effective only when the inflammation is already present, such as in asthmatics.

6.9.6 Changes in Host Defense Capability Following Ozone Exposures

A number of studies clearly show that a single acute exposure (1 to 4 h) of humans to moderate concentrations of O_3 (0.2 to 0.6 ppm) while exercising at moderate to heavy levels results in a number of cellular and biochemical changes in the lung including an inflammatory response characterized by increased numbers of PMNs, increased permeability of the epithelial cells lining the respiratory tract, cell damage, and production of proinflammatory cytokines and prostaglandins. This response can be detected as early as 1 h after exposure (Koren et al., 1991;

Schelegle et al., 1991) and persists for at least 18 h (Aris et al., 1993; Koren et al., 1989a). The response profile of these mediators is not defined adequately, although it is clear that the time course of response varies for different mediators and cells (Devlin et al., 1997; Schelegle et al., 1991). These changes also occur in humans exposed to 0.08 and 0.10 ppm O_3 for 6 to 8 h (Devlin et al., 1991; Peden et al., 1997). Decrements in the ability of AMs to phagocytose microorganisms have also been reported. Ozone also causes inflammatory changes in the nose, as indicated by increased levels of PMNs and albumin, a marker for increased epithelial cell permeability. Nasal lavage analyses, however, are not necessarily parallel to BAL analyses.

There appears to be no strong correlation between any of the measured cellular and biochemical changes and changes in lung function measurements, suggesting that different mechanisms may be responsible for these processes (Balmes et al., 1996; Devlin et al., 1991). The idea of different mechanisms is supported by a study in which ibuprofen, a cyclooxygenase inhibitor, blunted the O_3-induced decrements in lung function without altering the O_3-induced increase in PMNs or epithelial cell permeability (Hazucha et al., 1996).

In vitro studies suggest that epithelial cells are the primary target of O_3 in the lung and that O_3 induces them to produce many of the mediators found in the BAL fluid of humans exposed to O_3. Although O_3 does not induce AMs to produce these compounds in large quantities, it does directly impair the ability of AMs to phagocytose and kill microorganisms.

Only two studies (Foster et al., 1987; Gerrity et al., 1993) have investigated the effect of O_3 exposure on mucociliary particle clearance in humans. Foster et al. (1987) measured clearance during and after a 2 h exposure to 0.4 ppm O_3. Gerrity et al. (1993) measured clearance at 2 h postexposure (0.4 ppm O_3), by which time, sRaw had returned to baseline and FVC was within 5% of baseline (versus an 11% decrement immediately postexposure). Foster et al. (1987) found a stimulatory effect of acute O_3 exposure on mucociliary clearance. Gerrity et al. (1993), who observed no effect on clearance, suggested that transient clearance increases are coincident to pulmonary function responses. Investigators in both studies suggested that O_3-induced increases in mucociliary clearance could be mediated by cholinergic receptors.

6.10 EXTRAPULMONARY EFFECTS OF OZONE

Ozone reacts rapidly on contact with respiratory system tissue and is not absorbed or transported to extrapulmonary sites to any significant degree as such. Human exposure studies discussed in the previous criteria documents (U.S. Environmental Protection Agency, 1986, 1996) failed to demonstrate any consistent extrapulmonary effects. More recently, some human exposure studies have attempted to identify specific markers of exposure to O_3 in blood. Foster et al. (1996) found a reduction in the serum levels of the free radical scavenger α-tocopherol after O_3 exposure. Liu et al. (1997, 1999) 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 0.12 and 0.40 ppm suggest that O_3 increases production of hydroxyl radical. The levels of DHBA were correlated with changes in spirometry.

Gong et al. (1998) monitored ECG, HR, cardiac output, blood pressure, oxygen saturation, and chemistries, as well as calculating other hemodynamic variables (e.g., stroke volume, vascular resistance, rate-pressure products) in both healthy and hypertensive adult males, 41 to 78 years of age. No major acute cardiovascular effects were found in either the normal or hypertensive subjects after exposure to 0.3 ppm O_3 for 3 h with intermittent exercise at 30 L/min. Statistically significant O_3 effects for both groups combined were a decrease in FEV_1, and increases in HR, rate-pressure product, and the alveolar-to-arterial PO_2 gradient, which might be more important in some patients with severe cardiovascular disease.

6.11 EFFECTS OF OZONE MIXED WITH OTHER POLLUTANTS

Over the past 10 years only a handful of human controlled studies have examined the effects of pollutant mixtures containing O_3. The results of a controlled study on children (Linn et al., 1997), designed to approximate exposure conditions of an epidemiologic study (Neas et al., 1995) by matching the population and exposure atmosphere (0.1 ppm O_3, 0.1 ppm SO_2 and 101 $\mu g/m^2$ H_2SO_4), did not support the findings of this epidemiologic study. The study points out the difficulties in attempting to link the outcomes of epidemiologic and controlled studies. Another vulnerable population, asthmatics, demonstrated enhanced airway reactivity to house dust mite following exposures to O_3, NO_2, and the combination of the two gases. Spirometric

response, however, was impaired only by O_3, and O_3+NO_2 at higher concentrations (Jenkins et al., 1999). It is plausible that uneven longitudinal absorption of SO_2, NO_2, and O_3 in the conducting airways may influence a response. Ozone has been found to be scrubbed more efficiently in proximal airways and to penetrate less into the distal airways than either SO_2 and NO_2 (Rigas et al., 1997). Inhalation of a mixture of $PM_{2.5}$ and O_3 by healthy subjects increased brachial artery tone and reactivity (Brook et al., 2002). Since no other cardiovascular endpoints were affected by the exposure, the pathophysiological importance of this observation remains unclear.

All in all, the contention that air pollutant mixtures elicit stronger pathophysiologic effects than individual pollutants of the mix is only weakly supported by human studies of either healthy or at-risk population. The studies summarized in this section complement the studies reviewed in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996). Regarding the latter, the mobile laboratory comparative studies of exercising athletes (Avol et al., 1984, 1985) with chamber exposures to oxidant-polluted ambient air (mean O_3 concentration of 0.153 ppm) and purified air containing a controlled concentration of generated O_3 at 0.16 ppm showed similar pulmonary function responses and symptoms. These results strongly suggest that acute exposures of coexisting ambient pollutants had minimal contribution to these responses under the typical summer ambient conditions in Southern California. However, no unifying conclusions can be reached since each study employed different mixtures and examined different aspects of a response [*The complexities of O_3 and co-pollutant exposures in animal studies are discussed in Chapter 5.4.4*).

6.12 CONTROLLED STUDIES OF AMBIENT AIR EXPOSURES

A large amount of informative O_3 exposure-effects data has been obtained in controlled laboratory exposure studies under a variety of different experimental conditions. However, laboratory simulation of the variable pollutant mixtures present in ambient air is not practical. Thus, the exposure effects of one or several artificially generated pollutants (i.e., a simple mixture) on pulmonary function and symptoms may not explain responses to ambient air where complex pollutant mixtures exist.

6.12.1 Mobile Laboratory Studies

Quantitatively useful information on the effects of acute exposure to photochemical oxidants on pulmonary function and symptoms responses from field studies using a mobile laboratory were presented in prior criteria documents (U.S. Environmental Protection Agency, 1986, 1996). Relative to controlled exposure studies, mobile laboratory ambient air studies suffer the additional limation of a dependence on ambient outdoor conditions. Consistent with controlled exposure studies, mobile studies in California demonstrated that pulmonary effects from exposure to ambient air in Los Angeles are related to O_3 concentration and level of exercise. Healthy subjects with a history of allergy also appeared to be more responsive to O_3 than "nonallergic" subjects (Linn et al., 1980, 1983b), although a standardized evaluation of atopic status was not performed.

6.12.2 Aircraft Cabin Studies

Respiratory symptoms and pulmonary function effects resulting from exposure to O_3 in commercial aircraft flying at high altitudes, and in altitude-simulation studies, have been assessed previously (U.S. Environmental Protection Agency, 1986, 1996). Commercial aircraft cabin O_3 levels were reported to be very low (average concentration 0.01 to 0.02 ppm) during 92 randomly selected smoking and nonsmoking flights in 1989 (Nagda et al., 1989). None of these flights recorded O_3 concentrations exceeding the 3-h time-weighted average (TWA) standard of 0.10 ppm promulgated by the U.S. Federal Aviation Administration (FAA, 1980), probably due to the use of O_3-scrubbing catalytic filters (Melton, 1990).

Ozone contamination aboard high-altitude aircraft also has been an interest to the U.S. Air Force because of complaints by crew members of frequent symptoms of dryness and irritation of the eyes, nose, and throat and an occasional cough (Hetrick et al., 2000). Despite the lack of ventilation system modifications as used in commercial aircraft, the O_3 concentrations never exceeded the FAA ceiling limit of 0.25 ppm and exceeded the 3-h TWA of 0.10 ppm only 7% of the total monitored flight time (43 h). The authors concluded that extremely low average relative humidity (12%) during flight operations was most likely responsible for the reported symptoms.

6.13 SUMMARY

Responses in humans exposed to ambient O_3 concentrations include decreased inspiratory capacity; mild bronchoconstriction; rapid, shallow breathing pattern during exercise; and symptoms of cough and pain on deep inspiration. Reflex inhibition of inspiration results in a decrease in forced vital capacity (FVC) and, in combination with mild bronchoconstriction, contributes to a decrease in the forced expiratory volume in 1 s (FEV_1). In addition to physiological pulmonary responses and respiratory symptoms, O_3 exposure also results in airway hyperresponsiveness, inflammation, immune system activation, and epithelial injury. With repeated O_3 exposures over several days, spirometric and symptom responses become attenuated, but this tolerance is lost after about a week without exposure. Airway responsiveness also appears to be attenuated with repeated O_3 exposures, but less than FEV_1.

Young healthy adults exposed to O_3 concentrations $\geq$ 0.08 ppm develop significant reversible, transient decrements in pulmonary function if minute ventilation ($\dot{V}_E$) or duration of exposure are increased sufficiently. O_3-induced decrements in FEV_1 do not appear to depend on gender, race, body surface area, height, lung size, or baseline FVC in young healthy adults. Healthy children experience similar spirometric responses but lesser symptoms from O_3 exposure relative to young adults. Beyond the age of 18 to 20 yrs, spirometric and symptom responses to O_3 exposure begin to decline with increasing age. There is a large degree of intersubject variability in physiologic and symptomatic responses of heathy adults exposed to O_3. However, responses tend to be reproducible within a given individual over a period of several months. With increasing O_3 concentration, the distribution FEV_1 decrements becomes asymmetrical with a few individuals experiencing large decrements.

There is a tendency for slightly increased spirometric responses in mild asthmatics and allergic rhinitics relative to healthy subjects. Spirometric responses in asthmatics appear to be affected by baseline lung function, i.e., responses increase with disease severity. With repeated daily O_3 exposures, spirometric responses of asthmatics become attenuated, however, airway responsiveness becomes increased in subjects with preexisting allergic airway disease (with or without asthma). Possibly due to patient age, O_3 exposure does not appear to cause significant pulmonary function impairment or evidence of cardiovascular strain in patients with cardiovascular disease or chronic obstructive pulmonary disease relative to healthy subjects.

Available information on recovery from O_3 exposure indicates that an initial phase of recovery in healthy individuals proceeds relatively rapidly, with acute spirometric and symptom response appears to occur within about 2 to 4 h. Small residual lung function effects are almost completely resolved within 24 hours. Effects of O_3 on the small airways, assessed by decrement in FEF_{25-75}, may be due in part to inflammation. Indeed, a prolonged recovery of residual spirometric decrements following the initial rapid recovery could be due to slowly resolving airway inflammation. In hyperresponsive individuals, this recovery takes longer, as much as 48 hours, to return to baseline values. Persistent spirometry changes observed for up to 48 h postexposure could plausibly be sustained by the inflammatory mediators. Cellular responses (e.g., release of immuno-modulatory cytokines) appear to still be active as late as 20 h postexposure. More slowly developing inflammatory and cellular changes may persist for up to 48 h, but the time course in humans has not been explored fully.

Soluble mediators of inflammation such as the cytokines (IL-6, IL-8) and arachidonic acid metabolites (e.g., PGE_2, $PGF_{2\alpha}$, thromboxane, and leukotrienes [LTs] such as LTB_4) have been measured in the BAL fluid of humans exposed to O_3. Many of these compounds have bronchoconstrictive properties and may be involved in increased airway responsiveness following O_3 exposure. Some indicators of inflammation (e.g., PMN influx, IL-6, PGE_2, BAL protein, fibronectin) are attenuated with repeated O_3 exposures. Indicating that tissue damage probably continues to occur during repeated O_3 exposure, however, other markers (LDH, IL-8, total protein, epithelial cells) did not show attenuation. There appears to be no strong correlation between any of the measured cellular and biochemical changes and changes in lung function measurements. Whether airway reactivity or inflammatory responses to O_3 are dependent on the age of the exposed individual, such as spirometric responses, has not been determined.

Dietary antioxidant supplementation attenuates O_3-induced spirometric responses but not the intensity of subjective symptoms nor inflammatory responses. Dietary antioxidants also afforded partial protection to asthmatics by attenuating postexposure bronchial hyperresponsiveness.

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ANNEX AX6. CONTROLLED HUMAN EXPOSURE STUDIES OF OZONE AND RELATED PHOTOCHEMICAL OXIDANTS

AX6.1 INTRODUCTION

In the previous chapter, results of ozone (O_3) studies in laboratory animals and in vitro test systems were presented. The extrapolation of results from animal studies is one mechanism by which information on potential adverse human health effects from exposure to O_3 is obtained. More direct evidence of human health effects due to O_3 exposure can be obtained through controlled human exposure studies of volunteer subjects or through field and epidemiologic studies of populations exposed to ambient O_3. Controlled human exposure studies, discussed in this chapter, typically use fixed concentrations of O_3 under carefully regulated environmental conditions and subject activity levels.

Most of the scientific information selected for review and evaluation in this chapter comes from the literature published since 1996 which, in addition to further study of physiological pulmonary responses and respiratory symptoms, has focused on mechanisms of inflammation and cellular responses to injury induced by O_3 inhalation. Older studies are discussed where only limited new data are available and where new and old data are conflicting. The reader is referred to both the 1986 and 1996 Air Quality Criteria documents (U.S. Environmental Protection Agency, 1986, 1996) for a more extensive discussion of older studies. Summary tables of the relevant O_3 literature are included for each of the major subsections.
In summarizing the human health effects literature, changes from control are described if statistically significant at a probability (p) value less than 0.05, otherwise trends are noted as such.

AX6.2 PULMONARY FUNCTION EFFECTS OF OZONE EXPOSURE IN HEALTHY SUBJECTS

AX6.2.1 Introduction

The responses observed in young healthy non-smoking human adults exposed to ambient O_3 concentrations include decreased inspiratory capacity; mild bronchoconstriction; rapid, shallow breathing pattern during exercise; and symptoms of cough and pain on deep inspiration. In addition, O_3 has been shown to result in airway hyperresponsiveness as demonstrated by an increased physiological response to a nonspecific bronchoconstrictor, as well as airway injury and inflammation assessed via bronchoalveolar lavage and biopsy. Reflex inhibition of inspiration and consequent decrease in inspiratory capacity 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 s (FEV_1). Given that both FEV_1 and FVC are subject to decrease with O_3 exposures, changes in the ratio (FEV_1/FVC) become difficult to interpret and so are not discussed.

The majority of controlled human studies have investigated the effects of exposure to variable O_3 concentrations in healthy subjects performing continuous exercise (CE) or intermittent exercise (IE) for variable periods of time. These studies have several important limitations: (1) the ability to study only short-term, acute effects; (2) the inability to link short-term effects with long-term consequences; (3) the use of a small number of volunteers that may not be representative of the general population; and (4) the statistical limitations associated with the small sample size. Nonetheless, studies reviewed in the 1996 EPA criteria document (U.S. Environmental Protection Agency, 1996) provided a large body of data describing the effects and dose-response characteristics of O_3 as function of O_3 concentration (C), minute ventilation ($\dot{V}_E$), and duration or time (T) of exposure. In most of these studies, subjects were exposed to O_3 and to filtered air (FA [reported as 0 ppm O_3]) as a control. The most salient observations from these studies were: (1) healthy subjects exposed to O_3 concentrations $\geq$ 0.08 ppm develop significant reversible, transient decrements in pulmonary function if $\dot{V}_E$ or T are increased sufficiently, (2) there is a large degree of intersubject variability in physiologic and symptomatic responses to O_3 and these responses tend to be reproducible within a given individual over a several months period, and (3) subjects exposed repeated to O_3 over several days develop a

tolerance to successive exposures, as demonstrated by an attention of responses, which is lost after about a week without exposure.

In this section, the effects of single O_3 exposures of 1- to 8-h in duration on pulmonary function in healthy nonsmoking subjects are examined by reviewing studies that investigate: (1) the O_3 exposure-response relationship; (2) intersubject variability, individual sensitivity, and the association between responses; and (3) mechanisms of pulmonary function responses and the relationship between tissue-level events and functional responses. Discussion will largely be limited to studies published subsequent to the 1996 EPA criteria document (U.S. Environmental Protection Agency, 1996)

AX6.2.2 Acute Ozone Exposures for Up to 2 Hours

At-Rest Exposures. Exposure studies investigating the effects of O_3 exposures on sedentary subjects were discussed in the 1986 EPA criteria document (U.S. Environmental Protection Agency, 1986). The lowest O_3 concentration at which significant reductions in FVC and FEV_1 were reported was 0.5 ppm (Folinsbee et al., 1978; Horvath et al., 1979). Averaging there results of these two studies and correcting for FA responses, exposing resting young adults (n=23, age=22) to 0.5 ppm O_3 results in an ~4% reduction in FVC and an ~7% reduction FEV_1. At lower O_3 concentrations of 0.25 to 0.3 ppm, resting exposures did not significantly affect lung function.

Exposures with Exercise. Collectively, the studies reviewed in the 1996 EPA criteria document (U.S. Environmental Protection Agency, 1996) demonstrated that healthy young adults performing moderate to heavy IE or CE of 1 to 2.5 h duration, exposed to 0.12 to 0.18 ppm O_3 experienced statistically significant decrements in pulmonary function and respiratory symptoms. As an example, 2 hr exposures to 0.12 and 0.18 ppm O_3 during heavy IE (exercise $\dot{V}_E$ = 65 L/min) have resulted in FEV_1 decrements of 2.0 ± 0.8% (mean ± SE; n = 40) and 9.5 ± 1.1% (n = 89), respectively (McDonnell and Smith, 1994). Significant decrements in pulmonary function have been reported in heavily exercising healthy adults exposed for 1 h with CE at O_3 concentrations of 0.12 ppm (Gong et al., 1986), 0.16 ppm (Avol et al., 1984), and 0.2 ppm (Adams and Schelegle, 1983; Folinsbee et al., 1984).

In an attempt to describe O_3 dose-response characteristics, many investigators modeled acute responses as a function of total inhaled O_3 dose ($C \times T \times \dot{V}_E$), which was found to be a

better predictor of response than O_3 concentration, $\dot{V}_E$, or T of exposure, alone. In an analysis of 6 studies with 1 to 2 h exposures to between 0.12 and 0.18 ppm O_3 with exercise, Folinsbee et al. (1988) reported a good correlation (r = 0.81) between total inhaled O_3 dose and FEV_1 decrements. For a given exposure duration, total inhaled O_3 dose can be increased by increases in C and/or $\dot{V}_E$. In exposures of fixed duration, results of several studies suggested that O_3 concentration was a more important predictor of response or explained more of the variability in response than $\dot{V}_E$ (Adams et al., 1981; Folinsbee et al, 1978; Hazucha, 1987). Based on a review of previously published studies, Hazucha (1987) noted that relative to the FEV_1 decrement occurring at a given C and $\dot{V}_E$, doubling C (e.g., from 0.1 to 0.2 ppm) would increase the FEV_1 decrement by 400%, whereas doubling the $\dot{V}_E$ (e.g., from an exercise $\dot{V}_E$ of 20 to 40 L/min) which would only increase the FEV_1 decrement by 190%. Thus, C appears to have a greater affect than $\dot{V}_E$ on FEV_1 responses even when total inhaled O_3 doses are equivalent.

New studies (i.e., not reviewed in the 1996 EPA criteria document) that provide spirometric responses for up to 2 h exposures are summarized in Table AX6-1. Most of these newer studies have investigated mechanisms affecting responses, inflammation, and/or effects in diseased groups versus healthy adults, accordingly their findings may be summarized differently in several sections of this chapter. Rather than a FA exposure, some of these studies use O_3 exposures with placebo as a control. Studies appearing in Table 1, but not discussed in this section, are discussed in other sections of this chapter as indicated within the table.

McDonnell et al. (1997) pooled the results of eight studies entailing 485 healthy male subjects exposed for 2 h on one occasion to one of six O_3 concentrations (0.0, 0.12, 0.18, 0.24, 0.30, or 0.40 ppm) at rest or one of two levels of IE ($\dot{V}_E$ of 25 and 35 L/min/m^2 BSA). FEV_1 was measured preexposure, after 1 h of exposure, and immediately postexposure. Decrements in FEV_1 were modeled by sigmoid-shaped curve as a function of subject age, O_3 concentration, $\dot{V}_E$, and T. The modeled decrements reach a plateau with increasing T and dose rate ($C \times \dot{V}_E$). That is, for a given O_3 concentration, exercise $\dot{V}_E$ level, and after a certain length of exposure, the FEV_1 response tends not to increase further with increasing duration of exposure. The modeled FEV_1 responses increased with $C \times \dot{V}_E$ and T, decreased with subject age, but were only minimally affected by body size corrections to $\dot{V}_E$. Fitted and experimental FEV_1 decrements

Table AX6-1. Controlled Exposure of Healthy Humans to Ozone for 1 to 2 Hours during Exercise[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration and Activity	Exposure Conditions[c]	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.0 0.4	0 784	2 h IE 4 × 15 min on bicycle, $\dot{V}_E$ = 30 L/min	NA	5 M, 4 F 6 M, 7 F	Healthy adults 25 ± 2 years old Mild atopic asthmatics 22 ± 0.7 years old	O_3-induced reductions in FVC (12%, 10%) and FEV_1 (13%, 11%) for asthmatic and healthy subjects. Significant reductions in mid-flows in both asthmatics and healthy subjects. Indomethacin pretreatment significantly decreased FVC and FEV_1 responses to O_3 in healthy but not asthmatic subjects. *See Section AX6.3.2 and Tables AX6-3 and AX6-13.*	Alexis et al. (2000)
0.0 0.2	0 392	2 h IE 4 × 15 min at $\dot{V}_E$ = 20 L/min/m² BSA	20 °C 50% RH	8 M, 5 F	Healthy NS median age 23 years	Median O_3-induced decrements of 70 mL, 190 mL, and 400 mL/s in FVC, FEV_1, and FEF_{25-75}, respectively. Spirometric responses not predicted of inflammatory responses. *See Sections AX6.2.5.2, AX6.5.6, and AX6.9.3 and Table AX6-12*.	Blomberg et al. (1999)
0.0 0.2	0 392	2 h IE 4 × 15 min at $\dot{V}_E$ = 20 L/min/m² BSA	20 °C 50% RH	10 M, 12 F	Healthy NS mean age 24 years	Significant O_3-induced decrement in FEV_1 immediately post-exposure but not significantly different from baseline 2 h later. No correlation between Clara cell protein (CC16) and FEV_1 decrement. CC16 levels, elevated by O_3 exposure, remained high at 6 h post-exposure, but returned to baseline by 18 h postexposure. *See Table AX6-12*	Blomberg et al. (2003)
0.0 0.12 0.18 0.24 0.30 0.40	0 235 353 471 589 784	2 h rest or IE (4 × 15 min at $\dot{V}_E$ = 25 or 35 L/min/m² BSA)	22 °C 40% RH	485 M (each subject exposed at one activity level to one O_3 concentration)	Healthy NS 18 to 36 years old mean age 24 years	Statistical analysis of 8 experimental chamber studies conducted between 1980 and 1993 by the U.S. EPA in Chapel Hill, NC. Decrement in FEV_1 described by sigmoid-shaped curve as a function of subject age, O_3 concentration, $\dot{V}_E$, and time. Response decreased with age, was minimally affected by body size corrections, and was not more sensitive to O_3 concentration than $\dot{V}_E$. *Also see Section AX6.5*	McDonnell et al. (1997)
0.4	784	2 h IE 20 min mild-mod. exercise, 10 min rest	NA	4 M, 5 F	Healthy NS 30 ± 3 years old	Subjects previously in Nightingale et al. (2000) study. Placebo-control: Immediately postexposure decrements in FVC (9%) and FEV_1 (14%) relative to pre-exposure values. FEV_1 decrement only 9% at 1 hr postexposure. By 3 h postexposure, recovery in FVC to 97% and FEV_1 to 98% of preexposure values. Significant increases in 8-isoprostane at 4 h postexposure. Budesonide for 2 wk prior to exposure did not affect responses.	Montuschi et al. (2002)

Table AX6-1 (con't). Controlled Exposure of Healthy Humans to Ozone for 1 to 2 Hours during Exercise[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration and Activity	Exposure Conditions[c]	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.0 0.2	392	2 h IE 4 × 15 min at $\dot{V}_E$ = 20 L/min/m² BSA	20 °C 50% RH	6 M, 9 F 9 M, 6 F	Healthy adults 24 years old Mild asthmatics 29 years old	O_3-induced FEV_1 decrement (8%, healthy adults; 3% asthmatics) and PMN increase (20.6%, healthy adults; 15.2% asthmatics). Primary goal was to investigate relationship between antioxidant defenses and O_3 responses in asthmatics and healthy adults. *See Tables* AX*6-3 and AX6-13.*	Mudway et al. (2001) Stenfors et al. (2002)
0.4	784	2 h IE 20 min mild-mod. exercise, 10 min rest	NA	6 M, 9 F	Healthy NS mean age ~31 years	Placebo-control: O_3 caused significant decrements in FEV_1 (13.5%) and FVC (10%) immediately following exposure, a small increase in Mch-reactivity, and increased PMNs and myeloperoxidase in induced sputum at 4 h postexposure. FEV_1 at 96% and FVC at 97% preexposure values at 3 h postexposure. Budesonide for 2 wk prior to exposure did not affect spirometric responses. *See Section AX6.2.5 and Table AX6-13*	Nightingale et al. (2000)
0.0 0.4	784	2 h IE 4 × 15 min at $\dot{V}_E$ =18 L/min/m² BSA 2 exposures: 25% subjects exposed to air-air, 75% to O_3-O_3	21 °C 40% RH	Weak responders 7 M, 13F Strong responders 21 M, 21 F	Healthy NS 20 to 59 years old	Significant O_3-induced decrements in spirometric lung function. Young adults (< 35 years) were significantly more responsive than older individuals (> 35 years). Sufentanil, a narcotic analgesic, largely abolished symptom responses and improved FEV_1 in strong responders. Naloxone, an opioid antagonist, did not affect O_3 effects in weak responders. *See Section AX6.2.5.1*	Passannante et al. (1998)
0.0 0.4	784	2 h IE 4 × 15 min at $\dot{V}_E$ = 20 L/min/m² BSA	20 °C 40% RH	Placebo group 15 M, 1 F Antioxidant group 13 M, 2 F	Healthy NS mean age 27 years	Placebo and antioxidant groups had O_3-induced decrements in FEV_1 (20 and 14%) and FVC (13 and 10%), respectively. Percent neutrophils and IL-6 levels in BAL fluid obtained 1 h post exposure were not different in the two treatment groups. *See Table AX6-13.*	Samet et al. (2001) Steck-Scott et al. (2004)
0.0 0.25	490	1 h CE $\dot{V}_E$ = 30 L/min	NA Face mask exposure	32 M, 28 F	Healthy NS 22.6 ± 0.6 years old	Mean O_3-induced FEV_1 decrements of 15.9% in males and 9.4% in females (gender differences not significant). FEV_1 decrements ranged from −4 to 56%; decrements >15% in 20 subjects and >40% in 4 subjects. Uptake of O_3 greater in males than females, but uptake not correlated with spirometric responses.	Ultman et al. (2004)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.
[c]Studies conducted in exposure chamber unless otherwise indicated.

following a 2 h exposure at three nominal levels of $\dot{V}_E$ are illustrated in Figure AX6-1 as a function of O_3 concentration. Their analysis indicated that C was marginally, but not significantly more important than $\dot{V}_E$ in predicting FEV_1 response. Additionally, the McDonnell et al. (1997) analysis revealed that some prior analyzes of IE protocols may have over estimated the relative importance of C over $\dot{V}_E$ in predicting FEV_1 responses by considering only the $\dot{V}_E$ during exercise and ignoring the $\dot{V}_E$ during periods of rest.

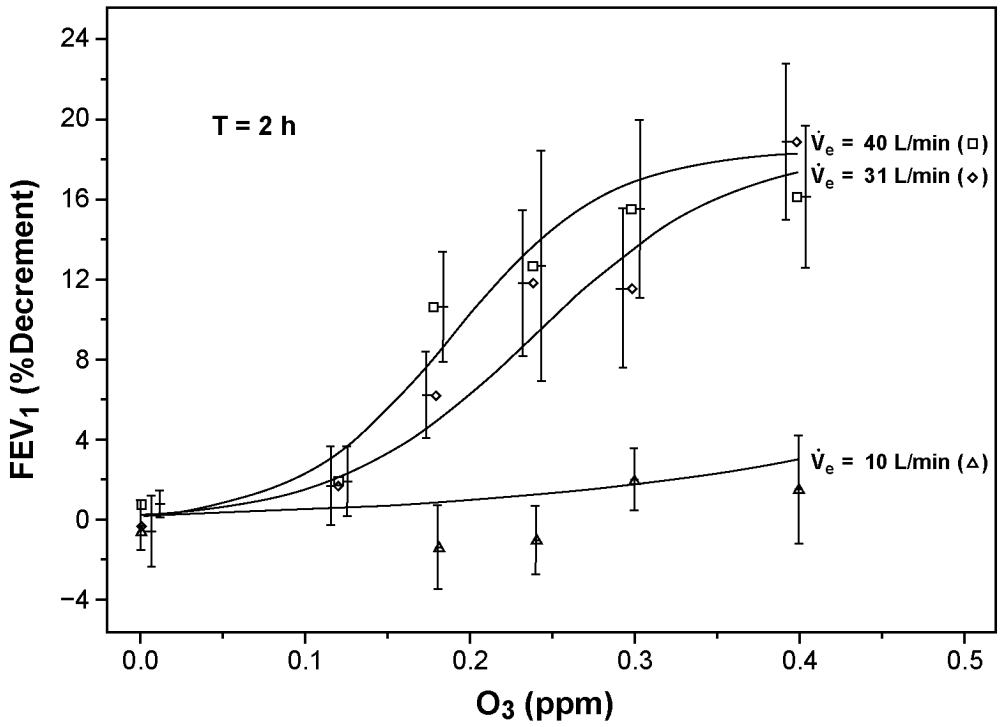

Figure AX6-1. FEV_1 decrements as a function of O_3 concentration following a 2 h exposure with incremental exercise (15 min intervals) or rest. Points are experimental data (mean ± SE) and lines are model predictions for each activity level. Minute ventilation ($\dot{V}_E$) represent average across intervals of rest and exercise.

Source: McDonnell et al. (1997).

Ultman et al. (2004) measured O_3 uptake and pulmonary responses in 60 young heathy non-smoking adults (32 M, 28 F). A bolus technique was used to quantify the uptake of O_3 as a function of the volume into the lung which the bolus penetrated. From these measurements, the volumetric depth at which 50% uptake occurred was calculated. This volumetric lung depth was correlated with conducting airways volume, i.e., a greater fraction of O_3 penetrated to deeper into the lungs of individuals have larger conducting airways volumes. Two weeks after the bolus measurements, subjects were exposed via a face mask to FA and subsequently two weeks later to 0.25 ppm O_3 for 1 h with CE at a target $\dot{V}_E$ of 30 L/min. The breath-by-breath uptake of O_3 was measured. There was a small but significant reduction in the breath-by-breath uptake of O_3 from 90.6% on average for the first 15 minutes to 87.3% on average for the last 15 minutes of exposure. The uptake fraction was significantly greater in males (91.4%) than females (87.1%), which is consistent with the larger f_B and smaller V_T of the females than males. Uptake was not correlated with spirometric responses. However, there was tendency for males to have greater O_3-induced FEV_1 decrements than females, 15.9% versus 9.4%, respectively. There was considerable intersubject variability in FEV_1 decrements which ranged from −4 to 56% with 20 subjects having decrements of >15% and 4 subjects with >40% decrements (*see Section AX6.4 for additional discussion regarding intersubject variability*).

AX6.2.3 Prolonged Ozone Exposures

Between 1988 and 1994, a number studies were completed that described the responses of subjects exposed to relatively low (0.08 to 0.16 ppm) O_3 concentrations for exposure durations of 4 to 8 h. These studies were discussed in the 1996 criteria document (U.S. Environmental Protection Agency, 1996) and only a select few are briefly discussed here. Table AX6-2 details newer studies of healthy subjects undergoing prolonged exposures at O_3 concentrations ranging from 0.06 to 0.20 ppm. In most of these studies, statistically significant changes in pulmonary function, symptoms, and airway responsiveness have been observed during and after exposures to O_3 concentrations of 0.08 ppm and higher. As with studies conducted at higher O_3 concentrations for shorter periods of time, there is considerable intersubject variability in response (*see Section AX6.4*).

Folinsbee et al. (1988) first reported the effects of a 6.6 h exposure to 0.12 ppm O_3 in ten young healthy adults (25 ± 4 yr) with quasi continuous exercise that was intended to simulate a

Table AX6-2. Pulmonary Function Effects after Prolonged Exposures to Ozone[a]

Ozone Concentration[b]		Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
ppm	µg/m^3						
Studies with 4 hr Exposures							
0.18	353	4 h IE (4 × 50 min) $\dot{V}_E$ = 35 L/min	23 °C 50% RH	2 M, 2 F	Adults NS, 21 to 33 years old	FVC decreased 19% and FEV_1 decreased 29% in these four pre-screened sensitive subjects.	Adams (2000a)
0.0 0.20	0 392	4 h IE (4 × 50 min cycle ergometry or treadmill running [$\dot{V}_E$ = 40 L/min])	20 °C 50% RH	FA: 11 M, 3 F O_3: 9 M, 3 F	Adult NS, 19 to 41 years old	Decrease in FVC, FEV_1, V_T, and SRaw and increase in f_B with O_3 exposure compared with FA; total cell count and LDH increased in isolated left main bronchus lavage and inflammatory cell influx occurred with O_3 exposure compared to FA exposure.	Aris et al. (1993)
0.2	392	4 h IE (4 × 50 min) $\dot{V}_E$ = 25 L/min/m^2 BSA	20 °C 50% RH	42 M, 24 F	Adults NS, 18 to 50 years old	FEV_1 decreased by 18.6%; Pre-exposure methacholine responsiveness was weakly correlated with the functional response to O_3 exposure. Symptoms were also weakly correlated with the FEV_1 response (r = -0.31 to −0.37)	Aris et al. (1995)
0.0 0.24	0 470	4 h IE (4 × 15 min) $\dot{V}_E$ =20 L/min	24°C 40% RH	10 M 9 M	Healthy NS, 60 to 69 years COPD 59 to 71 years	Healthy: small, 3.3%, decline in FEV_1 (p=0.03 [not reported in paper], paired-t on O_3 versus FA pre-post FEV_1). COPD: 8% decline in FEV_1 (p=ns, O_3 versus FA). Adjusted for exercise, ozone effects did not differ significantly between COPD patients and healthy subjects. *See Section AX6.5.1.*	Gong et al. (1997a)
Studies with >6 hr Exposures							
0.0 0.04 0.08 0.12	0 78 157 235	6.6 h IE (6 × 50min) $\dot{V}_E$ = 20 L/min/m^2 BSA	23 °C 50% RH	15 M, 15 F	Healthy NS, 22.4 ± 2.4 yrs old	FEV_1 and total symptoms at 6.6 h exposure to 0.04 ppm not significantly different from FA. FEV_1 (−6.4%) and total symptoms significant at 6.6 h exposure to 0.08 ppm. FEV_1 (−15.4%) at 6.6 h not significantly different between chamber and face mask exposure to 0.12 ppm.	Adams (2002)
0.12	235	3 day-6.6h/day IE (6 × 50 min) $\dot{V}_E$ = 17 L/min/m^2, 20 L/min/m^2 BSA, and 23 L/min/m^2 BSA	23 °C 50% RH	15 M, 15 F	Healthy NS, 18 to 31 years old	FEV_1 at 6.6 h decreased significantly by 9.3%, 11.7%, and 13.9%, respectively at three different exercise $\dot{V}_E$ rates, but were not significantly different from each other. Total symptoms at the highest $\dot{V}_E$ protocol were significantly greater than for the lowest $\dot{V}_E$ protocol beginning at 4.6 h. Largest subjects (2.2 m^2 BSA) had significantly greater average FEV_1 decrement for the three protocols, 18.5% compared to the smallest subjects (1.4 m^2 BSA), 6.5%.	Adams (2000b)

Table AX6-2 (cont'd). Pulmonary Function Effects after Prolonged Exposures to Ozone[a]

Ozone Concentration[b]		Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
ppm	µg/m^3						
(a) 0.08 (b) 0.08 (mean) varied from 0.03 to 0.15	235 235 (mean)	6.6 h IE (6 × 50 min) $\dot{V}_E$ = 20 L/min/m^2 BSA	23 °C 50% RH	15 M 15 F	Healthy NS, 18 to 25 years old	(a) FEV_1 decreased 6.2% after 6.6 h in square-wave exposures. Total symptoms significantly increased at 5.6 and 6.6 h. (b) FEV_1 decreased 5.6 to 6.2% after 4.6 to 6.6 h, respectively, in varied exposure; total symptoms significantly increased also after 4.6 to 6.6 h. No significant difference between face mask and chamber exposures.	Adams (2003a)
(a) 0.08	157	6.6 h IE (6 × 50 min) $\dot{V}_E$ = 20 L/min/m^2 BSA	23 °C 50% RH	15 M 15 F	Healthy NS, 18 to 25 years old	Significantly greater FEV_1 decrement (12.4%) for 2-h, 0.30 ppm exposure than for 6.6-h, 0.08 ppm exposure (3.6%).	Adams (2003b)
(b) 0.30	588	2 h IE (4 × 15 min) $\dot{V}_E$ = 35 L/min/m^2 BSA					
(a) 0.12 (b) 0.12 (mean) varied from 0.07 to 0.16 (c) 0.12 (mean) varied from 0.11 to 0.13 (d) 0.12	235 235 (mean) 235 235	6.6 h IE (6 × 50 min) (a,b,c) $\dot{V}_E$ = 20 L/min/m^2 BSA (d) $\dot{V}_E$ = 12 L/min/m^2 BSA	23 °C 50% RH	6 M, 6 F	Healthy NS, 19 to 25 years old	(a) FEV_1 decreased 11% at 6.6 h in square-wave exposure. Total symptoms significant from 4.6 to 6.6 h. (b) FEV_1 decreased 13% at 6.6 h; not significantly different from square-wave exposure. Total symptoms significant from 4.6 to 6.6 h. (c) FEV_1 decreased 10.3% at 6.6 h; not significantly different from square-wave exposure. Total symptoms significant from 4.6 to 6.6 h. (d) FEV_1 decreased 3.6% at 6.6 h; significantly less than for 20 L/min/m^2 BSA protocols.	Adams and Ollison (1997)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.

full workday of heavy physical labor. Except for a 35-min lunch break after 3 h, the subjects exercised at a moderate level ($\dot{V}_E \approx 40$ L/min) for 50 min of each hour. Ignoring the lunch break during which lung function did not change appreciably, approximately linear decreases were observed in FVC, FEV_1, and FEV_{25-75} with duration of O_3 exposure. Correcting for FA responses, decrements of 8.2, 14.9, and 26.8% in FVC, FEV_1, and FEV_{25-75} occurred as a result of the O_3 exposure. Using the same 6.6 h protocol, but a lower O_3 concentration of 0.08 ppm, Horstman et al. (1990) and McDonnell et al. (1991) observed decrements corrected for FA (and averaged across studies) of 5, 8, and 11% in FVC, FEV_1, and FEV_{25-75}, respectively, in 60 young adults (25 ± 5 years old). Horvath et al. (1991) observed a 4% ($p = 0.03$)[1] decrement in FEV_1 using the forementioned protocol (i.e., 6.6 h and 0.08 ppm O_3) in 11 healthy adults (37 ± 4 yr). The smaller decrement observed by Horvath et al. (1991) versus Horstman et al. (1990) and McDonnell et al. (1991) is consistent with response decreasing as subject age increases (*see Section AX6.5.1*).

AX6.2.3.1 Effect of Exercise Ventilation Rate on FEV_1 Response to 6.6 h Ozone Exposure

It is well known that response to O_3 exposure is a function of $\dot{V}_E$ in studies of 2 h or less in duration (*See Section AX6.2.2*). It is reasonable to expect that response to a prolonged 6.6-h O_3 exposure is also function of $\dot{V}_E$, although quantitative analyzes are lacking.

In an attempt to quantify this effect, Adams and Ollison (1997) exposed 12 young adults to an average O_3 concentration of 0.12 ppm for 6.6 h at varied exercise $\dot{V}_E$. They observed a mean FEV_1 decrements of 10 to 11% in two protocols having a mean exercise $\dot{V}_E$ of 33 L/min and a 14% decrement in a protocol with a mean exercise $\dot{V}_E$ of 36 L/min. These FEV_1 decrements were significantly greater than the average decrement of 3.6% (not significantly different from FA response) observed at an exercise $\dot{V}_E$ of only 20 L/min. In a subsequent study of 30 healthy adults (Adams, 2000b), the effect of smaller exercise $\dot{V}_E$ differences on pulmonary function and symptoms responses to 6.6 h exposure to 0.12 ppm O_3 was examined. FEV_1 decrements of 9.3, 11.7, and 13.9% were observed for the exercise $\dot{V}_E$ of 30.2, 35.5, and 40.8 L/min, respectively. Along with the tendency for FEV_1 responses to increase with $\dot{V}_E$, total symptoms severity was

[1]Based on two-tailed paired t-test of data in Table III of Horvath et al. (1991).

found to be significantly greater at the end of the highest $\dot{V}_E$ protocol relative to the lowest $\dot{V}_E$ protocol. Although the FEV_1 responses were not significantly different from each other, the power of the study to detect differences between the three $\dot{V}_E$ was not reported and no analysis was performed using all of the data (e.g., a mixed effects model). Data from the Adams and Ollison (1997) and Adams (2000b) studies are illustrated in Figure AX6-2 with data from three older studies. There are a paucity of data below an exercise $\dot{V}_E$ of 30 L/min. Existing data for exposure to 0.12 ppm O_3 suggests that FEV_1 responses increase with increasing exercise $\dot{V}_E$ until at least 35 L/min.

AX6.2.3.2 Exercise Ventilation Rate as a Function of Body/Lung Size on FEV_1 Response to 6.6 h Ozone Exposure

Typically, with the assumption that the total inhaled O_3 dose should be proportional to the lung size of each individual, exercise $\dot{V}_E$ in 6.6 h exposures has been set as a multiple of body surface area (BSA) (McDonnell et al., 1991) or as a product of eight times FVC (Folinsbee et al., 1988; Frank et al., 2001; Horstman et al., 1990). Utilizing previously published data, McDonnell et al. (1997) developed a statistical model analyzing the effects of O_3 concentration, $\dot{V}_E$, duration of exposure, age, and body and lung size on FEV_1 response. They concluded that any effect of BSA, height, or baseline FVC on percent decrement in FEV_1 in this population of 485 young adults was small if it exists at all. This is consistent with Messineo and Adams (1990), who examined pulmonary function responses in young adult women having small (n = 14) or large (n = 14) lung sizes (mean FVC of 3.74 and 5.11 L, respectively). Subject were exposed to 0.30 ppm O_3 for 1 h with CE ($\dot{V}_E$ = 47 L/min). There was no significant difference between the group FEV_1 decrements (22.1 and 25.6% for small and large lung, respectively). In addition, Messineo and Adams (1990) also did a retrospective analysis of 36 young adult males who each had completed similar 1 h exposures to 0.30 ppm O_3 with CE ($\dot{V}_E \approx$ 70 L/min) and found lung size was not realted with FEV_1 response.

Adams (2000b) studied a group of 30 young adult men and women exposed to 0.12 ppm O_3 for 6.6 h on three occasions while exercising 50 min of each hour at one of three different $\dot{V}_E$ levels (viz., 17, 20, and 23 1/min/m^2 BSA). Their postexposure FEV_1 responses were regressed as a function of BSA (which was directly related to the absolute amount of $\dot{V}_E$ during exercise

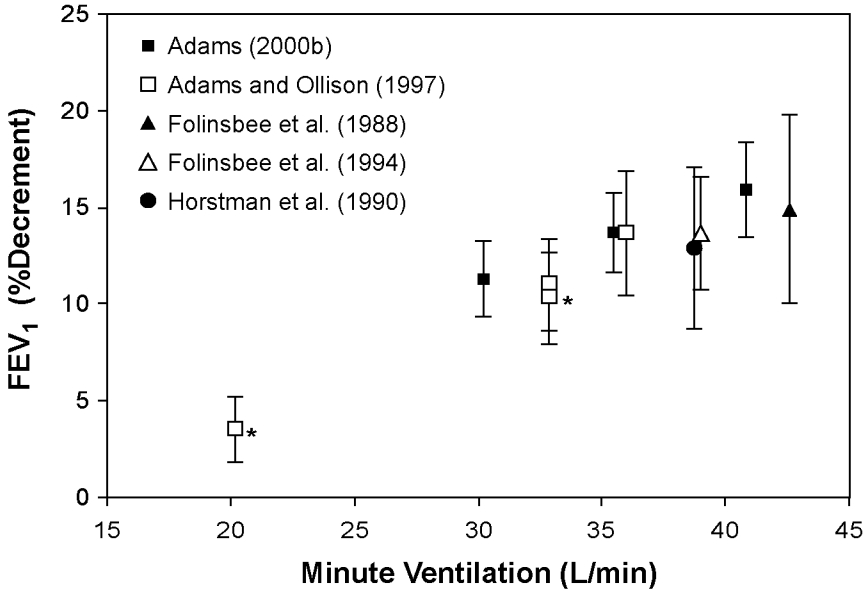

Figure AX6-2. Average FEV_1 decrements (±SE) for prolonged 6.6 h exposures to 0.12 ppm O_3 as a function of exercise $\dot{V}_E$. Since age affects response to O_3 exposure, selected studies had subjects with mean ages between 22 and 25 years. FEV_1 decrements were calculated as mean O_3 responses minus mean air responses. Unless provided in papers, SE were estimated from variability in post O_3 exposure responses. In one case, the SE for $\dot{V}_E$ of 33 L/Min (10.3% decrement) was taken as the SE of data from protocol with $\dot{V}_E$ of 33 L/min (11% decrement). All studies used a constant 0.12 ppm O_3 exposure except two (∗) which used 0.115 ppm O_3 for hours 1-2 and 5-6 and 0.13 ppm O_3 for hours 3-4 of exposure.

and, thus, primarily responsible for individual differences in total inhaled O_3 dose). The slope was significantly different from zero ($p = 0.01$), meaning that the smallest subjects, who had the lowest exercise $\dot{V}_E$ (≈ 26 L/min), had a lower FEV_1 decrement (−5%) than the largest subjects (−17%), whose exercise $\dot{V}_E$ was ≈44 L/min. This relationship was not a gender-based difference, as the mean female's FEV_1 decrement was −11.2%, which was not significantly different from the male's −12.2% mean value. Similarly, when total symptoms severity response was regressed against BSA, the slope was significantly different than zero ($p = 0.0001$), with lower values for smaller subjects than for larger subjects. Results of this study suggest that

for the O_3 concentration and exposure duration used, responses are more closely related to $\dot{V}_E$ than $\dot{V}_E$ normalized to BSA. Further, this observation is in agreement with McDonnell et al. (1997), who observed no evidence that measurements of lung or body size were significantly related to FEV_1 response in 2 h IE exposures. These authors state that the absence of an observed relationship between FEV_1 response and BSA, height, or FVC may be due to the poor correlation between these variables and airway caliber (Collins et al., 1986; Martin et al., 1987). Also, the O_3 dosimetry study of Bush et al. (1996) indicated that normalization of the O_3 dose would be more appropriately applied as a function of anatomic dead space.

AX6.2.3.3 Comparison of 6.6 h Ozone Exposure Pulmonary Responses to Those Observed in 2 h Intermittent Exercise Ozone Exposures

It has been shown that greater O_3 concentration (Horstman et al., 1990) and higher $\dot{V}_E$ (Adams, 2000b) each elicit greater FEV_1 response in prolonged, 6.6-h exposures, but data on the relative effect of O_3 concentration, $\dot{V}_E$, and T in prolonged exposures are very limited and have not been systematically compared to data from shorter (< 2-h) exposures. In a recent study (Adams, 2003b), the group mean FEV_1 response for a 2-h IE exposure to 0.30 ppm O_3 was −12.4%, while that for a 6.6-h exposure to 0.08 ppm O_3 was −3.5%. The total inhaled O_3 dose (as the simple product of $C \times T \times \dot{V}_E$) was 1358 ppm·L for the 2-h exposure and 946 ppm·L for the 6.6-h exposure. Thus, the FEV_1 decrement was 3.5 times greater and the total inhaled O_3 dose was 1.44 times greater for the 2-h exposure compared to the 6.6-h exposure. This difference illustrates the limitations of utilizing the concept of total O_3 dose for comparisons between studies of vastly different exposure durations.

Adams (2003b) also examined whether prolonged 6.6 h exposure to a relatively low O_3 concentration (0.08 ppm) and the 2-h IE exposure at a relatively high O_3 concentration (0.30 ppm) elicited consistent individual subject effects, i.e, were those most or least affected in one exposure also similarly affected in the other? Individual subject O_3 exposure reproducibility was first examined via a regression plot of the postexposure FEV_1 response to the 6.6-h chamber exposure as a function of postexposure FEV_1 response to the 2-h chamber exposure. The R^2 of 0.40, although statistically significant, was substantially less than that observed in a comparison of individual FEV_1 response to two 2-h IE exposures by chamber and face mask, respectively ($R^2 = 0.83$). The Spearman rank order correlation for the chamber 6.6-h and chamber 2-h

exposure comparison was also substantially less (0.49) than that obtained for the two 2-h exposures (0.85). The primary reason for the greater variability in the chamber 6.6-h exposure FEV_1 response as a function of that observed for the two 2-h IE exposures is very likely related to the increased variability in response upon repeated exposure to O_3 concentrations lower than 0.18 ppm (R = 0.57, compared to a mean R of 0.82 at higher concentrations) reported by McDonnell et al. (1985a). This rationale is supported by the lower R (0.60) observed by Adams (2003b) for the FEV_1 responses found in 6.6 h chamber and face mask exposures to 0.08 ppm O_3, compared to an R of 0.91 observed for responses found at 0.30 ppm O_3.

AX6.2.4 Triangular Ozone Exposures

To further explore the factors that determine responsiveness to O_3, Hazucha et al. (1992) designed a protocol to examine the effect of varying, rather than constant, O_3 concentrations. In this study, subjects were exposed to a constant level of 0.12 ppm O_3 for 8 h and to an O_3 level that increased linearly from 0 to 0.24 ppm for the first 4 h and then decreased linearly from 0.24 to 0 over the second 4 h of the 8 h exposure (triangular concentration profile). Subjects performed moderate exercise ($\dot{V}_E$ ~40 L/min) during the first 30 minutes of each hour. The total inhaled O_3 dose (i.e., C x T x $\dot{V}_E$) for the constant versus the triangular concentration profile was almost identical. FEV_1 responses are illustrated in Figure AX6-3. With exposure to the constant 0.12 ppm O_3, FEV_1 declined approximately 5% by the fifth hour of exposure and then remained at that level. This observation clearly indicates a response plateau as suggested in other prolonged exposure studies (Horstman et al., 1990; McDonnell et al., 1991). However, with the triangular O_3 concentration profile after a minimal initial response over the first 3 h, Hazucha et al. (1992) observed a substantial decrease in FEV_1 corresponding to the higher average O_3 concentration that reached a nadir after 6 h (−10.3%). Despite 2 h of continued exposure to a lower O_3 concentration (0.12 to 0.00 ppm, mean = 0.06 ppm), FEV_1 improved and was only reduced by 6.3% (relative to the preexposure FEV_1) at the end of the 8-h exposure. The authors concluded that total inhaled O_3 dose ($C \times \dot{V}_E \times T$) was not a sufficient index of O_3 exposure and that, as observed by others (Adams et al., 1981; Folinsbee et al., 1978; Hazucha, 1987; Larsen et al., 1991), O_3 concentration appears to be more important in determining exposure effects than is either duration or the volume of air breathed during the exposure. However, it should be noted that the mean O_3 concentration for Hazucha et al.'s triangular exposure profile was 0.12 ppm

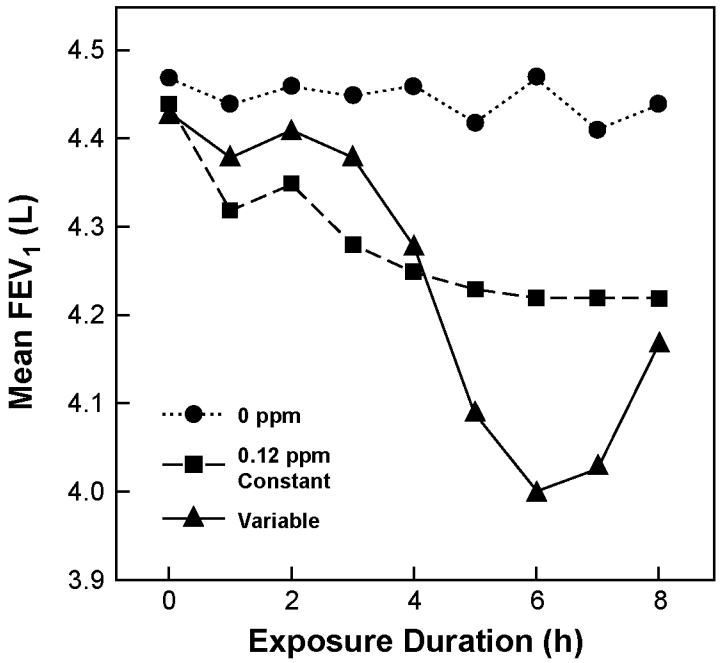

Figure AX6-3. The forced expiratory volume in 1 s (FEV_1) is shown in relation to exposure duration (hours) under three exposure conditions. Subjects exercised (minute ventilation ≈ 40 L/min) for 30 min during each hour; FEV_1 was measured at the end of the intervening rest period. Standard error of the mean for these FEV_1 averages (not shown) ranged from 120 to 150 mL.

Source: Hazucha et al. (1992).

at 4 h, 0.138 ppm at 5 h, 0.14 ppm at 6 h, and 0.133 ppm at 7 h, before falling to 0.12 ppm at 8 h. The FEV_1 responses of the last 4 hours (Figure AX6-3) follow a closely similar pattern as the total mean O_3 concentration over the same time period.

It has become increasingly well realized that laboratory simulations of air-pollution risk-assessment need to employ O_3 concentration profiles that more accurately mimic those encountered during summer daylight ambient air pollution episodes (Adams and Ollison, 1997; Lefohn and Foley, 1993; Rombout et al., 1986). Neither square-wave O_3 exposures or the one 8-h study by Hazucha et al. (1992) that utilized a triangular shaped varied O_3 exposure described above closely resembles the variable diurnal daylight O_3 concentration pattern observed in many urban areas experiencing air-pollution episodes (Lefohn and Foley, 1993). Recently, 6.6 h less

abrupt triangular O_3 exposure profiles at lower concentrations more typical of outdoor ambient conditions have been examined (Adams 2003a; Adams and Ollison, 1997).

Using a face-mask inhalation system, Adams and Ollison (1997) observed no significant differences in postexposure pulmonary function responses or symptoms between the 6.6-h, 0.12 ppm O_3 square-wave exposure; and those observed for a triangular O_3 profile in which concentration was increased steadily from 0.068 ppm to 0.159 ppm at 3.5 h and then decreased steadily to 0.097 ppm at end exposure. Further, no attenuation in FEV_1 response during the last 2 h was observed in either the 6.6 h square-wave or the triangular exposures. In a subsequent study (Adams, 2003a), no significant difference was observed in pulmonary function responses or symptoms between face-mask and chamber exposure systems either for a 6.6-h, 0.08 ppm O_3 square-wave profile or for the triangular O_3 exposure beginning at 0.03 ppm, increasing steadily to 0.15 ppm in the fourth hour, and decreasing steadily to 0.05 ppm at 6.6 h (mean = 0.08 ppm). For the chamber-exposure comparison, postexposure values for FEV_1 and symptoms were not significantly different from the responses for the square-wave 0.08 ppm O_3 exposure. However, analysis showed that FEV_1 response for the square-wave protocol did not become statistically significant until the 6.6-h postexposure value, while that for the triangular exposure protocol was significant at 4.6 h (when O_3 concentration was 0.15 ppm). Earlier significant FEV_1 responses for the triangular protocol were accompanied by significant increases in symptoms at 4.6 h, continuing on through the fifth and sixth hours when the mean O_3 concentration was 0.065 ppm. Symptoms for the square-wave 0.08 ppm exposure did not become statistically significant until 5.6 h. The rate of FEV_1 response to the triangular exposure did not decrease as was observed by Hazucha et al. (1992) during the last two hours of their 8-h triangular exposure (Figure AX6-3). Rather, FEV_1 responses for the triangular exposure showed clear signs of plateauing during the last 2 h; i.e., −5.46% at 4.6 h, −6.27% at 5.6 h, and −5.77% at 6.6 h. The most probable reason for differences in the triangular O_3 profile observations of Hazucha et al. (1992) and those of Adams (2003a) is that the increase and decrease in Hazucha et al.'s study (i.e., 0 to 0.24 ppm and back to 0) encompassed a much greater range of O_3 concentrations than those used by Adams (2003a), viz., 0.03 ppm to 0.15 ppm from 3.6 to 4.6 h, then decreasing to 0.05 ppm at 6.6 h. Nonetheless, the greatest FEV_1 decrement was observed at 6 h of Hazucha et al.'s 8 h triangular exposure (Figure AX6-3) corresponding to the time when total mean O_3 concentration was highest (0.14 ppm), with a very similar response at 7 h when total mean O_3 concentration was

0.138 ppm. Adams (2003a) observed the greatest FEV_1 decrement at 5.6 h (−6.27% with total mean O_3 concentration of 0.086 ppm), which was not significantly different than the 4.6-h value of −5.46% (total mean O_3 concentration = 0.0875 ppm).

Whereas FEV_1 decrements during square-wave O_3 exposures between 0.08 to 0.12 ppm tend to increase with time of exposure (i.e., with steadily increasing total inhaled dose), FEV_1 decrements during triangular exposures (Hazucha et al., 1992; Adams, 2003a) occurred 1 to 2 h after the peak O_3 concentration and 1 h to 2 h before the maximal total O_3 inhaled dose occurred at end exposure. This difference, especially because O_3 concentration profiles during summer daylight air-pollution episodes rarely mimic a square-wave, implies that triangular O_3 exposure profiles most frequently observed during summer daylight hours merit further investigation.

AX6.2.5 Mechanisms of Pulmonary Function Responses

Inhalation of O_3 for several hours while physically active elicits both subjective respiratory tract symptoms and acute pathophysiologic changes. The typical symptomatic response consistently reported in studies is that of tracheobronchial airway irritation. This is accompanied by decrements in lung capacities and volumes, bronchoconstriction, airway hyperresponsiveness, airway inflammation, immune system activation, and epithelial injury. The severity of symptoms and the magnitude of response depend on inhaled dose, O_3 sensitivity of an individual and the extent of tolerance resulting from previous exposures. The development of effects is time dependent during both exposure and recovery periods with considerable overlap of evolving and receding effects.

Exposure to O_3 initiates reflex responses manifested as a decline in spirometric lung function parameters ($\downarrow$FVC, $\downarrow FEV_1$, $\downarrow FEF_{25-75}$), bronchoconstriction ($\uparrow$SRaw) and altered breathing pattern ($\downarrow V_T$, $\uparrow f_B$), which becomes more pronounced as exposure progresses and symptoms of throat irritation, cough, substernal soreness and pain on deep inspiration develop. The spirometric lung function decline and the severity of symptoms during a variable (ramp) exposure profile seem to peak a short time (about 1 to 2 h) following the highest concentration of O_3 (Hazucha et al., 1992; Adams, 2003a). Exposure to a uniform O_3 concentration profile elicits the maximum spirometric response at the end of exposure (Hazucha et al., 1992; Adams, 2003a). Regardless of exposure concentration profile, as the exposure to O_3 progresses airway inflammation begins to develop and the immune response at both cellular and subcellular level is

activated. Airway hyperreactivity develops slower than pulmonary function effects, while neutrophilic inflammation of the airways develops even more slowly and reaches the maximum 3 to 6 h postexposure. The cellular responses (e.g., release of immunoregulatory cytokines) appear to still be active as late as 20 h postexposure (Jörres et al., 2000). Following cessation of exposure, the recovery in terms of breathing pattern, pulmonary function and airway hyperreactivity progresses rapidly and is almost complete within 4 to 6 hours in moderately responsive individuals. Persisting small residual lung function effects are almost completely resolved within 24 hours. Following a 2 h exposure to 0.4 ppm O_3 with IE, Nightingale et al. (2000) observed a 13.5% decrement in FEV_1. By 3 h postexposure, however, only a 2.7% FEV_1 decrement persisted. As illustrated in Figure AX6-4, a similar postexposure recovery in FVC was observed. In hyperresponsive individuals, the recovery takes longer and as much as 48 hours to return to baseline values. More slowly developing inflammatory and cellular changes persist for up to 48 hours. The time sequence, magnitude and the type of responses of this complex series of events, both in terms of development and recovery, indicate that several mechanisms, activated at different time of exposure must contribute to the overall lung function response (U.S. Environmental Protection Agency, 1996).

AX6.2.5.1 Pathophysiologic Mechanisms

Breathing pattern changes

Human studies consistently report that inhalation of O_3 alters the breathing pattern without significantly affecting minute ventilation. A progressive decrease in tidal volume and a "compensatory" increase in frequency of breathing to maintain steady minute ventilation during exposure suggests a direct modulation of ventilatory control. These changes parallel a response of many animal species exposed to O_3 and other lower airway irritants (Tepper et al., 1990). Although alteration of a breathing pattern could be to some degree voluntary, the presence of the response in animals and the absence of perception of the pattern change by subjects, even before appearance of the first subjective symptoms of irritation, suggests an involuntary reflex mechanism.

Direct recording from single afferent vagal fibers in animals convincingly demonstrated that bronchial C-fibers and rapidly adapting receptors are the primary vagal afferents responsible for O_3-induced changes in ventilatory rate and depth (Coleridge et al., 1993; Hazucha and

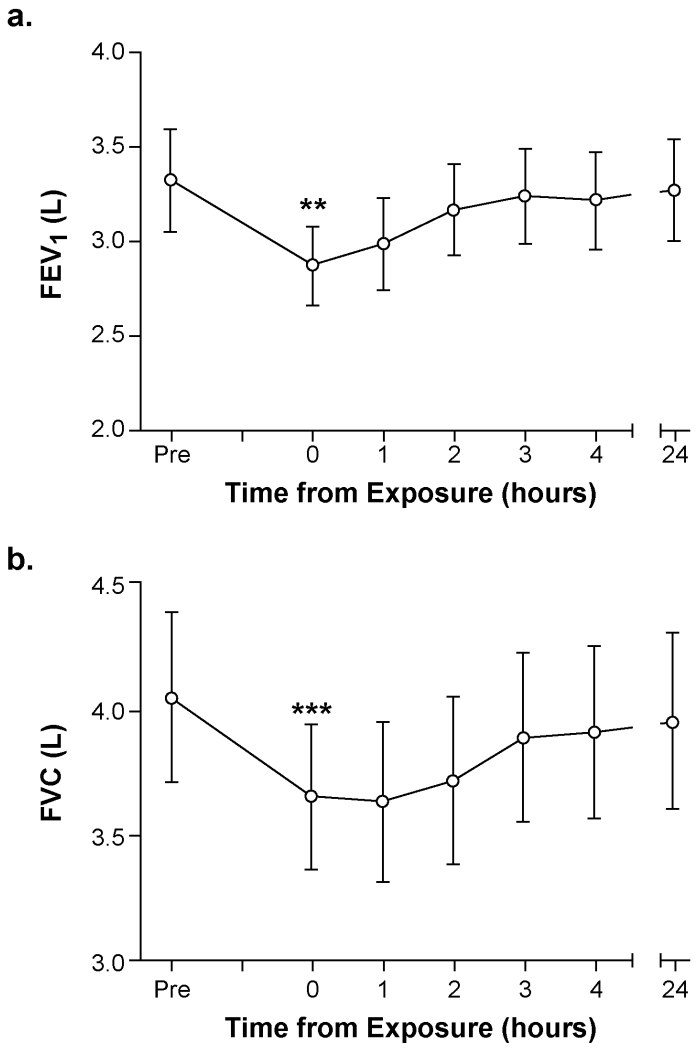

Figure AX6-4a,b. Recovery of spirometric responses following a 2 h exposure to 0.4 ppm O_3 with IE. Immediately postexposure, there were significant decrements (p < 0.001, ***p < 0.0005) in FVC (10%) and FEV_1 (13.5%) compared to preexposure values. At 3 h postexposure, FVC and FEV_1 were at 96 and 97% of preexposure values, respectively.**

Adapted from Nightingale et al. (2000).

Sant'Ambrogio, 1993). In spontaneously breathing dogs, an increase in V_T/T_i (T_i decreased more than V_T) was attributed to an increased inspiratory drive due to stimulation of rapidly adapting receptors and bronchial C-fibers by O_3 (Schelegle et al., 1993). Folinsbee and Hazucha (2000) also observed similar changes in V_T/T_i and other breath-timing parameters in humans exposed to

O_3 implying activation of the same mechanisms. They also reported that $Pm_{0.1}$ (pressure at mouth at 0.1 sec of inspiration against a transiently occluded mouthpiece which is considered an index of inspiratory drive) increased during controlled hypercapnia without a change in the slope of $Pm_{0.1}$ versus pCO_2 relation suggesting that the primary mechanism is an increased inspiratory drive. Since no significant within-individual differences in ventilatory response to CO_2 between air exposure and O_3 exposure were found, the CO_2 chemoreceptors did not modulate the response. Therefore, the principal peripheral mechanism modulating changes in breathing pattern appears to be direct and indirect stimulation of lung receptors and bronchial C-fibers by O_3 and/or its oxidative products. The activity of these afferents, centrally integrated with input from other sensory pathways, drives the ventilatory controller, which determines the depth and the frequency of breathing.

The potential modulation of breathing pattern by activation of sensory afferents located in extrathoracic airways by O_3 has not yet been studied in humans. Laboratory animal studies have shown that the larynx, pharynx, and nasal mucosa are densely populated by free-ending, unmyelinated sensory afferents resembling nociceptive C-fibers (Spit et al., 1993; Sekizawa and Tsubone, 1994). They are almost certainly stimulated by O_3 and likely contribute to overall ventilatory and symptomatic responses. Nasal only exposure of rats produced O_3-induced changes in breathing pattern that are similar to changes found in humans (Kleinman et al., 1999).

Symptoms and lung function changes

As already discussed, in addition to changes in ventilatory control, O_3 inhalation by humans will also induce a variety of symptoms, reduce vital capacity (VC) and related functional measures, and increase airway resistance. Hazucha et al. (1989) postulated that a reduction of VC by O_3 is due to a reflex inhibition of inspiration and not due to a voluntary reduction of inspiratory effort. Recently, Schelegle et al. (2001) convincingly demonstrated that a reduction of VC due to O_3 is indeed reflex in origin and not a result of subjective discomfort and consequent premature voluntary termination of inspiration. They reported that inhalation of an aerosolized topical anesthetic tetracaine substantially reduced if not abolished O_3-induced symptoms that are known to be mediated in part by bronchial C-fibers. Yet, such local anesthesia of the upper airway mucosa had a minor and irregular effect on pulmonary function decrements and tachypnea, strongly supporting neural mediation, i.e., stimulation of both

bronchial and pulmonary C-fibers, and not voluntary inhibition of inspiration (due to pain) as the key mechanism.

The involvement of nociceptive bronchial C-fibers modulated by opioid receptors in limiting maximal inspiration and eliciting subjective symptoms in humans was studied by Passannante et al. (1998). The authors hypothesized that highly variable responses among individuals might reflect the individual's inability or unwillingness to take a full inspiration. Moreover, development of symptoms of pain on deep inspiration, cough and substernal soreness suggested that nociceptive mechanism(s) might be involved in O_3-induced inhibition of maximal inspiration. If this were so, pain suppression or inhibition by opioid receptor agonists should partially or fully reverse symptoms and lung functional impairment. Subjects for this study were pre-screened with exposure to 0.42 ppm O_3 and classified either as "weak" ($FEV_1 \geq 95\%$ of preexposure value), "strong" ($FEV_1 \leq 85\%$ of preexposure value), or "moderate" responders. Sixty two (28 M, 34 F) healthy volunteers (18 to 59 yrs old), known from the previous screening to be "weak" (n = 20) or "strong" (n = 42) O_3-responders, participated in this double-blind crossover study. Subjects underwent either two 2 h exposures to air, or two 2 h exposures to 0.42 ppm O_3, with 15 min IE at 17.5 l/min/m^2 BSA. Immediately following postexposure spirometry the "weak" responders were given (in random order) either the potent opioid receptor antagonist naloxone (0.15 mg/kg) or saline, while "strong" responders received (in random order) either the potent, rapid-acting opioid agonist and analgesic sufentanil (0.2 μg/kg), or physiologic saline administered through an indwelling catheter. Administration of saline or naloxone had no significant effect on the relatively small decrements in FEV_1 observed in "weak" responders. However, as hypothesized, sufentanil rapidly reversed both the O_3-induced symptomatic effects and spirometric decrements (FEV_1; $p < 0.0001$) in "strong" responders (Figure AX6-5). All the same, the reversal was not complete and the average post-sufentanil FEV_1 remained significantly below (−7.3%) the preexposure value suggesting involvement of non-opioid receptor modulated mechanisms as well. Uneven suppression of symptoms has implied involvement of both A-δ and bronchial C-fibers. The plasma β-endorphin (a potent pain suppressor) levels, though substantially elevated immediately postexposure and post-drug administration, were not related to individuals' O_3 responsiveness. These observations have demonstrated that nociceptive mechanisms play a key role in modulating O_3-induced inhibition of inspiration. Moreover, these findings are consistent with and further support the concept that

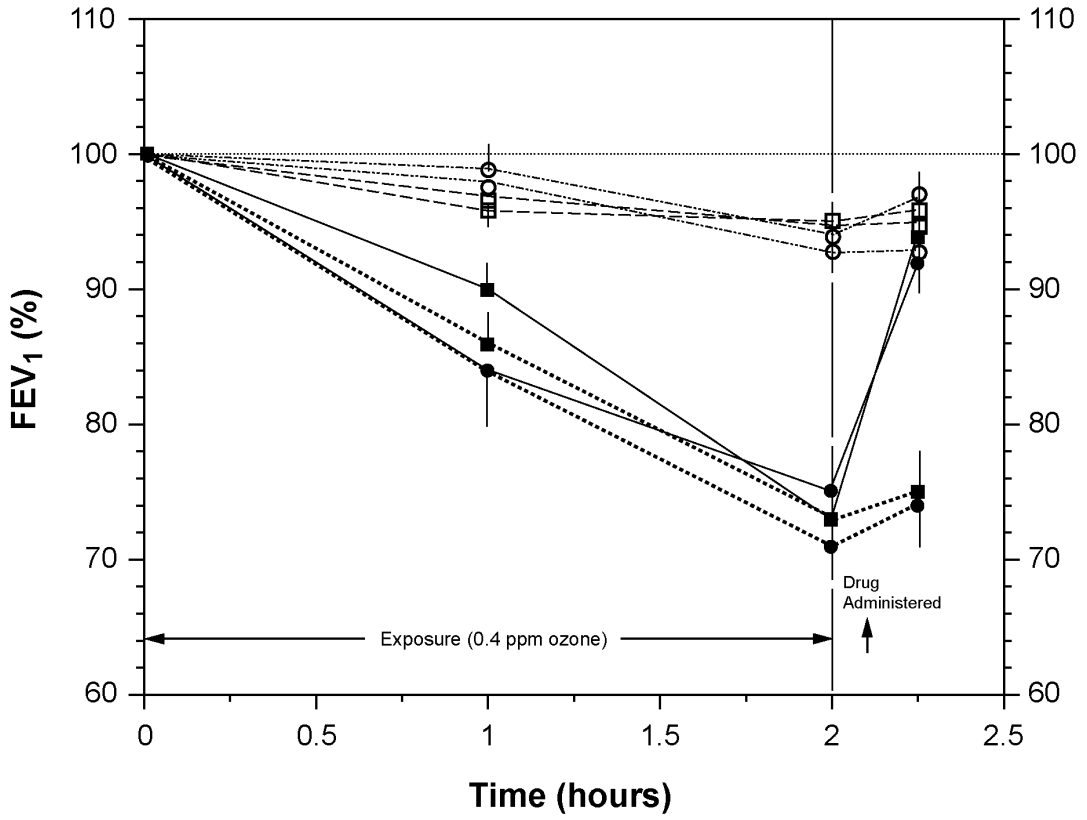

Figure AX6-5. Plot of the mean FEV_1 (% baseline) vs. time for ozone exposed cohorts. Solid lines represent data for "strong" males (n = 14; solid squares) and females (n = 15; solid circles) that received sufentanil and dotted lines represent data for the same cohorts after receiving saline. Dashed lines represent data for "weak" males (n = 5; open squares) and females (n = 10; open circles) that received naloxone and dot-dash lines represent data for the same cohorts after receiving saline. The arrow denotes the time of drug administration (~2.1 hrs). Vertical bars associated with the symbols are one-sided SEM.

Source: Adapted from Passannante et al. (1998).

the primary mechanism of O_3-induced reduction in inspiratory lung function, is an inhibition of inspiration elicited by stimulation of the C-fibers. The absence of effect of naloxone in "weak" responders shows that the weak response is not due to excessive endorphin production in those individuals. However, other neurogenic mechanisms not modulated by opioid receptors may have some though limited role in inspiratory inhibition.

Airway hyperreactivity

In addition to limitation of maximal inspiration and its effects on other spirometric endopoints, activation of airway sensory afferents also plays a role in receptor-mediated bronchoconstriction and an increase in airway resistance. Despite this common mechanism, post-O_3 pulmonary function changes and either early or late bronchial hyperresponsiveness (BHR) to inhaled aerosolized methacholine or histamine are poorly correlated either in time or magnitude. Fentanyl and indomethacin, the drugs that have been shown to attenuate O_3-induced lung function decrements in humans, did not prevent induction of BHR when administered to guinea pigs prior to O_3 exposure (Yeadon et al., 1992). Neither does post-O_3 BHR seem to be related to airway baseline reactivity. These findings imply that the mechanisms are either not related or are activated independently in time. Animal studies (with limited support from human studies) have suggested that an early post-O_3 BHR is, at least in part, vagally mediated (Freed, 1996) and that stimulation of C-fibers can lead to increased responsiveness of bronchial smooth muscle independently of systemic and inflammatory changes which may be even absent (Joad et al., 1996). In vitro study of isolated human bronchi have reported that O_3-induced airway sensitization involves changes in smooth muscle excitation-contraction coupling (Marthan, 1996). Characteristic O_3-induced inflammatory airway neutrophilia which at one time was considered a leading BHR mechanism, has been found in a murine model, to be only coincidentally associated with BHR and there was no cause and effect relationship (Zhang et al., 1995). However, this observation does not rule out involvement of other cells such as eosinophils or T-helper cells in BHR modulation. There is some evidence that release of inflammatory mediators by these cells can sustain BHR and bronchoconstriction. In vitro and animal studies have also suggested that airway neutral endopeptidase activity can be a strong modulator of BHR (Marthan et al., 1996; Yeadon et al., 1992). Late BHR observed in some studies is plausibly due to a sustained damage of airway epithelium and continual release of inflammatory mediators (Foster et al., 2000). Thus, O_3-induced BHR appears to be a product of many mechanisms acting at different time periods and levels of the bronchial smooth muscle signaling pathways. [*The effects of O_3 on BHR are described in Section AX6.8.*]

AX6.2.5.2 Mechanisms at a Cellular and Molecular Level

Stimulation of vagal afferents by O_3 and reactive products, the primary mechanism of lung function impairment is enhanced and sustained by what can be considered in this context to be secondary mechanisms activated at a cellular and molecular level. The complexity of these mechanisms is beyond the scope of this section and the reader is directed to Section AX6.9 of this chapter for greater details. A comprehensive review by Mudway and Kelly (2000) discusses the cellular and molecular mechanisms of O_3-induced pulmonary response in great detail.

Neurogenic airway inflammation

Stimulation of bronchial C-fibers by O_3 not only inhibits maximal inspiration but, through local axon reflexes, induces neurogenic inflammation. This pathophysiologic process is characterized by release of tachykinins and other proinflammatory neuropeptides. Ozone exposure has been shown to elevate C-fiber-associated tachykinin substance P in human bronchial lavage fluid (Hazbun et al. 1993) and to deplete neuropeptides synthesized and released from C-fibers in human airway epithelium rich in substance P-immunoreactive axons. Substance P and other transmitters are known to induce granulocyte adhesion and subsequent transposition into the airways, increase vascular permeability and plasma protein extravasation, cause bronchoconstriction, and promote mucus secretion (Solway and Leff, 1991). Although the initial pathways of neurogenic, antigen-induced, and generally immune-mediated inflammation are not the same, they eventually converge leading to further amplification of airway inflammatory processes by subsequent release of cytokines, eicosanoids, and other mediators. Significantly negative correlations between O_3-induced leukotriene ($LTC_4/D_4/E_4$) production and spirometric decrements (Hazucha et al., 1996), and an increased level of postexposure PGE_2, a mediator known to stimulate bronchial C-fibers, show that these mediators play an important role in attenuation of lung function due to O_3 exposure (Mohammed et al., 1993; Hazucha et al., 1996). Moreover, because the density of bronchial C-fibers is much lower in the small than large airways, the reported post O_3 dysfunction of small airways assessed by decrement in FEF_{25-75} (Weinman et al., 1995; Frank et al., 2001) may be due in part to inflammation. Also, because of the relative slowness of inflammatory responses as compared to reflex effects, O_3-triggered inflammatory mechanisms are unlikely to initially contribute to progressive lung function reduction. It is plausible, however, that when fully activated, they sustain and possibly

further aggravate already impaired lung function. Indeed, a prolonged recovery of residual spirometric decrements following the initial rapid improvement after exposure termination could be due to slowly resolving airway inflammation. Bronchial biopsies performed 6 h postexposure have shown that O_3 caused a significant decrease in immunoreactivity to substance P in the submucosa (Krishna et al., 1997a). A strong negative correlation with FEV_1 also suggests that the release of substance P may be a contributing mechanism to persistent post-O_3 bronchoconstriction (Krishna et al., 1997a). Persistent spirometry changes observed for up to 48 h postexposure could plausibly be sustained by the inflammatory mediators, many of which have bronchoconstrictive properties (Blomberg et al., 1999).

AX6.3 PULMONARY FUNCTION EFFECTS OF OZONE EXPOSURE IN SUBJECTS WITH PREEXISTING DISEASE

This section examines the effects of O_3 exposure on pulmonary function in subjects with preexisting disease by reviewing O_3 exposure studies that utilized subjects with (1) chronic obstructive pulmonary disease (COPD), (2) asthma, (3) allergic rhinitis, and (4) ischemic heart disease. Studies of subjects with preexisting disease exposed to O_3, published subsequent to or not included in the 1996 Air Quality Criteria Document (U.S. Environmental Protection Agency, 1996), are summarized in Table AX6-3. Studies examining increased airway responsiveness after O_3 exposure are discussed in Section AX6.8.

AX6.3.1 Subjects with Chronic Obstructive Pulmonary Disease

Five studies of O_3-induced responses in COPD patients were available for inclusion in the 1996 criteria document (U.S. Environmental Protection Agency, 1996). The COPD patients in these studies were exposed during light IE (4 studies) or at rest (1 study) for 1 to 2 hours to O_3 concentrations between 0.1 and 0.3 ppm. None of theses studies found significant O_3-induced changes in pulmonary function. Of the four studies examining arterial oxygen saturation, two reported small but statistically significant O_3-induced decreases in the COPD patients. These limited data suggest COPD patients experience minimal O_3-induced effects for 0.3 ppm O_3 exposures less than 2 hours in duration. These findings are also consistent decreasing O_3 effects with increasing age (*see Section AX6.5.1*).

Table AX6-3. Ozone Exposure in Subjects with Preexisting Disease[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration and Activity	Exposure Condition	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Subjects with Chronic Obstructive Pulmonary or Heart Disease							
0.0 0.24	0 472	4 h IE 15 min exercise 15 min rest $\dot{V}_E \approx 20$ L/min	24 °C 40% RH	9 M 10 M	COPD patients Age-matched healthy NS All subjects 59-71 years old	No significant changes in FEV_1, FVC, or SRaw due to ozone in COPD patients. Equivocal SaO_2 decrement during 2nd and 3rd hours of ozone exposure in COPD patients. Adjusted for exercise, ozone effects did not differ significantly between COPD patients and healthy subjects.	Gong et al. (1997a) Gong and Tierney (1995)
0.3	588	3 h IE $\dot{V}_E = 30$ L/min	22 °C 50% RH	10 M 6 M	Hypertension 42-61 years old Healthy 41-49 years old	No major cardiovascular effects in either healthy or hypertensive subjects.	Gong et al. (1998)
Subjects with Allergic Rhinitis							
0.0 0.2	0 392	1 h CE at $\dot{V}_E = 25$ L/min/m² BSA	20 °C 50% RH	13 M, 1 F	Dust mite sensitized asthmatics mean age 29 ± 5 years	FEV_1 decrement following O_3 of 10% not significantly different from the 4% decrement following FA. Subjects received dust mite antigen challenge at 0.5 h FA and O_3 postexposures and were lavaged 6 h post-challenge. Amount of allergen producing 15% FEV_1 decrement was decreased by O_3 compared to FA in 9 of 14 subjects. PMN in proximal airway lavage tended to be greater after O_3 than FA (p=0.06).	Chen et al. (2004)
0.125 0.250 0.125	245 490 245	3h IE (10 min rest, 15 min exercise on bicycle) $\dot{V}_E = 30$ L/min 3h IE × 4 days	27 °C 50 % RH	5 F, 6 M 6 F, 16 M	Mild bronchial asthma 20-53 years old Allergic rhinitis 19-48 years old	Mean early-phase FEV_1 response and number of ≥ 20% reductions in FEV_1 were significantly greater after 0.25 ppm O_3 or 4 × 0.125 ppm O_3. Most of the ≥ 15% late-phase FEV_1 responses occurred after 4 days of exposure to 0.125 ppm O_3, as well as significant inflammatory effects, as indicated by increased sputum eosinophils (asthma and allergic rhinitis) and increased sputum lymphocytes, mast cell tryptase, histamine, and LDH (asthma only).	Holz et al. (2002)
0.0 0.25	0 490	3 h IE, $\dot{V}_E = 30$ L/min 15 min ex/10 min rest/5 min no O_3; every 30 min.	27 ˚C 54% RH mouthpiece exposure	13 M, 11 F 6 M, 6 F 5 M, 5 F	Atopic mild asthma Positive allergen and IgE tests Healthy NS	O_3-induced FEV_1 decrements of 12.5, 14.1, and 10.2% in asthmatics, allergic rhinitics and healthy subjects, respectively (group differences not significant). Methacholine responsiveness increased in asthmatics. Allergen responsiveness: increased significantly after O_3 exposure in asthmatics (≈ 2 dose shift) and a smaller shift is rhinitics. No change in healthy. Neither allergen or methacholine response correlated with lung function and were not correlated with each.	Jörres et al. (1996)

Table AX6-3 (cont'd). Ozone Exposure in Subjects with Preexisting Disease[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Subjects with Asthma							
0.4	784	2h IE (15 min rest, 15 min exercise on bicycle) $\dot{V}_E$ = 30 L/min	NA	4 F, 5 M 7 F, 6 M	Healthy (25 ± 2 years old) Mild atopic asthma; beta agonists only (22 ± 0.7 years old)	Significant reductions in FVC (12%, 10%) and FEV_1 (13%, 11%) for asthmatic and healthy subjects, respectively; attenuated by indomethacin in healthy subjects only. Significant reductions in mid-flows which tended to be greater in asthmatics than healthy subjects. Indomethacin treatment attenuated mid-flow-reductions somewhat more in asthmatics than healthy subjects.	Alexis et al. (2000)
0.0 0.4	0 784	2h IE 4 × 15 min on bicycle, $\dot{V}_E$ = 40 L/min	NA	15 9	Healthy adults 18-40 years old Mild atopic asthmatics 18-40 years old	Sputum collected 24 h before and 4-6 h post O_3 exposure. Baseline CD11b expression positively correlated with O_3-induced PMN. Increased expression of mCD14 on macrophages following O_3 compared to FA. Asthmatic PMN response similar to healthy subjects (also see Table AX6-3). No spirometric data available.	Alexis et al. (2004)
0.12	236	Rest	22 ˚C 40% RH	10 M, 5 F	atopic asthma	No effect of O_3 on airway response to grass allergen.	Ball et al. (1996)
0.0 0.2	0 392	6 h 30 min rest/30 min exercise $\dot{V}_E \approx$ 25 L/min	22 °C 50% RH	5 M 5 M	Healthy NS Asthmatics, physician diagnosed, All 18-45 years	Similar spirometric responses in asthmatic and healthy. However, preexposure FEV1 and FVC were both ~0.4 L lower on O_3-day than FA day. More PMN's in asthmatics. IL-8 and IL-6 higher in asthmatics exposed to O_3. No relationship of spirometry and symptoms to inflammation.	Basha et al. (1994)
0.4	784	3h 6x15 min cycle ergometer $\dot{V}_E \approx$ 32L/min 5 consecutive days	31 ˚C 35% RH	8 M , 2 F	Asthmatic NS adults beta-agonist use only 19-48 years old ATS criteria for asthma	FEV_1 decreased 35% on first exposure day. Methacholine reactivity increased about ten-fold. *Also see Table AX6-7 for repeated exposure results.*	Gong et al. (1997b)
0.0 0.12	0 235	1 h rest air-antigen O_3-antigen	NA	9 M, 6 F	Mild allergic asthma; 18 to 49 years of age.	No effect of O_3 on airway response to grass or ragweed allergen.	Hanania et al. (1998)
0.4	784	2 h IE 15 min exercise 15 min rest $\dot{V}_E \approx$ 20L/min	Head mask exposure ≈18 ˚C 60% RH	5 M, 1 F 6 M	Healthy adults Atopic asthmatics	FEV_1 responses of healthy and asthmatic similar (≈ 15% decrease). Maximal FEV_1 response to methacholine increased similarly in both groups (12 h postexposure). Larger increase in PC_{20} in healthy subjects. Both groups had increased PMN's in sputum no correlation of PMN's and lung function.	Hiltermann et al. (1995)

Table AX6-3 (cont'd). Ozone Exposure in Subjects with Preexisting Disease[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Adult Subjects with Asthma (cont'd)							
0.0 0.16	0 314	7.6 h 25 min treadmill, 25 min cycle/10 min rest per hour. V_E =27-32 L/min	18° C 40% RH	13 M 7 M, 10 F	Healthy NS, age 19-32 years. Moderate Asthmatics, physician diagnosed, beta agonist users, age 19-32 years.	FEV_1 decreased 19% in asthmatics and only 10% in non-asthmatics. High responders had worse baseline airway status. More wheeze in asthmatics after O_3.	Horstman et al. (1995)
0.0 0.25	0 490	3 h IE, $\dot{V}_E$ = 30 L/min 15 min ex/10 min rest/5 min no O_3; every 30 min.	27 ˚C 54% RH mouthpiece exposure	13 M, 11 F 6 M, 6 F 5 M, 5 F	Atopic mild asthma Positive allergen and IgE tests Healthy NS	O_3-induced FEV_1 decrements of 12.5, 14.1, and 10.2% in asthmatics, allergic rhinitics and healthy subjects, respectively (group differences not significant). Methacholine responsiveness increased in asthmatics. Allergen responsiveness increased after O_3 exposure in asthmatics (= 2 dose shift), a smaller shift occurred in rhinitics, no change occurred in healthy subjects. Neither allergen nor methacholine responses were correlated with each other or with lung function.	Jörres et al. (1996)
0.16	314	7.6 h 25 min treadmill, 25 min cycle/10 min rest per hour. $\dot{V}_E$ = 25 L/min	22 °C 40 % RH	4 M, 5 F	Mild atopic asthma; no meds 12 h pre-exposure 20-35 years old	Significant FEV_1 decrease of 9.1 % following O_3 exposure; marked individual variability with responses ranging from 2 % to 26 %.	Kehrl et al. (1999)
0.25 0.40	490 784	$\dot{V}_E$ = 25-45 L/min	NA	8 M, 4 F 8 M, 10 F 22 M, 16 F	Asthmatics Allergic rhinitics Healthy adults All < 26 years old	Healthy 12.2% decrease in FEV_1, Rhinitics 10.1%, asthmatics 12.4%	Magnussen et al. (1994)
0.0 0.2	0 392	2 h IE 4 × 15 min at $\dot{V}_E$ = 20 L/min/m² BSA	20 °C 50% RH	6 M, 9 F 9 M, 6 F	Healthy adults 24 years old Mild asthmatics 29 years old	O_3-induced FEV_1 decrement (8%, healthy adults; 3% asthmatics) and PMN increase (20.6%, healthy adults; 15.2% asthmatics). Primary goal was to investigate relationship between antioxidant defenses and O_3 responses in asthmatics and healthy adults (see Tables AX6-3 andAX6 -13).	Mudway et al. (2001) Stenfors et al. (2002)
0.2	396	2h IE (15 min rest, 15 min exercise on bicycle) $\dot{V}_E$ = 20 L/min/m² BSA	22 °C 40 % RH	5 F, 4 M	Mild atopic asthma; no meds 8 h pre-exposure 21-42 years old	Significant decrease in FEV_1 and a trend toward decreases in mean inspiratory flow, FEF_{25}, and FEF_{75} after O_3 exposure. No significant differences in FEF_{50}, FVC, TLC, Raw, or sRaw. No correlation between sputum neutrophils at 6 h postexposure and FEV_1 immediately after exposure.	Newson et al. (2000)

Table AX6-3 (cont'd). Ozone Exposure in Subjects with Preexisting Disease[a]

Ozone Concentration[b]		Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
ppm	µg/m^3						
Adult Subjects with Asthma (cont'd)							
0.4	784	2 h rest	21 ˚C 40% RH	11 M , 11 F	Asthmatics sensitive to D Farinae, physician diagnosed, 18 to 35 years	Ozone resulted in nasal inflammation (increased PMN's) and caused augmented response to nasal allergen challenge.	Peden et al. (1995)
0.16	314	7.6 h 25 min treadmill, 25 min cycle/ every hour.	18° C 40% RH	8 M	Mild asthmatics, physician diagnosed, reactive to dust mite D. Farinae.	Increased eosinophils and PMN's after O_3 exposure more in initial (bronchial) fraction. No correlation of eosinophils and PMN's, FEV_1 & FVC decreased 14% and 9% respectively.	Peden et al. (1997)
0.0 0.2	0 392	4h 50 min exercise, 10 min rest each hour. $\dot{V}_E \approx$ 45-50 L/min	21 °C 50% RH	12 M, 6 F	18 adult mild asthmatics mostly beta agonist users.	FVC, FEV_1 decreased 17.6% and 25% respectively. Trend for larger increase in SRaw in asthmatics. Larger increase in PMN's and protein in asthmatics indicating more inflammation. No increase in eosinophils. Spirometry changes in asthmatics similar to healthy subjects (Aris et al., 1995; Balmes et al., 1997).	Scannell et al. (1996)

[a]See Appendix A for abbreviations and acronyms.
[b]Grouped by rest and exercise; within groups listed from lowest to highest O_3 concentration.

More recently, Gong et al. (1997a) exposed 9 COPD patients (age range, 59 to 71 years; mean age 66 ± 4 years) and 10 healthy NS (age range, 60 to 69 years; mean age 65 ± 3 years) to 0.24 ppm for 4 h with interment light exercise (≈20 L/min). COPD patients had decreases in FEV_1 following both clean air (−11%, $p = 0.06$) and O_3 (−19%, $p < 0.01$) exposures. These FEV_1 decrements, presumably due to exercise, were primarily attributable to four of the patients who lost greater than 14% of their FEV_1 following both the air and O_3 exposures. Relative to clean air, O_3 caused a statistically insignificant FEV_1 decrement of −8% in COPD patients which was not statistically different from the decrement of −3% in healthy subjects. Ozone-induced symptoms, sRaw, S_aO_2, and postexposure bronchial activity also exhibited little or no difference between the COPD patients and the healthy subjects.

AX6.3.2 Subjects with Asthma

Based on studies reviewed in the 1996 criteria document (U.S. Environmental Protection Agency, 1996) asthmatics appear to be at least as sensitive to acute effects of O_3 as healthy nonasthmatic subjects. At rest, neither adolescent asthmatics nor healthy controls had significant responses as a result of an hour exposure to 0.12 ppm O_3. Exposure of adult asthmatics to 0.25 ppm O_3 for 2 h at rest also caused no significant responses. Preexposure to between 0.10 and 0.25 ppm O_3 for 1 hr with light IE does not appear to exacerbate exercise-induced asthma (Fernandes et al., 1994; Weymer et al., 1994). At higher exposures (0.4 ppm O_3 with heavy IE for 2 h), Kreit et al. (1989) and Eschenbacher et al. (1989) demonstrated significantly greater FEV_1 and FEF_{25-75} decrements in asthmatics than in healthy controls. With longer duration exposures to lower O_3 levels (0.12 ppm with moderate IE for 6.5 h), asthmatics have also shown a tendency for greater FEV_1 decrements than healthy non-asthmatics (Linn et al., 1994). Newer studies (see Table AX6-3) continue to suggest that asthmatics are at least as sensitive as healthy controls to O_3-induced responses.

Studies of less than 3 h duration have reported similar or tendencies for increased O_3-induced spirometric responses up to O_3 concentrations of 0.4 ppm. Similar group decrements in FEV_1 and FVC were reported by Hiltermann et al. (1995), who exposed 6 asthmatics and 6 healthy subjects to 0.4 ppm O_3 for 2 h with light IE. Alexis et al. (2000) exposed 13 mild atopic asthmatics and 9 healthy subjects for 2 h to 0.4 ppm O_3 with IE ($\dot{V}_E$ = 30 L/min). Similar O_3-induced group decrements in FEV_1 and FVC were also reported by these investigators.

A tendency, however, for an increased O_3-induced reduction in mid-flows (viz., FEF_{25}, FEF_{50}, FEF_{60p}, FEF_{75}) was reported for the asthmatics relative to the healthy subjects. In a larger study, Jörres et al. (1996) exposed 24 asthmatics, 12 allergic rhinitis, and 10 healthy subjects to 0.25 ppm O_3 for 3 h with IE. Statistically significant O_3-induced decreases in FEV_1 occurred in all groups, but tended to be lower in healthy controls (allergic rhinitis, −14.1%; asthmatics, −12.5%; healthy controls, −10.2%). One study reported that asthmatics tended to have less of an FEV_1 response to O_3 than healthy controls (Mudway et al., 2001). In that study, however, the asthmatics also tended to be older than the healthy subjects which could partially explain their lesser response.

Studies between 4 and 8 h duration, with O_3 concentrations of 0.2 ppm or less, also suggest a tendency for increased O_3-induced pulmonary function responses in asthmatics relative to healthy subjects. Scannell et al. (1996) exposed 18 asthmatics to 0.2 ppm O_3 for 4 h with IE ($\dot{V}_E \approx 25$ L/min/m^2 BSA). Baseline and hourly pulmonary function measurements of FEV_1, FVC, and sRaw were obtained. Asthmatic responses were compared to 81 healthy subjects who underwent similar experimental protocols (Aris et al., 1995; Balmes et al., 1996). Asthmatic subjects experienced a significant O_3-induced increase in sRaw, FEV_1 and FVC. The O_3-induced increase in sRaw tended to be greater in asthmatics than the healthy subjects, whereas similar group decrements in FEV_1 and FVC were observed. Basha et al. (1994) also reported similar spirometric responses between 5 asthmatic and 5 healthy subjects exposed to 0.2 ppm O_3 for 6 h with IE. However, the mean preexposure FEV_1 in the asthmatics was about 430 mL less (i.e., ~12% decreased) on the O_3-day relative to the air-day. In a longer exposure duration (7.6 h) study, Horstman et al. (1995) exposed 17 asthmatics and 13 healthy controls to 0.16 ppm O_3 or FA with alternating periods of exercise (50 min, $\dot{V}_E \approx 30$ L/min) and rest (10 min). Both groups had significant O_3-induced decrements in FEV_1, FVC, and FEV_{25-75}. The asthmatic and healthy subjects had similar O_3-induced reductions in FVC. The FEV_1 decrement experienced by the asthmatics was significantly greater in the healthy controls (19% versus 10%, respectively). There was also tendency for a greater O_3-induced decrease in FEF_{25-75} in asthmatics relative to the healthy subjects (24% versus 15%, respectively).

With repeated O_3 exposures asthmatics, like healthy subjects (*see Section AX6.6*) develop tolerance. Gong et al. (1997b) exposed 10 asthmatics to 0.4 ppm O_3, 3 h per day with IE ($\dot{V}_E \approx 32$ L/min), for 5 consecutive days. Symptom and spirometric responses were greatest on

the first (−35 % FEV_1) and second (−34 % FEV_1) exposure days, and progressively diminished toward baseline levels (−6 % FEV_1) by the fifth exposure day. Similar to healthy subjects, asthmatics lost their tolerance 4 and 7 days later.

Other published studies with similar results (e.g., McBride et al., 1994; Basha et al., 1994; Peden et al., 1995, 1997; Peden, 2001a; Scannell et al., 1996; Hiltermann et al., 1997, 1999; Michelson et al., 1999; Vagaggini et al., 1999; Newson et al., 2000; Holz et al., 2002) also reported that asthmatics have a reproducible and somewhat exaggerated inflammatory response to acute O_3 exposure (*see Section AX6.9*). For instance, Scannell et al. (1996) performed lavages at 18 h post O_3 exposure to assess inflammatory responses in asthmatics. Asthmatic responses were compared to healthy subjects who underwent a similar experimental protocol (Balmes et al., 1996). Ozone-induced increases in BAL neutrophils and total protein were significantly greater in asthmatics than healthy subjects. There was also a trend for an ozone related increased IL-8 in the asthmatics relative to healthy subjects. Inflammatory responses do not appear to be correlated with lung function responses in either asthmatic or healthy subjects (Balmes et al., 1996, 1997; Holz et al., 1999). This lack of correlations between inflammatory and spirometric responses may be due to differences in the time kinetics of these responses (Stenfors et al., 2002). In addition, airway responsiveness to inhaled allergens is increased by O_3 exposure in subjects with allergic asthma for up to 24 h (*see Section AX6.8*).

One of the difficulties in comparing O_3-induced spirometric responses of healthy subjects versus asthmatics is the variability in responsiveness of asthmatics. Most of the asthma studies were conducted on subjects with a clinical history of mild disease. However, classification asthma severity is not only based on functional assessment (e.g., percent predicted FEV_1), but also on clinical symptoms, signs, and medication use (Table AX6-4). Although "mild atopic asthmatics" are frequently targeted as an experimental group, the criteria for classification has varied considerably within and across the available published studies. Although the magnitude of group mean changes in spirometry may not be significantly different between healthy and asthmatic subjects, many of the studies have reported clinically significant changes in some individuals.

Alexis et al. (2000) explored the possibility that the mechanisms of O_3-induced spirometric responses may differ between asthmatics and healthy subjects. Physician-diagnosed mild atopic asthmatics and healthy subjects were pretreated with 75 mg/day of indomethacin (a COX

Table AX6-4. Classification of Asthma Severity[1]

Classification	Step	Days with symptoms	Nights with symptoms	Lung Function[2]		Medications[3]	
				FEV1 or PEF % predicted oral	PEF variability (%)	Daily	Quick relief
Severe persistent	4	Continual	Frequent	≤ 60	> 30	High-dose inhaled steroids (ICS) and long-acting inhaled β2-agonist If needed, add oral steroids	Short-acting inhaled β2-agonist, as needed; oral steroids may be required
Moderate persistent	3	Daily	> 1/week	between 60 and 80	> 30	Low-to-medium-dose ICS and long-acting β2-agonist (preferred) Or Medium-dose ICS (another preferred option for children ages < 5 years) Or Low-to-medium-dose ICS and either leukotriene modifier or theophylline	Short-acting inhaled β2-agonist, as needed; oral steroids may be required
Mild persistent	2	> 2/week, but < 1 time/day	> 2/week	≥ 80	20-30	Low-dose inhaled steroids (preferred) Or Cromolyn leukotriene modifier, or (except for children aged < 5 years) nedocromil or sustained release theophylline to serum concentration of 5-15 µg/mL	Short-acting inhaled β2-agonist, as needed; oral steroids may be required
Mild intermittent	1	≤ 2/week	< 2/month	≥ 80	< 20	No daily medicine needed	Short-acting inhaled β2-agonist, as needed; oral steroids may be required

[1] Sources: Centers for Disease Control (2003); National Heart, Lung, and Blood Institute (1997, 2003).
[2] For adults and children aged > 5 years who can use a spirometer or peak flow meter.
[3] The medications listed here are appropriate for treating asthma at different levels of severity. The preferred treatments, dosage, and type of medication recommended vary for adults and children and are detailed in the *EPR-Update 2002* stepwise approach to therapy. The stepwise approach emphasizes that therapy should be stepped up as necessary and stepped down when possible to identify the least amount of medication required to achieve goals of therapy. The stepwise approach to care is intended to assist, not replace, the clinical decision-making required to meet individual patient needs.

inhibitor) or placebo and then exposed for 2 h to 0.4 ppm O_3 or to FA during mild IE ($\dot{V}_E$ = 30 L/m). The number and severity of O_3-induced symptoms were significantly increased in both asthmatics and healthy subjects. These symptom responses were similar between the subject groups and unaffected by indomethacin pretreatment. Asthmatics and healthy subjects also had similar O_3-induced reductions in FVC and FEV_1. These restrictive-type responses, occurring due to the combined effects of bronchoconstriction and reflex inhibition of inspiration (*see Section AX6.2.1*), were attenuated by indomethacin in the healthy subjects but not the asthmatics. Thus, in healthy subjects but not asthmatics, COX metabolites may contribute to O_3-induced reductions in FVC and FEV_1. As assessed by the magnitude of reductions in mid-flows (viz. FEF_{25}, FEF_{50}, FEF_{60p}, FEF_{75}), the small airways of the asthmatics tended to be more affected than the healthy subjects. This suggests asthmatics may be more sensitive to small airway effects of O_3, which is consistent with the observed increases in inflammation and airway responsiveness. Indomethacin pretreatment attenuated some of these O_3-induced small airways effects (FEF_{50} in healthy subjects, FEF_{60p} in asthmatics).

AX6.3.3 Subjects with Allergic Rhinitis

Most O_3 exposure studies in humans with existing respiratory disease have focused on lung diseases like COPD and asthma. However, chronic inflammatory disorders of the nasal airway, especially allergic rhinitis, are very common in the population. People with allergic rhinitis have genetic risk factors for the development of atopy that predispose them to increased upper airway responsiveness to specific allergens as well as nonspecific air pollutants like O_3. Studies demonstrating the interaction between air pollutants and allergic processes in the human nasal airways and rhinoconjunctival tissue have been reviewed by Peden (2001b) and Riediker et al. (2001), respectively. Ozone exposure of subjects with allergic rhinitis has been shown to induce nasal inflammation and increase airway responsiveness to nonspecific bronchoconstrictors, although to a lesser degree than experienced by asthmatics.

McDonnell et al. (1987) exposed non-asthmatic adults with allergic rhinitis to 0.18 ppm O_3. The allergic rhinitics were no more responsive to O_3 than healthy controls, based on symptoms, spirometry, or airway reactivity to histamine although they had a small but significantly greater increase in SRaw. The data on subjects with allergic rhinitis and asthmatic

subjects suggest that both of these groups have a greater rise in Raw to O_3 with a relative order of airway responsiveness to O_3 being normal < allergic < asthmatic.

Bascom et al. (1990) studied the upper respiratory response to acute O_3 inhalation, nasal challenge with antigen, and the combination of O_3 plus antigen in subjects with allergic rhinitis. Exposure to O_3 caused significant increases in upper and lower airway symptoms, a mixed inflammatory cell influx with a seven-fold increase in nasal lavage PMNs, a 20-fold increase in eosinophils, and a 10-fold increase in mononuclear cells, as well as an apparent sloughing of epithelial cells. McBride et al. (1994) also observed increased nasal PMN's after O_3 exposure in atopic asthmatics. Peden et al. (1995), who studied allergic asthmatics exposed to O_3, found that O_3 causes an increased response to nasal allergen challenge in addition to nasal inflammatory responses. Their data suggested that allergic subjects have an increased immediate response to allergen after O_3 exposure. In a follow-up study, Michelson et al. (1999) reported that 0.4 ppm O_3 did not promote early-phase-response mediator release or enhance the response to allergen challenge in the nasal airways of mild, asymptomatic dust mite-sensitive asthmatic subjects. Ozone did, however, promote an inflammatory cell influx, which helps induce a more significant late-phase response in this population.

Jörres et al. (1996) found that O_3 causes an increased response to bronchial allergen challenge in subjects with allergic rhinitis. This study also compared responses in subjects with mild allergic asthma (*see Sections AX6.3.2 and AX6.8*). The subjects were exposed to 0.25 ppm O_3 for 3 h with IE. Airway responsiveness to methacholine was determined 1 h before and after exposure; responsiveness to allergen was determined 3 h after exposure. Statistically significant decreases in FEV_1 occurred in subjects with allergic rhinitis (13.8%) and allergic asthma (10.6%), and in healthy controls (7.3%). Methacholine responsiveness was statistically increased in asthmatics, but not in subjects with allergic rhinitis. Airway responsiveness to an individual's historical allergen (either grass and birch pollen, house dust mite, or animal dander) was significantly increased after O_3 exposure when compared to FA exposure. In subjects with asthma and allergic rhinitis, a maximum percent fall in FEV_1 of 27.9 % and 7.8%, respectively, occurred 3 days after O_3 exposure when they were challenged with of the highest common dose of allergen. The authors concluded that subjects with allergic rhinitis, but without asthma, could be at risk if a high O_3 exposure is followed by a high dose of allergen.

Holz et al. (2002) extended the results of Jörres et al. (1996) by demonstrating that repeated daily exposure to lower concentrations of O_3 (0.125 ppm for 4 days) causes an increased response to bronchial allergen challenge in subjects with preexisting allergic airway disease, with or without asthma. There was no major difference in the pattern of bronchial allergen response between subjects with asthma or rhinitis, except for a 10-fold increase in the dose of allergen required to elicit a similar response ($\geq$ 20% decrease in FEV_1) in the asthmatic subjects. Early phase responses were more consistent in subjects with rhinitis and late-phase responses were more pronounced in subjects with asthma. There also was a tendency towards a greater effect of O_3 in subjects with greater baseline response to specific allergens chosen on the basis of skin prick test and history (viz., grass, rye, birch, or alder pollen, house dust mite, or animal dander). These data suggest that the presence of allergic bronchial sensitization, but not a history of asthma, is a key determinant of increased airway allergen responsiveness with O_3. [*A more complete discussion of airway responsiveness is found in Section AX6.8*]

AX6.3.4 Subjects with Cardiovascular Disease

Superko et al. (1984) exposed six middle-aged males with angina-symptom-limited exercise tolerance for 40 min to FA and to 0.2 and 0.3 O_3 while they were exercising continuously according to a protocol simulating their angina-symptom-limited exercise training prescription (mean $\dot{V}_E$ = 35 L/min). No significant pulmonary function impairment or evidence of cardiovascular strain induced by O_3 inhalation was observed. Gong et al. (1998) exposed hypertensive and healthy adult males, 41 to 78 years of age, to 0.3 ppm O_3 for 3 h with IE at 30 L/min. The ECG was monitored by telemetry, blood pressure by cuff measurement, and a venous catheter was inserted for measurement of routing blood chemistries and cardiac ezymes. Pulmonary artery and radial artery catheters were placed percutaneously for additional blood sampling and for measurement of hemodynamic pressures, cardiac output, and S_aO_2. Other hemodynamic variables were calculated, including cardiac index, stroke volume, pulmonary and systemic vascular resistance, left and right ventricular stroke-work indices, and rate-pressure product. Spirometric volumes (FVC, FEV_1) and respiratory symptoms were measured before and after the O_3 exposures. The overall results did not indicate any major acute cardiovascular effects of O_3 in either the hypertensive or normal subjects. Statistically significant O_3 effects for both groups combined were a decrease in FEV_1 and increases in HR, rate-pressure product, and

the alveolar-to-arterial PO_2 gradient, suggesting that impaired gas exchange was being compensated for by increased myocardial work. These effects might be more important in some patients with severe cardiovascular disease.

AX6.4 INTERSUBJECT VARIABILITY AND REPRODUCIBILITY OF RESPONSE

Analysis of the factors that contribute to intersubject variability is important for the understanding of individual responses, mechanisms of response, and health risks associated with acute O_3 exposures. Bates et al. (1972) noted that variation between individuals in sensitivity and response was evident in respiratory symptoms and pulmonary function following O_3 exposure. A large degree of intersubject variability in response to O_3 has been consistently reported in the literature (Adams et al., 1981; Aris et al., 1995; Folinsbee et al., 1978; Kulle et al., 1985; McDonnell et al., 1983). Kulle et al. (1985) noted that the magnitude of variability between individuals in FEV_1 responses increases with O_3 concentration. Similarly, McDonnell et al. (1983) observed FEV_1 decrements ranging from 3 to 48% (mean 18%) in 29 young adult males exposed to 0.40 ppm O_3 for 2 h during heavy IE. At a lower O_3 concentration of 0.18 ppm, 20 similarly exposed subjects had FEV_1 decrements ranging from 0 o 23% (mean = 6%), while those exposed to FA (n = 20) had decrements ranging from −2% to 6% (mean = 1%) (McDonnell et al., 1983). All of the subjects in these studies were young adult males. (*Intersubject variability related to age and gender is discussed in Sections AX6.5.1 and AX6.5.2, respectively*.)

More recently, McDonnell (1996) examined the FEV_1 response data from three 6.6 h exposure studies of young adult males conducted at the EPA Health Effects Research Laboratory in Chapel Hill, NC (Folinsbee et al., 1988; Horstman et al., 1990; McDonnell et al., 1991). The response distributions for subjects at each of four O_3 concentrations (0.0, 0.08, 0.10, and 0.12 ppm) are illustrated in Figure AX6-6. It is apparent that the FEV_1 responses in FA are small with most tightly grouped around zero. With increasing O_3 concentration, the mean response increases as does the variability about the mean. At higher O_3 concentrations, the distribution of response becomes asymmetric with a few individuals experiencing large FEV_1 decrements. The response distribution in Figure AX6-6 allows estimates of the number or

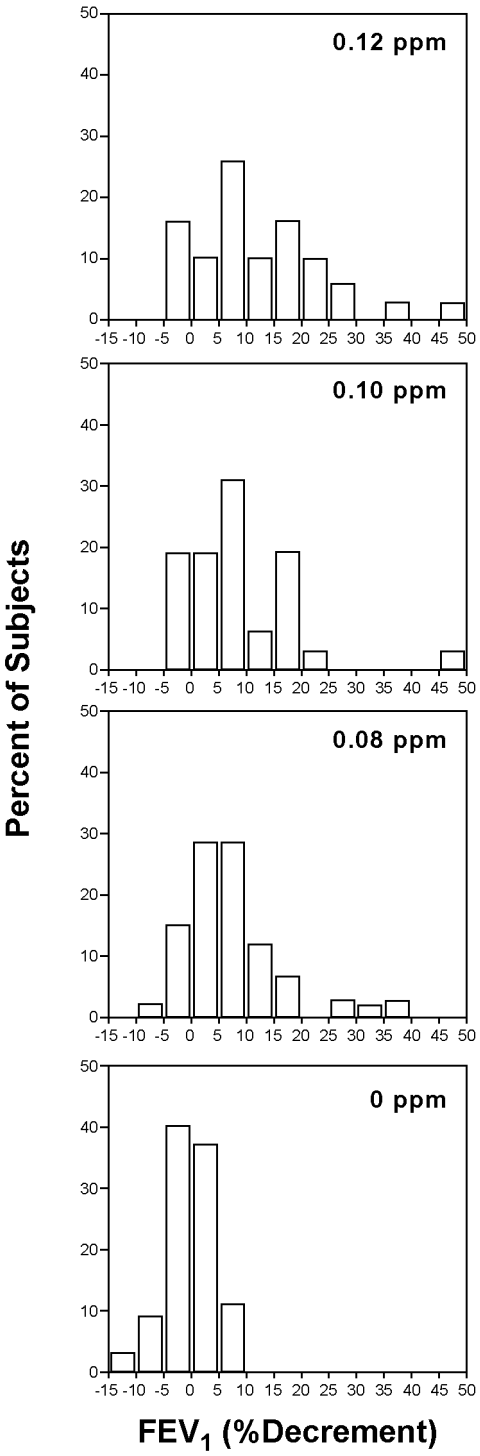

Figure AX6-6. Frequency distributions of percent decrements in FEV_1 for 6.6-h exposure to four concentrations of ozone.

Source: McDonnell (1996).

percentage of subjects responding in excess of a certain level. With FA exposure, none of 87 subjects had a FEV_1 decrement in excess of 10%; however, 26%, 31%, and 46% exceeded a 10% decrement at 0.08, 0.10, and 0.12 ppm, respectively. FEV_1 decrements as large as 30 to 50% were even observed in some individuals. In 6.6-h face mask exposures of young adults (half women) to 0.08 ppm O_3, Adams (2002) found that 6 of 30 subjects (20%) had > 10% decrements in FEV_1. The response distributions in Figure AX6-6 underlines the wide range of response to O_3 under prolonged exposure conditions and reinforces the observations by others consequent to 2 h IE exposures at higher O_3 concentrations (Horvath et al.,1981; McDonnell et al., 1983).

Some of the intersubject variability in response to O_3 inhalation may be due to intrasubject variability, i.e., how reproducible the measured responses are in an individual between several O_3 exposures. The more reproducible the subject's response, the more precisely it indicates his/her intrinsic responsiveness. McDonnell et al. (1985a) examined the reproducibility of individual responses to O_3 in healthy human subjects (n = 32) who underwent repeated exposures within a period of 21 to 385 days (mean = 88 days; no median reported) at one of five O_3 concentrations ranging from 0.12 to 0.40 ppm. Reproducibility was assessed using the intraclass correlation coefficient (R). The most reproducible responses studied were FVC (R = 0.92) and FEV_1 (R = 0.91). However, at the lowest concentration, 0.12 ppm, relatively poor FEV_1 reproducibility was observed (R = 0.58) due, in part, to a lack of specific O_3 response or a uniformly small response in the majority of subjects. McDonnell et al. (1985a) concluded that for 2 h IE O_3 exposures equal to or greater than 0.18 ppm, the intersubject differences in magnitude of change in FVC and FEV_1 are quite reproducible over time and likely due to differences in intrinsic responsiveness of individual subjects. Hazucha et al. (2003) exposed 47 subjects on three occasions for 1.5 h, with moderate intensity IE, to 0.40 to 0.42 ppm O_3. Reproducibility of FEV_1 responses was related to the length of time between re-exposures, with a Spearman correlation R of 0.54 obtained between responses for exposures 1 and 2 (median = 105 days), and an R of 0.85 between responses for exposures 2 and 3 (median = 7 days).

Identification of mechanisms of response and health risks associated with acute O_3 exposures are complicated by a poor association between various O_3-induced responses. For example, McDonnell et al. (1983) observed a very low correlation between changes in sRaw and FVC ($r = -0.16$) for 135 subjects exposed to O_3 concentrations ranging from 0.12 to

0.40 ppm for 2.5 h with IE. In a retrospective study of 485 male subjects (ages 18 to 36 yrs) exposed for 2 h to one of six O_3 concentrations at one of three activity levels, McDonnell et al. (1999) observed significant, but low, Spearman rank order correlations between FEV_1 response and symptoms of cough (R = 0.39), shortness of breath (R = 0.41), and pain on deep inspiration (R = 0.30). The authors concluded from their data that the O_3-induced responses are related mechanistically to some degree, but that there is not a single factor which is responsible for the observed individual differences in O_3 responsiveness across the spectrum of symptom and lung function responses. This conclusion is supported by differences in reproducibility observed by McDonnell et al., (1985a). Compared to the intraclass correlation coefficient for FEV_1 (R = 0.91), relatively low but statistically significant R values for symptoms ranged from 0.37 to 0.77, with that for sRaw being 0.54. The reproducibility correlations for f_B (R = −0.20) and V_T (R = −0.03) were not statistically significant.

The effect of this large intersubject variability on the ability to predict individual responsiveness to O_3 was demonstrated by McDonnell et al. (1993). These investigators analyzed the data of 290 male subjects (18 to 32 years of age) who underwent repeat 2 h IE exposures to one or more O_3 concentrations ranging from 0.12 to 0.40 ppm in order to identify personal characteristics (i.e., age, height, baseline pulmonary functions, presence of allergies, and past smoking history) that might predict individual differences in FEV_1 response. Only age contributed significantly to intersubject responsiveness (younger subjects were more responsive), accounting for just 4% of the observed variance. Interestingly, O_3 concentration accounted for only 31% of the variance, strongly suggesting the importance of as yet undefined individual characteristics that determine FEV_1 responsiveness to O_3. A more general form of this model was developed to investigate the O_3 exposure FEV_1 response relationship (McDonnell et al., 1997). These authors used data from 485 male subjects (age = 18 to 36 years) exposed once for 2 h to one of six O_3 concentrations (ranging from 0.0 to 0.40 ppm) at one of 3 activity levels (rest, n= 78; moderate IE, n = 92; or heavy IE, n = 314). In addition to investigating the influence of subject's age, the model focused on determining whether FEV_1 response was more sensitive to changes in C than to changes in $\dot{V}_E$, and whether the magnitude of responses is independent of differences in lung size. It was found that the unweighted proportion of the variability in individual responses explained by C, $\dot{V}_E$, T, and age was 41%, with no evidence that the sensitivity of FEV_1 response to $\dot{V}_E$ was different than changes in C, and no evidence that

magnitude of response was related to measures of body or lung size. The authors concluded that much inter-individual variability in FEV_1 response to O_3 remains unexplained.

AX6.5 INFLUENCE OF AGE, GENDER, ETHNIC, ENVIRONMENTAL AND OTHER FACTORS

AX6.5.1 Influence of Age

On the basis of results reported from epidemiologic studies, children and adolescents are considered to be at increased risk, but not necessarily more responsive, to ambient oxidants than adults. However, findings of controlled laboratory studies that have examined the acute effects of O_3 on children and adolescents do not completely support this assertion (Table AX6-5). Children experience about the same decrements in spirometric endpoints as young adults exposed to comparable O_3 doses (McDonnell et al., 1985b; Avol et al., 1987). In contrast to young adults, however, they had no symptomatic response, which may put them at an increased risk for continued exposure. Similarly, young adults (Linn et al., 1986; Avol et al., 1984) have shown comparable spirometric function response when exposed to low O_3 dose under similar conditions. Among adults, however, it has been repeatedly demonstrated that older individuals respond to O_3 inhalation with less intense lung function changes than younger adults. Thus, children, adolescents, and young adults appear to be about equally responsive to O_3, but more responsive than middle-aged and older adults when exposed to a comparable dose of O_3 (U.S. Environmental Protection Agency, 1996).

Gong et al. (1997a) studied ten healthy men (60 to 69 years old) and nine COPD patients (59 to 71 years old) from the Los Angeles area who were exposed to 0.24 ppm O_3 while intermittently exercising every 15 min at a light load (~20 L/min) for 4 h. [2]Healthy subjects showed a small but significant O_3-induced FEV_1 decrement of 3.3% ($p = 0.03$ [not reported in paper] paired-t on O_3 versus FA pre-post FEV_1). Small but statistically nonsignificant changes were also observed for respiratory symptoms, airway resistance and arterial O_2 saturation. In the COPD patients, there was an 8% FEV_1 decrement due to O_3 exposure which was not

[2]Personal communication from authors, correction to Table 2 in Gong et al. (1997a), the $\%FEV_1$ change at the end of the ozone exposure for subject ID 2195 should read 4.9 and not the published value of −4.3, the mean and standard deviation reported in the table are correct.

Table AX6-5. Age Differences in Pulmonary Function Responses to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.40 0.42	784 823	2 h IE (15' ex/15' rest) $\dot{V}_E \approx$ 33-45 L/min (47 subjects only) 1.5 h IE (20' ex/10' rest) $\dot{V}_E \approx$ 33-45 L/min (All subjects)	≈22 °C 40% RH treadmill	146 M 94 F	Healthy NS 18 to 60 years old	Young individuals of both gender (< 35 years) significantly more responsive than older subjects. Strong responses are less common over the age of 35 years, especially in women. The variability of an individual's responsiveness to repeated exposures to O_3 decreases with age.	Hazucha et al. (2003)
0.0 0.40	0 784	2 h, IE (15' ex/15' rest) $\dot{V}_E \approx$ 18 L/min/m² BSA 2 exposures: 25% of subj. exposed to air-air, 75% exposed to O_3-O_3	21 °C 40% RH treadmill	28 M 34 F	Healthy NS 18 to 57 years old Healthy NS 18 to 59 years old	Significant decrements in spirometric lung function in all groups. Young males and females (< 35 years) were significantly more responsive than older individuals (> 35 years).	Passannante et al. (1998)
0.0 0.24	0 470	4 h, IE (15' ex/ 15' rest) $\dot{V}_E$ =20 L/min	24°C 40% RH	10 M 9 M	Healthy NS 60 to 69 years old COPD 59 to 71 years old	Healthy: small, 3.3%, decline in FEV_1 (p=0.03 [not reported in paper], paired-t on O_3 versus FA pre-post FEV_1). COPD: 8% decline in FEV_1 (p=ns, O_3 versus FA). Adjusted for exercise, ozone effects did not differ significantly between COPD patients and healthy subjects.	Gong et al. (1997a)
0.0 0.12 0.18 0.24 0.30 0.40	0 235 353 471 589 784	2 h rest or IE (4 × 15 min at $\dot{V}_E$ = 25 or 35 L/min/m² BSA)	22 °C 40% RH	485 WM (each subject exposed at one activity level to one O_3 concentration)	Healthy NS 18 to 36 years old mean age 24 years	Statistical analysis of 8 experimental chamber studies conducted between 1980 and 1993 by the U.S. EPA in Chapel Hill, NC. O_3-induced decrement in FEV_1 predicted to decrease with age. FEV_1 response of a 30 year old predicted to be 50% the response of a 20 year old. *Also see Table 6-1*	McDonnell et al. (1997)
0.0 0.12 0.18 0.24 0.30 0.40	0 235 353 471 589 784	2.33 h IE (4 × 15 min at $\dot{V}_E$ = 25 L/min/m² BSA)	22 °C 40% RH	371 (WM, BM, WF, BF; ~25% per group) each subject exposed to one O_3 concentration	Healthy NS 18 to 35 years old mean age 24 years	Statistical analysis of experimental data collected between 1983 and 1990 by the U.S. EPA in Chapel Hill, NC. O_3-induced decrement in FEV_1 predicted to decrease with age. FEV_1 response of a 30 year old predicted to be 65% the response of a 20 year old. No effect of menstrual cycle phase on FEV_1 response. Inconsistent effect of social economic status on FEV_1 response.	Seal et al. (1996)

Table AX6-5 (cont'd). Age Differences in Pulmonary Function Responses to Ozone[a]

Ozone Concentration[b]		Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
ppm	µg/m³						
0.18 0.24 0.30 0.40	353 470 588 784	2.33 h IE $\dot{V}_E$ = 20 L/min/m² BSA	NA	48 WF, 55 BF	Healthy NS, 18 to 35 years old, black and white	Older women had smaller changes in FEV_1 than younger women. No age- related differences in SRaw or cough score.	Seal et al. (1993)
0.45	882	1 h, CE $\dot{V}_E \approx$ 26 L/min 2 h, IE $\dot{V}_E \approx$ 26 L/min	≈23 °C 58% RH cycle/treadmill	7 M 5 F	Healthy NS, 60 to 79 years old (all in 60s except one 79 years old)	Comparison of 1-h CE protocol and 2-h IE protocol indicated no difference between the changes in pulmonary function following the two protocols.	Drechsler-Parks et al. (1990)
0.45	882	2 h, IE (20' ex/20' rest) Male: $\dot{V}_E$ = 28.5 L/min Female: $\dot{V}_E$ = 26.1 L/min	23 °C 46% RH cycle/treadmill	10 M, 6 F	Healthy NS, 60 to 89 years old	Mean decrement in FEV_1 = 5.7%; eight subjects had a 5% or greater difference between their response to O_3 and FA, and the other eight had less than a 5% difference between their responses to FA and 0.45 ppm O_3.	Bedi et al. (1989)
0.45	882	2 h, IE (20' ex/20' rest) $\dot{V}_E \approx$ 26 L/min	≈24 °C 63% RH cycle	8 M 8 F	Healthy NS, 51 to 69 years old Healthy NS, 56 to 76 years old	13 subjects had decrements in FEV_1 on three separate exposures to 0.45 ppm within 5% of their mean response to the three exposures. The other three subjects were not reproducible. Symptom reports did not correlate well with pulmonary function changes.	Bedi et al. (1988)
0.12	235	1 h IE (mouthpiece) $\dot{V}_E$ = 4 to 5 × resting	22 °C 75% RH treadmill	5 M, 7 F	Healthy NS, 12 to 17 years old	No significant changes in any pulmonary function in healthy subjects.	Koenig et al. (1988)
0.20 0.30	392 588	1 h (mouthpiece) 50' rest/10' ex for first 7 males, 20' rest/10' ex for remaining subjects Male: $\dot{V}_E \approx$ 29 L/min Female: $\dot{V}_E \approx$ 23 L/min	≈22 °C ≥ 75% RH treadmill	9 M, 10 F	Healthy NS, 55 to 74 years old	No spirometic changes for either group. Females had 13% increase in R_T at 3 and 22 min after 0.30-ppm exposure.	Reisenauer et al. (1988)
0.113[c] + other ambient pollutants	221	1 h CE (bicycle) $\dot{V}_E \approx$ 22 L/min	32.7 °C ≈43% RH cycle	33 M, 33 F	NS for both groups, mean age = 9.4 years old	No differences in responses of boys and girls. Similar decrements (< 5% on average) following both purified air and ambient air (O_3 at 0.11 ppm) exposures.	Avol et al. (1987)

Table AX6-5 (cont'd). Age Differences in Pulmonary Function Responses to Ozone[a]

Ozone Concentration[b]		Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
ppm	µg/m³						
0.45	882	2 h, IE (20' ex/20' rest) $\dot{V}_E \approx 26$ L/min	≈23 °C 53% RH cycle	8 M, 8 F	Healthy NS, 51 to 76 years old	Mean decrement in $FEV_1 = 5.6 \pm 13\%$; range of decrements = 0 to 12%.	Drechsler-Parks et al. (1987a,b)
0.12	235	40 min (mouthpiece) IE, 10 min exercise at $\dot{V}_E = 32.6$ L/min	NA treadmill	3 M, 7 F	Healthy NS, 14 to 19 years old	No significant change in FEV_1; increased R_T with exposure to 0.18 ppm O_3. Some subjects responded to 5 to 10 mg/mL methacholine after 0.18-ppm O_3 exposure, whereas none responded to 25 mg/mL methacholine at baseline bronchochallenge.	Koenig et al. (1987)
0.18	353	40 min (mouthpiece) IE, 10 min exercise at $\dot{V}_E = 41.3$ L/min		4 M, 6 F			

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.
[c]Ozone concentration is the mean of a range of ambient concentrations.

significantly different from the response in the healthy subjects. The authors have concluded that typical ambient concentrations of O_3 are unlikely to induce "a clinically significant acute lung dysfunction" in exposed older men. However, they also acknowledged that the "worst case" scenario of O_3 exposure used in their study causes acute spirometric responses.

Although Gong et al. (1997a) and others (see Table 6-5) have examined responses to O_3 exposure in subjects of various ages, the exposure conditions differ between most studies so that age effects remain uncertain. Three recent studies, which analyzed large data sets ($\geq$ 240 subjects) of similarly exposed subjects, show clearly discernable changes in FEV_1 responses to O_3 as a function of age.

Seal et al. (1996) analyzed O_3-induced spirometric responses in 371 young nonsmokers (18 to 35 years of age). The subject population was approximately 25% white males, 25% white females, 25% black males, and 25% black females. Each subject was exposed once to 0.0, 0.12, 0.18, 0.24, 0.30, or 0.40 ppm ozone for 2.3 h during IE at a $\dot{V}_E$ of 25 L/min/m^2 BSA. A logistic function was used to model and test the significance of age, socioeconomic status (SES), and menstrual cycle phase as predictors of FEV_1 response to O_3 exposure. Menstrual cycle phase was not a significant. SES was inconsistent with the greatest response observed in the medium SES and the lowest response in high SES. FEV_1 responses decreased with subject age. On average, regardless of the O_3 concentration, the response of 25, 30, and 35 year old individuals are predicted to be 83, 65, and 48% (respectively) of the response in 20 year olds. For example, in 20 year old exposed to 0.12 ppm ozone (2.3 h IE, $\dot{V}_E$ = 25 L/min/m^2 BSA) a 5.4% decrement in FEV_1 is predicted, whereas, a similarly exposed 35 yr old is only predicted to have a 2.6% decrement. The Seal et al. (1996) model is limited to predicting FEV_1 responses immediately postexposure in individuals exposed for 2.3 h during IE at a $\dot{V}_E$ of 25 L/min/m^2 BSA.

McDonnell et al. (1997) examined FEV_1 responses in 485 healthy white males (18 to 36 years of age) exposed once for 2 h to an O_3 concentration of 0.0, 0.12, 0.18, 0.24, 0.30, or 0.40 ppm at rest or one of two levels of IE ($\dot{V}_E$ of 25 and 35 L/min/m^2 BSA). FEV_1 was measured preexposure, after 1 h of exposure, and immediately postexposure. Decrements in FEV_1 were modeled by sigmoid-shaped curve as a function of subject age, O_3 concentration, $\dot{V}_E$, and duration of exposure. Regardless of the O_3 concentration or duration of exposure, the average responses of 25, 30, and 35 year old individuals are predicted to be 69, 48, and 33%

(respectively) of the response in 20 year olds. The McDonnell et al. (1997) model is best suited to predicting FEV_1 responses in while males exposed to O_3 for 2 h or less under IE conditions.

Hazucha et al. (2003) analyzed the distribution of O_3 responsiveness in subjects (146 M, 94 F) between 18 and 60 years of age. Subjects were exposed to 0.42 ppm O_3 for 1.5 h with IE at $\dot{V}_E$ = 20 L/min/m^2 BSA. Figure AX6-7 illustrates FEV_1 responses to O_3 exposure as a function of subject age. Consistent with the discussion in Section 6.4, a large degree of intersubject variability is evident in Figure AX6-7. Across all ages, 18% of subjects were weak responders (≤5% FEV_1 decrement), 39% were moderate responders, and 43% were strong responders (≥15% FEV_1 decrement). Younger subjects (≤35 years of age) were predominately strong responders, whereas, older subjects (>35 years of age) were mainly weak responders. In males, the FEV_1 responses of 25, 35, and 50 year olds are predicted to be 94, 83, and 50% (respectively) of the average response in 20 year olds. In females, the FEV_1 responses of 25, 35, and 50 year olds are predicted to be 82, 46, and 18% (respectively) of the average response in 20 year olds. The Hazucha et al. (1996) model is limited to predicting FEV_1 responses immediately postexposure in individuals exposed to 0.42 ppm O_3 for 1.5 h during IE at a $\dot{V}_E$ of 20 L/min/m^2 BSA.

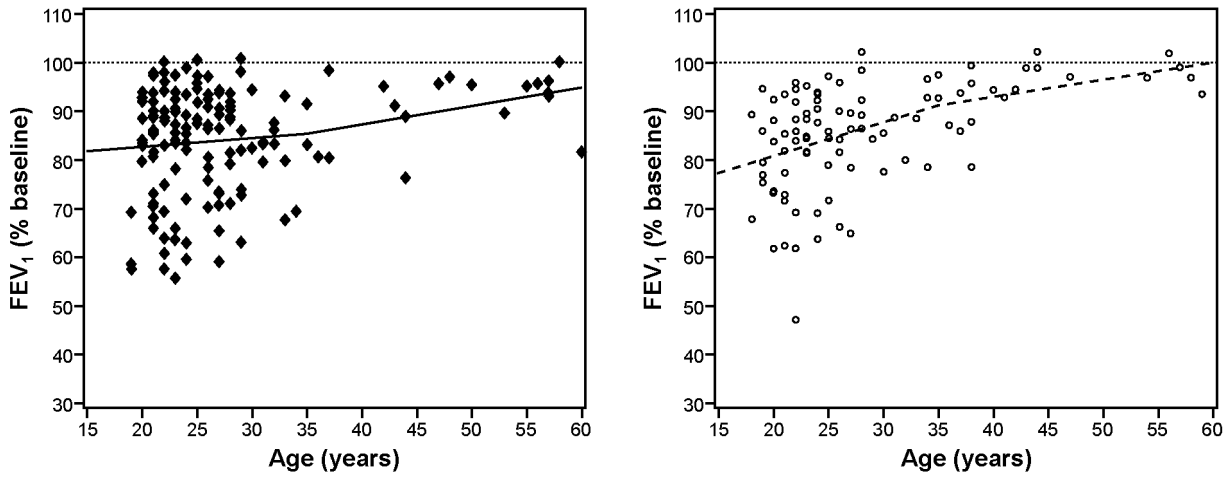

Figure AX6-7. Effect of O_3 exposure (0.42 ppm for 1.5 h with IE) on FEV_1 as a function of subject age. Left panel data for males (n = 146; 19 to 60 yrs old), right panel data for females (n = 94; 18 to 59 yrs old).

Source: Adapted from Hazucha et al. (2003).

The pathophysiologic mechanisms behind the pronounced age-dependent, gender-differential rate of loss of O_3 responsiveness are unclear. Passannante et al. (1998) have previously demonstrated that O_3-induced spirometric decrements (FEV_1) in healthy young and middle-aged adults are principally neural in origin, involving opioid-modulated sensory bronchial C-fibers. (*The methodological details of this study are presented in Section AX6.2.3 of this chapter*.) The peripheral afferents are most likely the primary site of action, which would be compatible with a reflex action as well as a cortical mechanism. The pattern of progressive decline, as well as the subsequent rate of recovery of spirometric lung function, suggest involvement of both direct and indirect (possibly by $PGE_{2\alpha}$) stimulation and/or sensitization of vagal sensory fibers. (*For details, see Section AX6.2.3.1 of this chapter*.)

The additional pulmonary function data published since the release of last O_3 criteria document (U.S. Environmental Protection Agency, 1996) and reviewed in this section reinforce the conclusions reached in that document. Children and adolescents are not more responsive to O_3 than young adults when exposed under controlled laboratory conditions. However, they are more responsive than middle-aged and older individuals. Young individuals between the age of 18 and 25 years appear to be the most sensitive to O_3. With progressing age, the sensitivity to O_3 declines and at an older age (> 60 yrs) appears to be minimal except for some very responsive individuals. Endpoints other than FEV_1 may show a different age-related pattern of responsiveness.

AX6.5.2 Gender and Hormonal Influences

The few late 1970 and early 1980 studies specifically designed to determine symptomatic and lung function responses of females to O_3 were inconsistent. Some studies have concluded that females might be more sensitive to O_3 than males, while others found no gender differences (U.S. Environmental Protection Agency, 1996). During the subsequent decade, seven studies designed to systematically explore gender-based differences in lung function following O_3 exposure were completed (Table AX6-6). Protocols included mouthpiece and chamber exposures, young and old individuals, normalization of ventilation to BSA or FVC, continuous and intermittent exercise, control for menstrual cycle phase, and the use of equivalent effective dose of O_3 during exposures. These studies have generally reported no statistically significant differences in pulmonary function between males and females (Adams et al., 1987; Drechsler-

Table AX6-6. Gender and Hormonal Differences in Pulmonary Function Responses to Ozone[a]

Ozone Concentration[b]		Exposure Duration and Activity	Exposure Conditions[c]	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
ppm	µg/m^3						
0.0 0.25	490	1 h CE $\dot{V}_E$ = 30 L/min	NA Face mask exposure	32 M, 28 F	Healthy NS 22.6 ± 0.6 years old	Mean O_3-induced FEV_1 decrements of 15.9% in males and 9.4% in females (gender differences not significant). FEV_1 decrements ranged from −4 to 56%; decrements >15% in 20 subjects and >40% in 4 subjects. Uptake of O_3 greater in males than females, but uptake not correlated with spirometric responses.	Ultman et al. (2004)
0.40 0.42	784 823	2 h, IE (15' ex/15' rest) $\dot{V}_E$ = 33-45 L/min 1.5 h IE (20' ex/10' rest) $\dot{V}_E$ = 33-45 L/min	22 °C 40% RH treadmill	146 M 94 F	Healthy NS, 18 to 60 years old	No significant gender differences in FEV_1 among young (< 35 years) and older individuals. Strong responses are less common over the age of 35 years, especially in women.	Hazucha et al. (2003)
0.0 0.35	0 686	1.25 h, IE (30' ex/15' rest/30' ex) $\dot{V}_E$ = 40 L/min	22 °C 40% RH treadmill	19 F	O_3 responders 22.1 ± 2.7 years old	FVC and FIVC changes about the same, −13%, FEV_1 −20%. Increased airway responsiveness to methacholine. Persistence of small effects on both inspired and expired spirometry past 18 h. Chemoreceptors not activated but ventilatory drive was accelerated.	Folinsbee and Hazucha (2000)
0.0 0.4	0 784	2 h, IE (15' ex/15' rest) $\dot{V}_E \approx$ 18 L/min/m^2 BSA 2 exposures: 25% of subj. exposed to air-air, 75% exposed to O_3-O_3	21 °C 40% RH treadmill	28 M 34 F	Healthy NS, 20-59 years old	Significant decrements in spirometric lung function. No significant differences in FEV_1 between young females and males and older females and males either in responders or non-responders subgroups.	Passannante et al. (1998)
0 0.12 0.24 0.30 0.40	0 235 470 588 784	2.33 h IE (15' ex/15' rest) $\dot{V}_E$ = 20 L/min/m^2 BSA one exposure per subject	22 °C 40% RH treadmill	48 WF, 55 BF	Healthy NS, 18 to 35 years old	Significant menstrual cycle phase × race interaction for FEV_1. No significant menstrual cycle phase effect when blacks and whites were analyzed separately. No significant menstrual phase effects for SRaw or cough score.	Seal et al. (1996)
0.0 0.35	0 686	2.15 h, IE (30' ex/30' rest)	19-24 °C 48-55% RH treadmill	12 M 12 F	Healthy NS, 5 F follicular and 7 luteal phase exposure, regular menstrual cycles, 18 to 35 years old	Changes in FVC, FEV_1, FEF_{25-75}, $\dot{V}_{max50\%}$, and $\dot{V}_{max25\%}$ were similar during both the follicular and luteal phases. No significant difference between males and females.	Weinmann et al. (1995)

Table AX6-6 (cont'd). Gender and Hormonal Differences in Pulmonary Function Responses to Ozone[a]

Ozone Concentration[b]		Exposure Duration and Activity	Exposure Conditions[c]	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
ppm	µg/m^3						
0.3	588	1 h CE $\dot{V}_E \approx 50$ L/min	NA	9 F	Healthy NS, regular menstrual cycles, 20 to 34 years old	FEV_1 decreased 13.1% during the mid-luteal phase and 18.1% during the follicular phase. Decrement in FEF_{25-75} was significantly larger during the follicular phase than the mid-luteal phase. Changes in FVC were similar in both phases.	Fox et al. (1993)
0 0.12 0.18 0.24 0.30 0.40	0 235 353 470 588 784	2.33 h (15' ex/15' rest) $\dot{V}_E = 25$ L/min/m^2 BSA (one exposure/subject)	22 °C 40% RH treadmill	30 to 33 F and 30 to 33 M in each concentration group; total of 372 individuals participated	Healthy NS, 18 to 35 years old, blacks and whites	Decrements in FEV_1, increases in SRaw and cough, correlated with O_3 concentration. There were no significant differences between the responses of males and females.	Seal et al. (1993)
0 0.18 0.30	0 353 588	1 h (mouthpiece), CE $\dot{V}_E \approx 47$ L/min exposures ≥ 4 days apart	21 to 25 °C 45 to 60% RH cycle	14 F 14 F	FVC = 5.11 ± 0.53 L, NS, 20 to 24 years old FVC = 3.74 ± 0.30 L, NS, 19 to 23 years old	Small lung group, FVC = 3.74 ± 0.30 L. Large lung group, FVC = 5.11 ± 0.53 L. Significant concentration-response effect on FVC and FEV_1; lung size had no effect on percentage decrements in FVC or FEV_1.	Messineo and Adams (1990)
0.0 0.45	0 882	2 h, IE (20' ex/20' rest) $\dot{V}_E = 28.5$ L/min for M $\dot{V}_E = 26.1$ L/min for F repeated O_3 exposures	23.1 °C 46.1% RH cycle/treadmill	10 M 6 F	Healthy NS, 60 to 89 years old Healthy NS, 64 to 71 years old	Mean decrement in FEV_1 = 5.7%. Decrements in FVC and FEV_1 were the only pulmonary functions significantly altered by O_3 exposure. No significant differences between responses of men and women.	Bedi et al. (1989)
0 0.20 0.30	0 392 588	1 h (mouthpiece) IE (50' rest/10' ex first 7 M) (20' rest/10' ex all others) $\dot{V}_E \approx 28$ L/min for M $\dot{V}_E \approx 23$ L/min for F	≈22 °C ≥ 75% RH treadmill	9 M, 10 F	Healthy NS, 55 to 74 years old	No change in any spirometic measure for either group. Females had 13% increase in R_T after 0.30-ppm exposure. Gender differences not evaluated.	Reisenauer et al. (1988)
0.3	588	1 h (mouthpiece), CE $\dot{V}_E \approx 70$ L/min for men $\dot{V}_E \approx 50$ L/min for women	21 to 25 °C 45 to 60% RH cycle	20 M 20 F	NS, 18 to 30 years old NS, 19 to 25 years old	Significant decrements in FVC, FEV_1, and FEF_{25-75} following O_3 exposure. No significant differences between men and women for spirometry or SRaw.	Adams et al. (1987)

Table AX6-6 (cont'd). Gender and Hormonal Differences in Pulmonary Function Responses to Ozone[a]

Ozone Concentration[b]		Exposure Duration and Activity	Exposure Conditions[c]	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
ppm	µg/m^3						
0.0 0.45	0 882	2 h, IE (20' rest/ 20' ex) $\dot{V}_E \approx 27.9$ L/min for M $\dot{V}_E \approx 25.4$ L/min for F repeated O_3 exposures	24 °C 58% RH cycle	8 M 8 F	Healthy NS, 51 to 69 years old Healthy NS, 56 to 76 years old	Range of responses in FEV_1: 0 to −12% (mean = −5.6%). No significant difference in responses of men and women. Tendency for women to have greater effects.	Drechsler-Parks et al. (1987a,b)
0.48	941	2 h, IE $\dot{V}_E \approx 25$ L/min	21 °C (WBGT) cycle	10 F	Healthy NS, 19 to 36 years old	Mean decrement in FEV_1 = 22.4%. Significant decrements in all spirometric measurements. Results not significantly different from a similar study on males (Drechsler-Parks et al., 1984).	Horvath et al. (1986)

[a] See Appendix A for abbreviations and acronyms.
[b] Listed from lowest to highest O_3 concentration.
[c] WBGT = 0.7 $T_{wet\ bulb}$ + 0.3 $T_{dry\ bulb\ or\ globe}$.

Parks, et al., 1987a; Messineo and Adams, 1990; Seal et al., 1993; Weinmann et al., 1995) although in some studies females appeared to experience a slightly greater decline then males (Drechsler-Parks et al., 1987a; Messineo and Adams, 1990). The comparative evaluations were based on responses that included spirometry, airway resistance, non-specific bronchial responsiveness (NSBR) determinations, and changes in frequency and severity of respiratory symptoms. However, depending on how the O_3 dose was calculated and normalized, the findings of at least three studies may be interpreted as showing that females are more sensitive to O_3 than males. The findings of the seven studies are presented in detail in Section 7.2.1.3 of the previous O_3 criteria document (U.S. Environmental Protection Agency, 1996).

Some support for a possible increased sensitivity of females to O_3 comes from a study of uric acid concentration in nasal lavage fluid (NLF). Housley et al. (1996) found that the NLF of females contains smaller amounts of uric acid than the NLF of males. The primary source of uric acid is plasma; therefore, lower nasal concentrations would reflect lower plasma concentrations of this antioxidant. The authors have speculated that in females, both lower plasma and NLF levels (of uric acid) can plausibly make them more susceptible to oxidant injury, since local antioxidant protection may not be as effective as with higher levels of uric acid, and consequently more free O_3 can penetrate deeper into the lung.

Several studies also have suggested that anatomical differences in the lung size and the airways between males and females, and subsequent differences in O_3 distribution and absorption, may influence O_3 sensitivity and potentially differential O_3 response. The study of Messinio and Adams (1990) have, however, convincingly demonstrated that the effective dose to the lung, and not the lung size, determines the magnitude of (FEV_1) response. Furthermore, the O_3 dosimetry experiments of Bush et al. (1996) have shown that despite gender differences in longitudinal distribution of O_3, the absorption distribution in conducting airways was the same for both sexes when expressed as a ratio of penetration to anatomic dead space volume. This implies that gender differences, if any, are not due to differences in (normal) lung anatomy. The data also have shown that routine adjustment of O_3 dose for body size and gender differences would be more important if normalized to anatomic dead space rather than the usual FVC or BSA.

One of the secondary objectives of a study designed to examine the role of neural mechanisms involved in limiting maximal inspiration following O_3 exposure has been to

determine if gender differences occur. A group of healthy males (n = 28) and females (n = 34) were exposed to 0.42 ppm O_3 for 2 h with IE. The methodological details of the study are presented in Section AX6.2.5.1 of this document. As Figure AX6-4 shows, the differences between males and females were, at any condition, measurement point, and O_3 sensitivity status only minimal and not significant (Passannante et al., 1998).

In another investigation, Folinsbee and Hazucha (2000) exposed a group of 19 O_3-responsive young females (average age of 22 years, prescreened for O_3 responsiveness by earlier exposure) to air and 0.35 ppm O_3. The randomized 75-min exposures included two 30-min exercise periods at a $\dot{V}_E$ of 40 L/min. In addition to standard pulmonary function tests, they employed several techniques used for the first time in human air pollution studies assessment of O_3 effects. The average lung function decline from a pre-exposure value was 13% for FVC, 19.9 % for FEV_1 , and 30% for FEF_{25-75}. The infrequently measured forced inspiratory vital capacity (FIVC) was the same as FVC suggesting that the lung volume limiting mechanisms are the same. The reduction in peak inspiratory flow (PIF) most likely reflects an overall reduction in inspiratory effort associated with neurally mediated inhibition of inspiration. Persistence of small inspiratory and expiratory spirometric effects, airway resistance, and airway responsiveness to methacholine for up to 18 h postexposure suggests that recovery of pulmonary function after O_3 exposure involves more than the simple removal of an irritant. Incomplete repair of damaged epithelium and still unresolved airway inflammation are the likely causes of the residual effects that in some individuals persisted beyond 24 h postexposure. However, by 42 hours no residual effects were detected. No significant changes were found in ventilatory response to CO_2 between air and O_3 exposures, suggesting that chemoreceptors were not affected by O_3. However, O_3 inhalation did result in accelerated timing of breathing and a modest increase in inspiratory drive. These observations are consistent with, and further supportive of, the primary mechanisms of O_3-induced reduction in inspiratory lung function, namely an inhibition of inspiration elicited by stimulation of the C-fibers and other pulmonary receptors. Because the measures of inspiratory and chemical drive to assess O_3 effects were not reported in any previous human study, no comparisons are possible. Because no male subjects were recruited for the study, it is not possible to compare gender effects. Despite being O_3-responsive, however, the average post-O_3 decline in expiratory lung function from preexposure (13% for FVC; 19.9% for FEV_1; 30% for FEF_{25-75}) was similar to that seen in female cohorts studied by

other investigators under similar conditions of exposure. These were the same studies that found no gender differences in O_3 sensitivity (Adams et al., 1987; Messineo and Adams, 1990).

The study by Hazucha et al. (2003), discussed in the previous section, has in addition to aging also examined gender differences in O_3 responsiveness. The male (n = 146) and female (n = 94) cohorts were classified into young (19 to 35 year-old) and middle-aged (35 to 60 year-old) groups. This classification was selected in order to facilitate comparison with data reported previously by other laboratories. Using a linear regression spline model (with a break point at 35 years), the authors reported that the rate of loss of sensitivity is about three times as high in young females as in young males ($p< 0.003$). In young females, the average estimated decline in FEV_1 response is 0.71% per year, while in young males it is 0.19% per year. Middle-aged groups of both genders show about the same rate of decline (0.36 to 0.39%, respectively). At 60 years of age, the model estimates about a 5% post-O_3 exposure decline in FEV_1 for males, but only a 1.3% decline for females. These observations suggest that young females lose O_3 sensitivity faster than young males, but by middle age, the rate is about the same for both genders. Descriptive statistics show that there were practically no differences in the mean value, standard error of the mean, and coefficient of variation for % FEV_1 decrement between the group of young males (n = 125; 83.7 ± 1.1%; CV = 13.5%) and young females (n = 73; 83.4 ± 1.25%; CV = 12.8%). A straight linear regression model of these data was illustrated in Figure AX6-7. The slopes, significant in both males ($r = 0.242$; $p = 0.003$) and females ($r = 0.488$; $p = 0.001$), represent the decline in responsiveness of 0.29% and 0.55% per year respectively, as assessed by FEV_1.

Two earlier studies of the effects of the menstrual cycle phase on O_3 responsiveness have reported conflicting results (U.S. Environmental Protection Agency, 1996). Weinmann et al. (1995) found no significant lung function effects related to menstrual cycle, although during the luteal phase the effects were slightly more pronounced than during the follicular phase; while Fox et al., (1993) reported that follicular phase enhanced O_3 responsiveness. In a more recent investigation of possible modulatory effects of hormonal changes during menstrual cycle on O_3 response, young women (n = 150) 18 to 35 years old were exposed once to one of multiple O_3 concentrations (0.0, 0.12, 0.18, 0.24, 0.30, 0.40 ppm) for 140 min with IE at 35 L/min/m^2 BSA. The women's menstrual cycle phase was determined immediately prior to O_3 exposure. Post-O_3, no significant differences in % predicted FEV_1 changes that could be related to the menstrual

cycle phase were found. Admittedly, a less precise method of determining menstrual cycle phase used in this study could have weakened the statistical power. Unfortunately, the direction and magnitude of O_3 response as related to the menstrual cycle phases were not reported (Seal et al., 1996). Considering the inconclusiveness of findings of this study and the inconsistency of results between the two earlier studies, it is not possible to make any firm conclusions about the influence of the menstrual cycle on responses to O_3 exposure.

Additional studies presented in this section clarify an open-ended conclusion reached in the previous O_3 criteria document (U.S Environmental Protection Agency, 1996) regarding the influence of age on O_3 responsiveness. Healthy young males and females are about equally responsive to O_3, although the rate of loss of sensitivity is higher in females than in males. Middle-aged men and women are generally much less responsive to O_3 than younger individuals. Within this range, males appear to be slightly more responsive than females, but the rate of age-related loss in FEV_1 is about the same. The O_3 sensitivity may vary during the menstrual cycle; however, this variability appears to be minimal.

AX6.5.3 Racial, Ethnic and Socioeconomic Status Factors

In the only laboratory study designed to compare spirometric responses of whites and blacks exposed to a range of O_3 concentrations (0 to 0.4 ppm), Seal et al. (1993) reported inconsistent and statistically insignificant FEV_1 differences between white and black males and females within various exposure levels. Perhaps, with larger cohorts the tendency for greater responses of black than white males may become significant. Thus, based on this study it is still unclear if race is a modifier of O_3 sensitivity, although the findings of epidemiologic studies reported in the previous criteria document "can be considered suggestive of an ethnic difference" (U.S. Environmental Protection Agency, 1996). However, as Gwynn and Thurston (2001) pointed out, it appears that it is more the socioeconomic status (SES) and overall quality of healthcare that drives PM_{10}- and O_3-related hospital admissions than an innate or acquired sensitivity to pollutants.

This assertion is somewhat supported by the study of Seal et al. (1996) who employed a family history questionnaire to examine the influence of SES on the O_3 responsiveness of 352 healthy, 18- to 35-year-old black and white subjects. Each subject was exposed once under controlled laboratory conditions to either air or 0.12, 0.18, 0.24, 0.30, 0.40 ppm O_3 for 140 min

with 15 min IE at 35 L/min/m^2 BSA. An answer to the "Education of the father" question was selected as a surrogate variable for SES status. No other qualifying indices of SES were used or potential bial examined. Of the three SES categories, individuals in the middle SES category showed greater concentration-dependent decline in % predicted FEV_1 (4-5% @ 0.4 ppm O_3) than low and high SES groups. The authors did not have an "immediately clear" explanation for this finding. The SES to %predicted FEV_1 relationship by gender-race group was apparently examined as well; however, these results were not presented. Perhaps a more comprehensive and quantitative evaluation of SES status would have identified the key factors and clarified the interpretation of these findings. With such a paucity of data it is not possible to discern the influence of racial or other related factors on O_3 sensitivity.

AX6.5.4 Influence of Physical Activity

Apart from the importance of increased minute ventilation on the inhaled dose of O_3 during increased physical activity, including work, recreational exercise, and more structured exercise like sports, no systematic effort has been made to study other potential physical factors that may modulate O_3 response. The typical physiologic response of the body to exercise is to increase both the rate and depth of breathing, as well as increase other responses such as heart rate, blood pressure, oxygen uptake, and lung diffusion capacity.

Physical activity increases minute ventilation in proportion to work load. At rest, and during light exercise, the dominant route of breathing is through the nose. The nose not only humidifies air, among other physiologic functions, but also absorbs O_3 thus decreasing the overall dose. As the intensity of exercise increases, the minute ventilation increases and the breathing switches from nasal to oronasal mode. There is considerable individual variation in the onset of oronasal breathing, which ranges from 24 to 46 L/min (Niinimaa et al., 1980). During heavy exercise, ventilation is dominated by oral breathing. Consequently, the residence time of inhaled air in the nose and the airways is shorter, reducing the uptake of O_3 (Kabel et al., 1994). Moreover, increasing inspiratory flow and tidal volume shifts the longitudinal distribution of O_3 to the peripheral airways, which are more sensitive to injury than the larger, proximal airways. Ozone uptake studies of human lung showed that at simulated quiet breathing, 50% of O_3 was absorbed in the upper airways, 50% in the conducting airways, and

none reached the small airways (Hu et al. 1994). With ventilation simulating heavy exercise (60 L/min), the respective O_3 uptakes were 10% (upper airways), 65% (conducting airways), and 25% (small airways). These observations imply that equal O_3 dose ($C \times T \times \dot{V}_E$) will have a greater effect on pulmonary function and inflammatory responses when inhaled during heavy physical activity than when inhaled during lighter activity. Although, Ultman et al. (2004) recently reported that spirometric response are not correlated with O_3 uptake. (*See Chapter 4 of this document for more information on the dosimetry of O_3.*)

Other physiologic factors activated in response to physical activity are unlikely to have as much impact on O_3 responsiveness as does minute ventilation; however, their potential influence has not been investigated.

AX6.5.5 Environmental Factors

Since the 1996 O_3 criteria document not a single human laboratory study has examined the potential influence of environmental factors such as rural versus urban environment, passive cigarette smoke exposure, and bioactive admixtures such as endotoxin on healthy individual's pulmonary function changes due to O_3 (U.S. Environmental Protection Agency, 1996).

Some of the unresolved issues, e.g., health effects of ETS and O_3 interaction, which need to be examined in human studies were explored very recently in laboratory animal studies (presented in a greater detail in Chapter 5). In one study on mice, preexposure of animals to sidestream cigarette smoke (ETS surrogate), which elicited no immediate effects, resulted in a potentiation of subsequent O_3-induced inflammatory response. This finding suggests that typical adverse effects of ETS do not necessarily have to elicit an immediate response to ETS, but may in fact potentiate the effects of a subsequent exposure to another pollutant like O_3 (Yu et al., 2002). The key mechanism by which smoke inhalation may potentiate subsequent oxidant injury appears to be damage to cell membranes and the resulting increase in epithelial permeability. Disruption of this protective layer may facilitate as well as accelerate injury to subepithelial structures when subsequently exposed to other pollutants (Bhalla, 2002). Although this may be a plausible mechanism in nonsmokers and acute smokers exposed to ETS and other pollutants, studies involving chronic smokers who most likely already have chronic airway inflammation do not seems to show exaggerated response with exposure to O_3.

More than 25 years ago, Hazucha et al. (1973) reported that the spirometric lung function of smokers declined significantly less than that of nonsmokers when exposed to 0.37 ppm O_3. The findings of this study have been confirmed and expanded (Table AX6-7). Frampton et al. (1997a) found that exposure of current smokers (n=34) and never smokers (n=56) to 0.22 ppm O_3 for 4 h with IE for 20 min of each 30 min period at 40 to 46 L/min, induced a substantially smaller decline in FVC, FEV_1 and SGaw of smokers than never smokers. Smokers also demonstrated a much narrower distribution of spirometric endpoints than never smokers. Similarly, nonspecific airway responsiveness to methacholine was decreased in smokers. However, both groups showed the consistency of response from exposure to exposure. It should be noted, that despite seemingly lesser response, the smokers were more symptomatic post air exposure than never smokers but the opposite was true for O_3 exposure. This would suggest that underlying chronic airway inflammation present in smokers has blunted stimulation of bronchial C-fibers and other pulmonary receptors, the receptors substantially responsible for post O_3 lung function decrements. In addition to desensitization, the other "protective" mechanisms active in smokers may be an increase in the mucus layer conferring not only a mechanical protection, but also acting as an O_3 scavenger. Another plausible explanation of a diminished responsiveness of smokers may be related to elevated levels of reduced glutathione (GSH), tissue antioxidant, found in epithelial lining fluid of chronic but not acute smokers (MacNee et al., 1996).

Despite some differences in a release of proinflammatory cytokines and subsequent recruitment of inflammatory cells, both smokers and nonsmokers developed airway inflammation following O_3 exposure. This was demonstrated by the Torres et al. (1997) study that involved exposures of about equal size cohorts of otherwise healthy young smokers, nonsmoker O_3 nonresponders (< 5% FEV_1 post O_3 decrement) and nonsmoker O_3 responders (> 15% FEV_1 post O_3 decrement) to air and two 0.22 ppm O_3 atmospheres for 4 hours, alternating 20 min of moderate exercise (25 L/min/m^2 BSA) with 10 min of rest. Both O_3 exposures were followed by nasal lavage (NL) and bronchoalveolar lavage (BAL) performed immediately post one of exposures and 18 hr later following the other exposure. Neither O_3 responsiveness nor smoking alters the magnitude or the time course of O_3-induced airway inflammation. The overall cell recovery was lower immediately post exposure but higher, particularly in nonsmokers, 18 h post O_3 exposure when compared to control (air) in all groups. Recovery of lymphocytes, PMNs and AMs in both alveolar and bronchial lavage fluid showed

Table AX6-7. Influence of Ethnic, Environmental, and Other Factors

Ozone Concentration		Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
ppm	µg/m^3						
0.0 0.4	0 780	2 h IE, 15' ex/15' rest $\dot{V}_E$ = 20 L/min/m^2 BSA	20°C 40% RH	15 M, 1 F 13 M, 2 F	Placebo group: healthy NS avg. age 27 yrs. Antiox. Suppl. Gr.: Healthy NS avg. age 27 yrs.	PF decrements in the supplementation group were signif. smaller for FVC ($p < 0.046$) and near significant for FEV_1 ($p < 0.055$). The inflammatory response (BAL) showed no significant differences between the two groups either in the recovery of cellular components or the concentrations and types of inflammatory cytokines.	Samet et al. (2001)
0.0 0.12	0 235	0.75 h IE, 15' ex/15' rest $\dot{V}_E$ = 40-46 L/min	60% RH	5 M, 12 F	Asthmatics sensitive to SO_2 19 to 38 yrs old	No significant differences due to O_3 between placebo and antioxidant supplement cohort in either PF or bronchial hyperresponsiveness to 0.1 ppm SO_2. The overall results interpreted as a demonstration of protective effect of antioxidants from O_3, particularly in "severe" asthmatics.	Trenga et al. (2001)
0.0 0.22 0.22	0 431 431	4 h IE, 20' ex/10' rest $\dot{V}_E$ = 40-46 L/min	21°C 37% RH	25 (M/F)	Healthy NS O_3 responders and nonresponders 18 to 40 yrs old	Glutathione peroxidase (GPx) activity and eGPx protein level were significantly ($p = 0.0001$) depleted in ELF for at least 18 h post-exp. In BAL both endpoints were elevated (ns). No association between cell injury, PF, or GPx activity.	Avissar et al. (2000)[b]
0.0 0.12-0.24[a]	0 235-470[a]	2.17 h IE, 10' ex/ 10' rest $\dot{V}_E$ = 36-39 L/min	22 °C or 30 °C 45-55% RH	5 M, 4 F	Healthy NS 24 to 32 yrs old	FEV_1 decreased signif. ($p < 0.5$) by ~8% at 22°C and ~ 6.5% at 30°C. 19 h post-exp decline of 2.3% still signif. ($p < 0.05$). SGaw signif. ($p < 0.05$) declined at 30°C but not at 22°C. The BHR assessed 19 h post-exp. as PC_{50} sGaw methacholine signif. ($p < 0.05$) higher at both temperatures.	Foster et al. (2000)

Table AX6-7 (cont'd). Influence of Ethnic, Environmental, and Other Factors

Ozone Concentration		Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
ppm	µg/m^3						
0.0 0.40	0 780	2 h IE, 20' ex/ 10' rest $\dot{V}_E$ = mild to mod.		6 M, 9 F	Healthy NS avg. age 31 yrs.	Corticosteroid pretreatment had no effects on post O_3 decline in PF, PMN response, and sputum cell count under both the placebo and treatment conditions. Methacholine PC_{20} FEV_1 was equally decreased in both cond. 4 h after exposure. No changes in exhaled NO and CO	Nightingale et al. (2000)
0.0 0.22	0 431	4 h IE 20' ex/10' rest $\dot{V}_E$ = 40-46 L/min	21°C 37% RH	90 M	56 never smokers 34 current smokers 18 to 40 yrs. old	Smokers are less responsive to O_3 as assessed by spirometric and plethysmographic variables. Neither age, gender, nor methacholine responsiveness were predictive of O_3 response.	Frampton et al. (1997a,c)[b]
0.0 0.22 0.22	0 431 431	4 h IE, 20' ex/10' rest $\dot{V}_E$ = 25 L/min/m^2 BSA	21°C 37% RH	10 M, 2 F 10 M, 3 F 11 M, 2 F	NS, O_3 nonresp., avg. age 25 yrs.; NS, O_3 resp., avg. age 25 yrs; smokers avg. age 28 yrs	Neither O_3 responsiveness nor smoking has altered the magnitude and the time course of O_3-induced airway inflammation. Inflammation involved all types of cells accessible by BAL. The recovery profile of these cells over time was very similar for all groups showing highest values 18 h postexposure.	Torres et al. (1997)[b] Frampton et al. (1997a,c)[b]

[a]Ramp exposure from 0.12 ppm to 0.24 ppm and back to 0.12 ppm at the end of exposure.
[b]Related studies, sharing of some subjects .

the largest increase in response to O_3 in all groups, with nonsmokers showing greater relative increases than smokers. Of the two cytokines, IL-6 and IL-8, IL-6 was substantially and significantly ($p < 0.0002$) elevated immediately post exposure but returned back to control 18 h later in all groups; but only nonsmokers' effects were significantly higher ($p < 0.024$). IL-8 showed a similar pattern of response but the increase in all groups, though still significant ($p < 0.0001$), was not as high as for IL-6. Between group differences were not significant. This inflammatory response involved all types of cells present in BAL fluid and the recovery profile of these cells over time was very similar for all groups. In contrast to BAL, NL did not prove to be a reliable marker of airway inflammation. The lack of association between lung function changes (spirometry) and airway inflammation for all three groups confirms similar observations reported from other laboratories. This divergence of mechanisms is further enhanced by an observation that a substantially different spirometric response between O_3 responders and nonresponders, the airway inflammatory response of the two groups was very similar, both in terms of magnitude and pattern (Torres et al., 1997).

The influence of ambient temperature on pulmonary effects induced by O_3 exposure in humans has been studied infrequently under controlled laboratory conditions. Several experimental human studies published more than 20 years ago reported additive effects of heat and O_3 exposure (see U.S. Environmental Protection Agency, 1986, 1996). In the study of Foster et al. (2000) 9 young (mean age 27 years) healthy subjects (4F/5M) were exposed for 130 min (IE 10 min @ 36 to 39 l/min) to filtered air and to ramp profile O_3 at 22° and 30 °C, 45-55% RH. The order of exposures was randomized. The O_3 exposure started at 0.12 ppm, reached the peak of 0.24 ppm mid-way through and subsequently declined to 0.12 ppm at the end of exposure. Ozone inhalation decreased V_T and increased f_B as compared to baseline at both temperatures. At the end of exposure FEV_1 decreased significantly ($p < 0.5$) by ~8% at 22°C and ~6.5% at 30 °C. One day (19 h) later, the decline of 2.3% from baseline was still significant ($p < 0.05$). FVC decrements were smaller and significant only at 22 °C immediately postexposure. SGaw significantly ($p < 0.05$) declined at 30°C but not at 22 °C. A day later, sGaw was elevated above the baseline for all conditions. The nonspecific bronchial responsiveness (NSBR) to methacloline assessed as PC_{50} sGaw was significantly ($p < 0.05$) higher one day following O_3 exposure at both temperatures but more so at 30 °C. Thus, these findings indicate that elevated temperature has partially attenuated spirometric response but

enhanced airway reactivity. Numerous studies have reported an increase in NSBR immediately after exposure to O_3. Whether the late NSBR reported in this study is a persistent residual effect of an earlier increase in airway responsiveness, or is a true one day lag effect cannot be determined from this study. Whatever the origin, however, a delayed increase in airway responsiveness raises a question of potentially increased susceptibility of an individual to respiratory impairment, particularly if the suggested mechanism of disrupted epithelial membrane holds true.

AX6.5.6 Oxidant-Antioxidant Balance

Oxidant-antioxidant balance has been considered as one of the determinants of O_3 responsiveness. Amateur cyclists who took antioxidant supplements (vitamins C, E,and β-carotene) for three months showed no decrements in spirometric lung function when cycling on days with high O_3 levels. In contrast, matched control group of cyclists not pretreated with vitamin supplements experienced an almost 2% decline in FVC and FEV_1 and > 5% reduction in PEF during the same activity period. Adjustment of data for confounders such as PM_{10} and NO_2 did not change the findings. Apparently, substantially elevated levels of plasma antioxidants may afford some protection against lung function impairment (Grievink et al.,1998, 1999).

Both laboratory animal and human studies have repeatedly demonstrated that antioxidant compounds present the first line of defense against the oxidative stress. Thus, upregulation of both enzymatic and nonenzymatic antioxidant systems is critical to airway epithelial protection from exposure to oxidants such as O_3 and NO_2 (*see Table AX6-7*). As an extension of an earlier study focused on pulmonary function changes (Frampton et al., 1997a), Avissar et al. (2000) hypothesized that concentration of glutathione peroxidase (GPx), one of the antioxidants in epithelial lining fluid (ELF), is related to O_3 and NO_2 responsiveness. They exposed healthy young nonsmokers (n=25), O_3-responders, and non-responders to filtered air and twice to 0.22 ppm O_3 for 4 h (IE, 20' ex /10' rest, @ $\dot{V}_E$ 40 to 46 L/min). In the NO_2 part of the study, subjects were exposed to air and twice to NO_2 (0.6 and 1.5 ppm) for 3 h, with IE of 10 min of each 30 min @ $\dot{V}_E$ of 40 L/min. Ozone exposure elicited a typical pulmonary function response with neutrophilic airway inflammation in both responders and non-responders. The GPx activity was significantly reduced ($p = 0.0001$) and eGPx protein significantly depleted ($p = 0.0001$) in epithelial lining fluid (ELF) for at least 18 h postexposure. In contrast, both GPx and eGPx were

slightly elevated in bronchoalveolar lavage fluid (BALF). However, neither of the two NO_2 exposures had a significant effect on pulmonary function, airway neutrophilia, epithelial permeability, GPx activity, or eGPx protein level in either ELF or BALF. The lack of a significant response to NO_2 has been attributed to the weak oxidative properties of this gas. No association has been observed between cell injury, assessed by ELF albumin, or pulmonary function and GPx activity for O_3 exposure. Thus, it is unclear what role antioxidants may have in modulation of O_3-induced lung function and inflammatory responses. The authors found a negative association between lower baseline eGPx protein concentration in ELF and post-O_3 neutrophilia to be an important predictor of O_3-induced inflammation; however, the causal relationship has not been established.

The effects of dietary antioxidant supplementation on O_3-induced pulmonary and inflammatory response of young healthy individuals has been investigated by Samet et al. (2001). Under controlled conditions, subjects received ascorbate restricted diet for three weeks. After the first week of prescribed diet, subjects were randomly assigned into two groups, and exposed to air (2 h, IE every 15 min at 20 L/min/m^2 BSA). Thereafter, one group received daily placebo pills and the other a daily supplement of ascorbate, α-tocopherol and a vegetable juice for the next two weeks. At the end of a two week period subjects were exposed to 0.4 ppm O_3 under otherwise similar conditions as in sham exposures. Serum concentration of antioxidants determined prior to O_3 exposure showed that subjects receiving supplements had substantially higher concentrations of ascorbate, tocopherol and carotenoid in blood than the control group. Plasma levels of glutathione and uric acid (cellular antioxidants) remained essentially the same. Ozone exposure reduced spirometric lung function in both groups; however, the average decrements in the supplementation group were smaller for FVC ($p = 0.046$) and FEV_1 ($p = 0.055$) when compared to the placebo group. There was no significant correlation between individual lung function changes and respective plasma levels of antioxidants. Individuals in both groups experienced typical post O_3 subjective symptoms of equal severity. Similarly, the inflammatory response as assessed by BALF showed no significant differences between the two groups either in the recovery of cellular components or the types and concentrations of inflammatory cytokines. Because of the complexity of protocol, the study was not designed as a cross-over type. However, it is unlikely that the fixed air-O_3 sequence of exposures influenced the findings in any substantial way. Although the study did not elucidate the protective

mechanisms, it has demonstrated the value of dietary antioxidants in attenuating lung function effects of O_3. This observation may appear to contradict the findings of Avissar's and colleagues study (2000) discussed above; however, neither study found association between lung function changes and glutathione levels. The lack of such association suggests that activation of antioxidant protective mechanisms is seemingly independent of mechanisms eliciting lung-function changes and that dietary antioxidants afford protection via a different pathway than tissue-dependent antioxidant enzymes. Moreover, the findings of this study have provided additional evidence that symptomatic, functional, inflammatory, and antioxidant responses are operating through substantially independent mechanisms.

Further evidence that the levels and activity of antioxidant enzymes in ELF may not be predictive or indicative of O_3-induced lung function or inflammatory effects has been provided by a study of Blomberg et al. (1999). No association was found between the respiratory tract lining fluid redox potential level, an indicator of antioxidants balance, and either spirometric or inflammatory changes induced by a moderate exposure of young individuals to O_3 (0.2 ppm/2 h, intermittent exercise at 20 L/min/m^2 BSA). However, O_3 exposure caused a partial depletion of antioxidants (uric acid, GSH, EC-SOD) in nasal ELF and a compensatory increase in plasma uric acid, affording at least some local protection (Mudway et al. 1999). More recently, Mudway et al. (2001) investigated the effect of baseline antioxidant levels on response to a 2-h exposure to 0.2 ppm O_3 in 15 asthmatic and 15 healthy subjects. In the BALF of 15 healthy subjects, significant O_3-induced reductions in ascorbate and increases in glutathione disulphide and EC-SOD were observed, whereas, levels were unaffected by O_3 exposure in the asthmatics. In both groups, BALF levels of uric acid and α-Tocopherol were unaffected by O_3.

Trenga et al. (2001) studied the potential protective effects of dietary antioxidants (500 mg vitamin C and 400 IU of vitamin E) on bronchial responsiveness of young to middle-aged asthmatics who were prescreened for their hyperreactivity to SO_2. Prior to the 1st exposure, subjects took either two supplements or two placebo pills at breakfast time for 4 weeks. They continued taking respective pills for another week when the 2nd exposure took place. The 45-min exposures to air and 0.12 ppm O_3 (15 min IE @ 3× resting $\dot{V}_E$) via mouthpiece were randomized. Each exposure was followed by two 10-min challenges to 0.10 and 0.25 ppm SO_2 with exercise to determine bronchial hyperresponsiveness. Due to variability of baseline lung function at different test sessions, and the way the data have been presented, it is difficult to

interpret the results. The authors have reported no significant differences due to O_3 between the placebo and supplemented cohort in either lung function or bronchial hyperresponsiveness to 0.1 ppm SO_2. However, post hoc classification of subjects into "mild" and "severe" asthmatics (based on responses to SO_2 during screening session) produced an unusual finding. In "severe" asthmatics, the challenge with 0.25 ppm SO_2 completely reversed O_3-induced whereas 0.1 ppm SO_2-enhanced decrements for PEF and FEF_{25-75}. The overall results of the study were interpreted by the investigators as a demonstration of the protective effect of antioxidants from O_3 exposure, particularly in "severe" asthmatics. It has been repeatedly demonstrated that pulmonary function response to O_3 is reflex in origin, involving stimulation of bronchial C-fibers (see Section AX6.2.5.1 for more information). With a relatively low O_3 dose used in this study the reflex response may be a dominant mechanism. Numerous animal and a few human studies used high concentration of SO_2 to "knock off" the lung receptors. It is plausible, therefore, that the reversal of O_3-induced spirometric decrements are due to a suppression of bronchial C-fibers activation and a subsequent reversal of a reflex response. Thus, the observed recovery of "severe" asthmatics following the second SO_2 challenge reported by Trenga and colleagues (2001) may not be related to antioxidant protection.

AX6.5.7 Genetic and Other Factors

It has been repeatedly postulated that genetic factors may play an important role in individual responsiveness to ozone. Recent studies (Bergamaschi et al., 2001; Corradi et al, 2002; Romieu et al, 2004) have indeed found that genetic polymorphisms of various enzymes, namely NAD(P)H:quinone oxidoreductase (NQO1) and glutathione-S-trasferase M1 (GSTM1), may play an important role in attenuating oxidative stress of airway epithelium. Bergamaschi and colleagues (2001) studied young nonsmokers (15 F, 9 M; mean age 28.5 years) who cycled for two hours on a cycling circuit in a city park on days with the average ozone concentration ranging from 32 to 103 ppb. There was no control study group nor the intensity of bicycling has been reported . Since spirometry was done within 30 min post-ride, it is difficult to gage how much of the statistically significant ($p = 0.026$) mean decrement of 160 ml in FEV_1 of 8/24 individuals with NQO1 wild type and GSTM1 null genotypes was due to ozone. Individuals with other genotype combinations including GSTM1 null had a mean post-ride decrement of FEV_1 of only 40 mL. The post-ride serum level of Clara cell protein (CC16), a biomarker of

airway permeability, has been elevated in both subgroups. Only a "susceptible" subgroup carrying NQO1 wild type in combination with GSTM1 null genotype, serum concentration of CC16 showed positive correlation with ambient concentration of ozone and negative correlation with FEV_1 changes. Despite some interesting observations, the study results should be interpreted cautiously.

Pretreatment of healthy young subjects with inhaled corticosteroids (2 × 800 µg/day budesonide, a maximal clinical dose) for 2 weeks prior to O_3 exposure (0.4 ppm/2 h, alternating 20 min exercise at 50W with 10 min rest) had no apparent effect on a typical lung function decline or inflammatory response to exposure. Because of the complexity of the protocol, the study was not a cross-over design and no control air exposures were conducted. Both the placebo and treatment conditions caused the same magnitude of changes. Similarly, nonspecific bronchial reactivity to methacholine (PC_{20} FEV_1) was increased about the same 4 h after exposure. Neither absolute nor relative sputum cell counts were affected by budesonide treatment and O_3 induced a typical neutrophilic response in both groups. Upregulation of pro-inflammatory mediators measured in sputum was not different between the groups either. The markers of inflammation and oxidative stress, exhaled NO and CO, as well as the reactive product nitrite measured in exhaled breath condensate, respectively, were not significantly influenced by budesonide. However, considering all these findings as a whole, budesonide seemed to have a moderating, although not statistically significant, effect on O_3-induced response (Nightingale et al., 2000). Budesonide is an antiinflammatory drug that in laboratory animal studies partially suppressed neutrophilic inflammation caused by O_3 (Stevens et al., 1994). Because the dose of budesonide was at therapeutic maximal levels, the pharmacologic action of this drug and the site of action of O_3 do not apparently coincide.

AX6.6 REPEATED EXPOSURES TO OZONE

Repeated daily exposure to O_3 in the laboratory for 4 or 5 days leads to attenuated changes in pulmonary function responses and symptoms (Hackney et al., 1977a; U.S. Environmental Protection Agency, 1986, 1996). A summary of studies investigating FEV_1 responses to repeated daily exposure for up to 5 days is given in Table AX6-8. The FEV_1 responses to repeated O_3 exposure typically have shown an increased response on the second exposure day

Table AX6-8. Changes in Forced Expiratory Volume in One Second After Repeated Daily Exposure to Ozone[a]

Ozone Concentration[b]		Exposure Duration and Activity[c]	Number and Gender of Subjects	Percent Change in FEV_1 on Consecutive Exposure Days					References[d]
ppm	µg/m³			First	Second	Third	Fourth	Fifth	
0.12	235	6.6 h, IE (40)	17 M	−12.79	−8.73	−2.54	−0.6	0.2	Folinsbee et al. (1994)
0.20	392	2 h, IE (30)	10 M	+1.4	+2.7	−1.6	—	—	Folinsbee et al. (1980)
0.20	392	2 h, IE (18 and 30)	8 M, 13 F	−3.0	−4.5	−1.1	—	—	Gliner et al. (1983)
0.20	392	2 h, IE (18 and 30)	9	−8.7	−10.1	−3.2	—	—	Gliner et al. (1983)
0.20	392	1 h, CE (60)	15 M	−5.02	−7.8	—	—	—	Brookes et al. (1989)
0.25	490	1 h, CE (63)	4 M, 2 F	−20.2	−34.8	—	—	—	Folinsbee and Horvath (1986)
			5 M, 2 F	−18.8	—	−22.3	—	—	
0.35	686	2 h, IE (30)	10 M	−5.3	−5.0	−2.2	—	—	Folinsbee et al. (1980)
0.35	686	1 h, CE (60)	8 M	−31.0	−41.0	−33.0	−25.0	—	Foxcroft and Adams (1986)
0.35	686	1 h, CE (60)	10 M	−16.1	−30.4	—	—	—	Schonfeld et al. (1989)
			10 M	−14.4	—	−20.6	—	—	
0.35	686	1 h, CE (60)	15 M	−15.9	−24.6	—	—	—	Brookes et al. (1989)
0.40	784	3 h, IE (4-5 × resting)	13 M[f]	−9.2	−10.8	−5.3	−0.7	−1.0	Kulle et al. (1982)
0.40	784	3 h, IE (4-5 × resting)	11 F[f]	−8.8	−12.9	−4.1	−3.0	−1.6	Kulle et al. (1982)
0.4	784	2 h, IE (65)	8 M	−18.0	−29.9	−21.1	−7.0	−4.4	Folinsbee et al. (1998)
0.4	784	3 h, IE (32)	8M, 2F[h]	−34.7	−31.1	−18.5	−12.0	−6.2	Gong et al. (1997b)
0.42	823	2 h, IE (30)	24 M	−21.1	−26.4	−18.0	−6.3	−2.3	Horvath et al. (1981)
0.45	882	2 h, IE (27)	1 M, 5 F	−13.3	—	−22.8	—	—	Bedi et al. (1985)
0.45	882	2 h, IE (27)	10 M, 6 F	−5.8	−5.6	−1.9	—	—	Bedi et al. (1989)
0.47	921	2 h, IE (3 × resting)	8M, 2F[g]	−11.4	−22.9	−11.9	−4.3	—	Linn et al. (1982)
0.5	980	2 h, IE (30)	8 M	−8.7	−16.5	−3.5	—	—	Folinsbee et al. (1980)
0.5	980	2.5 h, IE (2 × resting)	6	−2.7	−4.9	−2.4	−0.7	—	Hackney et al. (1977a)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.
[c]Exposure duration and intensity of IE or CE were variable; $\dot{V}_E$ (number in parentheses) given in liters per minute or as a multiple of resting ventilation.
[d]For a more complete discussion of these studies, see Table AX6-9 and U.S. Environmental Protection Agency (1986).
[e]Subjects were especially sensitive on prior exposure to 0.42 ppm O_3 as evidenced by a decrease in FEV_1 of more than 20%. These nine subjects are a subset of the total group of 21 individuals used in this study.
[f]Bronchial reactivity to a methacholine challenge also was studied.
[g]Seven subjects completed entire experiment.
[h]Subjects had mild asthma.

(Day 2) compared to the initial (Day 1) exposure response. This is readily apparent in repeated exposures to a range of concentrations from 0.4 to 0.5 ppm O_3 accompanied by moderate exercise (Folinsbee et al., 1980; Horvath et al., 1981; Linn et al., 1982), and at lower concentrations, 0.20 to 0.35 ppm, when accompanied by heavy exercise (Brookes et al., 1989; Folinsbee and Horvath, 1986; Foxcroft and Adams, 1986; Schonfeld et al., 1989). Mechanisms for enhanced pulmonary function responses on Day 2 have not been established, although persistence of acute O_3-induced damage for greater than 24 h may be important (Folinsbee et al., 1993). An enhanced Day 2 FEV_1 response was less obvious or absent in exposures at lower concentrations or those that caused relatively small group mean O_3-induced decrements. For example, Bedi et al. (1988) found no enhancement of the relatively small pulmonary function responses in older subjects (median age, 65 years) exposed repeatedly to O_3. Three reports (Bedi et al., 1985; Folinsbee and Horvath, 1986; Schonfeld et al., 1989) demonstrated that enhanced pulmonary function responsiveness was present within 12 h, lasted for at least 24 h and possibly 48 h, but was absent after 72 h.

After 3 to 5 days of consecutive daily exposures to O_3, FEV_1 responses are markedly diminished or absent. One study (Horvath et al., 1981) suggested that the rapidity of this decline in FEV_1 response was related to the magnitude of the subjects' initial responses to O_3 or their "sensitivity." A summary of studies examining the effects of repeated exposures to O_3 on FEV_1 and other pulmonary function, symptoms, and airway inflammation is given in Table AX6-9. Studies examining persistence of the attenuation of pulmonary function responses following 4 days of repeated exposure (Horvath et al., 1981; Kulle et al., 1982; Linn et al., 1982) indicate that attenuation is relatively short-lived, being partially reversed within 3 to 7 days and typically abolished within 1 to 2 weeks. Repeated exposures separated by 1 week (for up to 6 weeks) apparently do not induce attenuation of the pulmonary function response (Linn et al., 1982). Gong et al. (1997b) studied the effects of repeated exposure to 0.4 ppm O_3 in a group of mild asthmatics and observed a similar pattern of responses as those seen previously in healthy subjects. The attenuation of pulmonary responses reached after 5 days of consecutive O_3 exposure was partially lost at 4 and 7 days post exposure.

In addition to the significant attenuation or absence of pulmonary function responses after several consecutive daily O_3 exposures, symptoms of cough and chest discomfort usually associated with O_3 exposure generally are substantially reduced or absent (Folinsbee et al., 1980,

Table AX6-9. Pulmonary Function Effects with Repeated Exposures to Ozone[a]

Ozone Concentration[b]		Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
ppm	µg/m^3						
0.25	490	2 h IE, (30 min rest, 30 min exercise), $\dot{V}_E$ = 39 L/min	21.4 °C 43.9% RH 4 days consecutive FA exposure; 4 days consecutive O_3 exposure	5 M, 3 F	Healthy, NS	FVC and FEV_1 decrements were significantly attenuated on Day 4 of O_3 exposure compared to day 1 of O_3 exposure. Significant small airway function depression accompanied by significant reutrophilia in BALF one day following the end of O_3 exposure.	Frank et al. (2001)
0.2	392	4 h IE (4 × 30 min exercise), $\dot{V}_E$ = 14.8 L/min/m^2 BSA	1 day FA, 1 day, O_3; 4 days consecutive exposure to O_3	15 M, 8 F	Healthy, NS 21 to 35 years old	FEV_1 decrement and symptoms significantly reduced on Day 4 of O_3 exposure compared to Day 1 of O_3 exposure. Airway inflammation of mucosa persisted on Day 4 although some inflammatory markers in BALF attenuated significantly.	Jörres et al. (2000)
0.2	392	4 h IE (4 × 30 min exercise), $\dot{V}_E$ = 25 L/min/m^2 BSA	20 °C 50% RH (1 day, O_3; 4 days consecutive exposure to O_3	9 M, 6 F	Healthy, NS 23 to 37 years old	Significant decrease in FVC, FEV_1, SRaw, and symptoms on Day 4 of O_3 exposure compared to a single day of O_3 exposure. Number of PMNs, fibronectin, and IL6 in BALF were significantly decreased on Day 4 compared to a single day of O_3 exposure.	Christian et al. (1998)
0.4	784	3h/day for 5 days IE (15 min rest, 15 min exercise) $\dot{V}_E$ = 32 L/min	31 °C 35% RH 5 consecutive days plus follow up @ 4 or 7 days	8 M, 2 F	Mild asthma adult	FEV_1 decreased 35% on day 1 and only 6% on day 5. Bronchial reactivity increased after day 1 and remained elevated. Adaptation of asthmatics is similar to healthy subjects but may be slower and less complete.	Gong et al. (1997b)
0.12	235	6.6 h 50 min exercise/10 min rest, 30 min lunch $\dot{V}_E$ = 38.8 L/min	18 °C 40% RH five consecutive daily exposures	17 M	Healthy NS	FEV_1 responses were maximal on first day of exposure (−13%), less on second day (−9%), absent thereafter. Symptoms only the first 2 days. Methacholine airway responsiveness was at least doubled on all exposure days, but was highest on the second day of O_3. Airway responsiveness was still higher than air control after 5 days of O_3 exposure. Trend to lessened response, but it was not achieved after 5 days.	Folinsbee et al. (1994)
0.4	784	2 h IE (15 min rest, 15 min exercise $\dot{V}_E \approx$ 60 L/min	5 days consecutive O_3 exposure	16 M	Healthy NS	O_3-exposure FEV_1 decrement was greater on day 2, 29.9%, than day 1, 18.0%, then decreased on day 3, 21.1%, day 4, 7% and day 5, 4.4%	Folinsbee et al. (1998) Devlin et al. (1997)

Table AX6-9 (cont'd). Pulmonary Function Effects with Repeated Exposures to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference

0.45	882	2 h
		IE
		(3 × 20 min exercise)
		$\dot{V}_E$ = 27 L/min
			23.3 °C
			63% RH
			Exposed for 3 consecutive days, not exposed for 2 days, then exposed to 0.45 ppm again for 1 day
				10 M, 6 F	Healthy NS
					60 to 89 years old
					median 65 years old;
					mean FVC = 3.99 L;
					mean FEV_1 = 3.01 L;
					FEV_1/FVC range = 61 to 85%
						Overall increase in symptoms, but no single symptom increased significantly. FVC decreased 111 mL and 104 mL on Days 1 and 2, respectively. FEV_1 fell by 171 and 164 mL, and FEV_3 fell by 185 and 172 mL. No significant changes on Days 3 and 4 or with FA. FEV_1 changes were −5.8, −5.6, −1.9, and −1.7% on the four O_3 days.	Bedi et al. (1989)

0.20/0.20 0.35/0.20 0.35/0.35	392/392 686/392 686/686	1 h CE at 60 L/min	21 to 25 °C 40 to 60% RH (three 2-day sets of exposures)	15 M	Healthy aerobically trained NS, FVC = 4.24 to 6.98 L	Consecutive days of exposure to 0.20 ppm produced similar FEV_1 responses on each day (−5.02, −7.80); 0.35/0.20 ppm pair caused increased response to 0.20 ppm on second day (−8.74); 0.35/0.35 ppm caused much increased response on Day 2 (−15.9, −24.6). Symptoms were worse on the second exposure to 0.35 ppm, but not with second exposure to 0.20 ppm.	Brookes et al. (1989)
0.35	686	60 min CE $\dot{V}_E$ = 60 L/min	21 to 25 °C 40 to 60% RH (two exposures for each subject separated by 24, 48, 72, or 120 h)	40 M (4 groups of 10)	NS; nonallergic, non-Los Angeles residents for > 6 mo; =25 years old	No differences between responses to exposures separated by 72 or 120 h. Enhanced FEV_1 response at 24 h (−16.1% vs. −30.4%). Possible enhanced response at 48 h (−14.4% vs. −20.6%). Similar trends observed for breathing pattern and SRaw.	Schonfeld et al. (1989)
0.45	882	2 h IE (3 × 20 min exercise) $\dot{V}_E$ = 26 L/min	23.3 °C 62.5% RH (three exposures with a minimum 1-week interval)	8 M, 8 F	Healthy NS, 61 years old for M and 65 years old for F (FVC = 4.97 L for M and 3.11 L for F)	Spirometric changes were not reproducible from time to time after O_3 exposure ($R < 0.50$). Repeat exposures to air yielded consistent responses.	Bedi et al. (1988)
0.18	353	2 h IE (heavy) $\dot{V}_E \approx$ 60 to 70 L/min (35 L/min/m² BSA)	31 °C 35% RH (screen exposures in spring 1986; second exposures in summer/fall 1986 and winter 1987 and spring 1987 for responders and nonresponders only)	59 adult Los Angeles residents 12 responsive 13 nonresponsive	Responders: 19 to 40 years old 6 atopic, 2 asthmatic, 4 normal Nonresponders: 18 to 39 years old, 13 normal	Responders had ΔFEV_1 = −12.4% after initial screening; nonresponders had no change. Responders had nonsignificant response in late summer or early winter, but were responsive again in early spring (spring 1986, −385 mL; Autumn 1986, −17 mL; winter 1987, +16 mL; spring 1987, −347 mL). Nonresponders did not change with season. Suggests that responders responses may vary with ambient exposure, but nonresponders generally remain nonresponsive.	Linn et al. (1988) (also see Hackney et al., 1989)

Table AX6-9 (cont'd). Pulmonary Function Effects with Repeated Exposures to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.45 (+ 0.30 PAN)	882	2 h IE (20 min rest, 20 min exercise) $\dot{V}_E$ = 27 L/min	22 °C 60% RH 5 days consecutive exposure to PAN + O_3	3 M, 5 F	Healthy NS, Mean age = 24 years	FEV_1 decreased ≈19% with O_3 alone, ≈15% on Day 1 of O_3 + PAN, ≈5% on Day 5 of O_3 + PAN, ≈7% 3 days after 5 days of O_3 + PAN, ≈15% after 5 days of O_3 + PAN. Similar to other repeated O_3 exposure studies, O_3 responses peaked after 2 days, were depressed 3 days later, and responses returned 7 days later. PAN probably had no effect on repeated to O_3 exposure responses.	Drechsler-Parks et al. (1987b) (also see Table AX6-14)
0.35	686	≈1 h CE (see paper for details)	22 to 25 °C 35 to 50% RH (1 day FA; 1 day O_3; 4 days consecutive exposure to O_3)	8 M	Aerobically trained healthy NS (some were known O_3 sensitive), 22.4 ± 2.2 years old	Largest FEV_1 decrease on second of 4 days O_3 exposure (−40% mean decrease). Trend for attenuation of pulmonary function response not complete in 4 days. $\dot{V}O_{2max}$ decreased with single acute O_3 exposure (−6%) but was not significant after 4 days of O_3 exposure (−4%). Performance time was less after acute O_3 (211 s) exposure than after FA (253 s).	Foxcroft and Adams (1986)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.

1994; Foxcroft and Adams, 1986; Linn et al., 1982). Airway responsiveness to methacholine is increased with an initial O_3 exposure (Holtzman et al., 1979; Folinsbee et al., 1988), may be further increased with subsequent exposures (Folinsbee et al.,1994), and shows a tendency for the increased response to diminish with repeated exposure (Dimeo et al., 1981; Kulle et al., 1982). The initially enhanced and then lessened response may be related to changes that occur during the repair of pulmonary epithelia damaged as a consequence of O_3 exposure. Inflammatory responses (Koren et al., 1989a), epithelial damage, and changes in permeability (Kehrl et al., 1987) might explain a portion of these responses. By blocking pulmonary function responses and symptoms with indomethacin pretreatment, Schonfeld et al. (1989) demonstrated that in the absence of an initial response, pulmonary function and symptoms effects were not enhanced on Day 2 by repeated exposure to 0.35 ppm O_3. These results suggest that airway inflammation and the release of cyclooxygenase products of arachidonic acid play a role in the enhanced pulmonary function responses and symptoms observed upon reexposure to O_3 within 48 h.

Response to laboratory O_3 exposure as a function of the season of the year in the South Coast Air Basin of Los Angeles, CA, has been examined in several studies (Avol et al., 1988; Hackney et al., 1989; Linn et al., 1988). Their primary purpose was to determine whether O_3 responsive subjects would remain responsive after regular ambient exposure during the "smog season". The subjects were exposed to 0.18 ppm O_3 for 2 h with heavy IE on four occasions, spring, fall, winter, and the following spring. The marked difference in FEV_1 response between responsive and nonresponsive subjects seen initially (−12.4% versus +1%) no longer was present after the summer smog season (fall test) or 3 to 5 months later (winter test). However, when the subjects were exposed to O_3 during the following spring, the responsive subjects again had significantly larger changes in FEV_1, suggesting a seasonal variation in response.

Brookes et al. (1989) and Gliner et al. (1983) tested whether initial exposure to one O_3 concentration could alter response to subsequent exposure to a different O_3 concentration. Gliner et al. (1983) showed that FEV_1 response to 0.40 ppm O_3 was not influenced by previously being exposed to 0.20 ppm O_3 for 2 h on 3 consecutive days. Brookes et al. (1989) found enhanced FEV_1 and symptoms upon exposure to 0.20 ppm after previous exposure to 0.35 ppm O_3. These observations suggest that, although preexposure to low concentrations of O_3 may not

influence responses to higher concentrations, preexposure to a high concentration of O_3 can significantly increase responses to a lower concentration on the following day.

Foxcroft and Adams (1986) demonstrated that decrements in exercise performance seen after 1 h of exposure to 0.35 ppm O_3 with heavy CE were significantly less after 4 consecutive days exposure than they were after a single acute exposure. Further, exercise performance, $\dot{V}O_{2max}$, $\dot{V}_{Emax}$ and HR_{max} were not significantly different after 4 days of O_3 exposure compared to those observed in a FA exposure. Despite the change in exercise performance, Foxcroft and Adams (1986) did not observe a significant attenuation of FEV_1 response, although symptoms were significantly reduced. However, these investigators selected known O_3-sensitive subjects whose FEV_1 decrements exceeded 30% on the first 3 days of exposure. The large magnitude of these responses, the trend for the responses to decrease on the third and fourth day, the decreased symptoms, and the observations by Horvath et al. (1981) that O_3-sensitive subjects adapt slowly, suggest that attenuation of response would have occurred if the exposure series had been continued for another 1 or 2 days. These observations support the contention advanced by Horvath et al. (1981) that the progression of attenuation of response is a function of initial "O_3 sensitivity."

Drechsler-Parks et al. (1987b) examined the response to repeated exposures to 0.45 ppm O_3 plus 0.30 ppm peroxyacetyl nitrate (PAN) in 8 healthy subjects and found similar FEV_1 responses to exposures to O_3 (−19%) and to O_3 plus PAN (−15%). Thus, PAN did not increase responses to O_3. Further, repeated exposure to the PAN plus O_3 mixture resulted in similar changes to those seen with repeated O_3 exposure alone. The FEV_1 responses fell to less than −5% after the fifth day, with the attenuation of response persisting 3 days after the repeated exposures, but being absent after 7 days. These observations suggest that PAN does not influence the attenuation of response to repeated O_3 exposure. If the PAN responses are considered negligible, this study confirms the observation that the attenuation of O_3 responses with chamber exposures lasts no longer than 1 week. [*More discussion on the interaction of O_3 with other pollutants can be found in Section AX6.11*.]

Folinsbee et al. (1993) exposed a group of 16 healthy males to 0.4 ppm O_3 for 2 h/day on 5 consecutive days. Subjects performed heavy IE ($\dot{V}_E$ = 60 to 70 L/min). Decrements in FEV_1 averaged 18.0, 29.9, 21.1, 7.0, and 4.4% on the 5 exposure days. However, baseline preexposure FEV_1 decreased from the first day's preexposure measurement and was depressed by an average

of about 5% by the third day. This study illustrates that, with high-concentration and heavy-exercise exposures, pulmonary function responses may not be completely recovered within 24 h. During this study, BALF also was obtained immediately after the Day 5 exposure, with results reported by Devlin et al. (1997). These authors found that some inflammation and cellular responses associated with acute O_3 exposure were also attenuated after 5 consecutive days of O_3 exposure (compared to historical data for responses after a single-day exposure), although indicators of epithelial cell damage—not seen immediately after acute exposure—were present in BALF after the fifth day of exposure. When reexposed again 2 weeks later, changes in BALF indicated that epithelial cells appeared fully repaired (Devlin et al., 1997).

Frank et al. (2001) exposed 8 healthy young adults to 0.25 ppm O_3 for 2 h with moderate IE (exercise $\dot{V}_E$ = 40 L/min) on 4 consecutive days. In addition to standard pulmonary function measures, isovolumetric FEF_{25-75}, $\dot{V}_{max50}$ and $\dot{V}_{max75}$ were grouped into a single value representing small airway function (SAW_{grp}). Exercise ventilatory pattern was also monitored each day, while peripheral airway resistance was measured by bronchoscopy followed by lavage on Day 5. The authors observed two patterns of functional response in their subjects— attenuation and persistent. Values of FVC and FEV_1 showed significant attenuation by Day 4 compared to Day 1 values. However, SAW_{grp} and rapid shallow breathing during exercise persisted on Day 4 compared to Day 1, and were accompanied by significant neutrophilia in BALF 1 day following the end of O_3 exposure. Frank et al. (2001) suggested that both types of functional response (i.e., attenuation and persistence) are linked causally to inflammation. They contend that the attenuation component is attributable at least in part to a reduction in local tissue dose during repetitive exposure that is likely to result from the biochemical, mechanical, and morphological changes set in motion by inflammation. They speculated that the persistent component represents the inefficiencies incurred through inflammation. Whether the persistent small airway dysfunction is a forerunner of more permanent change in the event that oxidant stress is extended over lengthy periods of time is unknown.

Early repeated multihour (6 to 8 h) exposures focused on exposures to low concentrations of O_3 between 0.08 and 0.12 ppm (Folinsbee et al., 1994; Horvath et al., 1991; Linn et al., 1994). Horvath et al. (1991) exposed subjects for 2 consecutive days to 0.08 ppm using the 6.6-h prolonged exposure protocol (see Table AX6-2). They observed small pre- to postexposure changes in FEV_1 (−2.5%) on the first day, but no change on the second day. Linn et al. (1994)

observed a 1.7% decrease in FEV_1 in healthy subjects after 6.6 h exposure to 0.12 ppm O_3. A second consecutive day exposure to O_3 yielded even smaller (< 1%) responses. In a group of asthmatics exposed under similar conditions (Linn et al., 1994), the FEV_1 response on the first day was −8.6% which was reduced to −6.7% on day 2, both significantly greater than those observed for the nonasthmatics group. The observations of Horvath et al. (1991) and Linn et al. (1994) elicited a somewhat different pattern of response (no enhancement of response after the first exposure) than that seen at higher concentrations in 2 h exposures with heavy exercise (Tables AX6-8 and AX6-9). However, the subjects studied by Horvath et al. (1991) were exposed only to 0.08 ppm O_3 and were somewhat older (30 to 43 yrs) than the subjects studied by Folinsbee et al. (1994), mean age of 25 yrs, while the nonasthmatic subjects studied by Linn et al. (1994) were also older (mean = 32 yrs), had lower exercise $\dot{V}_E$ (−20%) and were residents of Los Angeles who often encountered ambient levels of O_3 at or above 0.12 ppm.

Folinsbee et al. (1994) exposed 17 subjects to 0.12 ppm O_3 for 6.6 h, with 50 min of moderately heavy exercise ($\dot{V}_E$ = 39 L/min) each hour, on 5 consecutive days. Compared with FA, the percentage changes in FEV_1 over the five days were −12.8%, −8.7%, −2.5%, −0.06%, and +0.18%. A parallel attenuation of symptoms was observed, but the effect of O_3 in enhancing airway responsiveness (measured by increase in SRaw upon methacholine challenge) over 5 days was not attenuated (3.67, 4.55, 3.99, 3.24, and 3.74, compared to 2.22 in FA control). Nasal lavage revealed no increases in neutrophils except on the first O_3 exposure day.

Christian et al. (1998) exposed 15 adults (6 females and 9 males; mean age = 29.1 yrs) to 4 consecutive days at 0.20 ppm O_3 for 4 h, with 30 mm of IE (exercise $\dot{V}_E$ = 25 L/min/m^2) each hour. Measures of FEV_1, FVC, and symptoms were all significantly reduced on Day 1, further decreased on Day 2, and then attenuated to near FA control values on Day 4. The pattern of SRaw response was similar, being greatest on Day 2 and no different from FA control on Day 4. BAL was done on Day 5 and showed that neutrophil recruitment to the respiratory tract was attenuated with repeated short-term exposures, compared to Day 1 control O_3 exposure, while airway epithelial injury appeared to continue as reflected by no attenuation of IL-6, IL-8, total protein, and LDH. The authors concluded that such injury might lead to airway remodeling, which has been observed in several animal studies (Brummer et al., 1977; Schwartz et al., 1976; Tepper et al., 1989; Van Bree et al., 1989). In a similar study to that of Christian et al. (1998), Jörres et al. (2000) exposed 23 adults (8 females and 15 males; mean age = 27.9 yrs) on

4 consecutive days to 0.20 ppm O_3 for 4 h, with 30 min of IE (exercise $\dot{V}_E$ = 26 L/min) each hour. The authors observed that FEV_1 was significantly reduced and symptoms were significantly increased on Day 1. On Day 2, FEV_1 was further decreased, while symptoms remained unchanged. By Day 4, both FEV_1 and symptoms were attenuated to near FA, control values. Twenty hours after the Day 4 exposure, BAL and bronchial mucosal biopsies were performed. These authors found via bronchial mucosal biopsies that inflammation of the bronchial mucosa persisted after repeated O_3 exposure, despite attenuation of some inflammatory markers in BALF and attenuation of lung function responses and symptoms. Further, Jörres et al. (2000) observed persistent although small decrease in baseline FEV_1 measured before exposure, thereby suggesting that there are different time scales of the functional responses to O_3, which may reflect different mechanisms. The levels of protein remaining elevated after repeated exposures confirms the findings of others (Christian et al., 1998; Devlin et al., 1997), and suggests that there is ongoing cellular damage irrespective of the attenuation of cellular inflammatory responses with the airways. [*Further discussion on the inflammatory responses to O_3 can be found in Section AX6.9*.]

Based on studies cited here and in the previous O_3 criteria documents (U.S. Environmental Protection Agency, 1986, 1996), several conclusions can be drawn about repeated 1- to 2-h O_3 exposures. Repeated exposures to O_3 can cause an enhanced (i.e., greater) lung function response on the second day of exposure. This enhancement appears to be dependent on the interval between the exposures (24 h is associated with the greatest increase) and is absent with intervals > 3 days. As shown in Figure AX6-8, an enhanced response also appears to depend on O_3 concentration and to some extent on the magnitude of the initial response. Small responses to the first O_3 exposure are less likely to result in an enhanced response on the second day of O_3 exposure. Repeated daily exposure also results in attenuation of pulmonary function responses, typically after 3 to 5 days of exposure. This attenuated response persists for less than 1 week or as long as 2 weeks. In temporal conjunction with the pulmonary function changes, symptoms induced by O_3, such as cough and chest discomfort, also are attenuated with repeated exposure. Ozone-induced changes in airway responsiveness attenuate more slowly than pulmonary function responses and symptoms. Attenuation of the changes in airway responsiveness appear to persist longer than changes in pulmonary function, although this has been studied only on a limited basis. In longer-duration (6.6 h), lower-concentration studies that do not cause an

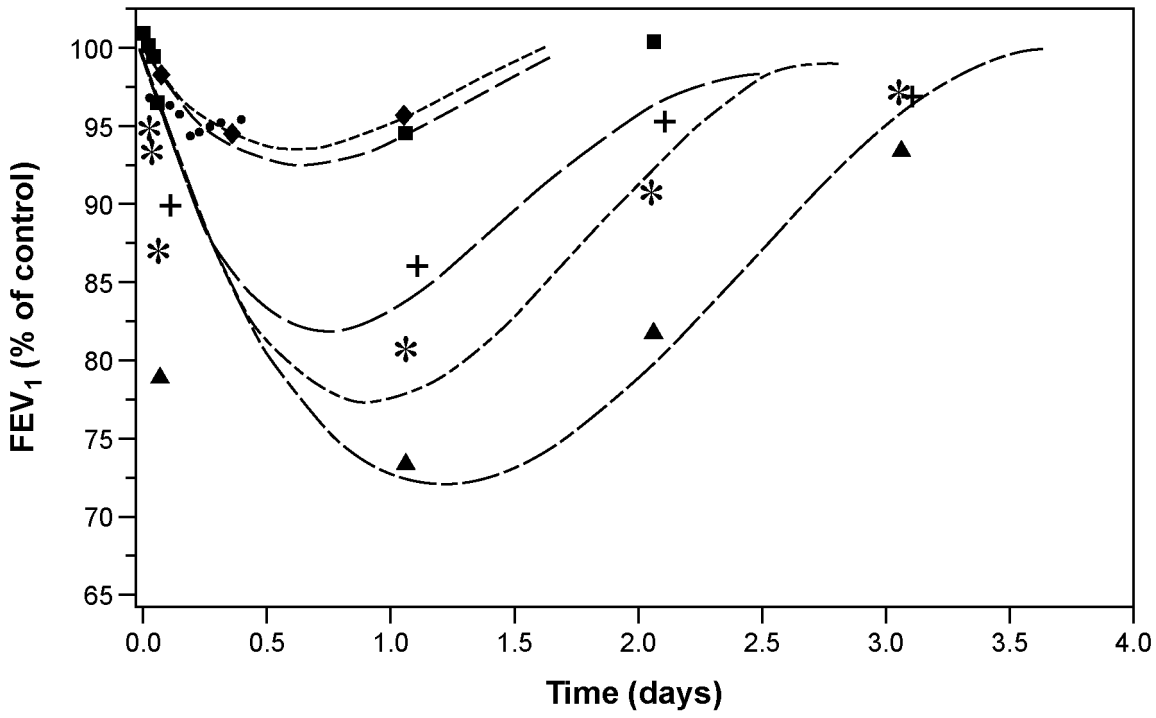

Figure AX6-8. Regression curves were fitted to day-by-day postexposure FEV_1 values obtained after repeated daily acute exposures to O_3 for 2 to 3 h with intermittent exercise at a V_E of 24 to 43 L/min (adaptation studies). Symbols represent the results from individual studies conducted at 0.2 ppm for 2 h (◆), 0.35 ppm for 2 h (■), 0.4 ppm for 2 h (✚), 0.5 ppm for 2 h (✱), and 0.54 ppm for 3 h (▲). Also shown for comparison are the FEV_1 values obtained after exposure to 0.12 ppm O_3 for 10 h (●).

Source: Modified from Hazucha (1993).

enhanced second-day response, the attenuation of response to O_3 appears to proceed more rapidly. Inflammatory markers from BALF on the day following both 2 h (Devlin et al., 1997) and 4 h (Christian et al., 1998; Jörres et al., 2000) repeated O_3 exposure for 4 days indicate that there is ongoing cellular damage irrespective of the attenuation of some cellular inflammatory responses of the airways, lung function responses and symptoms.

AX6.7 EFFECTS ON EXERCISE PERFORMANCE

AX6.7.1 Introduction

In an early epidemiologic study examining race performances in Los Angeles area high school cross-country runners, Wayne et al. (1967) observed that endurance exercise performance was depressed by inhalation of ambient oxidant air pollutants. The authors concluded that the detrimental effects of oxidant air pollutants on race performance might have been related to the associated discomfort in breathing, thus limiting the runners' motivation to perform at high levels, although physiologic effects limiting O_2 availability could not be ruled out. Subsequently, the effects of acute O_3 inhalation on endurance exercise performance have been examined in numerous controlled laboratory studies. These studies were discussed in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1996) in two categories: (1) those that examined the effects of acute O_3 inhalation on maximal oxygen uptake ($\dot{V}O_{2max}$) and (2) those that examined the effects of acute O_3 inhalation on the ability to complete strenuous continuous exercise protocols of up to 1 h in duration. In this section, major observations in these studies are briefly reviewed with emphasis on reexamining the primary mechanisms causing decrements in $\dot{V}O_{2max}$ and endurance exercise performance consequent to O_3 inhalation. A summary of major studies of O_3 inhalation effects on endurance exercise performance, together with observed pulmonary function and symptoms of breathing discomfort responses, is given in Table AX6-10.

AX6.7.2 Effect on Maximal Oxygen Uptake

Three early studies (Folinsbee et al., 1977; Horvath et al., 1979; Savin and Adams, 1979) examining the effects of acute O_3 exposures on $\dot{V}O_{2max}$ were reviewed in an earlier O_3 criteria document (U.S. Environmental Protection Agency, 1986). Briefly, Folinsbee et al. (1977) observed that $\dot{V}O_{2max}$ was significantly decreased (10.5%) following a 2-h exposure to 0.75 ppm O_3 with light (50 Watts) IE. Reduction in $\dot{V}O_{2max}$ was accompanied by a decrease in maximal ventilation, maximal heart rate, and a large decrease in maximal tidal volume. In addition, the 2-h IE O_3 exposure resulted in a 22.3% decrease in FEV_1 and significant symptoms of cough and chest discomfort. In contrast, Horvath et al. (1979) did not observe a change in $\dot{V}O_{2max}$ or other maximal cardiopulmonary endpoints in subjects exposed for 2 h at rest to either 0.50 or

Table AX6-10. Ozone Effects on Exercise Performance[a]

Ozone Concentration[b]		Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
ppm	µg/m³						
0.06-0.07 0.12-0.13	120-140 245-260	CE ($\dot{V}_E$ = 30 to 120 L/min) 16 to 28 min progressive maximum exercise protocol	23 to 24.5 °C 50 to 53% RH	12 M, 12 F	Athletic	Reduced maximum performance time and increased symptoms of breathing discomfort during O_3 exposure.	Linder et al. (1988)
0.18	353	1 h CE or competitive simulation at mean $\dot{V}_E$ = 94 L/min	NA	# not given; all males	Well-trained distance runners	Maximal treadmill run time reduced from 71.7 min in FA to 66.2 min during O_3 exposure with no decease in arterial O_2 saturation.	Folinsbee et al. (1986)
0.35	686	50 min CE $\dot{V}_E$ = 60 L/min	22 to 25 °C 35 to 50% RH	8 M	Trained nonathletes	V_T decreased, f_B increased with 50-min O_3 exposures; decrease in FVC, FEV_1, FEF_{25-75}, performance time, $\dot{V}O_{2max}$, $\dot{V}_{Emax}$, and HR_{max} from FA to 0.35-ppm O_3 exposure.	Foxcroft and Adams (1986)
0.12 0.20	235 392	1 h CE $\dot{V}_E$ = 89 L/min	31 °C	15 M, 2 F	Highly trained competitive cyclists	Decrease in $\dot{V}_{Emax}$, $\dot{V}O_{2max}$, V_{Tmax}, workload, ride time, FVC, and FEV_1 with 0.20 ppm O_3 exposure, but not significant with 0.12-ppm O_3 exposure, as compared to FA exposure.	Gong et al. (1986)
0.12 0.18 0.24	235 353 470	1 h competitive simulation exposures at mean $\dot{V}_E$ = 87 L/min	23 to 26 °C 45 to 60% RH	10 M	Highly trained competitive cyclists	Decrease in exercise time of 7.7 min and 10.1 min for subjects unable to complete the competitive simulation at 0.18 and 0.24 ppm O_3, respectively; decrease in FVC and FEV_1 for 0.18-and 0.24-ppm O_3 exposure compared with FA exposure.	Schelegle and Adams (1986)
0.21	412	1 h CE at 75% $\dot{V}O_2$max	19 to 21 °C 60 to 70% RH	6 M, 1 F	Well-trained cyclists	Decrease in FVC, FEV_1, FEF_{25-75}, and MVV with 0.21 ppm O_3 compared with FA exposure.	Folinsbee et al. (1984)
0.20 0.35	392 686	1 h CE or competitive simulation at mean $\dot{V}_E$ = 77.5 L/min	23 to 26 °C 45 to 60% RH	10 M	Well-trained distance runners	V_T decreased and f_B increased with continuous 50-min O_3 exposures; decrease in FVC, FEV_1, and FEF_{25-75} from FA to 0.20 ppm and FA to 0.35-ppm O_3 exposure in all conditions; three subjects unable to complete continuous and competitive protocols at 0.35 ppm O_3.	Adams and Schelegle (1983)
0.25 0.50 0.75	490 980 1,470	2 h rest	NA	8 M, 5 F		FVC decreased with 0.50- and 0.75-ppm O_3 exposure compared with FA; 4% nonsignificant decrease in mean $\dot{V}O_{2max}$ following 0.75 ppm O_3 compared with FA exposure.	Horvath et al. (1979)
0.15 0.30	294588	~30 min, progressively incremented exercise to voluntary exhaustion	23 °C 50% RH	9 M	Healthy, NS 21 to 44 years old	Exposure to 0.15 and 0.30 ppm O_3 did not decrease maximal exercise performance or $\dot{V}O_{2max}$ compared to FA. No significant pulmonary function or symptom responses were observed, although a trend ($P < .10$) was evident.	Savin and Adams (1979)
0.75	1,470	2 h IE (4 × 15 min light [50 W] bicycle ergometry)	NA	13 M	4 light S, 9 NS	Decrease in FVC, FEV_1, ERV, IC, and $FEF_{50\%}$ after 1-h 0.75-ppm O_3 exposure; decrease in $\dot{V}O_{2max}$, $\dot{V}_{Tmax}$, $\dot{V}_{Emax}$, maximal workload, and HRmax following 0.75-ppm O_3 exposure compared with FA.	Folinsbee et al. (1977)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.

0.75 ppm, although FVC was significantly decreased 10% following the latter exposure. Without preliminary exposure to O_3, Savin and Adams (1979) examined the effects of a 30-min exposure to 0.15 and 0.30 ppm O_3 while performing a progressively incremented exercise test to volitional fatigue (mean = 31.5 min in FA). No significant effect on maximal work time or $\dot{V}O_{2max}$ was observed compared to that observed upon FA exposure. Further, no significant effect on pulmonary function, maximal heart rate, and maximal tidal volume was observed, although maximal $\dot{V}_E$ was significantly reduced 7% in the 0.30 ppm O_3 exposure. Results of these early studies suggest that $\dot{V}O_{2max}$ is reduced if the incremented maximal exercise test is preceded by an O_3 exposure of sufficient total inhaled dose of O_3 to result in significant pulmonary function decrements and symptoms of breathing discomfort.

Using trained nonathletes, Foxcroft and Adams (1986) observed significant ($p < 0.05$) reductions in rapidly incremented $\dot{V}O_{2max}$ exercise performance time (−16.7%), $\dot{V}O_{2max}$ (−6.0%), maximal $\dot{V}_E$ (−15.0%), and maximal heart rate (−5.6%) immediately following an initial 50-min exposure to 0.35 ppm O_3 during heavy CE ($\dot{V}_E$ = 60 L/min). These decrements were accompanied by a significant reduction in FEV_1 (−23%) and the occurrence of marked symptoms of breathing discomfort. Similarly, Gong et al. (1986) found significant reductions in rapidly incremented $\dot{V}O_{2max}$ exercise performance time (−29.7%), $\dot{V}O_{2max}$ (−16.4%), maximal $\dot{V}_E$ (−18.5%), and maximal workload (−7.8%) in endurance cyclists immediately following a 1-h exposure to 0.20 ppm O_3 with very heavy exercise ($\dot{V}_E$ 89 L/min), but not following exposure to 0.12 ppm. Gong et al. (1986) observed only a 5.6% FEV_1 decrement and mild symptoms following exposure to 0.12 ppm, but a large decrement in FEV_1 (−21.6%) and substantial symptoms of breathing discomfort following the 0.20 ppm exposure, which the authors contended probably limited maximal performance and $\dot{V}O_{2max}$.

AX6.7.3 Effect on Endurance Exercise Performance

A number of studies of well trained endurance athletes exposed to O_3 have consistently observed an impairment of 1-h continuous heavy exercise performance of some individuals (Adams and Schelegle, 1983; Avol et al., 1984; Folinsbee et al., 1984; Gong et al., 1986). The performance impairment is indicated by an inability to complete the prescribed O_3 exposures (even at concentrations as low as 0.16 ppm) that subjects were able to complete in FA (Avol

et al., 1984). Other indications of impaired endurance exercise performance upon exposure to O_3 include a –7.7% reduced endurance treadmill running time when exposed to 0.18 ppm O_3 (Folinsbee et al., 1986), which was accompanied by significantly decreased FEV_1 and significantly elevated symptoms of breathing discomfort. Another study (Schelegle and Adams, 1986) observed the failure of some trained endurance athletes to complete a 1-h competitive simulation protocol upon exposure to O_3 (30 min warm-up, followed immediately by 30 min at the maximal workload that each subject could just maintain in FA; mean $\dot{V}_E$ = 120 L/min). In this study, all subjects (n = 10) completed the FA exposure, whereas one, five, and seven subjects could not complete the 0.12, 0.18, and 0.24 ppm O_3 exposures, respectively. Following the 0.18 ppm and 0.24 ppm O_3 exposures, but not the 0.12 ppm exposure, FEV_1 was reduced significantly and symptoms were significantly increased. Linder et al. (1988) also observed small decrements in performance time (1 to 2 min) during a progressive maximal exercise test (mean = 21.8 min) at O_3 concentrations of 0.065 and 0.125 ppm. These small effects were accompanied by a significant increase in subjective perception of overall effort at 0.125 ppm, but with no significant reduction in FEV_1 at either O_3 concentration. Collectively, reduced endurance exercise performance and associated pulmonary responses are clearly related to the total inhaled dose of O_3 (Adams and Schelegle, 1983; Avol et al., 1984; Schelegle and Adams, 1986).

Mechanisms limiting $\dot{V}O_{2max}$ and maximal exercise performance upon O_3 exposure have not been precisely identified. Schelegle and Adams (1986) observed no significant effect of O_3 on cardiorespiratory responses, and there was no indirect indication that arterial O_2 saturation was affected. The latter is consistent with the observation that measured arterial O_2 saturation at the end of a maximal endurance treadmill run was not affected by O_3 (Folinsbee et al., 1986). In studies in which O_3 inhalation resulted in a significant decrease in $\dot{V}O_{2max}$, and/or maximal exercise performance, significantly decreased FEV_1 and marked symptoms of breathing discomfort were observed (Adams and Schelegle, 1983; Avol et al., 1984; Folinsbee et al., 1977, 1984, 1986; Foxcroft and Adams, 1986; Gong et al., 1986; Schelegle and Adams, 1986). However, Gong et al. (1986) observed rather weak correlations between FEV_1 impairment and physiological variable responses during maximal exercise (R = 0.26 to 0.44). Rather, these authors concluded that substantial symptoms of breathing discomfort consequent to 1 h of very heavy exercise while exposed to 0.20 ppm O_3, probably limited maximal performance and

$\dot{V}O_{2max}$ either voluntarily or involuntarily (Gong et al., 1986). Strong support for this contention is provided by the observation of significant increases in $\dot{V}O_{2max}$ (4.7%) and maximal performance time (8.8%) following four consecutive days of 1 h exposure to 0.35 ppm O_3 with heavy exercise ($\dot{V}_E$ = 60 L/min) compared to initial O_3 exposure (Foxcroft and Adams, 1986). These improvements, which were not significantly different from those for FA, were accompanied by a significant reduction in symptoms of breathing discomfort with no significant attenuation of FEV_1 and other pulmonary function responses. In this regard, Schelegle et al. (1987) observed a disparate effect of indomethacin pretreatment (an inhibitor of the cyclo-oxygenation of arachidonic acid to prostaglandins associated with inflammatory responses) on O_3-induced pulmonary function response (significant reduction) and an overall rating of perceived exertion and symptoms of pain on deep inspiration and shortness of breath (no significant effect).

AX6.8 EFFECTS ON AIRWAY RESPONSIVENESS

Increased airway responsiveness, also called airway hyperresponsiveness (AHR) or bronchial hyperreactivity, indicates that the airways are more reactive to bronchoconstriction induced by a variety of stimuli (e.g., specific allergens, exercise, SO_2, cold air) than they would be when normoreactive. In order to determine the level of airway responsiveness, airway function (usually assessed by spirometry or plethysmography) is measured after the inhalation of small amounts of an aerosolized specific (e.g., antigen, allergen) or nonspecific (e.g., methacholine, histamine) bronchoconstrictor agent or measured stimulus (e.g., exercise, cold air). The dose or concentration of the agent or stimulus is increased from a control, baseline level (placebo) until a predetermined degree of airway response, such as a 20% drop in FEV_1 or a 100% increase in Raw, has occurred (Cropp et al., 1980; Sterk et al., 1993). The dose or concentration of the bronchoconstrictor agent that produced the increased responsiveness often is referred to as the "$PD_{20}FEV_1$" or "$PC_{20}FEV_1$" (i.e., the provocative dose or concentration that produced a 20% drop in FEV_1) or the "PD_{100}SRaw" (i.e., the provocative dose that produced a 100% increase in SRaw). A high level of bronchial responsiveness is a hallmark of asthma. The range of nonspecific bronchial responsiveness, as expressed by the PD_{20} for example, is at least 1,000-fold from the most sensitive asthmatics to the least sensitive healthy subjects.

Unfortunately, it is difficult to compare the $PD_{20}FEV_1$ or $PD_{100}SRaw$ across studies because of the many different ways of presenting dose response to bronchoconstrictor drugs, for example, by mg/mL, units/mL, and molar solution; or by cumulative dose (CIU or CBU) and doubling dose (DD). Other typical bronchial challenge tests with nonspecific bronchoconstrictor stimuli are based on exercise intensity or temperature of inhaled cold air.

Increases in nonspecific airway responsiveness were previously reported as an important consequence of exposure to O_3 (e.g., Golden et al., 1978; Table AX6-11). König et al. (1980) and Holtzman et al. (1979) found the increased airway responsiveness after O_3 exposure in healthy subjects appeared to be resolved after 24 h. Because atopic subjects had similar increases in responsiveness to histamine after O_3 exposure as non-atopic subjects, Holtzman et al. (1979) concluded that the increased nonspecific bronchial responsiveness after O_3 exposure was not related to atopy. Folinsbee and Hazucha (1989) showed increased airway responsiveness in 18 female subjects 1 and 18 h after exposure to 0.35 ppm O_3. Taken together, these studies suggest that O_3-induced increases in airway responsiveness usually resolve 18 to 24 h after exposure, but may persist in some individuals for longer periods.

Gong et al. (1986) found increased nonspecific airway responsiveness in elite cyclists exercising at competitive levels with O_3 concentrations as low as 0.12 ppm. Folinsbee et al. (1988) found an approximate doubling of the mean methacholine responsiveness in a group of healthy volunteers exposed for 6.6 h to 0.12 ppm O_3. Horstman et al. (1990) demonstrated significant decreases in the $PD_{100}SRaw$ in 22 healthy subjects immediately after a 6.6-h exposure to concentrations of O_3 as low as 0.08 ppm. No relationship was found between O_3-induced changes in airway responsiveness and changes in FVC or FEV_1 (Folinsbee et al., 1988; Aris et al., 1995), suggesting that changes in airway responsiveness and spirometric volumes occur by different mechanisms.

Dimeo et al. (1981) were the first to investigate attenuation of the O_3-induced increases in nonspecific airway responsiveness after repeated O_3 exposure. Over 3 days of a 2 h/day exposure to 0.40 ppm O_3, they found progressive attenuation of the increases in airway responsiveness such that, after the third day of O_3 exposure, histamine airway responsiveness was no longer different from the sham exposure levels. Kulle et al. (1982) found that there was a significantly enhanced response to methacholine after the first 3 days of exposure, but this response slowly normalized by the end of the fifth day. Folinsbee et al. (1994) found a more

TABLE AX6-11. Airway Responsiveness Following Ozone Exposures[a]

Ozone Concentration[b]		Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
ppm	µg/m^3						
0.125 0.250	245 490	3h IE (10 min rest, 15 min exercise on bicycle) $\dot{V}_E$ = 30 L/min	27 °C 50 % RH	5 F, 6 M 20-53 years old 6 F, 16 M 19-48 years old	Mild bronchial asthma Allergic rhinitis	Mean early-phase FEV_1 response and number of ≥ 20% reductions in FEV_1 were significantly greater after 0.25 ppm O_3 or 4 × 0.125 ppm O_3. Most of the ≥ 15% late-phase FEV_1 responses occurred after exposure to 4 × 0.125 ppm O_3, as well as significant inflammatory effects, as indicated by increased sputum eosinophils (asthma and allergic rhinitis) and increased sputum lymphocytes, mast cell tryptase, histamine, and LDH (asthma only).	Holz et al. (2002)
0.125	245	3h IE × 4 days					
0.4	784	2 h IE $\dot{V}_E$ = 20 L/min/m^2 BSA	NA	6 F 1 M 19-26 years old	Stable mild asthma; no meds 8 h preexposure	Increased bronchial responsiveness to methacholine 16 h after exposure; inhaled apocynin treatment significantly reduced O_3-induced airway responsiveness	Peters et al. (2001)
0.12	235	45 min IE 15 min exercise $\dot{V}_E$ = 3 x resting	60% RH for test atmospheres	12 F 5 M 19-38 years old	Physician diagnosed asthma; SO_2-induced airway hyperreactivity	Dietary supplementation with 400 IU Vit E + 500 mg Vit C reduced airway responsiveness to 0.10 and 0.25 ppm SO_2 challenge	Trenga et al. (2001)
0.2	392	4 h IE $\dot{V}_E$ = 25 L/min/m^2 BSA	20° C 62% RH	4F 8M 23-47 years old	Healthy nonsmokers	Increased sputum total cells, % neurtophils, IL-6, and IL-8 at 18 h after exposure; increased airway responsiveness to methacholine 2 h after postexposure FEV_1 returned to 5% of base-line; no anti-inflammatory effect of azithromycin	Criqui et al. (2000)
0.4	784	2 h IE 40 min/h @ 50 W	NA	15 healthy subjects ; 9 F, 6 M; 31.1 ± 2.1 years old	Healthy; nonatopic	Decreased FEV_1 and FVC; increased bronchial reactivity to methacholine 4 h post-exposure; no protection from inhaled corticosteroid, budesonide	Nightingale et al. (2000)
0.16	314	7.6 h IE 50 min/h $\dot{V}_E$ ≅25 l/min	22˚C 40% RH	5 F 4 M	Mild atopic asthma, HMD sensitive, 20-35 years old	Mean 9.1% FEV_1 decrease 18 h after O_3 exposure; provocative dose of dust mite allergen decreased from 10.3 to 9.7 dose units.	Kehrl et al. (1999)

TABLE AX6-11 (cont'd). Airway Responsiveness Following Ozone Exposures[a]

Ozone Concentration[b]		Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
ppm	µg/m^3						
0.2	392	4 h IE 40 min/h @ 50 W	NA	10 asthmatic (6 F, 4 M), 26.6 ± 2.3 years old; 10 healthy (4 F, 6 M), 27.3 ± 1.4 years old.	Mild atopic asthma; non-atopic healthy subjects; no meds 8 weeks pre-exposure	Decreased FEV_1 in asthmatic (9.3%) and healthy (6.7%) subjects; increased sputum neutrophils in both groups (NS); no change in methacholine airway reactivity 24 h post-exposure	Nightingale et al. (1999)
0.12 Air-antigen	235	1 h rest	NA	6 F 9 M	Mild allergic asthma; 18 to 49 years if age	No effect of O3 on airway response to grass or ragweed allergen.	Hanania et al. (1998)
0.4	784	2 h IE $\dot{V}_E$= 20 L/min/m^2 BSA	NA	5F 1M 18-27 years old	Stable mild asthma; no meds 8 h preexposure	Increased airway responsiveness to methacholine 16 h postexposure; no effect of proteinase inhibitor, rALP	Hiltermann et al. (1998)
0.2	392	4 h IE 50 min/h $\dot{V}_E$ = 25 L/min/m^2 BSA	20° C 50% RH	6 F 12 M 18-36 years old	Physician-diagnosed mild asthma; no meds prior to exposure	Decreased FEV_1 and FVC, increased SRaw; lower respiratory Sx; increased % neutrophils, total protein, LDH, fibronectin, IL-8, GM-CSF, and MPO in BAL. Correlation between pre-exposure methacholine challenge and O_3-induced SRaw increase.	Balmes et al. (1997); Scannell et al. (1996)
0.4	784	3 h/d for 5 days; alternating 15 min of rest and exercise at $\dot{V}_E$ = 32 L/min	31°C 35% RH	2 F 8 M 19-48 years old	Mild asthma requiring only occasional bronchodilator therapy	Significant FEV_1 and Sx response on 1st and 2nd O_3 exposure days, then diminishing with continued exposure; tolerance partially lost 4 and 7 days postexposure; bronchial reactivity to methacholine peaked on 1st O_3 exposure day, but remained elevated with continued exposure	Gong et al. (1997b)
0.12	236	Rest	22 ˚C 40% RH	5 F 10 M	atopic asthma	No effect of O_3 on airway response to grass allergen.	Ball et al. (1996)
0.25	490	3 h IE $\dot{V}_E$ = 30 L/min 15 min ex/ 10 min rest/ 5 min no O_3; every 30 min.	27 ˚C 54% RH mouthpiece exposure	24 mild asthmatics 11 F / 13 M 12 allergic rhinitics 6 M / 6 F	atopic mild asthmatic NS	Increased allergen responsiveness afer O_3 exposure.	Jörres et al. (1996)

TABLE AX6-11 (cont'd). Airway Responsiveness Following Ozone Exposures[a]

Ozone Concentration[b]		Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
ppm	µg/m^3						
0.2	392	4h IE 50 min/10 min exercise/rest each hour	22 °C 50% RH	42 M/24 F	18-50 years NS healthy	FEV_1(−18.6%), FVC (−14.6%), decreased after O_3. Baseline PC_{100} for methacholine was not related to changes in FVC, FEV_1, a weak association was seen for PC_{100} and increased SRaw.	Aris et al. (1995)
0.12	235	1 h R	Ambient T & RH for exposure; 23 °C & 50% RH for exercise challenge	8 F 7 M 19-45 years old	Mild stable asthma	No significant difference in % fall FEV_1 or V_{40p}; no increase in bronchial responsiveness to exercise challenge	Fernandes et al. (1994)
0.12	235	6.6 h, IE x 5 days 50 min exercise/10 min rest, 30 min lunch $\dot{V}_E$ = 38.8 L/min	18 °C 40% RH	17 M 25 ± 4 years old	Healthy nonsmokers	FEV_1 responses were maximal on 1st day of exposure (−13%), less on second day (−9%), absent thereafter. Sx responses only the first 2 days. Methacholine airway responsiveness was at least doubled on all exposure days, but was highest on the second day of O_3.	Folinsbee et al. (1994)
0.10 0.25 0.40	196 490 785	1 h light IE 2 × 15 min on treadmill $\dot{V}_E$ = 27 L/min	21 °C 40% RH	9 F 12 M 19-40 years old	Stable mild asthmatics with FEV_1 > 70% and methacholine responsiveness	No significant differences in FEV_1 or FVC were observed for 0.10 and 0.25 ppm O_3-FA exposures or postexposure exercise challenge; 12 subjects exposed to 0.40 ppm O_3 showed significant reduction in FEV_1.	Weymer et al. (1994)
Air-antigen 0.12 ppm O_3-antigen		1 h at rest	NA	4 M, 3 F	Asthmatic, 21 to 64 years old	Increased bronchoconstrictor response to inhaled ragweed or grass after O_3 exposure compared to air.	Molfino et al. (1991)
0.08 0.10 0.12	157 196 235	6.6 h IE at ≈39 L/min	18 °C 40% RH	22 M	Healthy NS, 18 to 32 years old	33, 47, and 55% decreases in cumulative dose of methacholine required to produce a 100% increase in SRaw after exposure to O_3 at 0.08, 0.10, and 0.12 ppm, respectively.	Horstman et al. (1990)

TABLE AX6-11 (cont'd). Airway Responsiveness Following Ozone Exposures[a]

Ozone Concentration[b]		Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
ppm	µg/m^3						
0.12 ppm O_3-100 ppb SO_2 0.12 ppm O_3-0.12 ppm O_3 Air-100 ppb SO_2		45 min in first atmosphere and 15 min in second IE	22 °C 75% RH	8 M, 5 F	Asthmatic, 12 to 18 years old	Greater declines in FEV_1 and $\dot{V}_{max50\%}$ and greater increase in respiratory resistance after O_3-SO_2 than after O_3-O_3 or air-SO_2.	Koenig et al. (1990)
0.35	686	70 min with IE at 40 L/min	NA	18 F	Healthy NS, 19 to 28 years old	PD_{100} decreased from 59 CIU after air exposure to 41 CIU and 45 CIU, 1 and 18 h after O_3 exposure, respectively.	Folinsbee and Hazucha (1989)
0.40	784	2 h with IE at $\dot{V}_E$ = 53 to 55 L/min	22 °C 50% RH	8 M, 10 F	9 asthmatics (5 F, 4 M), 9 healthy (5 F, 4 M), 18 to 34 years old	Decreased $PC_{100SRaw}$ from 33 mg/mL to 8.5 mg/mL in healthy subjects after O_3. $PC_{100SRaw}$ fell from 0.52 mg/mL to 0.19 mg/mL in asthmatic subjects after exposure to O_3 and from 0.48 mg/mL to 0.27 mg/mL after exposure to air.	Kreit et al. (1989)
0.12	235	6.6 h with IE at ≈25 L/min/m^2 BSA	NA	10 M	Healthy NS, 18 to 33 years old	Approximate doubling of mean methacholine responsiveness after exposure. On an individual basis, no relationship between O_3-induced changes in airway responsiveness and FEV_1 or FVC.	Folinsbee et al. (1988)
0.12 0.20	235 392	1 h at $\dot{V}_E$ = 89 L/min followed by 3 to 4 min at ≈150 L/min	31 °C 35% RH	15 M, 2 F	Elite cyclists, 19 to 30 years old	Greater than 20% increase in histamine responsiveness in one subject at 0.12 ppm O_3 and in nine subjects at 0.20 ppm O_3.	Gong et al. (1986)
0.40	784	3 h/day for 5 days in a row		13 M, 11 F	Healthy NS, 19 to 46 years old	Enhanced response to methacholine after first 3 days, but this response normalized by Day 5.	Kulle et al. (1982)
0.20 0.40 0.40	392 784 784	2 h with IE at 2 × resting 2 h with IE at 2 × resting 2 h/day for 3 days	22 °C 55% RH	12 M, 7 F	Healthy NS, 21 to 32 years old	110% increase in ΔSRaw to a 10-breath histamine (1.6%) aerosol challenge after exposure to O_3 at 0.40 ppm, but no change at 0.20 ppm. Progressive adaptation of this effect over 3-day exposure.	Dimeo et al. (1981)

TABLE AX6-11 (cont'd). Airway Responsiveness Following Ozone Exposures[a]

Ozone Concentration[b]		Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
ppm	µg/m^3						
0.10 0.32 1.00	196 627 1,960	2 h	NA	14	Health NS, 24 ± 2 years old	Increased airway responsiveness to methacholine immediately after exposure at the two highest concentrations of O_3.	König et al. (1980)
0.60	1,176	2 h with IE at 2 × resting	22 °C 55% RH	11 M, 5 F	9 atopic, 7 nonatopic, NS, 21 to 35 years old	Ten-breath methacholine or histamine challenge increased SRaw ≥ 150% in 16 nonasthmatics after O_3. On average, the atopic subjects had greater responses than the nonatopic subjects. The increased responsiveness resolved after 24 h. Atropine premedication blocked the O_3-induced increase in airway responsiveness.	Holtzman et al. (1979)
0.6	1,176	2 h at rest	NA	5 M, 3 F	Healthy NS, 22 to 30 years old	300% increase in histamine-induced ΔRaw 5 min after O_3 exposure; 84 and 50% increases 24 h and 1 week after exposure ($p > 0.05$), respectively. Two subjects had an increased response to histamine 1 week after exposure.	Golden et al. (1978)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.

persistent effect of O_3 on airway responsiveness which was only partially attenuated after 5 consecutive days of O_3 exposure.

The occurrence and duration of increased nonspecific airway responsiveness following O_3 exposure could have important clinical implications for asthmatics. Kreit et al. (1989) investigated changes in airway responsiveness to methacholine that occur after O_3 exposure in mild asthmatics. They found that the baseline $PC_{100}SRaw$ declined from 0.52 to 0.19 mg/mL after a 2-h exposure to 0.40 ppm O_3, as compared to a decline from 0.48 to 0.27 mg/mL after air exposure; however, because of the large variability in responses of the asthmatics, the percent decrease from baseline in mean $PC_{100}SRaw$ was not statistically different between healthy and asthmatic subjects (74.2 and 63.5%, respectively).

Two studies examined the effects of preexposure to O_3 on exacerbation of exercise-induced bronchoconstriction (Fernandes et al., 1994; Weymer et al., 1994). Fernandes et al. (1994) preexposed subjects with stable mild asthma and a history of > 15% decline in FEV_1 after exercise to 0.12 ppm O_3 for 1 h at rest followed by a 6-min exercise challenge test and found no significant effect on either the magnitude or time course of exercise-induced bronchoconstriction. Similarly, Weymer et al. (1994) observed that preexposure to either 0.10 or 0.25 ppm O_3 for 60 min while performing light IE did not enhance or produce exercise-induced bronchoconstriction in otherwise healthy adult subjects with stable mild asthma. Although the results suggested that preexposure to O_3 neither enhances nor produces exercise-induced asthma in asthmatic subjects, the relatively low total inhaled doses of O_3 used in these studies limit the ability to draw any definitive conclusions.

Gong et al. (1997b) found that subjects with asthma developed tolerance to repeated O_3 exposures in a manner similar to normal subjects; however, there were more persistent effects of O_3 on airway responsiveness, which only partially attenuated when compared to filtered air controls. Volunteer subjects with mild asthma requiring no more than bronchodilator therapy were exposed to filtered air or 0.4 ppm O_3, 3 h/d for 5 consecutive days, and follow-up exposures 4 and 7 days later. Symptom and FEV_1 responses were large on the 1st and 2nd exposure days, and diminished progressively toward filtered air responses by the 5^{th} exposure day. A methacholine challenge was performed when postexposure FEV_1 returned to within 10% of preexposure baseline levels. The first O_3 exposure significantly decreased $PD_{20}FEV_1$ by an order of magnitude and subsequent exposures resulted in smaller decreases, but they were still

significantly different from air control levels. Thus, the effects of consecutive O_3 exposures on bronchial reactivity differ somewhat from the effects on lung function. The same conclusion was drawn by Folinsbee et al. (1994) after consecutive 5-day O_3 exposures in healthy subjects, despite a much lower bronchial reactivity both before and after O_3 exposure.

A larger number of studies examined the effects of O_3 on exacerbation of antigen-induced asthma. Molfino et al. (1991) were the first to report the effects of a 1-h resting exposure to 0.12 ppm O_3 on the response of subjects with mild, stable atopic asthma to a ragweed or grass allergen inhalation challenge. Allergen challenges were performed 24 h after air and O_3 exposure. Their findings suggested that allergen-specific airway responsiveness of mild asthmatics is increased after O_3 exposure. However, Ball et al. (1996) and Hanania et al. (1998) were unable to confirm the findings of Molfino et al. (1991) in a group of grass-sensitive mild allergic asthmatics exposed to 0.12 ppm O_3 for 1 h. The differences between Hanania et al. (1998) and Molfino et al. (1991), both conducted in the same laboratory, were due to better, less variable control of the 1 h 0.12 ppm O_3 exposure and better study design by Hanania and colleagues. In the original, Molfino et al. (1991) study, the control (air) and experimental (O_3) exposures were not randomized after the second subject because of long-lasting (3 months), O_3-induced potentiation of airway reactivity in that subject. For safety reasons, therefore, the air exposures were performed prior to the O_3 exposures for the remaining 5 of 7 subjects being evaluated. It is possible that the first antigen challenge caused the significant increase in the second (post-O_3) antigen challenge.

Jörres et al. (1996) later confirmed that higher O_3 concentrations cause increased airway reactivity to specific antigens in subjects with mild allergic asthma, and to a lesser extent in subjects with allergic rhinitis, after exposure to 0.25 ppm O_3 for 3 h. The same laboratory repeated this study in separate groups of subjects with asthma and rhinitis and found similar enhancement of allergen responsiveness after O_3 exposure (Holz et al., 2002); however, the effects of a 3-h exposure to 0.25 ppm O_3 were more variable, most likely due to performing the allergen challenges 20 h after exposure, rather than the 3 h used in the first study.

The timing of allergen challenges in O_3-exposed subjects with allergic asthma is important. Bronchial provocation with allergen, and subsequent binding with IgE antibodies on mast cells in the lungs, triggers the release of histamine and leukotrienes and a prompt early-phase contraction of the smooth muscle cells of the bronchi, causing a narrowing of the lumen of the

bronchi and a decrease in bronchial airflow (i.e., decreased FEV_1). In many asthma patients, however, the release of histamine and leukotrienes from the mast cells also attracts an accumulation of inflammatory cells, especially eosinophils, followed by the production of mucus and a late-phase decrease in bronchial airflow for 4 to 8 h.

A significant finding from the study by Holz et al. (2002) was that clinically relevant decreases in FEV_1 ($\geq$ 20%) occurred during the early-phase allergen response in subjects with rhinitis after a consecutive 4-day exposure to 0.125 ppm O_3. Kehrl et al. (1999) previously found an increased reactivity to house dust mite antigen in asthmatics 16 to 18 h after exposure to 0.16 ppm O_3 for 7.6 hours. These important observations indicate that O_3 not only causes immediate increases in airway-antigen reactivity, but that this effect may persist for at least 18 to 20 h. Ozone exposure, therefore, may be a clinically important co-factor in the response to airborne bronchoconstrictor substances in individuals with pre-existing allergic asthma. It is plausible that this phenomenon could contribute to increased symptom exacerbations and, even, consequent increased physician or ER visits, and possible hospital admissions (*see Chapter 7*).

A number of human studies, especially more recent ones, have been undertaken to determine various aspects of O_3-induced increases in nonspecific airway responsiveness, but most studies have been conducted in laboratory animals (*See the toxicology chapter, Section 5.3.4.4*.). In humans, increased airway permeability (Kehrl et al., 1987; Molfino et al., 1992) could play a role in increased airway responsiveness. Inflammatory cells and mediators also could affect changes in airway responsiveness. The results of a multiphase study (Scannell et al., 1996; Balmes et al., 1997) showed a correlation between preexposure methacholine responsiveness in healthy subjects and increased SRaw caused by a 4 h exposure to 0.2 ppm O_3, but not with O_3-induced decreases in FEV_1 and FVC. The O_3-induced increase in SRaw, in turn, was correlated with O_3-induced increases in neutrophils and total protein concentration in BAL fluid. Subjects with asthma had a significantly greater inflammatory response to the same O_3 exposures, but it was not correlated with increased SRaw, and nonspecific airway provocation was not measured. Therefore, it is difficult to determine from this series of studies if underlying airway inflammation plays a role in increased airway responsiveness to nonspecific bronchoconstrictors. The study, however, confirmed an earlier observation (e.g., Balmes et al., 1996) that O_3-induced changes in airway inflammation and lung volume measurements are not correlated.

Hiltermann et al. (1998) reported that neutrophil-derived serine proteinases associated with O_3-induced inflammation are not important mediators for O_3-induced nonspecific airway hyperresponsiveness. Subjects with mild asthma, prescreened for O_3-induced airway responsiveness to methacholine, were administered an aerosol of recombinant antileukoprotease (rALP) or placebo at hourly intervals two times before and six times after exposure to filtered air or 0.4 ppm O_3 for 2 h. Methacholine challenges were performed 16 h after exposure. Treatment with rALP had no effect on the O_3-induced decrease in FEV_1 or $PC_{20}FEV_1$ in response to methacholine challenge. The authors speculated that proteinase-mediated tissue injury caused by O_3 may not be important in the development of airway hyperresponsiveness of asthmatics to O_3. In a subsequent study using a similar protocol (Peters et al., 2001), subjects with mild asthma were administered an aerosol of apocynin, an inhibitor of NADPH oxidase present in inflammatory cells such as eosinophils and neutrophils, or a placebo. In this study, methacholine challenge performed 16 h after O_3 exposure showed treatment-related effects on $PC_{20}FEV_1$, without an effect on FEV_1. The authors concluded that apocynin could prevent O_3-induced bronchial hyperresponsiveness in subjects with asthma, possibly by preventing superoxide formation by eosinophils and neutrophils in the larger airways.

Nightingale et al. (1999) reported that exposures of healthy subjects and subjects with mild atopic asthma to a lower O_3 concentration (0.2 ppm) for 4 h caused a similar neutrophilic lung inflammation in both groups but no changes in airway responsiveness to methacholine measured 24 h after O_3 exposure in either group. There were, however, significant decreases in FEV_1 of 6.7 and 9.3% immediately after O_3 exposure in both healthy and asthmatic subjects, respectively. In a subsequent study, a significant increase in bronchoresponsiveness to methacholine was reported 4 h after healthy subjects were exposed to 0.4 ppm O_3 for 2 h (Nightingale et al., 2000). In the latter study, preexposure treatment with inhaled budesonide (a corticosteroid) did not protect against O_3-induced effects on spirometry, methacholine challenge, or sputum neutrophils. These studies also confirm the earlier reported findings that O_3-induced increases in airway responsiveness usually resolve by 24 h after exposure.

Ozone-induced airway inflammation and hyperresponsiveness were used by Criqui et al. (2000) to evaluate anti-inflammatory properties of the macrolide antibiotic, azithromycin. In a double-blind, cross-over study, healthy volunteers were exposed to 0.2 ppm O_3 for 4 h after pretreatment with azithromycin or a placebo. Sputum induction 18 h postexposure resulted in

significantly increased total cells, percent neutrophils, IL-6, and IL-8 in both azithromycin- and placebo-treated subjects. Significant pre- to post-exposure decreases in FEV_1 and FVC also were found in both subject groups. Airway responsiveness to methacholine was not significantly different between azithromycin-treated and placebo-treated subjects when they were challenged 2 h after postexposure FEV_1 decrements returned to within 5 % of baseline. Thus, azithromycin did not have anti-inflammatory effects in this study.

The effects of dietary antioxidants on O_3-induced bronchial hyperresponsiveness were evaluated in adult subjects with asthma by Trenga et al. (2001). Recruited subjects were pretested for responsiveness to a provocative SO_2 challenge (0.10 and 0.25 ppm) while exercising on a treadmill and selected for study if they experienced a > 8% decrease in FVC. The rationale for this environmental challenge approach is based on previously published work by this laboratory (Koenig et al., 1990). In a placebo-controlled, double-blind crossover study, subjects took two vitamin supplements (400 IU vitamin E and 500 mg vitamin C) or two placebos once a day for 4 weeks, and were exposed to filtered air and to 0.12 ppm O_3 for 45 min during intermittent exercise at three times resting ventilation. Blood samples were used to verify placebo and vitamin treatment levels. Provocative airway challenges with SO_2 were performed immediately after O_3 exposure. Ozone exposure potentiated the SO_2 challenge in asthmatics, and subjects given antioxidant supplementation responded less severely to the airway challenge than subjects given the placebo. The protective effect of antioxidants was even more pronounced among subjects with more severe asthma and higher sensitivity to SO_2.

AX6.9 EFFECTS ON INFLAMMATION AND HOST DEFENSE

AX6.9.1 Introduction

In general, inflammation can be considered as the host response to injury, and the induction of inflammation can be accepted as evidence that injury has occurred. Several outcomes are possible: (1) inflammation can resolve entirely; (2) continued acute inflammation can evolve into a chronic inflammatory state; (3) continued inflammation can alter the structure or function of other pulmonary tissue, leading to diseases such as fibrosis or emphysema; (4) inflammation can alter the body's host defense response to inhaled microorganisms; and (5) inflammation can alter the lung's response to other agents such as allergens or toxins.

At present, it is known that short-term exposure of humans to O_3 can cause acute inflammation and that long-term exposure of laboratory animals results in a chronic inflammatory state (see Chapter 5). However, the relationship between repetitive bouts of acute inflammation in humans caused by O_3 and the development of chronic respiratory disease is unknown.

Bronchoalveolar lavage (BAL) using fiberoptic bronchoscopy has been utilized to sample cells and fluids lining the respiratory tract primarily from the alveolar region, although the use of small volume lavages or balloon catheters permits sampling of the airways. Cells and fluid can be retrieved from the nasal passages using nasal lavage (NL) and brush or scrape biopsy.

Several studies have analyzed BAL and NL fluid and cells from O_3-exposed humans for markers of inflammation and lung damage (see Tables AX6-12 and AX6-13). The presence of neutrophils (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. It is apparent, however, that inflammation within airway tissues may persist beyond the point that inflammatory cells are found in BAL fluid. Soluble mediators of inflammation such as the cytokines (IL-6, IL-8) and arachidonic acid metabolites (e.g., PGE_2, $PGF_{2\alpha}$, thromboxane, and leukotrienes [LTs] such as LTB_4) have been measured in the BAL fluid of humans exposed to O_3. In addition to their role in inflammation, many of these compounds have bronchoconstrictive properties and may be involved in increased airway responsiveness following O_3 exposure.

Some recent evidence suggests that changes in small airways function may provide a sensitive indicator of O_3 exposure and effect (*see Section AX6.2.5*), despite the fact that inherent variability in their measurement by standard spirometric approaches make their assessment difficult. Observations of increased functional responsiveness of these areas relative to the more central airways, and of persistent effects following repeated exposure, may indicate that further investigation of inflammatory processes in these regions is warranted.

Under normal circumstances, the epithelia lining the large and small airways develop tight junctions and restrict the penetration of exogenous particles and macromolecules from the airway lumen into the interstitium and blood, as well as restrict the flow of plasma components into the airway lumen. O_3 disrupts the integrity of the epithelial cell barrier in human airways, as measured by markers of plasma influx such as albumin, immunoglobulin, and other proteins into the airways. Markers of epithelial cell damage such as lactate dehydrogenase (LDH) also have been measured in the BAL fluid of humans exposed to O_3. Other soluble factors that have been

Table AX6-12. Studies of Respiratory Tract Inflammatory Effects from Controlled Human Exposure to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s)	Reference
Upper Airway Studies						
0.4	784	2 h	At rest	12 mild, asymptomatic dust mite-sensitive asthmatics; 18-35 years of age	Release of early-onset mast cell-derived mediators into NL in response to allergen not enhanced following O_3 exposure. Neutrophil and eosinophil inflammatory mediators were not increased after O_3 exposure or enhanced after allergen challenge. O_3 increased eosinophil influx following allergen exposure.	Michelson et al. (1999)
0.2	392	2 h	IE (15 min/30 min); ($\dot{V}_E$) ≈ 20 L/min/m² BSA	8 M, 5 F healthy NS 20-31 years of age	No neutrophilia in NL samples by 1.5 h post exposure. Depletion of uric acid in NL fluid by 30% during h 2 of exposure with increase in plasma uric acid levels. No depletion of ascorbic acid, reduced glutathione, extracellular superoxide dismutase.	Mudway et al. (1999)
0.4	980	2 h	At rest	10 mild NS asthmatics 18-35 years old	Response to allergen increased (NS). PMN and eosinophils increased after O_3 plus allergen challenge. Ozone alone increased inflammation in the nose.	Peden et al. (1995)
0.12 0.24	235 470	1.5 h	IE (20 L/min) at 15-min intervals	5 M, 5 F, asthmatic; 4 M, 4 F, nonasthmatic; 18 to 41 years old	NL done immediately and 24 h after exposure. Increased number of PMNs at both times in asthmatic subjects exposed to 0.24 ppm O_3; no change in nonasthmatic subjects. No change in lung or nasal function.	McBride et al. (1994)
0.5	980	4 h	Resting	6 M, 6 F, allergic rhinitics, 31.4 ± 2.0 (SD) years old	NL done immediately after exposure. Increased upper and lower respiratory symptoms and increased levels of PMNs, eosinophils, and albumin in NL fluid.	Bascom et al. (1990)
0.4	784	2 h	IE (70 L/min) at 15-min intervals	11 M, 18 to 35 years old	NL done immediately before, immediately after, and 22 h after exposure. Increased numbers of PMNs at both times after exposure; increased levels of tryptase, a marker of mast cell degranulation, immediately after exposure; increased levels of albumin 22 h after exposure.	Graham and Koren (1990) Koren et al. (1990)
0.5	980	4 h on 2 consecutive days	Resting	41 M (21 O_3-exposed, 20 air-exposed), 18 to 35 years old	NL done immediately before and after each exposure and 22 h after the second exposure. Increased levels of PMNs at all times after the first exposure, with peak values occurring immediately prior to the second exposure.	Graham et al. (1988)

Table AX6-12 (cont'd). Studies of Respiratory Tract Inflammatory Effects from Controlled Human Exposure to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s)	Reference
Lower Airway Studies						
0.2	392	2 h	IE (15 min/30 min); ($\dot{V}_E$) ≈ 20 L/min/m² BSA	6M, 6F healthy, nonatopic and 9 M, 6F mild asthmatic subjects, 19-48 years of age	Significantly higher baseline expression of IL-4 and IL-5 in bronchial mucosal biopsies from asthmatic vs. healthy subjects 6 h post-exposure. Following O_3 exposure, epithelial expression of IL-5, GM-CSF, ENA-78, and IL-8 increased significantly in asthmatics, as compared to healthy subjects.	Bosson et al. (2003)
0.1	196	2 h	mild IE	12 M, 10 F healthy subjects mean age ~30 years	Markers of exposure in exhaled breath condensate, including increased 8-isoprostane, TBARS and LTB-4, and a marker of ROS-DNA interaction in peripheral blood leukocytes (8-OHdG), were increased in a sub-set of subjects bearing the wild genotype for NAD(P)H:quinone oxidoreductase and the null genotype for glutathione-S-transferase M1.	Corradi et al. (2002)
0.2	392	2 h	IE (15 min/30 min); ($\dot{V}_E$) ≈ 20 L/min/m² body surface area	6M, 9F healthy subjects and 9 M, 6F mild asthmatics	No evidence seen for increased responsiveness to the inflammatory effects of O_3 in mild asthmatics versus healthy subjects at 6 h following exposure. Used neutrophil recruitment and exacerbation of pre-existing inflammation.	Stenfors et al. (2002)
0.27	529	2 h	IE (20 min/ 60 min); ($\dot{V}_E$) ≈ 25 L/min/m² BSA	12 subjects with intermittent-mild asthma exhibiting a dual response; 18-37 years of age	Exposure to O_3 24 h following allergen challenge resulted in a significant decrease in FEV1, FVC and VC and increase in symptom scores compared to air exposure. The percentage of eosinophils, but not neutrophils, in induced sputum was higher 6 h after O_3 than after air.	Vagaggini et al. (2002)
0.22	431	4 h	IE (15 min/30 min); ($\dot{V}_E$) = 25 L/min/m² BSA	12 nonsmoker, nonresponders; 13 nonsmoker, responders; 13 smokers; 18-40 years of age	Recovery of AM was approximately 3-fold higher in BAL from smokers versus nonsmokers. Unstimulated AM from smokers released ~2-fold greater amounts of superoxide anion than from nonsmokers at 30 min and 18 h post-exposure, but release was not further enhanced by stimulation of the cells. ROS generation by AM from non-smokers decreased following exposure at 18 h; markers of epithelial permeability increased. No relationship was found between measures of ROS production and lung function responsiveness to O_3.	Voter et al. (2001)

Table AX6-12 (cont'd). Studies of Respiratory Tract Inflammatory Effects from Controlled Human Exposure to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s)	Reference
Lower Airway Studies (cont'd)						
0.2	392	2 h	IE (15 min/30 min); ($\dot{V}_E$) ≈ 20 L/min/m² BSA	8M, 5F healthy nonsmokers; 20-31 years of age	Early (1.5 h post-exposure) increase in adhesion molecule expression, submucosal mast cell numbers and alterations in lining fluid redox status. No clear relationship between early markers of response and lung function deficits. 2.5-fold increase in % human leukocyte antigen (HLA)-DR+ alveolar macrophages in BAL.	Blomberg et al. (1999)
0.4	784	2 h	IE (15 min/30 min); ($\dot{V}_E$) ≈ 20 L/min/m² BSA	10M, 6F subjects with intermittent asthma; 19-35 years of age	In a cross-over study, levels of eosinophil cationic protein, IL-8 and percentage eosinophils were found to be highly correlated in induced sputum and BAL 16 h following O_3 exposure.	Hiltermann et al. (1999)
0.4	784	2 h	At rest	12 mild, asymptomatic dust mite-sensitive asthmatics; 18-35 years of age	Release of early-onset mast cell-derived mediators into NL in response to allergen not enhanced following O_3 exposure. Neutrophil and eosinophil inflammatory mediators were not increased after O_3 exposure or enhanced after allergen challenge. O_3 increased eosinophil influx following allergen exposure.	Michelson et al. (1999)
0.4	784	1 h	Continuous exercise; ($\dot{V}_E$) ≈ 30 L/min/m² BSA	4 healthy subjects	Apoptosis was observed in cells obtained by airway lavage 6 h following exposure. AM obtained by BAL showed the presence of a 4-hydroxynonenal (HNE) protein adduct and the stress proteins, 72-kD heat shock protein and ferritin. These effects were replicated by *in vitro* exposure of AM to HNE.	Hamilton et al. (1998)
0.2	392	2 h		15 healthy nonsmokers	Increased numbers of CD3+, CD4+, and CD8+ T lymphocyte subsets, in addition to neutrophils, in BAL 6 h post-exposure.	Blomberg et al. (1997)
0.4	784	2 h/day for 5 days, 2 h either 10 or 20 days later	IE (40 L/min) at 15-min intervals	16 M; 18 to 35 years of age	BAL done immediately after fifth day of exposure and again after exposure 10 or 20 days later. Most markers of inflammation (PMNs, IL-6, PGE_2, LDH, elastase, fibronectin) showed complete attenuation; markers of damage did not. Reversal of attenuation was not complete for some markers, even after 20 days.	Devlin et al. (1997)

Table AX6-12 (cont'd). Studies of Respiratory Tract Inflammatory Effects from Controlled Human Exposure to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s)	Reference
Lower Airway Studies (cont'd)						
0.22	431	4 h	IE 20 min ex/19 min rest ($\dot{V}_E$) ≈ 39-45 l/min	31M, 7F smokers and nonsmokers	Post O_3 exposure FEV_1 in 3 groups: Smokers (−13.9%); non-responders (−1.4%) and responders (−28.5%). PMN's increased immediately and at 18 h in all groups. Eosinophils and lymphocytes increased after O_3. IL-6 increased more in non-smokers. No relationship of symptoms with inflammation, lung function changes not related to inflammation. Nasal lavage indicators did not predict bronchial or alveolar inflammation.	Frampton et al. (1997a) Torres et al. (1997)
0.12	235	2 h	IE (15 min/30 min); ($\dot{V}_E$) ≈ 20 L/min/m² BSA	9M, 3F healthy nonsmokers; mean age ~ 28 years	Increase in the percentage of vessels expressing P-selectin in bronchial biopsies at 1.5 h post-exposure. No changes in FEV_1, FVC, inflammatory cells or markers in BAL, or vessels expressing VCAM-1, E-selectin or ICAM-1 in biopsies.	Krishna et al. (1997b)
0.16	314	7.6 h	IE 50 min/hr ($\dot{V}_E$) = 25 L/min	8 asthmatics sensitive to dust mites	Increased numbers of eosinophils in BAL after O_3 exposure.	Peden et al. (1997)
0.2	392	4 h T = 20 °C RH = 50%	IE (50 min/60 min); ($\dot{V}_E$) ≈ 44 L/min	14 M, 6 F healthy NS	Ozone increased PMN, protein, IL-8, for all subjects. No relationship of inflammation with spirometric responses.	Balmes et al. (1996)
0.4	784	2 h T = 22 °C RH = 50%	15 min rest 15 min exercise cycle ergometer ($\dot{V}_E$) ≈ 55 l/min	11 healthy nonsmokers; 18-35 years	Mean FEV_1, change = −10%. BAL occurred at 0, 2, or 4 h post-exposure. Small n limits statistical inference. Trend for PMN's to be highest at 4 h. LTC_4 increased at all time points. No change in PGE_2 or thromboxane.	Coffey et al. (1996)
0.4	784	2 h 15 min, ex/15 min, rest	($\dot{V}_E$) = 66 l/min	8 M healthy nonsmokers	Comparison of BAL at 1 h post-exposure vs. 18 h post-exposure. At 1 h, PMN's, total protein, LDH, α1-antitrypsin, fibronectin, PGE_2, thromboxane B_2, $C3_a$, tissue factor, and clotting factor VII were increased. IL-6 and PGE_2 were higher after 1 h than 18 h. Fibronectin and tissue plasminogen activator higher after 18 h. No time differences for PMN and protein.	Devlin et al. (1996) (compare with Koren et al. (1989a)
0.2	392	4 h T = 20 °C RH = 50%	IE (50 min/60 min); ($\dot{V}_E$) ≈ 44 L/min	17 M, 6F mild asthmatics	Increased PMN, protein, IL-8, LDH, in BAL. Inflammatory responses were greater than a group of non-asthmatics (Balmes et al., 1996)	Scannell et al. (1996)

Table AX6-12 (cont'd). Studies of Respiratory Tract Inflammatory Effects from Controlled Human Exposure to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s)	Reference
Lower Airway Studies (cont'd)						
0.4	784	2 h mouthpiece exposure 20 °C 42% RH	15 min exercise 15 min rest ($\dot{V}_E$) ≈ 40 l/min	5M, 5F healthy; age ≈ 30	Sputum induction 4 h after O_3 exposure 3-fold increase in neutrophils and a decrease in macrophages after O_3 exposure. IL-6, IL-8, and myeleperoxidase increased after O_3. Possible relationship of IL-8 and PMN levels.	Fahy et al. (1995)
0.2	392	4 h	IE (50 min/60 min); ($\dot{V}_E$) = 40 L/min	15 M, 13 F, 21 to 39 years old	Bronchial lavage, bronchial biopsies, and BAL done 18 h after exposure. BAL shows changes similar to other studies. Airway lavage shows increased cells, LDH, IL-8. Biopsies show increased number of PMNs.	Aris et al. (1993)
0.08 0.10	157 196	6.6 h	IE (50 min/60 min) + 35 min lunch; ($\dot{V}_E$) = 40 L/min	18 M, 18 to 35 years of age	BAL fluid 18 h after exposure to 0.1 ppm O_3 had significant increases in PMNs, protein, PGE_2, fibronectin, IL-6, lactate dehydrogenase, and α-1 antitrypsin compared with the same subjects exposed to FA. Similar but smaller increases in all mediators after exposure to 0.08 ppm O_3 except for protein and fibronectin. Decreased phagocytosis of yeast by alveolar macrophages was noted at both concentrations.	Devlin et al. (1990, 1991) Koren et al. (1991)
0.4	784	2 h	IE (15 min/30 min); ($\dot{V}_E$) = 70 L/min	10 M, 18 to 35 years old	BAL fluid 1 h after exposure to 0.4 ppm O_3 had significant increases in PMNs, protein, PGE_2, TXB_2, IL-6, LDH, α-1 antitrypsin, and tissue factor compared with the same subjects exposed to FA. Decreased phagocytosis of yeast by alveolar macrophages.	Koren et al. (1991)
0.3	588	1 h (mouth-piece)	CE (60 L/min)	5 M	Significantly elevated PMNs in the BAL fluid 1, 6, and 24 h after exposure, with peak increases at 6 h.	Schelegle et al. (1991)
0.40	784	2 h	IE (15 min/30 min); ($\dot{V}_E$) = 70 L/min	11 M, 18 to 35 years old	Macrophages removed 18 h after exposure had changes in the rate of synthesis of 123 different proteins as assayed by computerized densitometry of two-dimensional gel protein profiles.	Devlin and Koren (1990)
0.40	784	2 h	IE (15 min/30 min); ($\dot{V}_E$) = 70 L/min	11 M, 18 to 35 years old	BAL fluid 18 h after exposure contained increased levels of the coagulation factors, tissue factor, and factor VII. Macrophages in the BAL fluid had elevated tissue factor mRNA.	McGee et al. (1990)
0.4	784	2 h	IE (15 min/30 min); ($\dot{V}_E$) = 70 L/min	11 M, 18 to 35 years old	BAL fluid 18 h after exposure had significant increases in PMNs, protein, albumin, IgG, PGE_2, plasminogen activator, elastase, complement C3a, and fibronectin.	Koren et al. (1989a,b)

Table AX6-12 (cont'd). Studies of Respiratory Tract Inflammatory Effects from Controlled Human Exposure to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s)	Reference
Repeated Exposure Studies						
0.125 0.25	245 490	3 h exposures to both O_3 concs. and to FA; 3 h on four consecutive days to 0.125; study arms separated by > 4 wks	IE (15 min/30 min)	5M, 6F allergic asthmatic and 16M, 6F allergic rhinitic subjects; 19-53 years of age	All subjects underwent 4 exposure arms and were challenged with allergen 20 h following the last exposure in each. Sputum was induced 6-7 h later. In rhinitics, but not asthmatics, the incidence and magnitude of early phase FEV_1 decrements to Ag were greater after 0.25 and 4x 0.125 ppm O_3. Repeated exposure caused increases in neutrophil and eosinophil numbers in both subject groups, as well as increased percentage and number of lymphocytes in the asthmatics.	Holz et al., (2002)
0.25	490	2 h on four consecutive days; O_3 and FA exposure study arms separated by ≥3 wks	IE (30 min/60 min); ($\dot{V}_E$) ≈ 8 times the FVC/min	5M, 3F healthy subjects; 25-31 years of age	Maximal mean reductions in FEV_1 and FVC were observed on day 2, and became negligible by day 4. FEF_{25-75}, Vmax50, and Vmax75 were combined into a single value representing small airway function (SAWgrp). This variable was the only one to show persistent depression of the 24 h post-exposure baseline from day 2 to day 5 measurements. Numbers of PMNs in BAL fluid on day 5 were significantly higher in subjects following O_3, compared to air, exposures.	Frank et al. (2001)
0.2	392	single, 4 h exposures to O_3 and to FA; 4 h on four consecutive days to O_3; study arms separated by > 4 wks	IE (15 min/30 min); (Mean $\dot{V}_E$) = 14.8 L/min/m² BSA	15M, 8F healthy subjects; 21-35 years of age	All subjects underwent 3 exposure arms with BAL and bronchial mucosal biopsies performed 20 h following the last exposure in each. After repeated exposure, functional and BAL cellular responses were not different from those after FA, whereas total protein, IL-6, IL-8, reduced glutathione and ortho-tyrosine remained elevated. Also at this time, macroscopic scores of inflammation and tissue neutrophils were increased in mucosal biopsies. IL-10 was detected only in BAL fluid following repeated O_3 exposure.	Jörres et al. (2000)
0.2	392	single, 4 h exposure; 4 h exposures on four consecutive days; study arms separated by > 4 wks	IE (30 min/60 min); (Mean $\dot{V}_E$) = 25 L/min/m² BSA	9M, 6F healthy NS 23-37 years of age	Subjects were randomly assigned to each of the exposure regimens in a crossover design. Compared to single exposure, repeated exposure resulted in an initial progression followed by an attenuation of decrements in FEV_1, FVC and specific airways resistance by day 4. Bronchial and BAL washings showed decreases in the numbers of PMNs and fibronectin levels and IL-6 was decreased in BAL fluid on day 4.	Christian et al. (1998)

Table AX6-12 (cont'd). Studies of Respiratory Tract Inflammatory Effects from Controlled Human Exposure to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s)	Reference
Repeated Exposure Studies (cont'd)						
0.4	784	2 h/day for 5 days, 2 h either 10 or 20 days later	IE (60 L/min) at 15-min intervals	16 M; 18 to 35 years of age	BAL done immediately after fifth day of exposure and again after exposure 10 or 20 days later. Most markers of inflammation (PMNs, IL-6, PGE_2, fibronectin) showed complete attenuation; markers of damage (LDH, IL-8, protein, α1-antitrypsin, elastase) did not. Reversal of attenuation was not complete for some markers, even after 20 days.	Devlin et al. (1997)
0.40 0.60	784	2 h	IE (83 W for women, 100 W for men) at 15-min intervals	7M, 3F 23 to 41 years of age	BAL fluid 3 h after exposure had significant increases in PMNs, PGE_2, TXB_2, and $PGF_{2\alpha}$ at both O_3 concentrations.	Seltzer et al. (1986)

[a] See Appendix A for abbreviations and acronyms.
[b] Listed from lowest to highest O_3 concentration.

Table AX6-13. Studies of Effects on Host Defense, on Drug Effects and Supportive *In Vitro* Studies Relating to Controlled Human Exposure to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s)	Reference
Host Defense						
0.2	392	2 h	IE (15 min/30 min); ($\dot{V}_E$) ≈ 20 L/min/m² BSA	4M, 5F mild atopic asthmatics; 21-42 years of age	A significant decline in FEV_1 and VC immediately following exposure. A 2-fold increase in percent PMNs, with no changes in other biomarkers, was observed at 6 h post exposure. By 24 h post-exposure, PMNs had decreased, but albumin, total protein, myeloperoxidase and eosinophil cationic protein had increased.	Newson et al. (2000)
0.3	588	6 h/day for 5 consecutive days	IE (light treadmill)	24 M (12 O_3, 12 air)	Subjects inoculated with type 39 rhinovirus prior to exposure. NL was performed on the morning of Days 1 to 5, 8, 15, and 30. No difference in virus titers in NL fluid of air and O_3-exposed subjects at any time tested. No difference in PMNs or interferon gamma in NL fluid, or in blood lymphocyte proliferative response to viral antigen.	Henderson et al. (1988)
0.2	382	2 h	IE (15 min/30 min); ($\dot{V}_E$) ≈ 30 L/min	10M, 2F healthy NS mean ~ 28 years of age	Subjects were exposed to O_3 and FA in a cross-over design and underwent BAL 6 h post-exposure. O_3 exposure induced a 3-fold increase in % PMNs and epithelial cells, and increased IL-8, Gro-α, and total protein in BAL fluid. % PMNs correlated positively with chemokine levels. Exposure also resulted in a significant decrease in the CD4+/CD8+ ratio and the % of activated CD4+ and CD8+ T cells in BAL fluid.	Krishna et al. (1998)
Host Defense - Mucous Clearance						
0.4	784	1 h	CE (40 L/min)	15 healthy NS 18 to 35 years old	Subjects inhaled radiolabeled iron oxide particles 2 h after exposure. No significant O_3-induced effect on clearance of particles during the next 3 h or the following morning.	Gerrity et al. (1993)
0.20 0.40	392 784	2 h	IE (light treadmill)	7 M, 27.2 ± 6.0 (SD) years old	Subjects inhaled radiolabeled iron oxide particles immediately before exposure. Concentration-dependent increase in rate of particle clearance 2 h after exposure, although clearance was confined primarily to the peripheral airways at the lower O_3 concentration.	Foster et al. (1987)

Table AX6-13 (cont'd). Studies of Effects on Host Defense, on Drug Effects and Supportive *In Vitro* Studies Relating to Controlled Human Exposure to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s)	Reference
Host Defense - Epithelial Permeability						
0.15 0.35	294 686	130 min	IE 10 exercise/ 10 rest ($\dot{V}_E$) ≈ 8×FVC	8M,1F NS	Subjects inhaled ^{99m}Tc-DTPA 19 h after exposure to O_3. Clearance was increased in the lung periphery. Clearance was not related to spirometry.	Foster and Stetkiewicz (1996)
0.5	784	2.25 h	IE (70 L/min) at 15-min intervals	16 M, 20 to 30 years old	Similar design and results as earlier study (Kehrl et al., 1987). For the combined studies the average rate of clearance was 60% faster in O_3-exposed subjects.	Kehrl et al. (1989)
0.4	784	2 h	IE (70 L/min) at 15-min intervals	8 M, 20 to 30 years old	Subjects inhaled ^{99m}Tc-DTPA 75 min after exposure. Significantly increased clearance of ^{99m}Tc-DTPA from the lung in O_3-exposed subjects. Subjects had expected changes in FVC and SRaw.	Kehrl et al. (1987)
Drug Effects on Inflammation						
0.4	784	2 h		23 healthy adults	Subjects were exposed to O_3 following random selection for a 2 wk daily regimen of antioxidants, including vegetable juice high in the carotenoid, lycopene, or placebo. Concentrations of lycopene in the lungs of supplemented subjects increased by 12% following treatment. Supplemented subjects showed a 20% decrease in epithelial cell DNA damage as assessed by the Comet Assay. Effects attributable to lycopene could not be separated from those of other antioxidants.	Arab et al. (2002)
0.0 0.4	0 784	2 h IE 20 min mild-mod. exercise, 10 min rest	4 M, 5 F	Healthy NS 30 ± 3 years old	Subjects previously in Nightingale et al. (2000) study. Placebo-control: Immediately postexposure decrements in FVC (9%) and FEV_1 (14%) relative to pre-exposure values. FEV_1 decrement only 9% at 1 hr postexposure. By 3 h postexposure, recovery in FVC to 97% and FEV_1 to 98% of preexposure values. Significant increases in 8-isoprostane at 4 h postexposure. Budesonide for 2 wk prior to exposure did not affect responses.	Montuschi et al. (2002)
0.2	392	2 h All exposures separated by at least 2 wks (mean ≈ 30d)	IE (15 min/30 min); ($\dot{V}_E$) ≈ 20 L/min/m² BSA	Healthy (6 M, 9 F) and mild asthmatic (9 M, 6 F) subjects	Comparison was made of responses in healthy subjects, who had higher basal ascorbate (ASC) levels and lower glutathione disulfide (GSSG) levels than those of asthmatics. 6 h after exposure, ASC levels were decreased and GSSG levels were increased in BAL fluid of normals, but not asthmatics. Despite these differences in basal antioxidant levels and response to O_3, decrements in FEV_1 and neutrophil influx did not differ in the two subject groups.	Mudway et al. (2001)

Table AX6-13 (cont'd). Studies of Effects on Host Defense, on Drug Effects and Supportive *In Vitro* Studies Relating to Controlled Human Exposure to Ozone[a]

Oz one Concentration[b]		Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s)	Reference
ppm	µg/m^3					
Drug Effects on Inflammation (cont'd)						
0.4	784	2 h	IE 15-min intervals; $\dot{V}_E \approx 20$ L/min/m^2 BSA	Placebo group 15 M, 1 F Antioxidant group 13 M, 2 F Mean age 27 years	All subjects were exposed to FA and then entered a 2 wk regimen of placebo or 250 mg Vit C, 50IU α-tocopherol, and 12 oz veg. cocktail/day prior to O_3 exposure. O_3-induced decrements in FEV_1 and FVC were 30% and 24% less, respectively, in supplemented subjects. Percent neutrophils and IL-6 levels in BAL fluid obtained 1 h post exposure were not different in the two treatment groups.	Samet et al. (2001) Stech-Scott et al. (2004)
0.27	529	2 h All exposures separated by at least 1 wk (mean ≈ 14 d)	Continuous exercise; ($\dot{V}_E$) ≈ 25 L/min/m^2 BSA	7 M, F subjects with mild asthma; 20-50 years of age	Subjects were randomly exposed to FA and to O_3 before and after 4 wks of treatment with 400 µg budesonide, b.i.d. Budesonide did not inhibit the decrement in FEV_1 or increase in symptom scores, but significantly reduced the increase in % neutrophils and IL-8 in sputum induced 6 h post-exposure.	Vagaggini, et al. (2001)
0.4	784	2 h	IE 15-min intervals $\dot{V}_E$ min ≈ 30 L/min	5 M, 4 F healthy 6 M, 7 F asthmatics	Subjects were pretreated for 3 days prior to exposure with indomethacin (75 mg/day) or placebo. Similar reductions in FEV_1 and FVC were seen in both groups following placebo, whereas mid-flows showed greater decline in asthmatics than normals. Indomethacin attenuated decrements in FEV_1 and FVC in normals, but not asthamtics. Attenuation of decrements was seen for FEF_{60p} in asthmatics and for FEF_{50} in normals.	Alexis et al. (2000)
0.4	784	2 h	IE (20 min/30 min); workload @ 50 watts	6 M, 9 F healthy NS mean ~ 31 years of age	Subjects were randomly exposed to FA and to O_3 before and after 2 wks of treatment with 800 µg budesonide, b.i.d. O_3 caused significant decrements in FEV_1 and FVC immediately following exposure, and a small increase in Mch-reactivity and increases in neutrophils and myeloperoxidase in sputum induced at 4 h post-exposure. No differences were detected between responses in the two treatment groups.	Nightingale et al. (2000)
0.0 0.4	784	2 h IE 4 × 15 min at $\dot{V}_E$ =18 L/min/m^2 BSA 2 exposures: 25% subjects exposed to air-air, 75% to O_3-O_3	21 °C 40% RH	Weak responders 7 M, 13F Strong responders 21 M, 21 F Healthy NS 20 to 59 years old	Significant O_3-induced decrements in spirometric lung function. Young adults (< 35 years) were significantly more responsive than older individuals (> 35 years). Sufentanil, a narcotic analgesic, largely abolished symptom responses and improved FEV_1 in strong responders. Naloxone, an opioid antagonist, did not affect O_3 effects in weak responders. *See Section AX6.2.5.1*	Passannante et al. (1998)

Table AX6-13 (cont'd). Studies of Effects on Host Defense, on Drug Effects and Supportive *In Vitro* Studies Relating to Controlled Human Exposure to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s)	Reference
Drug Effects on Inflammation (cont'd)						
0.4	784	2 h	IE (60 L/min) at 15-min intervals	10 M	Subjects given 800 mg ibuprofen or placebo 90 min before exposure. Subjects given ibuprofen had less of a decrease in FEV_1 after O_3 exposure. BAL fluid 1 h after exposure contained similar levels of PMNs, protein, fibronectin, LDH, α-1 antitrypsin, LTB_4, and C3a in both ibuprofen and placebo groups. However, subjects given ibuprofen had decreased levels of IL-6, TXB_2, and PGE_2.	Hazucha et al. (1996)
0.4	784	2 h	IE (15 min/ 30 min); ($\dot{V}_E$) = 30 L/min/m² BSA	13 healthy male subjects	Four days prior to O_3 exposure, subjects received either no treatment, placebo or 150 mg indomethacin/day. Indomethacin treatment attenuated the O_3-induced decrease in FEV_1, but had no effect on the O_3-induced increase in Mch responsiveness.	Ying et al. (1990)
0.35	686	1 h	Continuous exercise; ($\dot{V}_E$) ≈ 60 L/min	14 healthy college-age males	In a placebo- and air-controlled random design, subjects were treated with 75 mg indomethacin every 12 h for 5 days prior to exposure. Indomethacin significantly reduced O_3-induced decrements in FEV_1 and FVC.	Schelegle et al. (1987)
Supportive In Vitro Studies						
0.01 to 0.10	19.6 to 196	6 h	bronchial epithelial cells	Nonatopic, nonasthmatic and atopic, mild asthmatic bronchial biopsy samples	Exposure to 0.01-0.10 ppm O_3 significantly decreased the electrical resistance of cells from asthmatic sources, compared to nonasthmatic sources. This range of O_3 concentrations also increased the movement of ^{14}C-BSA across the confluent cultures of "asthmatic" cells to an extent that was greater than that in "nonasthmatic" cells.	Bayram et al. (2002)
0.1	196	24 h	Nasal mucosa	Allergic and nonallergic patients	Increased concentrations of neurokinin A and substance P in medium following O_3 exposure. Levels of release of both neuropeptides were higher from tissues derived from allergic compared to nonallergic patients.	Schierhorn et al. (2002)
0.2	392	3 h	Nasal epithelial cells and airway epithelial cell line		Synergistic effect of O_3 exposure on rhinovirus-induced release of IL-8 at 24 h through mechanisms abrogated by antioxidant pretreatment. Additive enhancement of ICAM-1 expression.	Spannhake et al. (2002)
1	1,690	4 h	Macrophage-like THP-1 cells		THP-1 cells were treated with samples of human surfactant protein A (SP-A) genetic variants (SP-A1 and SP-A2) that had been previously exposed to O_3. O_3-exposed variants differed in their ability to stimulate the production of TNFα and IL-8 by these cells.	Wang et al. (2002)

Table AX6-13 (cont'd). Studies of Effects on Host Defense, on Drug Effects and Supportive *In Vitro* Studies Relating to Controlled Human Exposure to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s)	Reference
Supportive In Vitro Studies (cont'd)						
0.01 to 0.10	19.6 to 196	6 h	bronchial epithelial cells	Nonatopic, nonasthmatic and atopic, mild asthmatic bronchial biopsy samples	No difference in constitutive release of IL-8, GM-CSF, sICAM-1 and RANTES from cells from nonasthmatic and asthmatic sources, except for detection of RANTES in latter cells only. Increased release of all mediators 24 h after 0.05 to 0.10 ppm O_3 in "asthmatic" cells, but only IL-8 and sICAM-1 in "nonasthmatic" cells.	Bayram et al. (2001)
0.12 0.24 0.50	235 470 980	3 h	Nasal epithelial cells		Small dose-response activation of NF-κB coinciding with O_3-induced production of free radicals assessed by electron spin resonance. Increased TNFα at two higher concentrations of O_3 at 16 h post-exposure.	Nichols et al. (2001)
0.06 to 0.20	118 to 392	24 h	Nasal mucosa	105 surgical samples from atopic and nonatopic patients	Increased histamine release correlated with mast cell degranulation. Increased release of IL-1, IL-6, IL-8 and TNFα following O_3 exposure at 0.10 ppm. Release of IL-4, IL-6, IL-8 and TNFα at this concentration was significantly greater from tissues from atopic versus nonatopic patients.	Schierhorn et al. (1999)
0.5	980	1 h	Lung fibroblast cell line with an airway epithelial cell line		BEAS-2B cells in the presence or absence of HFL-1 cells were exposed and incubated for 11 or 23 h. Steady-state mRNA levels of alpha 1 procollagens type I and II, as well as TGFβ1, were increased in O_3-exposed co-cultured fibroblasts compared to air controls. Data support interactions between the cell types in the presence and the absence of O_3-exposure.	Lang et al. (1998)
0.5	980	1 h	tracheal epithelial cells		O_3 exposure caused an increase in ROS formation and a decline in PGE_2 production. No differences in mRNA and protein levels of prostaglandin endoperoxide G/H synthase 2 (PGHS-2) or the rate of its synthesis were detected, suggesting a direct effect of O_3-generated oxidants on PGHS-2 activity.	Alpert et al. (1997)
0.4	784	1 h	Lung fibroblasts; airway epithelial cell line		Cells incubated with O_3-exposed arachidonic acid (AA) were found to contain DNA single strand breaks. Pretreatment of the exposed AA solution with catalase eliminated the effect on DNA, indicating its dependence on H_2O_2 production. The effect was potentiated by the non-carbonyl component of ozonized AA.	Kozumbo et al. (1996)

Table AX6-13 (cont'd). Studies of Effects on Host Defense, on Drug Effects and Supportive *In Vitro* Studies Relating to Controlled Human Exposure to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s)	Reference
Supportive In Vitro Studies (cont'd)						
0.25 0.50	490 980	6 h	Human nasal epithelial cells		Increased in ICAM-1, IL-6, IL-1, and TNF expression at 0.5 ppm. No increase in IL-8 expression. No increases at 0.25 ppm.	Beck et al. (1994)
0.25 0.50 1.00	490 980 1,960	1 h	Airway epithelial cell line and alveolar macrophages		Increased secretion of IL-6, IL-8, and fibronectin by epithelial cells, even at lowest O_3 concentration. No O_3-induced secretion of these compounds by macrophages.	Devlin et al. (1994)
0.20 to 1.0	392 to 1960	2 h or 4 h	Airway epithelial cell line		O_3 caused a dose-related loss in cellular replicative activity at exposure levels that caused minimal cytotoxicity. DNAsingle strand breaks were not detected. These effects were different from those of H_2O_2 and, thus, not likely related to production of this oxidant within the cells.	Gabrielson et al. (1994).
0.25 0.50 1.00	490 980 1,960	1 h	Airway epithelial cell line		Concentration-dependent increased secretion of PGE_2, TXB_2, $PGF_{2\alpha}$, LTB_4, and LTD_4. More secretion basolaterally than apically.	McKinnon et al. (1993)
0.30 1.00	588 1,960	1 h	Alveolar macrophages		Concentration-dependent increases in PGE_2 production, and decreases in phagocytosis of sheep erythrocytes. No O_3-induced secretion of IL-1, TNF, or IL-6.	Becker et al. (1991)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.

studied include those involved with fibrin deposition and degradation (Tissue Factor, Factor VII, and plasminogen activator), potential markers of fibrogenesis (fibronectin, platelet derived growth factor), and components of the complement cascade (C3a).

Inflammatory cells of the lung such as alveolar macrophages (AMs), monocytes, and PMNs also constitute an important component of the pulmonary host defense system. Upon activation, they are capable of generating free radicals and enzymes with microbicidal capabilities, but they also have the potential to damage nearby cells. More recently published studies since the last literature review (U.S. Environmental Protection Agency, 1996) observed changes in T lymphocyte subsets in the airways following exposure to O_3 that suggest components of the immune host defense also may be affected.

AX6.9.2 Inflammatory Responses in the Upper Respiratory Tract

The nasal passages constitute the primary portal for inspired air at rest and, therefore, the first region of the respiratory tract to come in contact with airborne pollutants. Nikasinovic et al. (2003) recently reviewed the literature of laboratory-based nasal inflammatory studies published since 1985. Nasal lavage (NL) has provided a useful tool for assessing O_3-induced inflammation in the nasopharynx. Nasal lavage is simple and rapid to perform, is noninvasive, and allows collection of multiple sequential samples. Graham et al. (1988) reported increased levels of PMNs in the NL fluid of humans exposed to 0.5 ppm O_3 at rest for 4 h on 2 consecutive days, with NL performed immediately before and after each exposure, as well as 22 h after the second exposure. Nasal lavage fluid contained elevated numbers of PMNs at all postexposure times tested, with peak values occurring immediately prior to the second day of exposure. Bascom et al. (1990) exposed subjects with allergic rhinitis to 0.5 ppm O_3 at rest for 4 h, and found increases in PMNs, eosinophils, and mononuclear cells following O_3 exposure. Graham and Koren (1990) compared inflammatory mediators present in both the NL and BAL fluids of humans exposed to 0.4 ppm O_3 for 2 h. Increases in NL and BAL PMNs were similar (6.6- and eightfold, respectively), suggesting a qualitative correlation between inflammatory changes in the lower airways (BAL) and the upper respiratory tract (NL), although the PMN increase in NL could not quantitatively predict the PMN increase in BAL. Torres et al. (1997) compared NL and BAL in smokers and nonsmokers exposed to 0.22 ppm O_3 for 4 h. In contrast to Graham and Koren (1990), they did not find a relationship between numbers or percentages of

inflammatory cells (PMNs) in the nose and the lung, perhaps in part due to the variability observed in their NL recoveries. Albumin, a marker of epithelial cell permeability, was increased 18 h later, but not immediately after exposure, as seen by Bascom et al. (1990). Tryptase, a constituent of mast cells, was also elevated after O_3 exposure at 0.4 ppm for 2 h (Koren et al., 1990). McBride et al. (1994) reported that asthmatic subjects were more sensitive than non-asthmatics to upper airway inflammation at an O_3 concentration (0.24 ppm (1.5 h)) that did not affect lung or nasal function or biochemical mediators. A significant increase in the number of PMNs in NL fluid was detected in the asthmatic subjects both immediately and 24 h after exposure. Peden et al. (1995) also found that O_3 at a concentration of 0.4 ppm had a direct nasal inflammatory effect, and reported a priming effect on the response to nasal allergen challenge, as well. A subsequent study in dust mite-sensitive asthmatic subjects indicated that O_3 at this concentration enhanced eosinophil influx in response to allergen, but did not promote early mediator release or enhance the nasal response to allergen (Michelson et al., 1999). Similar to observations made in the lower airways, the presence of O_3 molecular "targets" in nasal lining fluid is likely to provide some level of local protection against exposure. In a study of healthy subjects exposed to 0.2 ppm O_3 for 2 h, Mudway and colleagues (1999) observed a significant depletion of uric acid in NL fluid at 1.5 h following exposure.

An increasing number of studies have taken advantage of advances in cell and tissue culture techniques to examine the role of upper and lower airway epithelial cells and mucosa in transducing the effects of O_3 exposure. Many of these studies have provided important insight into the basis of observations made *in vivo*. One of the methods used enables the cells or tissue samples to be cultured at the air-liquid interface (ALI), allowing cells to establish apical and basal polarity, and both cells and tissue samples to undergo exposure to O_3 at the apical surfaces as would occur *in vivo*. Nichols and colleagues (2001) examined human nasal epithelial cells grown at the ALI for changes in free radical production, based on electron spin resonance, and activation of the NF-κB transcription factor following exposure to O_3 at 0.12 to 0.5 ppm for 3 h. They found a dose-related activation of NF-κB within the cells that coincided with O_3-induced free radical production and increased release of TNFα at levels above 0.24 ppm. These data confirm the importance of this oxidant stress-associated pathway in transducing the O_3 signal within nasal epithelial cells, and suggest its role in directing the inflammatory response. In a study of nasal mucosal biopsy plugs, Schierhorn, et al. (1999) found that tissues exposed to O_3 at

a concentration of 0.1 ppm induced release of IL-4, IL-6, IL-8, and TNFα that was significantly greater from tissues from atopic patients compared to nonatopic controls. In a subsequent study, this same exposure regimen caused the release of significantly greater amounts of the neuropeptides, neurokinin A and substance P, from allergic patients, compared to nonallergic controls, suggesting increased activation of sensory nerves by O_3 in the allergic tissues (Schierhorn et al., 2002).

AX6.9.3 Inflammatory Responses in the Lower Respiratory Tract

Seltzer et al. (1986) were the first to demonstrate that exposure of humans to O_3 resulted in inflammation in the lung. Bronchoalveolar lavage fluid (3 h post-exposure) from subjects exposed to O_3 contained increased PMNs as well as increased levels of PGE_2, $PGF_{2\alpha}$, and TXB_2 compared to fluid from air-exposed subjects. Koren et al. (1989a,b) described inflammatory changes 18 h after O_3 exposure. In addition to an eightfold increase in PMNs, Koren et al. reported a two-fold increase in BAL fluid protein, albumin, and immunoglobulin G (IgG) levels, suggestive of increased epithelial cell permeability. There was a 12-fold increase in IL-6 levels, a two-fold increase in PGE_2, and a two-fold increase in the complement component, C3a. Evidence for stimulation of fibrogenic processes in the lung was shown by significant increases in coagulation components, Tissue Factor and Factor VII (McGee et al., 1990), urokinase plasminogen activator and fibronectin (Koren et al., 1989a). Subsequent studies by Lang, et al. (1998), using co-cultures of cells of the BEAS-2B bronchial epithelial line and of the HFL-1 lung fibroblast line, provided additional information about O_3-induced fibrogenic processes. They demonstrated that steady-state mRNA levels of both alpha 1 and procollagens type I and III in the fibroblasts were increased following O_3 exposure and that this effect was mediated by the O_3-exposed epithelial cells. This group of studies demonstrated that exposure to O_3 results in an inflammatory reaction in the lung, as evidenced by increases in PMNs and proinflammatory compounds. Furthermore, they demonstrated that cells and mediators capable of damaging pulmonary tissue are increased after O_3 exposure, and provided early suggestion of the potential importance of the epithelial cell-myofibroblast "axis" in modulating fibrotic and fibrinolytic processes in the airways.

Isolated lavage of the mainstream bronchus using balloon catheters or BAL using small volumes of saline have been used to assess O_3-induced changes in the large airways. Studies

collecting lavage fluid from isolated airway segments after O_3 exposure indicate increased neutrophils in the airways (Aris et al., 1993; Balmes et al., 1996; Scannell et al., 1996). Other evidence of airway neutrophil increase comes from studies in which the initial lavage fraction ("bronchial fraction") showed increased levels of neutrophils (Schelegle et al., 1991; Peden et al., 1997; Balmes et al., 1996; Torres et al., 1997). Bronchial biopsies show increased PMNs in airway tissue (Aris et al.,1993) and, in sputum collected after O_3 exposure, neutrophil numbers are elevated (Fahy et al., 1995).

Increased BAL protein, suggesting O_3-induced changes in epithelial permeability (Koren et al., 1989a, 1991 and Devlin et al., 1991) supports earlier work in which increased epithelial permeability, as measured by increased clearance of radiolabled diethylene triamine pentaacetic acid (^{99m}Tc-DTPA) from the lungs of humans exposed to O_3, was demonstrated (Kehrl et al., 1987). In addition, Foster and Stetkiewicz (1996) have shown that increased permeability persists for at least 18-20 h and the effect is greater at the lung apices than at the base. In a study of mild atopic asthmatics exposed to 0.2 ppm O_3 for 2 h, Newson, et al. (2000) observed a 2-fold increase in the percentage of PMNs present at 6 hours post exposure, with no change in markers of increased permeability as assessed by sputum induction. By 24 h, the neutrophilia was seen to subside while levels of albumin, total protein, myeloperoxidase, and eosinophil cationic protein increased significantly. It was concluded that the transient PMN influx induced by acute exposure of these asthmatic subjects was followed by plasma extravasation and the activation of both PMNs and eosinophils within the airway tissues. Such changes in permeability associated with acute inflammation may provide better access of inhaled antigens, particulates, and other substances to the submucosal region.

Devlin et al. (1991) reported an inflammatory response in subjects exposed to 0.08 and 0.10 ppm O_3 for 6.6 h. Increased numbers of PMNs and levels of IL-6 were found at both O_3 concentrations, suggesting that lung inflammation from O_3 can occur as a consequence of prolonged exposure to ambient levels while exercising. Interestingly, those individuals who had the largest increases in inflammatory mediators in this study did not necessarily have the largest decrements in pulmonary function, suggesting that separate mechanisms underlie these two responses. The absence of a relationship between spirometric responses and inflammatory cells and markers has been reported in several studies, including Balmes et al., 1996; Schelegle et al., 1991; Torres et al., 1997; Hazucha et al., 1996; Blomberg et al., 1999. These observations relate

largely to disparities in the times of onset and duration following single exposures. The relationship between inflammatory and residual functional responses following repeated or chronic exposures may represent a somewhat different case (see Section AX6.9.4).

As indicated above, a variety of potent proinflammatory mediators have been reported to be released into the airway lumen following O_3 exposure. Studies of human alveolar macrophages (AM) and airway epithelial cells exposed to O_3 *in vitro* suggest that most mediators found in the BAL fluid of O_3-exposed humans are produced by epithelial cells. Macrophages exposed to O_3 *in vitro* showed only small increases in PGE_2 (Becker et al., 1991). In contrast, airway epithelial cells exposed *in vitro* to O_3 showed large concentration-dependent increases in PGE_2, TXB_2, LTB_4, LTC_4, and LTD_4 (McKinnon et al., 1993) and increases in IL-6, IL-8, and fibronectin at O_3 concentrations as low as 0.1 ppm (Devlin et al., 1994). Macrophages lavaged from subjects exposed to 0.4 ppm (Koren et al., 1989a) showed changes in the rate of synthesis of 123 different proteins, whereas AMs exposed to O_3 *in vitro* showed changes in only six proteins, suggesting that macrophage function was altered by mediators released from other cells. Furthermore, recent evidence suggests that the release of mediators from AMs may be modulated by the products of O_3-induced oxidation of airway lining fluid components, such as human surfactant protein A (Wang et al., 2002).

Although the release of mediators has been demonstrated to occur at exposure concentrations and times that are minimally cytotoxic to airway cells, potentially detrimental latent effects have been demonstrated in the absence of cytotoxicity. These include the generation of DNA single strand breaks (Kozumbo et al., 1996) and the loss of cellular replicative activity (Gabrielson et al., 1994) in bronchial epithelial cells exposed *in vitro*, and the formation of protein and DNA adducts. A highly toxic aldehyde formed during O_3-induced lipid peroxidation is 4-hydroxynonenal (HNE). Healthy human subjects exposed to 0.4 ppm O_3 for 1 h underwent BAL 6 h later. Analysis of lavaged alveolar macrophages by Western blot indicated increased levels of a 32-kDa HNE-protein adduct, as well as 72-kDa heat shock protein and ferritin, in O_3- versus air-exposed subjects (Hamilton et al., 1998). In a recent study of healthy subjects exposed to 0.1 ppm O_3 for 2 h (Corradi et al., 2002), formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a biomarker of reactive oxidant species (ROS)-DNA interaction, was measured in peripheral blood lymphocytes. At 18 h post exposure, 8-OHdG was significantly increased in cells compared to pre-exposure levels, presumably linked to concurrent

increases in chemical markers of ROS. Of interest, the increase in 8-OHdG was only significant in a subgroup of subjects with the wild genotype for NAD(P)H:quinone oxidoreductase and the null genotype for glutathione-S-transferase M1, suggesting that polymorphisms in redox enzymes may confer "susceptibility' to O_3 in some individuals. The generation of ROS following exposure to O_3 has been shown to be associated with a wide range of responses. In a recent study, ROS production by alveolar macrophages lavaged from subjects exposed to 0.22 ppm for 4 h was assessed by flow cytometry (Voter et al., 2001). Levels were found to be significantly elevated 18 h post exposure and associated with several markers of increased permeability. An *in vitro* study of human tracheal epithelial cells exposed to O_3 indicated that generation of ROS resulted in decrease in synthesis of the bronchodilatory prostaglandin, PGE_2, as a result of inactivation of prostaglandin endoperoxide G/H synthase 2 (Alpert et al., 1997). These and similar studies indicate that the responses to products of O_3 exposure in the airways encompass a broad range of both stimulatory and inhibitory activities, many of which may be modulated by susceptibility factors upstream in the exposure process, at the level of compensating for the imposed oxidant stress.

The inflammatory responses to O_3 exposure also have been studied in asthmatic subjects (Basha et al., 1994; Scannell et al., 1996; Peden et al., 1997). In these studies, asthmatics showed significantly more neutrophils in the BAL (18 h post-exposure) than similarly exposed healthy individuals. In one of these studies (Peden et al., 1997), which included only allergic asthmatics who tested positive for Dematophagoides farinae antigen, there was an eosinophilic inflammation (2-fold increase), as well as neutrophilic inflammation (3-fold increase). In a study of subjects with intermittent asthma that utilized a 2-fold higher concentration of O_3 (0.4 ppm) for 2 h, increases in eosinophil cationic protein, neutrophil elastase and IL-8 were found to be significantly increased 16 h post-exposure and comparable in induced sputum and BAL fluid (Hiltermann et al, 1999). In two studies (Basha et al., 1994; Scannell et al., 1996), IL-8 was significantly higher in post-O_3 exposure BAL in asthmatics compared to non-asthmatics, suggesting a possible mediator for the increased neutrophilic inflammation in those subjects. In a recent study comparing the neutrophil response to O_3 at a concentration and exposure time similar to those of the latter three studies, Stenfors and colleagues (2002) were unable to detect a difference in the increased neutrophil numbers between 15 mild asthmatic and 15 healthy subjects by bronchial wash at the 6 h post-exposure time point. These results suggest that, at

least with regard to neutrophil influx, differences between healthy and asthmatic individuals develop gradually following exposure and may not become evident until later in the process. In another study, mild asthmatics who exhibited a late phase underwent allergen challenge 24 hrs before a 2 h exposure to 0.27 ppm O_3 or filtered air in a cross-over design (Vagaggini et al., 2002). At 6 h post-exposure, eosinophil numbers in induced sputum were found to be significantly greater after O_3 than after air. Studies such as these suggest that the time course of eosinophil and neutrophil influx following O_3 exposure can occur to levels detectable within the airway lumen by as early as 6 h. They also suggest that the previous or concurrent activation of proinflammatory pathways within the airway epithelium may enhance the inflammatory effects of O_3. For example, in an *in vitro* study of epithelial cells from the upper and lower respiratory tract, cytokine production induced by rhinovirus infection was enhanced synergistically by concurrent exposure to O_3 at 0.2 ppm for 3 h (Spannhake et al, 2002). The use of bronchial mucosal biopsies has also provided important insight into the modulation by O_3 of existing inflammatory processes within asthmatics. In a study of healthy and allergic asthmatic subjects exposed to 0.2 ppm O_3 or filtered air for 2 h, biopsies were performed 6 hr following exposure (Bosson et al., 2003). Monoclonal antibodies were used to assess epithelial expression of a variety of cytokines and chemokines. At baseline (air exposure), asthmatic subjects showed significantly higher expression of interleukins (IL)-4 and -5. Following O_3 exposure, the epithelial expression of IL-5, IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF) and epithelial cell-derived neutrophil-activating peptide 78 (ENA-78) was significantly greater in asthmatic subjects, as compared to healthy subjects. *In vitro* studies of bronchial epithelial cells derived by biopsy from nonatopic, nonasthmatic subjects and asthmatic subjects also demonstrated the preferential release of GM-CSF and also of regulated on activation, normal T cell-expressed and -secreted (RANTES) from asthmatic cells following O_3 exposure.

The time course of the inflammatory response to O_3 in humans has not been explored fully. Nevertheless, studies in which BAL was performed 1-3 h (Devlin et al., 1990; Koren et al., 1991; Seltzer et al., 1986) after exposure to 0.4 ppm O_3 demonstrated that the inflammatory response is quickly initiated, and other studies (Koren et al., 1989a,b; Torres et al., 1997; Scannell et al., 1996; Balmes et al., 1996) indicated that, even 18 h after exposure, inflammatory mediators such as IL-6 and PMNs were still elevated. However, different markers show peak responses at different times. Ozone-induced increases in IL-8, IL-6, and PGE_2 are greater

immediately after O_3 exposure, whereas BAL levels of fibronectin and plasminogen activator are greater after 18 h. PMNs and some products (protein, Tissue Factor) are similarly elevated both 1 and 18 h after O_3 exposure (Devlin et al., 1996; Torres et al., 1997). Schelegle et al. (1991) found increased PMNs in the "proximal airway" lavage at 1, 6, and 24 h after O_3 exposure, with a peak response at 6 h. In a typical BAL sample, PMNs were elevated only at the later time points. This is consistent with the greater increase 18 h after exposure seen by Torres et al. (1997). In addition to the influx of PMNs and (in allergic asthmatics) eosinophils, lymphocyte numbers in BAL were also seen to be elevated significantly at 6 h following exposure of healthy subjects to 0.2 ppm O_3 for 2 h (Blomberg et al., 1997). Analysis of these cells by flow cytometry indicated the increased presence of CD3+, CD4+ and CD8+ T cell subsets. This same laboratory later demonstrated that within 1.5 h following exposure of healthy subjects to the same O_3 regimen, expression of human leukocyte antigen (HLA)-DR on lavaged macrophages underwent a significant, 2.5-fold increase (Blomberg et al., 1999). The significance of these alterations in immune system components and those in IL-4 and IL-5 expression described above in the studies of Bosson et al. (2003) has not been fully explored and may suggest a role for O_3 in the modulation of immune inflammatory processes.

AX6.9.4 Effects of Repeated Exposures and Adaptation of Responses

Residents of areas with high oxidant concentrations tend to have somewhat blunted pulmonary function responses and symptoms to O_3 exposure (Hackney et al., 1976, 1977b, 1989; Avol et al., 1988; Linn et al., 1988). Animal studies suggest that while inflammation may be diminished with repeated exposure, underlying damage to lung epithelial cells continues (Tepper et al., 1989). Devlin et al. (1997) examined the inflammatory responces of humans repeatedly exposed to 0.4 ppm O_3 for 5 consecutive days. Several indicators of inflammation (e.g., PMN influx, IL-6, PGE_2, fibronectin, macrophage phagocytosis) were attenuated after 5 days of exposure (i.e., values were not different from FA). Several markers (LDH, IL-8, total protein, epithelial cells) did not show attenuation, indicating that tissue damage probably continues to occur during repeated exposure. The recovery of the inflammatory response occurred for some markers after 10 days, but some responses were not normalized even after 20 days. The continued presence of markers of cellular injury indicates a persistent but not necessarily perceived response to O_3. Christian et al. (1998) randomly subjected heathy subjects to a single

exposure and to 4 consecutive days of exposure to 0.2 ppm O_3 for 4 h. As reported by others, they found an attenuation of FEV_1, FVC and specific airway resistance when comparing the single exposure with day 4 of the multiday exposure regimen. Similarly, both "bronchial" and "alveolar" fractions of the BAL showed decreased numbers of PMNs and fibronectin concentration at day 4 versus the single exposure, and a decrease in IL-6 levels in the alveolar fraction. Following a similar study design and exposure parameters, but with single day filtered air controls, Jörres et al. (2000) found a decrease in FEV_1 and increases in the percentages of neutrophils and lymphocytes, in concentrations of total protein, IL-6, IL-8, reduced glutathione, ortho-tyrosine and urate in BAL fluid, but no changes in bronchial biopsy histology following the single exposure. Twenty hours after the day 4 exposure, both functional and BAL cellular responses to O_3 were abolished. However, levels of total protein, IL-6, IL-8, reduced glutathione and ortho-tyrosine were still increased significantly. In addition, following the day 4 exposure, visual scores for bronchitis, erythema and the numbers of neutrophils in the mucosal biopsies were increased. Their results indicate that, despite reduction of some markers of inflammation in BAL and measures of large airway function, inflammation within the airways persists following repeated exposure to O_3. In another study, Frank and colleagues (2001) exposed healthy subjects to filtered air and to O_3 (0.25 ppm, 2 h) on 4 consecutive days each, with pulmonary function measurements being made prior to and following each exposure. BAL was performed on day 5, 24 h following the last exposure. On day 5, PMN numbers remained significantly higher in the O_3 arm compared to air control. Of particular note in this study was the observation that small airway function, assessed by grouping values for isovolumetric FEF_{25-75}, Vmax50 and Vmax75 into a single value, showed persistent reduction from day 2 through day 5. These data suggest that methods to more effectively monitor function in the most peripheral airway regions, which are known to be the primary sites of O_3 deposition in the lung, may provide important information regarding the cumulative effects of O_3 exposure. It is interesting to note that Alexis et al. (2000) reported that, following exposure of normals and asthmatics to 0.4 ppm O_3 for 2 h, variables representing small airways function (viz., FEF_{25}, FEF_{50}, FEF_{60P}, FEF_{75}) demonstrated the greatest O_3-induced decline in the asthmatic subjects. Holz, et al. (2002) made a comparison of early and late responses to allergen challenge following O_3 in subjects with allergic rhinitis or allergic asthma. With some variation, both early and late FEV_1

and cellular responses in the two subject groups were significantly enhanced by 4 consecutive days of exposure to 0.125 ppm O_3 for 3 h.

AX6.9.5 Effect of Anti-Inflammatory and other Mitigating Agents

Studies have shown that indomethacin, a non-steroidal anti-inflammatory agent (NSAID) that inhibits the production of cyclooxygenase products of arachidonic acid metabolism, is capable of blunting the well-documented decrements in pulmonary function observed in humans exposed to O_3 (Schelegle et al., 1987; Ying et al., 1990). In the latter study, indomethacin did not alter the O_3-induced increase in bronchial responsiveness to methacholine. Pretreatment of healthy subjects and asthmatics with indomethacin prior to exposure to 0.4 ppm for 2 h significantly attenuated decreases in FVC and FEV_1 in normals, but not asthmatics (Alexis et al., 2000). Subjects have also been given ibuprofen, another NSAID agent that blocks cyclooxygenase metabolism, prior to O_3 exposure. Ibuprofen blunted decrements in lung function following O_3 exposure (Hazucha et al., 1996). Subjects given ibuprofen also had reduced BAL levels of the cyclooxygenase product PGE_2 and thromboxane B_2, as well as IL-6, but no decreases were observed in PMNs, fibronectin, permeability, LDH activity, or macrophage phagocytic function. These studies suggest that NSAIDs can blunt O_3-induced decrements in FEV_1 with selective (perhaps drug-specific) affects on mediator release and other markers of inflammation.

At least two studies have looked at the effects of the inhaled corticosteroid, budesonide, on the effects of O_3, with differing outcome perhaps associated with the presence of preexistent disease. Nightingale and colleagues (2000) exposed healthy nonsmokers to 0.4 ppm O_3 for 2 h following 2 wk of treatment with budesonide (800 micrograms, twice daily) or placebo in a blinded, randomized cross-over study. This relatively high exposure resulted in significant decreases in spirometric measures and increases in methacholine reactivity and neutrophils and myeloperoxidase in induced sputum. No significant differences were observed in any of these endpoints following budesonide treatment versus placebo. In contrast, Vagaggini et al. (2001) compared the effects of treatment with budesonide (400 micrograms, twice daily) for 4 wk on the responses of mild asthmatic subjects to exposure to 0.27 ppm O_3 for 2 h. Prior to exposure, at the midpoint and end of exposure, and at 6 h post exposure, FEV_1 was measured and a symptom questionnaire was administered; at 6 h post exposure, sputum was induced.

Budesonide treatment did not inhibit the decrement in FEV_1 or alter symptom score, but significantly blunted the increase in percent PMNs and concentration of IL-8 in the sputum. The difference in subject health status between the two studies (healthy versus mild asthmatic) may suggest a basis for the differing outcomes; however, because of differences in the corticosteroid dosage and O_3 exposure levels, that basis remains unclear.

Because the O_3 exerts its actions in the respiratory tract by virtue of its strong oxidant activity, it is reasonable to assume that molecules that can act as surrogate targets in the airways, as constituents of either extracellular fluids or the intracellular milieus, could abrogate the effects of O_3. Some studies have examined the ability of dietary "antioxidant" supplements to reduce the risk of exposure of the lung to oxidant exposure. In a study of healthy, nonsmoking adults, Samet and colleagues (2001) restricted dietary ascorbate and randomly treated subjects for 2 weeks with a mixture of vitamin C, α-tocopherol and vegetable cocktail high in carrot and tomato juices or placebo. Responses to 0.4 ppm O_3 for 2 h were assessed in both groups at the end of treatment. O_3-induced decrements in FEV_1 and FVC were significantly reduced in the supplemented group, whereas the inflammatory response, as assessed by percentage neutrophils and levels of IL-6 in BAL fluid, were unaffected by antioxidant supplementation. In a study that focused on supplementation with a commercial vegetable cocktail high in the carotenoid, lycopene, healthy subjects were exposed for 2 h to 0.4 ppm O_3 after 2 wk of antioxidant supplementation or placebo (Arab et al., 2002). These investigators observed that lung epithelial cell DNA damage, as measured by the Comet Assay, decreased by 20 % in supplemented subjects. However, the relationships between the types and levels of antioxidants in airway lining fluid and responsiveness to O_3 exposure is likely to be complex. In a study in which differences in ascorbate and glutathione concentrations between healthy and mild asthmatic subjects were exploited, no relationship between antioxidant levels and spirometric or cellular responses could be detected (Mudway, et al., 2001).

AX6.9.6 Changes in Host Defense Capability Following Ozone Exposure

Concern about the effect of O_3 on human host defense capability derives from numerous animal studies demonstrating that acute exposure to as little as 0.08 ppm O_3 causes decrements in antibacterial host defenses (see Chapter 5). A study of experimental rhinovirus infection in susceptible human volunteers failed to show any effect of 5 consecutive days of O_3 exposure on

the clinical evolution of, or host response to, a viral challenge (Henderson et al., 1988). Healthy men were nasally inoculated with type 39 rhinovirus (10^3 $TCID_{50}$). There was no difference between the O_3-exposed and control groups in rhinovirus titers in nasal secretions, in levels of interferon gamma or PMNs in NL fluid, or in blood lymphocyte proliferative response to rhinovirus antigen. However, subsequent findings that rhinovirus can attach to the intracellular adhesion molecule (ICAM)-1 receptor on respiratory tract epithelial cells (Greve et al., 1989) and that O_3 can up-regulate the ICAM-1 receptor on nasal epithelial cells (Beck et al., 1994) suggest that more studies are needed to explore the possibility that prior O_3 exposure can enhance rhinovirus binding to, and infection of, the nasal epithelium.

In a single study, human AM host defense capacity was measured *in vitro* in AMs removed from subjects exposed to 0.08 and 0.10 ppm O_3 for 6.6 h while undergoing moderate exercise. Alveolar macrophages from O_3-exposed subjects had significant decrements in complement-receptor-mediated phagocytosis of *Candida albicans* (Devlin et al., 1991). The impairment of AM host defense capability could potentially result in decreased ability to phagocytose and kill inhaled microorganisms *in vivo*. A concentration-dependent decrease in phagocytosis of AMs exposed to 0.1 to 1.0 ppm O_3 *in vitro* has also been shown Becker et al. (1991). Although the evidence is inconclusive at present, there is a concern that O_3 may render humans and animals more susceptible to a subsequent bacterial challenge.

Only two studies (Foster et al., 1987; Gerrity et al., 1993) have investigated the effect of O_3 exposure on mucociliary particle clearance in humans. Foster et al. (1987) had seven healthy subjects inhale radiolabeled particles (5 µm MMAD) and then exposed these subjects to FA or O_3 (0.2 and 0.4 ppm) during light IE for 2 h. Gerrity et al. (1993) exposed 15 healthy subjects to FA or 0.4 ppm O_3 during CE (40 L/min) for 1 h; at 2 h post O_3 exposure, subjects then inhaled radiolabeled particles (5 µm MMAD). Subjects in both studies had similar pulmonary function responses (average FVC decrease of 11 to 12%) immediately post exposure to 0.4 ppm O_3. The Foster et al. (1987) study suggested there is a stimulatory affect of O_3 on mucociliary clearance; whereas, Gerrity et al. (1993) found that in the recovery period following O_3 exposure, mucus clearance is similar to control, i.e., following a FA exposure. The clearance findings in these studies are complementary not conflicting. Investigators in both studies suggested that O_3-induced increases in mucociliary clearance could be mediated by cholinergic receptors. Gerrity et al. (1993) further suggested that transient clearance increases might be coincident to

pulmonary function responses; this supposition based on the return of sRaw to baseline and the recovery of FVC to within 5% of baseline (versus an 11% decrement immediately postexposure) prior to clearance measurements.

Insofar as the airway epithelial surface provides a barrier to entry of biological, chemical and particulate contaminants into the submucosal region, the maintenance of barrier integrity represents a component of host defense. Many of the studies of upper and lower respiratory responses to O_3 exposure previously cited above have reported increases in markers of airway permeability after both acute exposures and repeated exposures. These findings suggest that O_3 may increase access of airborne agents. In a study of bronchial epithelial cells obtained from nonatopic and mild atopic asthmatic subjects (Bayram et al., 2002), cells were grown to confluence and transferred to porous membranes. When the cultures again reached confluence, they were exposed to 0.01-0.1 ppm O_3 or air and their permeability was assessed by measuring the paracellular flux of ^{14}C-BSA. The increase in permeability 24 h following O_3 exposure was observed to be significantly greater in cultures of cells derived from asthmatics, compared to healthy subjects. Thus, the late increase in airway permeability following exposure of asthmatic subjects to O_3, of the sort described by Newson et al. (2000), may be related to an inherent susceptibility of 'asthmatic' cells to the barrier-reducing effects of O_3.

As referenced in Section 6.9.3, the O_3-induced increase in the numbers of CD8+ T lymphocytes in the airways of healthy subjects reported by Blomberg, et al. (1997) poses several interesting questions regarding possible alterations in immune surveillance processes following exposure. In a subsequent study from the same group, Krishna et al. (1998) exposed healthy subjects to 0.2 ppm O_3 or filtered air for 2 h followed by BAL at 6 h. In addition to increased PMNs and other typical markers of inflammation, they found a significant decrease in the CD4+/CD8+ T lymphocyte ratio and in the proportion of activated CD4+ and CD8+ cells. Studies relating to the effects of low-level O_3 exposure on the influx and activity of immuno-competent cells in the upper and lower respiratory tracts may shed additional light on modulation of this important area of host defense.

AX6.10 EXTRAPULMONARY EFFECTS OF OZONE

Ozone reacts rapidly on contact with respiratory system tissue and is not absorbed or transported to extrapulmonary sites to any significant degree as such. Laboratory animal studies suggest that reaction products formed by the interaction of O_3 with respiratory system fluids or tissues may produce effects measured outside the respiratory tract—either in the blood, as changes in circulating blood lymphocytes, erythrocytes, and serum, or as changes in the structure or function of other organs, such as the parathyroid gland, the heart, the liver, and the central nervous system. Very little is known, however, about the mechanisms by which O_3 could cause these extrapulmonary effects. (*See Section 5.4 for a discussion of the systemic effects of* O_3 *observed in laboratory animals*.)

The results from human exposure studies discussed in the previous criteria documents (U.S. Environmental Protection Agency, 1986, 1996) failed to demonstrate any consistent extrapulmonary effects. Early studies on peripheral blood lymphocytes collected from human volunteers did not find any significant genotoxic or functional changes at O_3 exposures of 0.4 to 0.6 ppm for up to 4 h/day. Limited data on human subjects indicated that 0.5 ppm O_3 exposure for over 2 h caused transient changes in blood erythrocytes and sera (e.g., erythrocyte fragility and enzyme activities), but the physiological significance of these studies remains questionable. The conclusions drawn from these early studies raise doubt that cellular damage or altered function is occurring to circulating cells at O_3 exposures under 0.5 ppm.

Other human exposure studies have attempted to identify specific markers of exposure to O_3 in blood. For example, Schelegle et al. (1989) showed that $PGF_{2\alpha}$ was elevated after O_3 exposure (0.35 ppm); however, no increase in α-1 protease inhibitor was observed by Johnson et al. (1986). Foster et al. (1996) found a reduction in the serum levels of the free radical scavenger α-tocopherol after O_3 exposure. Vender et al. (1994) failed to find any changes in indices of red blood cell antioxidant capacity (GSH, CAT) in healthy male subjects exposed to 0.16 ppm O_3 for 7.5 h while intermittently exercising. Liu et al. (1997, 1999) 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 0.12 and 0.40 ppm suggest that O_3 increases production of hydroxyl radical. The levels of DHBA were correlated with changes in spirometry.

Only a few experimental human studies have examined O_3 effects in other non-pulmonary organ systems besides blood. Early studies on the central nervous system (Gliner et al., 1979, 1980) were not able to find significant effects on motor activity or behavior (vigilance and psychomotor performance) from O_3 exposures at rest up to 0.75 ppm (U.S. Environmental Protection Agency, 1986). Drechsler-Parks et al. (1995) monitored ECG, HR, cardiac output, stroke volume, and systolic time intervals in healthy, older subjects (56 to 85 years of age) exposed to 0.45 ppm O_3 using a noninvasive impedance cardiographic method. No changes were found at this high O_3 concentration after 2 h of exposure while the subjects exercised intermittently at 25 L/min. Gong et al. (1998) monitored ECG, HR, cardiac output, blood pressure, oxygen saturation, and chemistries, as well as calculating other hemodynamic variables (e.g., stroke volume, vascular resistance, rate-pressure products) in both healthy and hypertensive adult males, 41 to 78 years of age. No major acute cardiovascular effects were found in either the normal or hypertensive subjects after exposure to 0.3 ppm O_3 for 3 h with intermittent exercise at 30 L/min. Statistically significant O_3 effects for both groups combined were a decrease in FEV_1, and increases in HR, rate-pressure product, and the alveolar-to-arterial PO_2 gradient, which might be more important in some patients with severe cardiovascular disease. [*See Section AX6.3 for a more detailed discussion of the effects of O_3 exposure in subjects with preexisting disease*.]

AX6.11 OZONE MIXED WITH OTHER POLLUTANTS

Controlled laboratory studies simulating conditions of ambient exposures have failed for the most part to demonstrate significant adverse effects either in healthy subjects, atopic individuals, or in young and middle-aged asthmatics.

AX6.11.1 Ozone and Sulfur Oxides

The difference in solubilities and other chemical properties of O_3 and SO_x seems to limit chemical interaction and formation of related species in the mixture of these pollutants either in liquid or gaseous phase. Laboratory studies reviewed in the previous O_3 criteria document (Table AX6-14) reported, except for one study (Linn et al., 1994), no significant effects on healthy individuals exposed to mixtures of O_3 and SO_2 or H_2SO_4 aerosol. In the study of Linn

Table AX6-14. Ozone Mixed with Other Pollutants[a]

Concentration[b] ppm	Concentration[b] µg/m³	Pollutant	Exposure Duration and Activity	Exposure Conditions[c]	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Sulfur-Containing Pollutants								
0.0 0.1 + 0.1 +	0 196[b] + 262[b] + 101[b]	Air O_3 + SO_2 + H_2SO_4	4 h IE 15' ex/ 15' rest V_E=22 L/min	25 °C 50% RH	8 M, 7 F 1 M, 4 F 10 M, 11 F	Healthy Asthmatic Allergic All NS, 9 to 12 yrs. old	Spirometry, PEFR and subjective symptoms score showed no meaningful changes between any condition for a total study population. The symptoms score reported by a subset of asthmatics/allergics were positively associated with inhaled concentration of H_2SO_4 (p = 0.01).	Linn et al. (1997)
0.2 0.3	392 564	O_3 NO_2 H_2SO_4	90 min. $\dot{V}_E \approx$ 32 L/min IE 3 × 15 min	21 °C 50% RH	24 (17 M, 7 F)	Asthmatic NS, 11 to 18 years old	$H_2SO_4/O_3/NO_2$, O_3/NO_2 and clean air produced similar responses	Linn et al. (1995)
0.12 0.30 0.05	235 564 70	O_3 NO_2 H_2SO_4 HNO_3	1.5 h with IE for 2 consecutive days; $\dot{V}_E \approx$ 23.2 L/min	22 °C 65% RH	22 completed study; 15 M, 7 F	Asthmatic NS, adolescents; NS, 12 to 19 years old	No significant pulmonary function changes following any exposure compared to response to clean air. Six additional subjects started the study, but dropped out due to uncomfortable symptoms.	Koenig et al. (1994)
0.12	235 100	O_3 H_2SO_4	6.5 h 2 consecutive days 50 min exercise/h $\dot{V}_E$ = 29 L/min	21 °C 50% RH	8 M, 7 F 13 M, 17 F	Nonasthmatic NS, 22 to 41 years old Asthmatic NS, 18 to 50 years old	Exposure to O_3 or $O_3 + H_2SO_4$ induced significant decrements in forced expiratory function. Differences between O_3 and $O_3 + H_2SO_4$ were, at best, marginally significant. O_3 is the more important pollutant for inducing respiratory effects. A few asthmatic and nonasthmatic subjects were more responsive to $O_3 + H_2SO_4$ than to O_3 alone.	Linn et al. (1994)
0.08 0.12 0.18	157 235 353 100 100	O_3 O_3 O_3 NaCl H_2SO_4	3-h exposure to aerosol, followed 24 h later by a 3-h exposure to O_3. IE (10 min per half hour) $\dot{V}_E$ = 4 times resting (30 to 364 min)	21 °C ≈40% RH	16 M, 14 F 10 M, 20 F	Nonasthmatic NS, 18 to 45 years old Asthmatic NS, 21 to 42 years old	No significant changes in symptoms or lung function with any aerosol/O_3 combination in the healthy group. In asthmatics, H_2SO_4 preexposure enhanced the small decrements in FVC that occurred following exposure to 0.18 ppm O_3. Asthmatics had no significant changes on FEV_1 with any O_3 exposures, but symptoms were greater.	Utell et al. (1994) Frampton et al. (1995)

Table AX6-14 (cont'd). Ozone Mixed with Other Pollutants[a]

Concentration[b] ppm	Concentration[b] µg/m³	Pollutant	Exposure Duration and Activity	Exposure Conditions[c]	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Sulfur-Containing Pollutants (cont'd)								
0.12 0.10	235 262	O_3 SO_2	1 h (mouthpiece) IE $\dot{V}_E \approx 30$ L/min 45-min exposure to air or O_3, followed by 15-min exposure to O_3 or SO_2	22 °C 75% RH	8 M, 5 F	Allergic asthmatics, 12 to 18 years old, medications withheld for at least 4 h before exposures	Prior exposure to O_3 potentiated pulmonary function responses to SO_2; decrements in FEV_1 were −3, −2, and −8% for the air/O_3, O_3/O_3, and O_3/SO_2 exposures, respectively.	Koenig et al. (1990)
0.25	490 1,200 to 1,600	O_3 H_2SO_4	2 h IE $\dot{V}_E$ = 30 to 32 L/min	35 °C 83% RH	9 M	Healthy NS, 19 to 29 years old	No significant effects of exposure to O_3 alone or combined with H_2SO_4 aerosol.	Horvath et al. (1987)
Nitrogen-Containing Pollutants								
0.0 0.2 0.4 0.2+0.4	0 392 752	Air O_3 NO_2 O_3+NO_2	3 h IE 10' ex/ 20' rest $\dot{V}_E$ =32 L/min		9 M, 2 F	Atopic asthmatics 22 to 41 yrs. old	Exposure to NO_2 alone had minimal effects on FEV_1. However, O_3 alone or in combination elicited significantly greater decline in FEV_1 in a short (3 h) exposure (higher concentrations) than a long (6 h) exposure where the effects were nonsignificant. Allergen challenge inhalation significantly reduced PD_{20} FEV_1 in all short but not the long exposures. No additive or potentiating effects have been observed.	Jenkins et al. (1999)
0.1 0.2 0.1+0.2	196 376	O_3 NO_2 O_3+NO_2	6 h IE 10' ex/ 20' rest $\dot{V}_E$ =32 L/min T = 25°C RH = 50%					
0.0 0.36 0.36 0.75 0.36	0 706 677 1,411 943	Air O_3 NO_2 NO_2 SO_2	2 h rest	Head only exposure	6 M, 6 F	Healthy NS 19 to 33 yrs. old	For NO_2 and SO_2 the absorbed fraction of O_3 increased relative (to baseline) whereas after O_3 exposure it decreased. The differences explained by an increased production of O_3-reactive substrate in ELF due to inflammation.	Rigas et al. (1997)
0.45 0.60	883 1,129	O_3 NO_2	2-h random exposures to FA, O_3, NO_2, and O_3 + NO_2; IE; $\dot{V}_E$ = 26-29 L/min	23.6 °C 62% RH	6 M 2 F	Healthy, NS, 56 to 85 years old	Exercise-induced cardiac output was smaller with O_3 + NO_2 exposure compared to FA or O_3 alone.	Drechsler-Parks et al. (1995)

Table AX6-14 (cont'd). Ozone Mixed with Other Pollutants[a]

Concentration[b] ppm	Concentration[b] µg/m³	Pollutant	Exposure Duration and Activity	Exposure Conditions[c]	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Nitrogen-Containing Pollutants (cont'd)								
0.30 0.60	589 1,129	O_3 NO_2	2-h exposure to NO_2 or FA, followed 3 h later by 2-h exposure to O_3, IE $\dot{V}_E$ = 20 L/min/m² BSA	21 °C 40% RH	21 F	Healthy NS, 18 to 34 years old	No significant effect of NO_2 exposures on any measured parameter. Sequential exposure of NO_2 followed by O_3 induced small but significantly larger decrements in FEV_1 and FEF_{25-75} than FA/O_3 sequence. Subjects had increased airway responsiveness to methacholine after both exposures, with significantly greater responsiveness after the NO_2/O_3 sequences than after the FA/O_3 sequence.	Hazucha et al. (1994)
0.2	392 500	O_3 HNO_3 H_2O	5 h IE (50 min/h exercise) $\dot{V}_E \approx$ 40 L/min 2 h HNO_3 or H_2O fog or air, followed by 1-h break, followed by 3 h O_3	20 °C 5% RH	6 M, 4 F	Healthy NS, minimum of 10% decrement in FEV_1 after 3 h exposure to 0.20 ppm O_3 with 50 min exercise/h	Exposure to HNO_3 or H_2O fog followed by O_3 induced smaller pulmonary function decrements than air followed by O_3.	Aris et al. (1991)
0.0 0.12 0.30 0.12+0.30	0 235 564	Air O_3 NO_2 $O_3 + NO_2$	1 h (mouthpiece) IE $\dot{V}_E$ = 33 L/min $\dot{V}_E$ = 35 L/min	22 °C 75% RH	5 M, 7 F 9 M, 3 F	Healthy NS, 12 to 17 years old Asthmatic 13 to 18 years old	Findings inconsistent across cohorts and atmospheres. No significant differences in FEV_1 and R_T between asthmatics and healthy, or between atmospheres and cohorts.	Koenig et al. (1988)
0.30 0.60	589 1,129	O_3 NO_2	1 h (mouthpiece) CE $\dot{V}_E \approx$ 70 L/min for men $\dot{V}_E \approx$ 50 L/min for women		20 M, 20 F	Healthy NS, 21.4 ± 1.5 (SD) years old for F, 22.7 ± 3.3 (SD) years old for M	No differences between responses to O_3 and $NO_2 + O_3$ for spirometric parameters. Increase in SRaw with $NO_2 + O_3$ was significantly less than for O_3 alone.	Adams et al. (1987)
0.30 0.30	589 564 200	O_3 NO_2 H_2SO_4	2 h CE for 20 min $\dot{V} \approx$ 25 L/min	28 to 29 °C 50 to 60% RH	6 M	Healthy subjects, some smokers	Possible small decrease in SG_{aw}	Kagawa (1986)
0.15 0.15	294 284 200	O_3 NO_2 H_2SO_4	2 h, 60 min total exercise $\dot{V} \approx$ 25 L/min		6 M		Possible small decrease in SG_{aw}	
0.15 0.15 0.15	294 282 393 200	O_3 NO_2 SO_2 H_2SO_4	2 h, 60 min total exercise $\dot{V} \approx$ 25 L/min		3 M		Possible small decrease in FEV_1	

Table AX6-14 (cont'd). Ozone Mixed With Other Pollutants[a]

Concentration[b] ppm	Concentration[b] µg/m³	Pollutant	Exposure Duration and Activity	Exposure Conditions[c]	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Peroxyacetyl Nitrate								
0.45 0.60 0.13	883 1,129 644	O_3 NO_2 PAN	2 h IE $\dot{V}_E \approx 25$ L/min	24 °C 55 to 58% RH	8 M, 8 F 8 M, 8 F	Healthy NS; 19 to 26 years old; 51 to 76 years old	No differences between responses to O_3 alone, $O_3 + NO_2$, O_3 + PAN, or $O_3 + NO_2$ + PAN.	Drechsler-Parks et al. (1989)
0.45 0.30	883 1,485	O_3 PAN	2 h IE $\dot{V}_E \approx 27$ L/min	22 °C 60% RH	3 M, 5 F	Healthy NS, mean age = 24 years	No differences between responses to exposure to O_3 alone and O_3 + PAN.	Drechsler-Parks et al. (1987b)
0.485 0.27	952 1,337	O_3 PAN	2 h IE $\dot{V}_E \approx 25$ L/min	21 °C WBGT	10 F	Healthy NS, 19 to 36 years old	Exposure to the mixture of PAN + O_3 induced decrements in FVC and FEV_1 averaging 10% greater than observed following exposure to O_3 alone.	Horvath et al. (1986)
Particle-Containing Pollutants								
0.0 0.12	0 235[b] + 153[b]	Air O_3 + $PM_{2.5}$	2-2.5 h rest	22 °C 30% RH	15 M, 10 F	Healthy NS 18 to 50 yrs. old	Neither systolic nor diastolic pressure has been affected by pollutants exposure despite a significant brachial artery constriction and a reduction in arterial diameter when compared to filtered air ($p = 0.03$). Absence of flow- and nitroglycerin-mediated brachial artery dilatation.	Brook et al. (2002)

[a]See Appendix A for abbreviations and acronyms.
[b]Grouped by pollutant mixture.
[c]$WBGT = 0.7\ T_{wet\ bult} + 0.3\ T_{dry\ bulb\ or\ globe}$.

et al. (1994), which was a repeated 6.5 h exposure protocol, O_3 alone and O_3 + H_2SO_4 induced significant spirometric decrements in healthy adults and asthmatics, but the magnitude of effects between exposure atmospheres was not significant. Asthmatic and atopic subjects showed somewhat enhanced or potentiated response to mixtures or sequential exposure, respectively; however, the observed effects were almost entirely attributable to O_3 (U.S. Environmental Protection Agency, 1996). Thus, in both healthy and asthmatic subjects, the interactive effects of O_3 and other pollutants were marginal and the response was dominated by O_3.

Since 1994, the only laboratory study that examined the health effects of a mixture of O_3 and sulfur oxides (SO_2 and H_2SO_4) has been that of Linn et al. (1997). In this study, the investigators closely simulated ambient summer haze air pollution conditions in Uniontown, PA as well as controlled the selection of study subjects with the objective to corroborate earlier reported findings of an epidemiologic study of Neas et al. (1995). The subjects were 41 children (22F/19M) 9 to 12 yrs old. Of these, 26 children had history of asthma or allergy. During a 14-day study period, children were exposed on the 4th and 11th day for 4 hrs (IE, 15 min @ avg. $\dot{V}_E$ 22 L/min) in random order to air and a mixture of 0.10 ppm O_3, 0.10 ppm SO_2 and 42 to 198 mg/m^3 H_2SO_4 (mean conc. 101 mg/m^3, 0.6 mm MMAD). The effects of controlled exposures were assessed by spirometry. Except for exposure days, children used diaries to record activity, respiratory symptoms, location, and PEFR. Thus, every exposure day was bracketed by 3 days of monitoring. Spirometry, PEFR, and respiratory symptoms score showed no meaningful changes between any condition for a total study population. The symptoms score reported by a subset of asthmatic/allergic subjects was positively associated with the inhaled concentration of H_2SO_4 ($p = 0.01$). However, the reported symptoms were different from the ones reported in the Uniontown study (Neas et al., 1995). Although retrospective statistical power calculations using these study observations for the symptoms score, PEFR, and spirometric endpoints were sufficient to detect with > 80% probability the same magnitude of changes as observed in Uniontown, the effects were minimal and not significant. The divergent observations of the two studies have been explained by the presence of an unidentified environmental factor in Uniontown, differences in physico-chemical properties of acid, differences in time course of exposure and history of previous exposure of children to pollutants, psychological and physiological factors related to chamber exposures, and by other conjectures.

AX6.11.2 Ozone and Nitrogen-Containing Pollutants

Nitrogen dioxide is a key component of the photooxidation cycle and formation of O_3. Both gases are almost invariably present in ambient atmosphere. Compared to O_3, NO_x species have limited solubility and moderate oxidizing capability. Both O_3 and NO_2 are irritants and tissue oxidants and exert their toxic actions through many common mechanisms. The regional dosimetry and the primary sites of action of O_3 and NO_2 overlap but are not the same. Since these gases are relatively insoluble in water, they will likely penetrate into the peripheral airways that are more sensitive to damage than better protected conducting airways. The controlled studies reviewed in the previous O_3 criteria document (Table AX6-14) generally reported only small pulmonary function changes after combined exposures of NO_2 or nitric acid (HNO_3) with O_3, regardless if the interactive effects were potentiating or additive. In two of these studies, the effects reached statistical significance, but they were not coherent. Preexposure with NO_2 potentiated both spirometric and nonspecific airway reactivity response following subsequent O_3 exposure (Hazucha et al., 1994); however, exposure to NO_2 + O_3 mixture blunted SRaw increase as compared to O_3 alone (Adams et al.,1987). As with O_3 and SO_x mixtures, the effects have been dominated by O_3 (U.S. Environmental Protection Agency, 1996).

Combined exposure to O_3 and NO_2 also blunted the exercise-induced increase in cardiac output found with FA and O_3 exposures alone (Drechsler-Parks, 1995). Eight healthy older subjects (56 to 85 years of age) were exposed for 2 h to FA, 0.60 ppm NO_2, 0.45 ppm O_3, and to 0.60 ppm NO_2 + 0.45 ppm O_3 while alternating 20-min periods of rest and exercise. Cardiac output, HR, stroke volume, and systolic time intervals were measured by noninvasive impedance cardiography at the beginning of each exposure, while the subjects were at rest, and again during the last 5 min of exercise. Metabolic exercise data ($\dot{V}_E$, $\dot{V}O_2$, f_B) also were measured. There were no statistically significant differences between exposures for HR, $\dot{V}_E$, $\dot{V}O_2$, f_B, stroke volume, or systolic time intervals. Exercise increased cardiac output after all exposures; however, the incremental increase over rest was significantly smaller for the combined O_3 and NO_2 exposures. The authors speculated that nitrate and nitrite reaction products from the interaction of O_3 and NO_2 cross the air/blood interface in the lungs, causing peripheral vasodilation and a subsequent drop in cardiac output. No major cardiovascular effects of O_3 only exposures have been reported in human subjects (*see Section AX6.10*).

Despite suggested potentiation of O_3 response by NO_2 in healthy subjects, it is unclear what response, and at what dose, either sequential or combined gas exposures will induce in asthmatics. Jenkins et al. (1999) exposed 11 atopic asthmatics in random order to air, 0.1 ppm O_3, 0.2 ppm NO_2, and 0.1 ppm O_3 + 0.2 ppm NO_2 for 6 h (IE for 10 min @ 32 L/min every 40 min). Two weeks later, 10 of these subjects were exposed for 3 h to doubled concentrations of these gases (i.e., 0.2 ppm O_3, 0.4 ppm NO_2, and 0.2 ppm O_3 + 0.4 ppm NO_2) employing the same exercise regimen. Immediately following each exposure, subjects were challenged with allergen (D. pteronyssinus) and PD_{20} FEV_1 was determined. Exposure to NO_2 alone had minimal effects on FEV_1 or airway responsiveness. However, O_3 alone or in combination with NO_2 elicited a significantly ($p < 0.05$) greater decline in FEV_1 in a short (3 h) exposure (higher concentrations) than the long (6 h) exposure, where the effects were not significant. Allergen challenge inhalation significantly ($p = 0.018$ to 0.002) reduced PD_{20} FEV_1 in all short, but not the long, exposures. No associations were observed between pollutant concentrations and physiologic endpoints. The statistical analyses of these data suggest that the combined effect (O_3 + NO_2) on lung function (FVC, FEV_1) was not significantly greater than the effect of individual gases for 6-h exposures, thus no additive or potentiating effects have been observed. Shorter 3-h exposures using twice as high NO_2 concentrations, however, showed significant FEV_1 decrements following exposures to atmospheres containing O_3. The analysis also suggests that it is the inhaled concentration, rather than total dose, that determines lung airway responsiveness to allergen.

The potential for interaction between O_3 and other gas mixtures was studied by Rigas et al. (1997). They used an O_3 bolus absorption technique to determine how exposures to O_3, NO_2, and SO_2 will affect distribution of O_3 adsorption by airway mucosa. The selected O_3 bolus volume was set to reach lower conducting airways. Healthy young nonsmokers (6F/6M) were exposed on separate days at rest in a head dome to 0.36 ppm O_3, 0.36 ppm NO_2, 0.75 ppm NO_2 and 0.75 ppm SO_2 for 2 h. The rationale for the selection of these gases was their differential absorption. Because O_3 and NO_2 are much less soluble in liquid (i.e., ELF) than SO_2, they are expected to penetrate deeper into the lung than SO_2 which is absorbed more quickly in the epithelial lining fluid of the upper airways. The actual experimental measurements have shown that during continuous NO_2 and SO_2 exposure the absorbed fraction of an O_3 bolus in lower conducting airways increased relative to baseline, whereas during continuous O_3 exposure the O_3

bolus fraction in lower conducting airways decreased. The authors attempted to explain the differences by suggesting that there may be increased production of an O_3-reactive substrate in epithelial lining fluid due to airway inflammation. As interpreted by the investigators, during NO_2 and SO_2 exposures the substrate was not depleted by these gases and so could react with the O_3 bolus, whereas during O_3 exposure the substrate was depleted, causing the fractional absorption of the O_3 bolus to decrease. Greater absorption in males than females for all gases was attributed to anatomical differences in the bronchial tree.

AX6.11.3 Ozone and Other Pollutant Mixtures Including Particulate Matter

Almost all of the studies published over the last twenty years investigating the health effects of mixtures of O_3 with other air pollutants involved peroxyacetyl nitrate (PAN). These studies on healthy individuals exposed under laboratory conditions came from the Horvath laboratory at UC Santa Barbara (Table AX6-13). In the last of this series of studies, Drechsler-Parks and colleagues (1989) found the same equivocal interaction of O_3 and PAN as in previous studies, which is attributable to O_3 exposure alone (U.S. Environmental Protection Agency, 1996). Subsequently, only a couple of studies have investigated the effects of more complex air pollutant mixtures on human pathophysiology under controlled conditions.

It is not only the interaction between air pollutants in ambient air; but, as Rigas et al. (1997) has found, an uneven distribution of O_3, SO_2, and NO_2 absorption in the lower conducting airways of young healthy subjects may modulate pathophysiologic response as well. Exposure to SO_2 and NO_2 increased, while exposure to O_3 decreased, the absorbing capacity of the airways for O_3. The authors have suggested that SO_2 or NO_2 -inflamed airways release additional substrates into the epithelial lining fluid that react with O_3, thus progressively removing O_3 from the airway lumen. This mechanism may explain findings of antagonistic response (e.g., Adams et al., 1987; Dreschler-Parks, 1995) when the two gases are combined in an exposure atmosphere.

The mechanisms by which inhalation exposure to other complex ambient atmospheres containing particulate matter (PM) and O_3 induce cardiac events frequently reported in epidemiologic studies are rarely studied in human subjects under laboratory conditions. Recently, Brook et al. (2002) have reported changes in brachial artery tone and reactivity in healthy nonsmokers following 2-h exposures to a mixture of 0.12 ppm O_3 and 153 $\mu g/m^3$ of

concentrated ambient $PM_{2.5}$, and a control atmosphere of filtered air with a trace of O_3, administered in random order. Neither systolic nor diastolic pressure was affected by pollutant exposure despite a significant brachial artery constriction and a reduction in arterial diameter when compared to filtered air ($p = 0.03$). The authors postulate that changes in arterial tone may be a plausible mechanism of air pollution-induced cardiac events. However, the observations of no changes in blood pressure, and an absence of flow- and nitroglycerin- mediated brachial artery dilatation, cast some doubt on the plausibility of this mechanism. A number of other proposed mechanisms advanced to establish a link between cardiac events due to pollution and changes in vasomotor tone based on the findings of this study are purely speculative.

AX6.12 CONTROLLED STUDIES OF AMBIENT AIR EXPOSURES

A large amount of informative O_3 exposure-effects data has been obtained in controlled laboratory exposure studies under a variety of different experimental conditions. However, laboratory simulation of the variable pollutant mixtures present in ambient air is not practical. Thus, the exposure effects of one or several artificially generated pollutants (i.e., a simple mixture) on pulmonary function and symptoms may not explain responses to ambient air where complex pollutant mixtures exist. Epidemiologic studies, which do investigate ambient air exposures, do not typically provide the level of control and monitoring necessary to adequately characterize short term responses. Thus, controlled exposures to ambient air using limited numbers of volunteers have been used to try and bridge the gap between laboratory and community exposures.

AX6.12.1 Mobile Laboratory Studies

As presented in previous criteria documents (U.S. Environmental Protection Agency, 1986; 1996), quantitatively useful information on the effects of acute exposure to photochemical oxidants on pulmonary function and symptoms responses originated from field studies using a mobile laboratory. These field studies involved subjects exposed to ambient air, FA without pollutants, or FA containing artificially generated concentrations of O_3 that are comparable to those measured in the ambient environment. As a result, measured pulmonary responses in ambient air can be directly compared to those found in more artificial or controlled conditions.

However, the mobile laboratory shares some of the same limitations of stationary exposure laboratories (e.g., limited number of both subjects and artificially generated pollutants for testing). Further, mobile laboratory ambient air studies are dependent on ambient outdoor conditions which can be unpredictable, uncontrollable, and not completely characterizable.

As summarized in Table AX6-15, investigators in California used a mobile laboratory and demonstrated that pulmonary effects of ambient air in Los Angeles residents are related to O_3 concentration and level of exercise (Avol et al., 1983, 1984, 1985a,b,c, 1987; Linn et al., 1980, 1983). Avol et al. (1987) observed no significant pulmonary function or symptoms responses in children (8 to 11 years) engaged in moderate continuous exercise for 1 h while breathing ambient air with an O_3 concentration of 0.113 ppm. However, significant pulmonary function decrements and increased symptoms of breathing discomfort were observed in healthy exercising (1 h continuous) adolescents (Avol et al., 1985a,b), athletes, (Avol et al., 1984, 1985c) and lightly exercising asthmatic subjects (Linn et al., 1980, 1983) at O_3 concentrations averaging from 0.144 to 0.174 ppm. Many of the healthy subjects with a history of allergy appeared to be more responsive to O_3 than "nonallergic" subjects (Linn et al., 1980, 1983), although a standardized evaluation of atopic status was not performed. Comparative studies of exercising athletes (Avol et al., 1984, 1985c) with chamber exposures to oxidant-polluted ambient air (mean O_3 concentration of 0.153 ppm) and purified air containing a controlled concentration of generated O_3 at 0.16 ppm showed similar pulmonary function responses and symptoms, suggesting that acute exposures to coexisting ambient pollutants had minimal contribution to these responses under the typical summer ambient conditions in Southern California. This contention is similar to, but extends, the laboratory finding of no significant difference in pulmonary function effects between O_3 and O_3 plus PAN exposures (Drechsler-Parks, 1987b). *Additional supporting evidence is provided in Section AX6.11.*

AX6.12.2 Aircraft Cabin Studies

Respiratory symptoms and pulmonary function effects resulting from exposure to O_3 in commercial aircraft flying at high altitudes, and in altitude-simulation studies, have been reviewed elsewhere (U.S. Environmental Protection Agency, 1986, 1996). Flight attendants, because of their physical activities at altitude, tend to receive higher exposures. In a series of hypobaric chamber studies of nonsmoking subjects exposed to 1,829 m (6,000 ft) and O_3 at

Table AX6-15. Acute Effects of Ozone in Ambient Air in Field Studies With a Mobile Laboratory[a]

Mean Ozone Concentration[b]		Ambient Temperature[c]	Exposure	Activity Level			
ppm	µg/m^3	(°C)	Duration	($\dot{V}_E$)	Number of Subjects	Observed Effect(s)	Reference
0.113. ± .033	221 ± 65	33 ± 1	1 h	CE (22 L/min)	66 healthy children, 8 to 11 years old	No significant changes in forced expiratory function and symptoms of breathing discomfort after exposure to 0.113 ppm O_3 in ambient air.	Avol et al. (1987)
0.144 ± .043	282 ± 84	32 ± 1	1 h	CE (32 L/min)	59 healthy adolescents, 12 to 15 years old	Small significant decreases in FVC (−2.1%), $FEV_{0.75}$ (−4.0%), FEV_1 (−4.2%), and PEFR (−4.4%) relative to control with no recovery during a 1-h postexposure rest; no significant increases in symptoms.	Avol et al. (1985a,b)
0.153 ± .025	300 ± 49	32 ± 2	1 h	CE (53 L/min)	50 healthy adults (competitive bicyclists)	Mild increases in symptoms scores and significant decreases in FEV_1 (−5.3%) and FVC; mean changes in ambient air were not statistically different from those in purified air containing 0.16 ppm O_3.	Avol et al. (1984, 1985c)
0.156 ± .055	306 ± 107	33 ± 4	1 h	CE (38 L/min)	48 healthy adults, 50 asthmatic adults	No significant changes for total symptom score or forced expiratory performance in normals or asthmatics; however, FEV_1 remained low or decreased further (−3%) 3 h after ambient air exposure in asthmatics.	Linn et al. (1983) Avol et al. (1983)
0.165 ± .059	323 ± 115	33 ± 3	1 h	CE (42 L/min)	60 "healthy" adults (7 were asthmatic)	Small significant decreases in FEV_1 (−3.3%) and FVC with no recovery during a 1-h postexposure rest; TLC decreased and ΔN_2 increased slightly.	Linn et al. (1983) Avol et al. (1983)
0.174 ± .068	341 ± 133	33 ± 2	2 h	IE (2 times resting) at 15-min intervals	34 "healthy" adults, 30 asthmatic adults	Increased symptom scores and small significant decreases in FEV_1 (−2.4%), FVC, PEFR, and TLC in both asthmatic and healthy subjects; however, 25/34 healthy subjects were allergic and "atypically" reactive to polluted ambient air.	Linn et al. (1980, 1983)

[a]See Appendix A for abbreviations and acronyms.
[b]Ranked by lowest level of O_3 in ambient air, presented as the mean ± SD.
[c]Mean ± SD.

concentrations of 0.2 and 0.3 ppm for 3 or 4 h (Lategola et al., 1980a,b), increased symptoms and pulmonary function decrements occurred at 0.3 ppm but not at 0.2 ppm.

Commercial aircraft cabin O_3 levels were reported to be very low (average concentration 0.01 to 0.02 ppm) during 92 randomly selected smoking and nonsmoking flights in 1989 (Nagda et al., 1989). None of these flights recorded O_3 concentrations exceeding the 3-h time-weighted average (TWA) standard of 0.10 ppm promulgated by the Federal Aviation Administration (FAA, 1980), probably due to the use of O_3-scrubbing catalytic filters (Melton, 1990). However, in-flight O_3 exposure can still occur because catalytic filters are not necessarily in continuous use during flight. Other factors to consider in aircraft cabins, however, are erratic temperature changes, lower barometric pressure and oxygen pressure, and lower humidity, often reaching levels between 4 and 17% (Rayman, 2002).

Ozone contamination aboard high-altitude aircraft also has been an interest to the U.S. Air Force because of complaints by crew members of frequent symptoms of dryness and irritation of the eyes, nose, and throat and an occasional cough (Hetrick et al., 2000). Despite the lack of ventilation system modifications as used in commercial aircraft, the O_3 concentrations never exceeded the FAA ceiling limit of 0.25 ppm and exceeded the 3-h TWA of 0.10 ppm only 7% of the total monitored flight time (43 h). The authors concluded that extremely low average relative humidity (12%) during flight operations was most likely responsible for the reported symptoms.

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7. EPIDEMIOLOGICAL STUDIES OF HUMAN HEALTH EFFECTS ASSOCIATED WITH AMBIENT OZONE EXPOSURE

7.1 INTRODUCTION

This chapter evaluates current epidemiologic literature on health and physiological effects of ambient O_3 exposure. Epidemiologic studies linking community ambient O_3 concentrations to health effects were reported in the 1996 Ozone Air Quality Criteria Document (O_3 AQCD; U.S. Environmental Protection Agency, 1996a). Many of those studies reported that pulmonary function decrements, hospital and emergency department admissions, and respiratory symptoms in human populations were associated with ambient levels of O_3. Numerous more recent epidemiologic studies discussed in this chapter evaluate the relationship of ambient O_3 to morbidity and mortality, and thereby provide an expanded basis for assessment of health effects associated with exposures to O_3 at concentrations currently encountered in the U.S.

As discussed elsewhere in this document (Chapters 5 and 6), a substantial amount of experimental evidence links O_3 exposure unequivocally with respiratory effects in laboratory animals and humans. These include structural changes in the bronchiolar-alveolar transition (centriacinar) region of the lung, biochemical evidence of acute cellular/tissue injury, inflammation, increased frequency and severity of experimental bacterial infection, and temporary reductions in mechanical lung function. These effects have been observed with exposure to O_3 at ambient or near-ambient concentrations. Thus, many of the reported epidemiologic associations of ambient O_3 with respiratory health effects have considerable biological credibility. Accordingly, the new epidemiologic studies of ambient O_3 assessed here are best considered in combination with information from the other chapters on ambient O_3 concentration and exposure (Chapter 3), and toxicological effects of O_3 in animals and humans (Chapters 5 and 6, respectively). The epidemiologic studies constitute important information on associations between health effects and exposures of human populations to "real-world" O_3 and also help to identify susceptible subgroups and associated risk factors.

7.1.1 Approach to Identifying O_3 Epidemiologic Studies

Numerous O_3 epidemiologic papers have been published since completion of the 1996 O_3 AQCD. The U.S. Environmental Protection Agency (NCEA-RTP) has implemented a systematic approach to identify relevant epidemiologic studies for consideration in this chapter. In general, an ongoing search has been employed in conjunction with other strategies to identify O_3 epidemiology literature pertinent to developing criteria for O_3 National Ambient Air Quality Standards (NAAQS). A publication base was established using Medline, Pascal, BIOSIS, NTIS, and Embase, and a set of search terms proven by prior use to identify pertinent literature. The search strategy was reexamined and modified to enhance identification of published papers. PubMed was added to the search regime.

While the above search regime provided good coverage of the relevant literature, additional approaches augmented the traditional search methods. First, a Federal Register Notice was issued requesting information and published papers from the public at large. Next, non-EPA chapter authors, expert in this field, identified literature on their own. NCEA-RTP staff also identified publications as an element of their assessment and interpretation of the literature. Finally, additional potentially relevant publications will be included following external review as a result of comments from both the public and CASAC. The combination of these approaches is believed to produce a comprehensive collection of studies appropriate for review and assessment here. The principal objective criteria used for selecting literature for the present assessment is to include all identified studies that evaluated the relationship between measured ambient O_3 levels and a human health outcome. All new studies published through October 2004, as identified using the search, have been included in this AQCD and additional efforts have been made to assess more recent studies.

7.1.2 Approach to Assessing Epidemiologic Evidence

Definitions of the various types of epidemiologic studies assessed have been provided in an earlier PM AQCD (U.S. Environmental Protection Agency, 1996b). Briefly, epidemiologic studies are generally divided into two groups, *morbidity* studies and *mortality* studies. *Morbidity* studies evaluate O_3 effects on a wide range of health endpoints, including the following: changes in pulmonary function, respiratory symptoms, and self-medication in asthmatics; respiratory- and cardiovascular-related emergency department visits and hospital admissions;

and changes in cardiovascular physiology/functions and airway inflammation. *Mortality* studies investigate O_3 effects on total (nonaccidental) mortality and cause-specific mortality, providing evidence related to a clearly adverse endpoint. The epidemiologic strategies most commonly used in O_3 health studies are of four types: (1) ecologic studies; (2) time-series semi-ecologic studies; (3) prospective cohort studies; and (4) case-control and crossover studies. All of these are observational studies rather than experimental studies.

The approach to assessing epidemiologic evidence has been eloquently stated most recently in the 2004 PM AQCD (U.S. Environmental Protection Agency, 2004a) and is adapted here. The critical assessment of epidemiologic evidence presented in this chapter is conceptually based upon consideration of salient aspects of the evidence of associations so as to reach fundamental judgments as to the likely causal significance of the observed associations. In so doing, it is appropriate to draw from those aspects initially presented in Hill's classic monograph (Hill, 1965) and widely used by the scientific community in conducting such evidence-based reviews. A number of these aspects are judged to be particularly salient in evaluating the body of evidence available in this review, including the aspects described by Hill as strength, experiment, consistency, plausibility, and coherence. Other aspects identified by Hill, including temporality and biological gradient, are also relevant and considered here (e.g., in characterizing lag structures and concentration-response relationships), but are more directly addressed in the design and analyses of the individual epidemiologic studies included in this assessment. As discussed below, these salient aspects are interrelated and considered throughout the evaluation of the epidemiologic evidence presented in this chapter, and are more generally reflected in the integrative synthesis presented in Chapter 8 of this AQCD.

In the following sections, the general evaluation of the strength of the epidemiological evidence reflects consideration not only of the magnitude of reported O_3 effect estimates and their statistical significance, but also of the precision of the effect estimates and the robustness of the effects associations. Consideration of the robustness of the associations takes into account a number of factors, including in particular the impact of alternative models and model specifications and potential confounding by copollutants, as well as issues related to the consequences of measurement error.

Consideration of the consistency of the effects associations, as discussed in the following sections, involves looking across the results of multi- and single-city studies conducted by

different investigators in different places and times. Relevant factors are known to exhibit much variation across studies, including, for example, the presence and levels of copollutants, the relationships between central measures of O_3 and exposure-related factors, relevant demographic factors related to sensitive subpopulations, and climatic and meteorological conditions. Thus, in this case, consideration of consistency and the related heterogeneity of effects are appropriately understood as an evaluation of the similarity or general concordance of results, rather than an expectation of finding quantitative results within a very narrow range.

Looking beyond the epidemiological evidence, evaluation of the biological plausibility of the O_3-health effects associations observed in epidemiologic studies reflects consideration of both exposure-related factors and dosimetric/toxicologic evidence relevant to identification of potential biological mechanisms. Similarly, coherence of health effects associations reported in the epidemiologic literature reflects consideration of information pertaining to the nature of the various respiratory- and cardiac-related mortality and morbidity effects and biological markers evaluated in toxicologic and human clinical studies. These broader aspects of the assessment are only touched upon in this chapter but are more fully integrated in the discussion presented in Chapter 8.

In identifying these aspects as being particularly salient in this assessment, it is also important to recognize that no one aspect is either necessary or sufficient for drawing inferences of causality. As Hill (1965) emphasized:

> None of my nine viewpoints can bring indisputable evidence for or against the cause-and-effect hypothesis and none can be required as a sine qua non. What they can do, with greater or less strength, is to help us to make up our minds on the fundamental question — is there any other way of explaining the set of facts before us, is there any other answer equally, or more, likely than cause and effect?

Thus, while these aspects frame considerations weighed in assessing the epidemiologic evidence, they do not lend themselves to being considered in terms of simple formulas or hard-and-fast rules of evidence leading to answers about causality (Hill, 1965). One, for example, cannot simply count up the numbers of studies reporting statistically significant results for the various O_3 indicators and health endpoints evaluated in this assessment and reach credible conclusions about the relative strength of the evidence and the likelihood of causality. Rather, these important considerations are taken into account and discussed throughout this assessment with

the goal of producing an objective appraisal of the evidence, informed by peer and public comment and advice, including weighing of alternative views on controversial issues, leading to conclusions and inferences that reflect the best judgements of the scientists engaged in this review.

In assessing the relative scientific quality of epidemiologic studies reviewed here and to assist in interpreting their findings, the following considerations were taken into account:

(1) To what extent are the aerometric data/exposure metrics used of adequate quality and sufficiently representative to serve as credible exposure indicators, well-reflecting geographic or temporal differences in study population pollutant exposures in the range(s) of pollutant concentrations evaluated?

(2) Were the study populations well-defined and adequately selected so as to allow for meaningful comparisons between study groups or meaningful temporal analyses of health effects results?

(3) Were the health endpoint measurements meaningful and reliable, including clear definition of diagnostic criteria utilized and consistency in obtaining dependent variable measurements?

(4) Were the statistical analyses used appropriate, and properly performed and interpreted, including accurate data handling and transfer during analyses?

(5) Were likely important covariates (e.g., potential confounders or effect modifiers) adequately controlled for or taken into account in the study design and statistical analyses?

(6) Were the reported findings internally consistent, biologically plausible, and coherent in terms of consistency with other known facts?

These guidelines provide benchmarks for judging the relative quality of various studies and in assessing the body of epidemiologic evidence. Detailed critical analysis of all epidemiologic studies on O_3 health effects, especially in relation to all of the above questions, is beyond the scope of this document. Of most importance for present purposes are those studies which provide useful qualitative or quantitative information on exposure-response relationships for health effects associated with ambient air levels of O_3 likely to be encountered in the U.S. among healthy and susceptible populations.

7.1.3 Study Designs and Analysis Methods Used to Assess O_3 Health Effects

Prior to discussing results from the recent O_3 studies, issues and questions arising from the study designs and analysis methods used in the assessment of O_3 effect estimates will be briefly presented. Air pollution time-series studies in particular have design and analysis aspects that complicate the interpretation of O_3 health effects. Analyses using administrative data (e.g., numbers of deaths and emergency hospital admissions) have inherent limitations as well as strengths (Virnig and McBean, 2001), however in this section we focus mainly on the topics of exposure assessment and model specification in time-series or longitudinal studies. Potential biases that may result from O_3 exposure measurement error, and choice of exposure index and lag period are first presented. A discussion of model specification issues and potential confounding by temporal factors, meteorological effects, seasonal trends, and copollutants follow. Integrative discussion of these topics is presented later in Section 7.6.

7.1.3.1 Exposure Assessment in Epidemiologic Studies

In general, the exposure of the participant is not directly observed, and the concentration of O_3 and other air pollutants at one or more stationary air monitors is used as a proxy for individual exposure to ambient air pollution. In an ideal situation, studies of air pollution health effects would be conducted at the individual level, with information on personal exposure to the various pollutants. However, determining accurate personal exposure information is difficult and often impractical. In many epidemiologic studies, especially time-series studies with administrative data on mortality and hospitalization outcomes, data from central ambient monitoring sites often are used as the estimate of exposure. Routinely collected ambient data, though readily available and convenient, may not represent true personal exposure. The use of ambient data tends to underestimate the effect of the air pollutant on health (Krzyzanowski, 1997). As discussed thoroughly in the 2004 PM AQCD (Section 8.4.5), the resulting exposure measurement error and its effect on the estimates of relative risk must be considered. In theory, there are three components to exposure measurement error: (1) the use of average population rather than individual exposure data; (2) the difference between the average personal ambient exposure and the ambient concentrations; and (3) the difference between the true and measured ambient concentrations. Zeger et al. (2000) indicated that the first and third error components are largely Berksonian errors and would not significantly bias the risk estimate. However, the

second error component resulting from the difference between the average personal ambient exposure and ambient concentration levels might introduce bias, especially if indoor sources are associated with ambient levels.

Several studies measured O_3 concentrations in a variety of indoor environments, including homes (Lee et al., 2004), schools (Linn et al., 1996), and the workplace (Liu et al., 1995). Indoor O_3 concentrations were, in general, approximately one-tenth of the outdoor concentrations in these studies. However, the specific contribution of indoor sources to indoor O_3 levels has not been investigated. Few indoor sources of O_3 exist, possible sources being office equipment (e.g., photocopiers, laser printers) and air cleaners. As described in Section 3.9 of this document, indoor O_3 exposure primarily results from infiltration of O_3 from the outdoors through ventilation and is noted as minimal.

The impact of measurement error on O_3 effect estimates was demonstrated in a study by Navidi et al. (1999). In this study, a simulation was conducted using data from the University of Southern California Children's Health Study of the long-term effects of air pollutants on children. The effect estimate from computed "true" O_3 exposure was compared to effect estimates from exposure determined using several methods: (1) ambient stationary monitors; (2) the microenvironmental approach (multiply concentrations in various microenvironments by time present in each microenvironment); and (3) personal sampling. Effect estimates based on all three exposure measures were biased towards the null. The bias that results when using the microenvironmental and personal sampling approach is due to nondifferential measurement error. Use of ambient monitors to determine exposure will tend to overestimate true personal O_3 exposure (assumes that subjects are outdoors 100% of their time), thus generally their use will result in effect estimates that are biased towards the null.

7.1.3.2 O_3 Exposure Indices Used

The results of studies of mortality and morbidity health outcomes from exposure to O_3 are usually presented in this document as a relative risk, or risk rate relative to a baseline mortality or morbidity rate. These relative risks are based on an incremental change in exposure. To enhance comparability between studies, presenting these relative risks by a uniform exposure increment is needed. However, determining a standard increment is complicated by the use of different O_3 exposure indices in the existing health studies. The three daily O_3 exposure indices

that most often appear in the literature are 1-h average maximum (1-h max), 8-h average maximum (8-h max), and 24-h average (24-h avg). As concentrations are lower and less variable for the longer averaging times, relative risks of adverse health outcomes for a specific numeric concentration range are not directly comparable across metrics. Using the nationwide distributional data for O_3 monitors in U.S. Metropolitan Statistical Areas, increments representative of a low-to-high change in O_3 concentrations were developed based on mean and upper percentile values in the dataset (Langstaff, 2003):

Daily Exposure Index	Exposure Increment (ppb)
1-h max O_3	40
8-h max O_3	30
24-h avg O_3	20

In the following discussion sections, efforts were made to standardize the O_3 excess risks using these increments, except as noted, so that the risk estimates could be compared across studies.

7.1.3.3 Lag of O_3 Exposure Used

Lags of exposure may reflect the distribution of effects across time in a population and the potential mechanisms of effects. However, simply choosing the most significant exposure lag may bias the air pollution risk estimates away from the null, as shown by a simulation by Lumley and Sheppard (2000) that used $PM_{2.5}$ as an example. This is especially true when the choice is made from a large number of lags. Most of the O_3 time-series studies examined relatively small numbers of lagged days, typically 0 through 3 days, and/or cumulative lags thereof (e.g., cumulative lag of 0 and 1 day). An examination of the "most significant" lags suggests that the majority of the single-day associations were immediate (0-day lag), not a random pattern in which associations could be observed on any of the lags examined with equal probabilities. However, the lags may vary by health outcome, as some effects are delayed (e.g., airway inflammation) and are captured in longer lag periods. Note that when associations are found at multiple days, presenting selected risk estimates from single-day lags may result in bias (i.e., typically toward underestimation of magnitude of overall risk).

7.1.3.4 Model Specification Issues

The relationships between daily numbers of deaths and hospital admissions, and levels of O_3 and related environmental factors have been analyzed widely over the past decade, yielding insights into the possible effects of O_3 on acute exacerbations of respiratory and cardiovascular diseases, and related mortality. These daily time-series studies exploit the high degree of day-to-day variability in ambient air pollution concentrations to develop quantitative estimates of impacts on daily health outcomes. The basic analytical approach used to estimate the effects of O_3 in this type of study is multiple regression. Because a given location is followed over time, many factors that might confound a multicity cross-sectional study do not affect time-series studies. Cross-sectional confounders include cigarette smoking, diet, occupation, and other risk factors that may vary across cities in ways that correlate with variations in air pollution levels. In contrast, these factors are unlikely to vary over time in a way that correlates with day-to-day variations in air pollution, thus confounding by these factors is minimized in a time-series study. Longer-term secular time trends, such as changes in morbidity due to improved clinical management of disease, also generally do not present a confounder problem in time-series studies because these trends are removed analytically. Other advantages of the daily time-series study design include the relatively large sample sizes in terms of person-days and the readily available data, making such studies convenient and economical to conduct in a wide variety of locations.

However, several challenges present themselves with respect to designing and interpreting time-series studies. The principal challenge facing the analyst in the daily time-series context is avoiding bias due to confounding by short-term temporal factors operating over time scales from days to seasons. In the current regression models used to estimate short-term effects of air pollution, there are two major potential confounders that need to be considered: (1) seasonal trend and other "long-wave" temporal trends; and (2) weather effects. Both of these variables tend to predict a significant fraction of fluctuations in time-series. Unfortunately, both of these terms are also highly correlated with O_3, as O_3 has strong seasonal cycles and is formed more at higher temperatures. The correlation of O_3 with these confounding terms tends to be higher than that for PM or other gaseous pollutants. In the U.S., the mass concentration of $PM_{2.5}$ generally does not have strong seasonal cycles like O_3 because $PM_{2.5}$ tends to reflect both primary emissions (throughout the year, but often higher in winter in most U.S. cities) and secondary

aerosols (higher in summer). Therefore, $PM_{2.5}$ and O_3 effect estimates from studies primarily designed to examine $PM_{2.5}$ health effects may not be comparable as model specifications that may be appropriate for $PM_{2.5}$ may not necessarily be adequate for O_3. The following section reviews the current methodologies used to control for potential confounding by temporal trends and weather effects.

7.1.3.5 Controlling for Temporal Trends and Meteorologic Effects

An examination of recent time-series studies indicates that several types of fitting approaches have been used to adjust for temporal trends and weather effects. The use of parametric and nonparametric smoothers with varying degrees of freedom per year has emerged as the prevailing approach. The use of larger degrees of freedom to adjust for potential confounding by time-varying factors may inadvertently result in ascribing more effects to these unmeasured potential confounders and take away the air pollution effect. Often smaller pollution effect estimates are observed when more degrees of freedom are used. Currently, the degrees of freedom used to adjust for temporal trends in time-series studies generally range from 4 to 12 degrees of freedom per year using either nonparametric or parametric smoothers. Statistical diagnostics such as Akaike's Information Criteria, residual autocorrelation, or dispersion of the regression model often are used to choose or evaluate the adequacy of the degrees of freedom for temporal trend, but these diagnostics do not provide epidemiological justification or interpretation of the fitted model.

The issue of model specifications to adjust for temporal trends and weather variables in time-series studies was a consideration of several researchers that conducted sensitivity analyses of PM data (HEI, 2003). The sensitivity of O_3 coefficients to model specifications for temporal trend adjustment has not been as well-studied. Only one recent multicity study examined the sensitivity of O_3 coefficients to the extent of smoothing for adjustment of temporal trends and meteorologic factors (Bell et al., 2004). Most, if not all, O_3 studies used the same model specifications to estimate the excess risks for PM and other gaseous pollutants. The relationship between a pollutant and the temporal trend or weather effect being fitted differs for each pollutant, and interpretation of the excess risk estimates needs to take into consideration this varying concurvity (nonlinear analogue of multiple correlation) across pollutants. As noted above, O_3 is expected to have the strongest correlation with both temporal (seasonal) trend and

weather effects. The strong annual cycle in O_3 concentrations presents a unique problem in time-series analyses where time trends are fitted simultaneously with pollution and other model terms (i.e., co-adjustment). In this setting, the annual O_3 cycle itself may compete with the smooth function of time to explain some of the annual, cyclic behavior in the health outcome, which can result in biased effect estimates for O_3 when data for all seasons are analyzed together.

Current weather models used in time-series analyses can be classified into: (1) quantile (e.g., quartile, quintile) indicators; (2) parametric functional forms such as V- or U-shape functions; and (3) parametric (e.g., natural splines) or nonparametric (e.g., locally estimated smoothing splines [LOESS]) smoothing functions. More recent studies tend to use smoothing functions. While these methods provide flexible ways to fit health outcomes as a function of temperature and other weather variables, there are two major issues that need further examination to enable more meaningful interpretation of O_3 morbidity and mortality effects.

The first issue is the interpretation of weather or temperature effects. Most researchers agree about the morbidity and mortality effects of extreme temperatures (i.e., heat waves or cold spells). However, as extreme hot or cold temperatures, by definition, happen rarely, much of the health effects occur in the mild or moderate temperature range. Given the significant correlation between O_3 and temperature, ascribing the association between temperature and health outcomes solely to temperature effects may underestimate the effect of O_3.

The second issue is that in most studies weather model specifications are fitted for year-round data. Such models will ignore the correlation structure that can change across seasons, resulting in inefficiency and model mis-specification. This is particularly important for O_3, which appears to change its relationship with temperature as well as with other pollutants across seasons. Ambient O_3 levels are typically higher in the summer or warm season, often referred to as the O_3 season. In the winter or colder months, O_3 levels tend to be much lower compared to the summer months. During the winter in some urban locations, O_3 mainly comes from the free troposphere and can be considered a tracer for relatively clean air (i.e., cold, clear air coming down from the upper atmosphere), as discussed in Chapter 3 of this AQCD. The clean air is associated with the passage of cold fronts and the onset of high-pressure conditions, which occur with colder temperatures. Thus, sunny clear winter days following a high-pressure system are the days when air pollution levels from primary emissions (e.g., NO_2, SO_2, and PM from local

sources) tend to be lower and O_3 is relatively higher. This can lead to negative correlations between O_3 and the primary pollutants in the winter. As shown in Figure 3-6 in the Chapter 3 Annex, the relationship between O_3 and $PM_{2.5}$ was U-shaped for the year-round data in Fort Meade, MD. The negative $PM_{2.5}/O_3$ slope was in the range of O_3 concentrations less than 30 ppb, providing supporting evidence of the aforementioned winter phenomenon.

This changing relationship between O_3 and temperature, as well as O_3 and other pollutants across seasons, and its potential implications to health effects modeling has not been examined thoroughly in the time-series literature. Even the flexible smoother-based adjustments for seasonal and other time-varying variables cannot fully take into account these complex relationships. One obvious way to alleviate or avoid this complication is to analyze data by season. While this practice reduces sample size, its extent would not be as serious as PM (which is collected only every 6th day in most locations) because O_3 is collected daily, though only in warm seasons in some states. An alternative approach is to include separate O_3 concentration variables for each season (by multiplying O_3 concentrations by a season indicator variable).

In locations where seasonal variability may be a factor, O_3 effect estimates calculated using year-round data can be misleading, as the changing relationship between O_3, temperature, and other pollutants across seasons may have a significant influence on the estimates. Analyses have indicated that confounding from seasonal variability may be controlled effectively by stratifying the data by season.

7.1.3.6 Confounding Effects of Copollutants

Extensive discussions on the issues related to confounding effects among air pollutants in time-series study design are provided in Section 8.4.3 of the 2004 PM AQCD. Since the general issues discussed in that document are applicable to all pollutants, such discussions are not repeated here. What was not discussed in the 2004 PM AQCD was the issue of changing relationships among air pollutants across seasons. For O_3, the confounder of main interest is PM, especially fine particles or sulfates that are high in summer, as other copollutants (e.g., CO, NO_2, SO_2) tend to be elevated in the colder season. As mentioned in the previous section, PM indices in some urban locations may be positively correlated with O_3 in the summer and negatively correlated in the winter. Thus the correlation between O_3 and PM for year-round data may be misleading. The high reactivity of O_3 with certain copollutants further complicates the

analysis. For example, the reaction between NO_x, emitted from motor vehicles, and O_3 results in reduced O_3 levels but increased NO_2 levels during high traffic periods.

Multipollutant models often are used to assess potential confounding by copollutants. The limitations of multipollutant regression models, including the potential transfer of "effects" from causal pollutant to noncausal pollutant in the presence of unequal measurement errors, are discussed in the 2004 PM AQCD (Sections 8.4.3 and 8.4.5). In addition, uncertainty remains as to the use of multipollutant regression models to assess the independent health effects of pollutants that are correlated. Particularly in the case of O_3, there remains concern as to whether multipollutant regression models for year-round data can adjust for potential confounding adequately due to the changing relationship between O_3 and other pollutants. Despite these limitations, multipollutant models are still the prevailing approach in most, if not all, studies of O_3 health effects and serve as an important tool in addressing the issue of confounding by copollutants, especially in season-stratified analyses.

7.1.3.7 Model Uncertainty and Multiple Testing

In the analyses of air pollution health effects, there is often uncertainty as to which model is most appropriate. In the case of PM, there were concerns that the positive associations found with mortality were the result of multiple testing and selection. Testing many models to identify the best fit can lead to an underestimation of uncertainty, thus there is a need for statistical methods that can identify the best model while properly accounting for model uncertainty. Standard methods of variable selection include Akaike Information Criterion and Bayes Information Criterion. Bayesian model averaging is a recent family of methods used to address these issues. In Bayesian model averaging, predictions and inferences are based on a set of models, rather than a single model, and each model contributes proportionally to the support it receives from the observed data (Clyde, 1999). While there remains concerns about the large enumeration of models, Bayesian model averaging is a computationally efficient way to incorporate model uncertainty into decision making.

Some researchers have used other methods to address the issue of multiple testing. Dominici et al. (2003) used a minimum number of tests in the U.S. 90 cities study, which minimized the uncertainty associated with multiple testing, but at the cost of possibly not identifying the best model. Another method used by Dominici et al. (2003) to evaluate the

model was sensitivity testing. Lumley and Sheppard (2000) used different control variables to check the bias in model identification. They found that the bias was small, but of the same magnitude as the estimated health impacts. Another approach is to use one set of data for model identification, and a second set of data for model fitting. Cross validation also sheds light on this issue.

With the currently available knowledge, multiple testing is unavoidable in air pollution health effects analyses. To address the issues of model uncertainty and multiple testing, further research leading to the development of standard methodology may be necessary.

7.1.3.8 Impact of GAM Convergence Issue on O_3 Risk Estimates

Generalized Additive Models (GAM) have been widely utilized for epidemiologic analysis of the health effects attributable to air pollution. The impact of the GAM convergence issue was thoroughly discussed in Section 8.4.2 of the 2004 PM AQCD. Reports have indicated that using the default convergence criteria in the Splus software package for the GAM function can lead to suboptimal regression estimates for PM and an underestimation of the standard error of that effect estimate (Dominici et al., 2002; Ramsay et al., 2003). GAM default convergence criterion has a convergence precision of 10^{-3} and a maximum number of 10 iterations. The more stringent convergence criterion refers to increased stringency of both the convergence precision and number of iterations. The default convergence criteria was found to be a problem when the estimated relative risks were small, and two or more nonparametric smoothing curves were included in the GAM (Dominici et al., 2002). The magnitude and direction of the bias depend in part on the concurvity of the independent variables in the GAM and the magnitude of the risk estimate. Most attention has been focused on the influence of the GAM function on effect estimates for PM. However, because O_3 covaries more strongly with both weather and time factors than does PM, the issue of GAM convergence criteria for O_3 needs to be considered.

A recent meta-analysis by Stieb et al. (2003) found a difference in O_3-mortality risk estimates between the GAM studies and non-GAM studies. In the single-pollutant models, the O_3-mortality risk estimates for the non-GAM studies and GAM studies were 1.8% (95% CI: 0.5, 3.1) and 2.2% (95% CI: 1.4, 2.8), respectively, per 40 ppb daily 1-h max O_3. In the multipollutant models, the pooled risk estimate was 1.0% (95% CI: −0.5, 2.6) for non-GAM studies and 0.5% (95% CI: −1.0, 1.9) for GAM studies.

A few GAM studies reanalyzed the O_3 risk estimates using more stringent convergence criteria or general linear models (GLM). Reanalysis of an asthma hospital admissions study in Seattle, WA (Sheppard et al., 1999; reanalysis Sheppard, 2003) indicated that there were only slight changes in the risk estimates when using more stringent convergence precision (10^{-8}) in GAM. The original GAM analysis indicated an excess risk of 9% (95% CI: 3, 17) whereas the stringent GAM analysis found an excess risk of 11% (95% CI: 3, 19) per 30 ppb increase in 8-h max O_3. Similar results were found using GLM with natural splines, 11% (95% CI: 2, 20). In the reanalysis of Santa Clara County, CA data, Fairley (1999; reanalysis Fairley, 2003) used the same methods as the original analysis except the convergence precision (ϵ) was increased from 10^{-4} to 10^{-12} and the maximum number of iterations (M) were increased from 10 to 10^7. The O_3 nonaccidental mortality risk estimates slightly increased from 2.8% using default GAM parameters to 2.9% using stringent GAM parameters per 30 ppb increase in 8-h max O_3. The O_3-mortality risk estimates further increased to 3.0% using GLM with natural cubic splines. In the reanalysis of the Netherlands data by Hoek et al. (2000; reanalysis Hoek, 2003), the O_3 nonaccidental mortality risk estimates increased from 1.3% (default GAM) to 1.5% (stringent GAM, $\epsilon = 10^{-8}$, $M = 10^3$) and 1.6% (GLM with natural splines) per 30 ppb increase in 8-h avg O_3 (12 p.m.-8 p.m.). In the analysis of the large 90 U.S. cities (Samet et al., 2000; reanalysis Dominici et al., 2003), the year-round combined estimate of O_3 nonaccidental mortality risk changed from a nonsignificant negative value of approximately −0.2% (default GAM) per 20 ppb change in 24-h avg O_3 to a significantly positive excess risk of 0.8% (stringent GAM, $\epsilon = 10^{-15}$, $M = 10^3$).

Most analyses comparing results using default GAM convergence criteria to results from stringent GAM convergence criteria and GLM have found little difference among the O_3 effect estimates. However, one study by Cifuentes et al. (2000) in Santiago, Chile observed a large difference in the O_3-mortality excess risks calculated using default GAM (2.4%) and GLM (0.3%). Therefore, the impact of the GAM convergence problem appears to vary depending on data sets, and likely depends upon the intercorrelation among covariates and the magnitude of the risk estimate. However, in the limited number of studies that have reanalyzed O_3 risk estimates, there is little evidence that default GAM analyses resulted in positively biased estimates as observed for PM. Generally it appears that the use of default convergence criteria in GAM tends to bias risk estimates towards the null, in addition to underestimating the standard

errors. In uniformity with the approach used in the 2004 PM AQCD, the results from studies that analyzed data using GAM with default convergence criteria and at least two nonparametric smoothing terms are generally not considered in this chapter, with a few exceptions as noted.

7.1.4 Approach to Presenting O_3 Epidemiologic Evidence

To produce a thorough appraisal of the evidence, we first concisely highlight key points derived from the 1996 O_3 AQCD assessment. Then pivotal information, including methodological features and results, from important new studies that have become available since the prior O_3 AQCD are presented in summary tables in Chapter 7 of the Annex. In the main body of the chapter, particular emphasis is focused on those studies and analyses considered to provide information most directly applicable for development of criteria. Not all studies should be accorded equal weight in the overall interpretive assessment of evidence regarding O_3-association health effects. Among well-conducted studies with adequate control for confounding, increasing scientific weight should be accorded in proportion to the precision of their effect estimates. Small-scale studies without a wide range of exposures generally produce less precise estimates compared to larger studies with an adequate exposure gradient. Therefore, the range of exposures, the size of the study as indicated by the length of the study period and total number of events, and the inverse variance of the principal effect estimate are all important indices useful in determining the likely precision of the health effect estimates and in according relative scientific importance to the findings of a given study. In any case, emphasis should be accorded to estimates from studies with narrow confidence bands.

Emphasis is placed on text discussion of (1) new multicity studies that employ standardized methodological analyses for evaluating O_3 effects across several or numerous cities and often provide overall effect estimates based on combined analyses of information pooled across multiple cities; (2) studies that consider O_3 as a component of a complex mixture of air pollutants, including in particular the gaseous criteria pollutants (CO, NO_2, SO_2) and PM; and (3) North American studies conducted in the U.S. or Canada. Multicity studies are of particular interest and value due to their evaluation of a wider range of O_3 exposures and large numbers of observations, thus possibly providing more precise effect estimates than most smaller scale independent studies of single cities. Another potential advantage of the multicity studies, compared to meta-analyses of multiple "independent" studies, is consistency in data handling

and model specifications that eliminates variation due to study design. Also, unlike regular meta-analyses, they do not suffer from potential omission of nonsignificant results due to "publication bias." Furthermore, geographic patterns of air pollution effects have the potential to provide especially valuable evidence regarding relative homogeneity and/or heterogeneity of O_3 health effects relationships across geographic locations. In accordance to the emphasis placed on the O_3 epidemiology studies in this chapter, the tables in the Chapter 7 Annex were organized by region with multicity studies in each region presented first.

In the coming sections, field/panel studies and studies of emergency department visits and hospital admissions, which contributed to the establishment of the revised 1997 NAAQS for O_3, are presented first. This is followed by a discussion of O_3-related mortality and chronic effects. The chapter ends with an integrative discussion providing a summary and conclusions.

7.2 FIELD STUDIES ADDRESSING ACUTE EFFECTS OF OZONE

7.2.1 Summary of Key Findings on Field Studies of Acute Effects From the 1996 O_3 AQCD

In the 1996 O_3 AQCD, individual-level camp and exercise studies provided useful quantitative information on the exposure-response relationships linking human lung function declines with O_3 exposure occurring in ambient air. The available body of evidence supported a dominant role of O_3 in the observed lung function decrements. Extensive epidemiologic evidence of pulmonary function responses to ambient O_3 came from camp studies. Six studies from three separate research groups provided a combined database on individual exposure-response relationships for 616 children (mostly healthy, nonasthmatic) ranging in age from 7 to 17 years, each with at least six sequential measurements of FEV_1 (forced expiratory volume in 1 second) while attending summer camps (Avol et al., 1990; Higgins et al., 1990; Raizenne et al., 1987, 1989; Spektor et al., 1988a, 1991). When analyzed together using consistent analytical methods, these data yielded an average relationship between afternoon FEV_1 and concurrent-hour O_3 concentration of −0.50 mL/ppb ($p < 0.0001$), with study-specific slopes ranging from −0.19 to −1.29 mL/ppb. Exposure in camp studies usually extended for multiple hours. Although the regression results noted above were based on one-hour O_3 levels, single- and multiple-hour averages were observed to be highly correlated, thus these results might

represent, to some extent, the influence of multihour exposures. In addition to the camp study results, two studies involving lung function measurements before and after well-defined exercise events in adults yielded exposure-response slopes of −0.4 mL/ppb (Selwyn et al., 1985) and −1.35 mL/ppb (Spektor et al., 1988b). Ozone concentrations during exercise events of approximately ½-hour duration ranged from 4 to 135 ppb in these studies.

Results from other field panel studies also supported a consistent relationship between ambient O_3/oxidant exposure and acute respiratory morbidity in the population. Respiratory symptoms (or exacerbation of asthma) and decrements in peak expiratory flow (PEF) occurred with increased ambient O_3 concentrations, especially in asthmatic children (Lebowitz et al., 1991; Krzyzanowski et al., 1992). The aggregate results showed greater responses in asthmatic individuals than in nonasthmatics (Lebowitz et al., 1991; Krzyzanowski et al., 1992), indicating that asthmatics might constitute a sensitive group in epidemiologic studies of oxidant air pollution. Since the 1996 O_3 AQCD, new research has examined a broad scope of field studies which are presented next.

7.2.2 Introduction to Recent Field Studies of Acute O_3 Effects

Numerous field studies carried out over the past decade have tested for and, in many cases, observed acute associations between measures of respiratory ill-health and O_3 concentrations in groups of subjects (Table AX7-1 in Chapter 7 Annex). Acute field studies are distinguished from other acute epidemiologic study designs in that they recruit and collect data from individual human subjects instead of utilizing administrative data on aggregate health outcomes such as daily mortality, hospital admissions, or emergency department visits. Because of the logistical burden associated with direct data collection from individual subjects, field/panel studies tend to be small in both numbers of subjects and in duration of follow-up. While this may limit the statistical power of field studies, it is compensated for by the ability to determine individual-level information on health outcomes, exposure levels, and other potentially confounding factors.

The most common outcomes measured in acute field studies on the effects of air pollution exposure are lung function and various respiratory symptoms. Other respiratory outcomes examined on a limited basis include inflammation and generation of hydroxyl radicals in the upper airways, and school absences. Several studies examined cardiovascular outcomes

including heart rate variability and risk of myocardial infractions. The first group of studies provides varying degrees of evidence supporting the conclusion that elevated O_3 levels can have negative impacts on lung function and symptoms, confirming and adding to the body of knowledge that is presented in the 1996 O_3 AQCD. Some emphasis has been placed in examining the independent role of O_3 in the presence of PM and other pollutants. The other new studies contribute information on cardiopulmonary outcomes which had not been as well-documented previously.

7.2.3 Acute O_3 Exposure and Lung Function

As discussed in the 1996 O_3 AQCD and in the earlier chapter of this document on controlled human exposure studies (Chapter 6), a large body of literature from clinical and field studies has clearly and consistently demonstrated reversible decrements in pulmonary function following acute O_3 exposure. Significant O_3-induced spirometric and symptom responses have been observed in clinical studies of exercising healthy young adults (see Section 6.2) and in some potentially susceptible subpopulations, namely asthmatics and children (see Sections 6.3.2 and 6.5.1). Field studies of acute O_3 exposure that examine pulmonary function fall into two distinct groupings, those that conduct spirometry (FEV_1 and FVC [forced vital capacity]) and those that measure PEF. Results from the previous O_3 AQCD and Chapter 6 of this document support the conclusion that the spirometric parameter FEV_1 is the stronger and more consistent measure of lung function. PEF is a useful clinical measure that is more feasible to perform in field studies, however its measurements are more variable and possibly less reliable than FEV_1 (Fuhlbrigge et al., 2001).

Studies of FEV_1 will be presented first, followed by a discussion of PEF studies. Other dividing aspects between these two major types of lung function studies include health status of subjects (e.g., healthy, mildly asthmatic, severely asthmatic), time spent outdoors, and exertion levels. Several studies brought these factors together to produce informative data. Some FEV_1 studies involved both increased outdoor O_3 exposure and higher exertion levels. The results from this group of subjects are comparable to those from the exercising subjects in the clinical studies discussed in Chapter 6.

7.2.3.1 Acute O_3 Studies with Spirometry (FEV_1)

Studies published over the past decade have provided some new insights on the acute effects of O_3 on FEV_1. Tables 7-1a, 7-1b, and 7-1c summarize the results of all studies that investigated quantitative O_3-related effects on FEV_1. Four studies of spirometry were not included in the tables; three studies did not provide quantitative O_3 data (Cuijpers et al., 1994; Delfino et al., 2004; Frischer et al., 1997) and one measured $FEV_{0.75}$ (forced expiratory volume in 0.75 seconds) (Scarlett et al., 1996). With few exceptions, the O_3 effect estimates showed decrements for FEV_1 across studies and several were statistically significant. These studies are discussed in further detail, starting with the O_3 effect on individuals with elevated exertion levels.

Exercise and outdoor worker panels

The current 8-hour NAAQS for O_3 has its original basis in the controlled human exposure studies, as discussed in Chapter 6. These field studies with subjects at elevated exertion levels

Table 7-1a. Field Studies that Investigated the Association between Acute Ambient O_3 Exposure and Changes in FEV_1

Reference	Study Location	Study Period	n[a]
Linn et al. (1996)	Rubidoux, Upland, and Torrance, CA	Fall-spring 1992-1993, 1993-1994	269
Korrick et al. (1998)	Mount Washington, NH	Summers 1991, 1992	530
Brauer et al. (1996)	Fraser Valley, British Columbia, Canada	Jun-Aug 1993	58
Höppe et al. (1995a)	Munich, Germany	Apr-Sep 1992-1994	208
Ulmer et al. (1997)	Freudenstadt and Villingen, Germany	Mar-Oct 1994	135
Castillejos et al. (1995)	SW Mexico City	Aug 1990-Oct 1991	40
Romieu et al. (1998)	Mexico City	Mar-May 1996; Jun-Aug 1996	47
Romieu et al. (2002)	Mexico City	Oct 1998-Apr 2000	158
Chen et al. (1999)	Sanchun, Taihsi, and Linyuan, Taiwan	May 1995-Jan 1996	895

[a] The number of the total study population is presented. Some of the effect estimates presented in Tables 7-1b and 7-1c were based on a subset of the total population.

Table 7-1b. Changes in FEV_1 (95% CI) Associated with Acute Ambient O_3 Exposures, Ordered by Size of the Estimate

	Reference	Study Population/Analysis	Mean O_3 Level (ppb)	Exposure Index	Change in FEV_1[a] (mL)
1	Brauer et al. (1996)	Berry pickers, next morning	40.3	1-h max	−180.0 (−227.0, −133.0)
2	Brauer et al. (1996)	Berry pickers, afternoon	40.3	1-h max	−152.0 (−183.4, −120.6)
3	Romieu et al. (1998)	Street workers on placebo (1st phase, lag 0-1)	123	1-h max	−117.2 (−207.4, −27.0)
4	Ulmer et al. (1997)	School children in Freudenstadt	50.6	½-h max	−87.5 (−143.2, −31.7)
5	Höppe et al. (1995a)	Juvenile asthmatics	74[b]	½-h max	−84.0 (−196.4, 28.4)
6	Romieu et al. (1998)	Street workers on placebo (1st phase, lag 0)	123	1-h max	−71.6 (−113.9, −29.3)
7	Höppe et al. (1995a)	Clerks	68[b]	½-h max	−63.2 (−108.8, −17.6)
8	Ulmer et al. (1997)	School boys in Freudenstadt and Villingen	41.4	½-h max	−61.4 (−122.9, 0.0)
9	Höppe et al. (1995a)	Athletes	71[b]	½-h max	−60.8 (−115.2, −6.4)
10	Ulmer et al. (1997)	School children in Freudenstadt and Villingen	41.4	½-h max	−56.6 (−101.3, −12.0)
11	Höppe et al. (1995a)	Forestry workers	64[b]	½-h max	−56.0 (−118.4, 6.4)
12	Ulmer et al. (1997)	School girls in Freudenstadt and Villingen	41.4	½-h max	−44.2 (−105.0, 16.7)
13	Romieu et al. (1998)	Street workers on supplement (1st phase, lag 0-1)	123	1-h max	−41.2 (−143.9, 61.5)
14	Chen et al. (1999)	Children, with NO_2 in model (lag 1)	19.7-110.3[c]	1-h max	−34.0 (−60.7, −7.3)
15	Chen et al. (1999)	Children (lag 1)	19.7-110.3[c]	1-h max	−25.6 (−49.1, −2.1)
16	Romieu et al. (2002)	Moderate to severe asthmatic children on placebo (lag 1)	102	1-h max	−18.8 (−34.2, −3.4)
17	Romieu et al. (2002)	Moderate to severe asthmatic children on placebo, with NO_2 and PM_{10} in model (lag 1)	102	1-h max	−18.4 (−35.5, −1.3)
18	Romieu et al. (1998)	Street workers on supplement (1st phase, lag 0)	123	1-h max	−17.6 (−68.6, 33.4)
19	Chen et al. (1999)	Children (lag 2)	N/A	24-h avg	−17.4 (−41.7, 6.9)

Table 7-1b (cont'd). Changes in FEV_1 (95% CI) Associated with Acute Ambient O_3 Exposures, Ordered by Size of the Estimate

	Reference	Study Population/Analysis	Mean O_3 Level (ppb)	Exposure Index	Change in FEV_1 [a] (mL)	
20	Chen et al. (1999)	Children (lag 2)	19.7-110.3[c]	1-h max	−16.0	(−44.2, 12.2)
21	Ulmer et al. (1997)	School children in Villingen	32.1	½-h max	−15.0	(−74.6, 44.5)
22	Chen et al. (1999)	Children (lag 1)	N/A	24-h avg	−13.6	(−33.2, 6.0)
23	Romieu et al. (1998)	Street workers on placebo (2nd phase, lag 0)	123	1-h max	−13.2	(−64.2, 37.8)
24	Chen et al. (1999)	Children (lag 7)	19.7-110.3[c]	1-h max	−12.4	(−31.2, 6.4)
25	Romieu et al. (1998)	Street workers on placebo (2nd phase, lag 0-1)	123	1-h max	−12.0	(−96.7, 72.7)
26	Chen et al. (1999)	Children (lag 7)	N/A	24-h avg	−6.0	(−20.9, 8.9)
27	Linn et al. (1996)	School children, next morning	23	24-h avg	−5.2	(−15.0, 4.6)
28	Linn et al. (1996)	School children, afternoon	23	24-h avg	−3.6	(−13.8, 6.6)
29	Romieu et al. (2002)	All asthmatic children on placebo (lag 1)	102	1-h max	−3.6	(−13.5, 6.3)
30	Romieu et al. (2002)	Moderate to severe asthmatic on supplement (lag 1)	102	1-h max	−0.7	(−15.1, 13.7)
31	Romieu et al. (2002)	Moderate to severe asthmatic on supplement, with NO_2 and PM_{10} in model (lag 1)	102	1-h max	−0.2	(−15.6, 15.1)
32	Romieu et al. (2002)	All asthmatic children on supplement (lag 1)	102	1-h max	0.8	(−9.8, 11.3)
33	Romieu et al. (1998)	Street workers on supplement (2nd phase, lag 0)	123	1-h max	6.0	(−23.8, 35.8)
34	Höppe et al. (1995a)	Seniors	70[b]	½-h max	13.6	(−26.8, 54.0)
35	Romieu et al. (1998)	Street workers on supplement (2nd phase, lag 0-1)	123	1-h max	27.2	(−25.3, 79.7)

[a] Change in FEV_1 is per standard unit ppb O_3 (40 ppb for ½-h max O_3 and 1-h max O_3, 30 ppb for 8-h max O_3, and 20 ppb for 24-hr avg O_3).
[b] Mean O_3 concentration on high O_3 days.
[c] Range of O_3 concentrations.

Table 7-1c. Cross-day Changes in FEV_1 Associated with Acute Ambient O_3 Exposures, Ordered by Size of the Estimate

	Reference	Study Population/ Analysis	Mean O_3 Level (ppb)	Exposure Index	Cross-day Change in FEV_1 [a] (mL)
1	Korrick et al. (1998)	Hikers with wheeze or asthma (post–pre-hike)	40	8-h avg	−182.5 [b] (−312.2, −52.9)
2	Korrick et al. (1998)	Hikers who hiked 8-12 hours (post–pre-hike)	40	8-h avg	−84.5 [b] (−154.1, −14.9)
3	Korrick et al. (1998)	Hikers age 28-37 years (post–pre-hike)	40	8-h avg	−82.1 [b] (−139.7, −24.4)
4	Korrick et al. (1998)	Hikers who never smoked (post–pre-hike)	40	8-h avg	−72.3 [b] (−132.3, −12.2)
5	Korrick et al. (1998)	Hikers male (post–pre-hike)	40	8-h avg	−67.4 [b] (−127.4, −7.3)
6	Korrick et al. (1998)	Hikers age 38-47 years (post–pre-hike)	40	8-h avg	−64.9 [b] (−127.3, −2.5)
7	Korrick et al. (1998)	All hikers (post–pre-hike)	40	8-h avg	−62.5 [b] (−115.3, −9.7)
8	Korrick et al. (1998)	All hikers, with $PM_{2.5}$ and acidity in model (post–pre-hike)	40	8-h avg	−58.8 [b] (−135.6, 18.0)
9	Korrick et al. (1998)	Hikers age 18-27 years (post–pre-hike)	40	8-h avg	−52.7 [b] (−117.5, 12.2)
10	Korrick et al. (1998)	Hikers female (post–pre-hike)	40	8-h avg	−47.8 [b] (−141.4, 45.9)
11	Korrick et al. (1998)	Hikers age 48-64 years (post–pre-hike)	40	8-h avg	−46.5 [b] (−125.8, 32.7)
12	Korrick et al. (1998)	Hikers without wheeze or asthma (post–pre-hike)	40	8-h avg	−44.1 [b] (−101.7, 13.5)
13	Korrick et al. (1998)	Hikers who hiked 2-8 hours (post–pre-hike)	40	8-h avg	−40.4 [b] (−110.0, 29.2)
14	Korrick et al. (1998)	Hikers who formerly smoked (post–pre-hike)	40	8-h avg	−29.4 [b] (−125.4, 66.6)
15	Linn et al. (1996)	School children (p.m.–a.m.)	23	24-h avg	−11.6 (−20.6, −2.6)
16	Castillejos et al. (1995)	Private primary school (post–pre-exercise)	112.3	1-h max	−9.1 [c] (−13.6, −4.7)
17	Brauer et al. (1996)	Berry pickers (post–pre-workshift)	40.3	1-h max	0 (−47.0, 47.0)

[a] Cross-day change in FEV_1 is per standard unit ppb O_3 (40 ppb for 1-h max O_3, 30 ppb for 8-h avg O_3, and 20 ppb for 24-h avg O_3).

[b] Korrick et al. presented % change in FEV_1. The data was transformed to FEV_1 units of mL by multiplying by the total population average FEV_1 of 4,083 mL.

[c] Castillejos et al. presented % change in FEV_1. The data was transformed to FEV_1 units of mL by multiplying by the total population average FEV_1 of 1,900 mL.

are of particular interest due to their similarities to the human chamber studies. The majority of human chamber studies have examined the effects of O_3 exposure in subjects performing continuous or intermittent exercise for variable periods of time (see Chapter 6 of this O_3 AQCD).

A study by Brauer and colleagues (1996) reported unusually large O_3 effects on lung function among outdoor workers. This study presented O_3 effects during an extended outdoor exposure period combined with elevated levels of exertion. The investigators repeatedly measured spirometric lung function before and after outdoor summer work shifts over 59 days on a group of 58 berry pickers in Fraser Valley, British Columbia, Canada. Subjects, both male and female, ranged from 10 to 69 years old, with a mean age of 44 years. Outdoor work shifts averaged 11 hours in duration. The mean 1-h max O_3 concentration was 40.3 ppb. Exertion levels were estimated using portable heart rate monitors carried over a period of four or more hours by a representative subset of subjects during 16 work shifts. Heart rates were essentially constant over the work shift, averaging 36% higher than resting levels. The authors estimated that minute ventilations may have averaged roughly 30 L/min during work. Post-shift FEV_1 and FVC showed large decreases as a function of O_3 concentration and those effects remained significant when $PM_{2.5}$ was included in the analysis. Significant declines in lung function also were observed on the morning following high O_3 exposure. The effects seen in this study are larger than have been reported previously. For example, afternoon FEV_1 was 3.8 mL lower per 1 ppb increase in O_3 concentrations, compared to the decline of 0.4 mL/ppb and 1.35 mL/ppb observed in the earlier adult exercise studies (Spektor et al., 1988b; Selwyn et al., 1985). Further, when data were restricted to days with 1-h max O_3 concentrations under 40 ppb, the O_3 effects on afternoon FEV_1 did not change in magnitude and remained significant.

In a Mexico City study of 47 outdoor street workers (Romieu et al., 1998), spirometry was performed repeatedly at the end of the workshift over a two month period. Subjects were exposed to outdoor ambient O_3 levels for a mean of 7.4 hours during the workday. Among those who had never taken an antioxidant supplement (subjects who received a placebo during the 1st phase of the study), same day O_3 concentrations were significantly associated with decreases in FEV_1. A mean decline of 71.6 mL (95% CI: 29.3, 113.9) was observed per 40 ppb increase in 1-h max O_3. The results from this study, in addition to those from the Canadian study of berry pickers (Brauer et al., 1996), indicate that outdoor workers are a potentially susceptible population that may need protection from O_3 exposures.

Höppe et al. (1995a) examined forestry workers (n = 41) for changes in pulmonary function attributable to O_3 exposure in Munich, Germany. In addition, athletes (n = 43) were monitored in the afternoon after a two-hour outdoor training period. Pulmonary function tests were conducted on days of both "high" (mean ½-h max O_3 of 64 to 74 ppb) and "low" (mean ½-h max O_3 of 32 to 34 ppb) ambient O_3 concentrations. From the average activity levels, ventilation rates were estimated. Athletes, who had a fairly high ventilation rate of 80 L/min, experienced a significant decrease of 60.8 mL (95% CI: 6.4, 115.2) in FEV_1 per 40 ppb increase in ½-h max O_3. Among the forestry workers, an O_3-related decline in FEV_1 also was observed (−56.0 mL), however the change was not statistically significant.

For the above studies that examine outdoor workers and athletes who train outdoors, Table 7-2 presents the estimated O_3 external doses and compares them to changes in FEV_1 associated with acute ambient O_3 exposures. The use of estimated O_3 external doses offers another potential for insight into studies that examine subjects with elevated ventilation rates and prolonged outdoor exposures at varying ambient O_3 concentrations. No consistent relationship between estimated O_3 external doses and changes in FEV_1 can be derived from the limited evidence.

One FEV_1 study appears to mirror the sort of outcome typically seen in clinical studies. In a study by Korrick et al. (1998), adult hikers (n = 530) of Mount Washington, NH performed spirometry before and after hiking for a mean of 8 hours (range: 2 - 12). The mean hourly O_3 concentration ranged from 21 to 74 ppb. After their hike, subjects experienced a mean decline of 1.5% (95% CI: 0.2, 28) in FEV_1 and 1.3% (95% CI: 0.5, 2.1) in FVC per 30 ppb increase in the mean of the hourly O_3 concentration during the hike. In addition, Korrick et al. (1998) compared hikers who hiked 8 to 12 hours to those who hiked 2 to 8 hours. Among those who hiked longer, the % change in FEV_1 was more than two-fold greater per ppb exposure compared to those who hiked only for 2 to 8 hours. Each hour hiked, which may reflect dose, was associated with a decline of 0.3% ($p = 0.05$) in FEV_1, after adjusting for O_3.

In a Mexico City study, the O_3 effect attributable to exercise was determined using a group of school children (n = 40) chronically exposed to moderate to high levels of O_3 (Castillejos et al., 1995). Children were tested up to 8 times between August 1990 and October 1991. Spirometry was performed by the children before and after a one-hour intermittent exercise session outdoors. Outdoor O_3 levels ranged up to 365 ppb, with a mean of 112.3 ppb. Linear

Table 7-2. Estimated O_3 External Doses and Changes in FEV_1 Associated with Acute Ambient O_3 Exposures in Outdoor Workers and Athletes

	Reference	Study Location	Study Period	N	Study Population	Age (years)
1	Brauer et al. (1996)	Fraser Valley, Canada	Jun-Aug 1993	58	Berry pickers	10-69
2	Höppe et al. (1995a)	Munich, Germany	Apr-Sep 1994	43	Athletes	13-38
3	Höppe et al. (1995a)	Munich, Germany	Apr-Sep 1993	41	Forestry Workers	20-60
4	Romieu et al. (1998)	Mexico City	Mar-May 1996	13	Street Workers [a]	18-58

	Mean O_3 Level (ppb)	Exposure Duration (h)	Ventilation Rate (L/min)	Exposure (mg/h)	Dose (mg)	Change in FEV_1 [b] (mL)
1	26.0	11	30	91	997	−152.0 (−183.4, −120.6)
2	71.0 [c]	2	80	660	1320	−60.8 (−115.2, −6.4)
3	64.0 [c]	7	40	298	2083	−56.0 (−118.4, 6.4)
4	67.3	9	28 [d]	219	1971	−71.6 (−113.9, −29.3)

[a] For the street workers in Romieu et al. (1998), only results from subjects who had never taken the antioxidant supplement (on placebo during 1st phase of study) are presented here.
[b] Change in FEV_1 is per 40 ppb increase in 1-h max O_3 or equivalent.
[c] Mean O_3 concentration during exposure period was not presented in Höppe et al. (1995). The ½-h max O_3 concentrations are shown here.
[d] Ventilation rate was not presented. The ventilation rate of 28 L/min was calculated for a male performing a heavy workload for 1/8 of the workday in Table B.17 of the International Commission on Radiological Protection (ICRP) Publication 66 (ICRP, 1994).

trend analyses of the relationship between quintiles of O_3 and % change in lung function were significant. However, stratified analyses indicated that statistically significant changes were observed only with higher quintiles of O_3 exposure (72-125 ppb and 183-365 ppb). Therefore, when exercising at higher O_3 levels, children experienced significant declines in pulmonary function despite the repeated daily exposure to moderate and high levels of O_3 in Mexico City.

Collectively, the above studies confirm and extend clinical observations that prolonged exposure periods, combined with elevated levels of exertion or exercise, may magnify the effect of O_3 on lung function. The most representative data comes from the Korrick et al. (1998) hiker study. This U.S. study provided outcome measures stratified by several factors (e.g., gender, age, smoking status, presence of asthma) within a population capable of more than normal exertion.

Panels of other risk groups

Höppe et al. (1995a,b) examined several potentially susceptible populations for changes in pulmonary function attributable to O_3 exposure in Munich, Germany. The forestry workers and athletes were discussed in the previous section. Senior citizens (n = 41) and juvenile asthmatics (n = 43) were also monitored on "low" O_3 and "high" O_3 days. Subjects were requested to stay outdoors for at least 2 hours just before the afternoon pulmonary function test. Clerks (n = 40) were considered the nonrisk control group. Although clerks spent the majority of their time indoors, their outdoor exposures on the "high" O_3 days were similar to that of the four other risk groups. The results showed no significant O_3 effects on the senior citizens, who had the lowest ventilation rate. Asthmatics and clerks experienced slight reductions in FEV_1 on high O_3 days. Among all risk groups, juvenile asthmatics experienced the largest O_3-related decline in FEV_1, though not statistically significant.

Several other panel studies performed spirometry in children, another potentially susceptible group (Chen et al., 1999; Cuijpers et al., 1994; Frischer et al., 1997; Linn et al., 1996; Romieu et al., 2002; Scarlett et al., 1996; Ulmer et al., 1997). All studies, with the exception of Scarlett et al. (1996), observed a statistically significant decrease in FEV_1 associated with O_3 exposure. One large study measured spirometric lung function in 895 school children in three towns in Taiwan (Chen et al., 1999). Lung function was measured only once for each subject. The authors reported statistically significant associations between diminished FEV_1 and FVC with a 1-day lag of O_3 concentrations. Effect sizes were typical of those observed in past studies, i.e., 0.5 to 1.0 mL decline in FEV_1 per ppb increase in O_3 concentration. Ozone was the only significant air pollutant in multipollutant models including SO_2, CO, PM_{10}, and NO_2. The O_3 associations became nonsignificant when days with O_3 above 60 ppb were excluded from the analysis, implying a practical threshold of around 60 ppb in this individual study.

Linn et al. (1996) repeatedly measured spirometric lung function among 269 school children in three southern California communities (Rubidoux, Upland, and Torrance). Lung function was measured over five consecutive days, once in each of three seasons over two school years. Between-week variability was effectively removed from the analysis by seasonal terms in the model. Statistical power was limited by the narrow range of exposures that were experienced within each week. In addition, the study was restricted to the school year, eliminating most of the "high" O_3 season from consideration. During the study period, 24-h

avg O_3 levels at the central monitoring site ranged up to 53 ppb (mean 23 ppb) while personal measurements ranged up to 16 ppb (mean 5 ppb). The difference between morning (tested near the beginning of the school day) and afternoon (tested following lunch) FEV_1 was significantly associated with same-day O_3 concentrations. Other associations (involving individual morning or afternoon FVC and FEV_1 measurements) went in the plausible direction but were not statistically significant.

Ulmer et al. (1997) examined 135 children aged 8 to 11 years in two towns in Germany from March to October 1994 for O_3 effects on pulmonary function at four time periods. The cross-sectional results at each of the four time points showed limited FVC and no FEV_1 associations. However, the longitudinal analysis, which combined data from all four periods, obtained a statistically significant negative association between O_3 exposure and both FVC and FEV_1 for the town with the higher O_3 levels (median ½-h max of 50.6 ppb versus 32.1 ppb). In the cross-sectional analysis, between-person variability could not be distinguished from within-person variability, limiting the statistical power. The longitudinal study design, in which subjects provided multiple days of measurements, had greater power as it provided information about both between- and within-subject responses.

7.2.3.2 Acute O_3 Studies of PEF

Many studies of the acute effect of O_3 on PEF examined PEF levels daily, both in the morning and afternoon. PEF follows a circadian rhythm with the highest values found during the late afternoon and lowest values during the night and early morning. Due to the diurnal variation in PEF, most studies analyzed their data after stratifying by time of day. The peak flow studies examined both asthmatic panels and healthy individuals. The asthma panels are discussed first.

Asthma panels

Asthmatics were examined in several panel studies. Several aspects of these studies affect the outcomes. For example, large panels have a greater opportunity to test the hypothesis of an O_3 effect on PEF. In addition, the severity of asthma in the panel subjects and the medications that they take may affect the results of the study. Figures 7-1a and 7-1b present % changes in morning and evening PEF outcomes from six panel studies of mostly asthmatic children. The

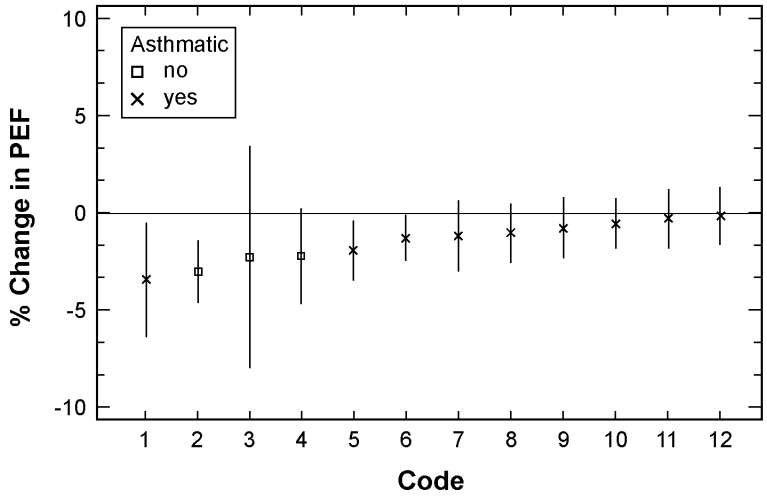


Figure 7-1a. Percent change (95% CI) in morning PEF in children per 40 ppb increase in 1-h max O_3 or equivalent, arranged by size of the effect estimate. Study codes are explained in the tables below. Information on study location and period, study population, and O_3 exposure is presented.

Code	Reference	Study Location	Study Period	N
1	Ross et al. (2002) [a]	East Moline, IL	May-Oct 1994	40
2	Gold et al. (1999)	SW Mexico City	Jan-Feb, Apr-May, Oct-Nov 1991	40
3	Neas et al. (1999)	Philadelphia, PA	Jul-Sep 1993	156
4	Neas et al. (1999)	Philadelphia, PA	Jul-Sep 1993	156
5	Romieu et al. (1996)	N Mexico City	Apr-Jul 1991, Nov 1991-Feb 1992	71
6	Gielen et al. (1997)	Amsterdam, the Netherlands	Apr-Jul 1995	61
7	Romieu et al. (1996)	N Mexico City	Apr-Jul 1991, Nov 1991-Feb 1992	71
8	Romieu et al. (1997)	SW Mexico City	Apr-Jul 1991, Nov 1991-Feb 1992	65
9	Romieu et al. (1997)	SW Mexico City	Apr-Jul 1991, Nov 1991-Feb 1992	65
10	Gielen et al. (1997)	Amsterdam, the Netherlands	Apr-Jul 1995	61
11	Romieu et al. (1997)	SW Mexico City	Apr-Jul 1991, Nov 1991-Feb 1992	65
12	Romieu et al. (1996)	N Mexico City	Apr-Jul 1991, Nov 1991-Feb 1992	71

Code	Population	Age (years)	Mean O_3 Level (ppb)	O_3 Exposure Index	Exposure Lag Days
1	asthmatic	5-49	41.5	8-h max	0-1
2	nonasthmatic	8-11	52	24-h avg	1-10
3	nonasthmatic	6-11	57.5 (SW); 55.9 (NE)	12-h avg [b]	1-5
4	nonasthmatic	6-11	57.5 (SW); 55.9 (NE)	12-h avg [b]	0
5	mildly asthmatic	5-13	190	1-h max	0
6	asthmatic	7-13	35	8-h max	2
7	mildly asthmatic	5-13	190	1-h max	2
8	mildly asthmatic	5-13	196	1-h max	0
9	mildly asthmatic	5-13	196	1-h max	2
10	asthmatic	7-13	35	8-h max	1
11	mildly asthmatic	5-13	196	1-h max	1
12	mildly asthmatic	5-13	190	1-h max	1

[a] Study population also includes adults.
[b] Percent PEF change is presented per 25 ppb increase in 12-h avg O_3. The standard units are used for other O_3 indices.

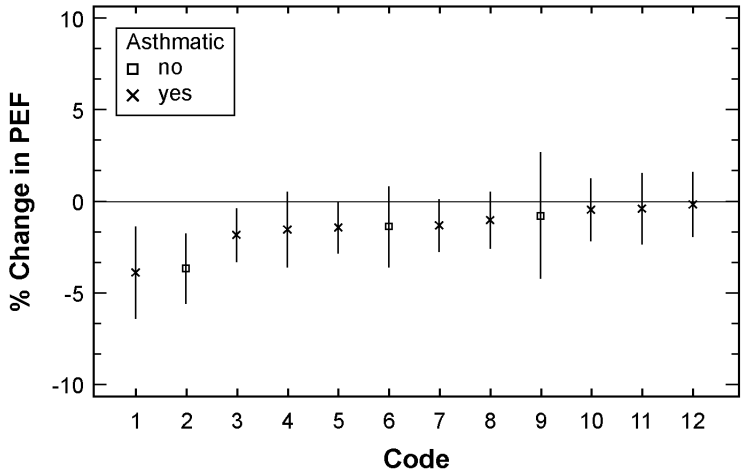


Figure 7-1b. Percent change (95% CI) in afternoon PEF in children per 40 ppb increase in 1-h max O_3 or equivalent, arranged by size of the effect estimate. Study codes are explained in the tables below. Information on study location and period, study population, and O_3 exposure is presented.

Code	Reference	Study Location	Study Period	N
1	Ross et al. (2002)[a]	East Moline, IL	May-Oct 1994	40
2	Gold et al. (1999)	SW Mexico City	Jan-Feb, Apr-May, Oct-Nov 1991	40
3	Romieu et al. (1997)	SW Mexico City	Apr-Jul 1991, Nov 1991-Feb 1992	65
4	Romieu et al. (1996)	N Mexico City	Apr-Jul 1991, Nov 1991-Feb 1992	71
5	Romieu et al. (1997)	SW Mexico City	Apr-Jul 1991, Nov 1991-Feb 1992	65
6	Neas et al. (1999)	Philadelphia, PA	Jul-Sep 1993	156
7	Gielen et al. (1997)	Amsterdam, the Netherlands	Apr-Jul 1995	61
8	Romieu et al. (1996)	N Mexico City	Apr-Jul 1991, Nov 1991-Feb 1992	6
9	Neas et al. (1999)	Philadelphia, PA	Jul-Sep 1993	156
10	Romieu et al. (1996)	N Mexico City	Apr-Jul 1991, Nov 1991-Feb 1992	71
11	Gielen et al. (1997)	Amsterdam, the Netherlands	Apr-Jul 1995	61
12	Romieu et al. (1997)	SW Mexico City	Apr-Jul 1991, Nov 1991-Feb 1992	65

Code	Population	Age (years)	Mean O_3 Level (ppb)	O_3 Exposure Index	Exposure Lag Days
1	asthmatic	5-49	41.5	8-h max	0
2	nonasthmatic	8-11	52	24-h avg	0-9
3	mildly asthmatic	5-13	196	1-h max	1
4	mildly asthmatic	5-13	190	1-h max	2
5	mildly asthmatic	5-13	196	1-h max	0
6	nonasthmatic	6-11	57.5 (SW); 55.9 (NE)	12-h avg[b]	0
7	asthmatic	7-13	35	8-h max	2
8	mildly asthmatic	5-13	190	1-h max	1
9	nonasthmatic	6-11	57.5 (SW); 55.9 (NE)	12-h avg[b]	1-5
10	mildly asthmatic	5-13	190	1-h max	0
11	asthmatic	7-13	35	8-h max	1
12	mildly asthmatic	5-13	196	1-h max	2

[a] Study population also includes adults.
[b] Percent PEF change is presented per 25 ppb increase in 12-h avg O_3. The standard units are used for other O_3 indices.

individual effect estimates are identified by study codes which are linked to the associated tables that provide study details. The tables present general information on the study location and period as well as specifics on the study population and O_3 exposure. Only single city studies that performed analyses stratified by time of day are included in the figure. Studies that examined cross-day changes and daily variability in PEF were excluded from the figure (e.g., Just et al., 2002; Thurston et al., 1997). Collectively, all of the studies indicated decrements of peak flow but most of the individual estimates were not statistically significant.

Mortimer et al. (2000, 2002) examined 846 asthmatic children from the National Cooperative Inner-City Asthma Study (NCICAS) for O_3-related changes in PEF. Children from eight urban areas in the U.S. (St. Louis, MO; Chicago, IL; Detroit, MI; Cleveland, OH; Washington, DC; Baltimore, MD; East Harlem, NY; and Bronx NY) were monitored from June through August 1993. This multicities study provides representative data for the U.S. Asthmatic children from urban areas are an important subgroup of asthmatics. Study children either had physician-diagnosed asthma and symptoms in the past 12 months or respiratory symptoms consistent with asthma that lasted > 6 weeks during the previous year. In a focused analysis, Mortimer et al. (2000) observed that the subpopulation of low birth weight and premature asthmatic children had significantly greater O_3-associated declines in PEF than normal birth weight children.

In the main study, Mortimer et al. (2002) further investigated changes in morning PEF associated with O_3 concentrations in the eight urban areas. The reductions in morning PEF were not significant in each individual city, however when the data from all eight cities were combined, a statistically significant change of −1.18% (95% CI: −2.10, −0.26) per 30 ppb increase in 8-h avg O_3 (10 a.m.-6 p.m.) was observed with a cumulative lag of 1 to 5 days. Figure 7-2 illustrates the probability density curves (or density curves) of the results from the city-stratified analysis and that from the pooled analysis of all eight cities. Summary density curves serve as a descriptive aid to the understanding of multiple effect estimates. These curves can be viewed as smoothed histograms. However, unlike a histogram, summary density curves account for varying standard errors of the individual mean effect estimates. Normal distribution functions can be calculated for each effect estimate and standard error. The density curve for the all cities analysis was calculated by taking the derivative of the normal distribution function from the analysis that pooled data from all eight cities. This density curve is a graphical

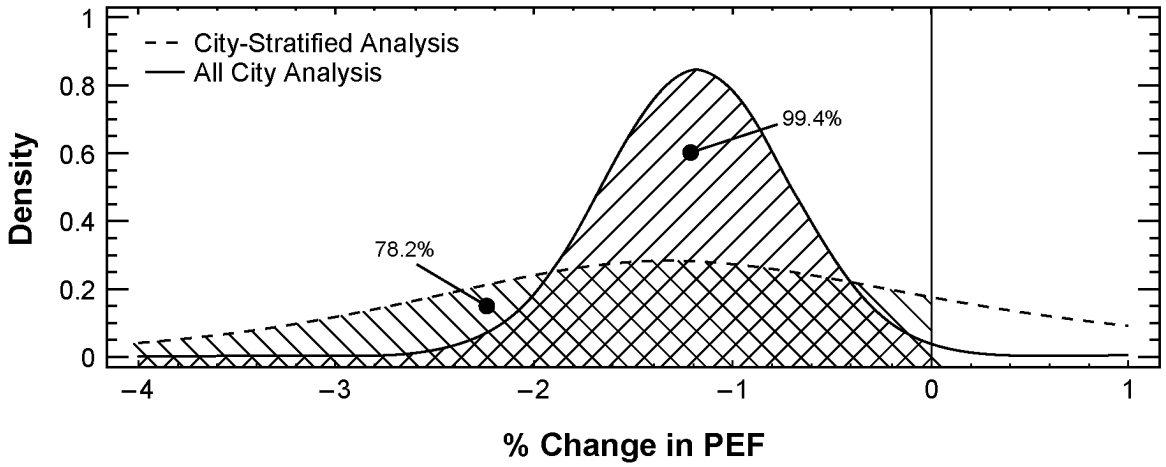


Figure 7-2. Density curves of the % change in PEF per 30 ppb increase in 8-h avg O_3 with a cumulative lag of 1 to 5 days for the individual eight NCICAS cities and the pooled average of all cities. Note that 99% and 78% of the areas under the curves are less than zero for the pooled cities analysis and individual cities analysis, respectively.

Source: Derived from Mortimer et al. (2002).

presentation of the all cities regression analysis presented by Mortimer et al. (2002) and only represents one effect estimate and corresponding standard error. The summary density curve for the city-stratified analysis was calculated by summing together the normal distribution functions for each of the eight cities, then taking the derivative of the summed function. The individual city estimates presented by Mortimer et al. (2002) were used to calculate the summary density curve. Both density curves graphically depict the mean and distribution of the % change in PEF per 30 ppb increase in 8-h avg O_3. The area under the density curve and to the left of a value on the x-axis is an estimate of the probability that the effect estimate will be less than or equal to that value. For example, the area under the density curve to the left of 0% change in PEF is 99% in the all cities analysis. As a statistically significant decline in PEF was observed in the all cities regression analysis, it is expected that more than 97.5% ($p < 0.05$) of this area would be less than zero. A wider distribution was observed in the city-stratified analysis, with only 78% of the area less than zero. The all cities analysis likely had a smaller standard error compared to the city-specific analysis as it was based upon more subjects and considered differences between cities to vary about the same mean effect. The regression analysis by Mortimer et al. (2002)

suggested a lack of heterogeneity by city, as indicated by the nonsignificant interaction term between O_3 effect and city. As shown in Figure 7-2, the summary density curve of the city-stratified analysis has a peak at about the same value as the curve of the all cities analysis, suggesting a common O_3 effect for all eight cities and small variation among them. The unimodal shape of the density curve of the city-stratified analysis also indicates the absence of outlying cities.

Mortimer et al. (2002) further noted that small declines in PEF may be of uncertain clinical significance, thus they calculated the incidence of ≥ 10% declines in PEF. A 5 to 15% change in FEV_1 has been expressed as having clinical importance to asthma morbidity (American Thoracic Society, 1991; Lebowitz et al., 1987; Lippmann, 1988). Although greater variability is expected in PEF measurements, a ≥ 10% change in PEF also likely has clinical significance. In Mortimer et al. (2002), the incidence of ≥ 10% declines in PEF was statistically significant, indicating that O_3 exposure may be associated with clinically significant changes in PEF in asthmatic children. This study also observed that excluding days when 8-h avg O_3 levels were less than 80 ppb provided effect estimates which were similar to those when all days were included in the analysis.

In Mexico City, two studies of asthmatic school children were carried out simultaneously in the northern (Romieu et al., 1996) and southwestern sections of the city (Romieu et al., 1997). In the northern study, 71 mildly asthmatic school children aged 5 to 13 years old, were followed over time for daily morning (before breakfast) and afternoon (bedtime) PEF. In single-pollutant models, O_3 at 0-, 1-, and 2-day lags was associated with diminished morning and afternoon PEF, but only the 0-day lag morning effect was statistically significant. The O_3 effect became nonsignificant when $PM_{2.5}$ was added to the model. In the southwestern study, 65 mildly asthmatic children aged 5 to 13 years old were followed during the summer and winter for daily morning and afternoon PEF. Significant effects at 0- and 1-day lag O_3 were observed on afternoon PEF, with effects larger with a 1-day lag. Associations involving O_3 were stronger than those involving PM_{10}. Several additional studies, both in the U.S. and in other countries, reported statistically significant associations between O_3 exposure and decrements in PEF among asthmatics (Gielen et al., 1997; Jalaludin et al., 2000; Just et al., 2002; Ross et al., 2002; Thurston et al., 1997).

Other epidemiologic studies did not find a significant O_3 effect on the lung function of asthmatics. Delfino et al. (1997a) examined morning and evening PEF among 22 asthmatics ranging in age from 9 to 46 years, living in Alpine, CA. Daily ambient 12-h avg O_3 (8 a.m.-8 p.m.) concentrations ranged from 34 to 103 ppb, with a mean value of 64 ppb. Unique to this study, personal O_3 exposures were measured using 12-h passive O_3 samplers that were worn by the subjects. The personal 12-h avg O_3 (8 a.m.-8 p.m.) concentrations, which had a mean value of 18 ppb, were much lower than the fixed-site ambient levels. Quantitative O_3 results were not reported but researchers stated that no significant O_3 effects were observed on morning and evening PEF. In Hiltermann et al. (1998), 60 nonsmoking adults aged 18 to 55 years in Bilthoven, the Netherlands, were followed between July and October 1995 with morning and afternoon PEF measurements. Ozone was associated with declines in PEF, but statistical significance was not achieved.

Results from the multicities study by Mortimer et al. (2002), as well as those from several regional studies provide evidence of a significant relationship between O_3 concentrations and PEF among asthmatics. Collectively, these studies indicate that O_3 may be associated with declines in lung function in this potentially susceptible population.

Panels of healthy subjects

The effect of O_3 on PEF in healthy subjects also was investigated in several studies. During the summer of 1990, Neas et al. (1995) examined 83 children in Uniontown, PA and reported twice daily PEF measurements. Researchers found that evening PEF was associated with O_3 levels weighted by hours spent outdoors. Using a similar repeated measures design, Neas et al. (1999) saw evidence for effects due to ambient O_3 exposure among 156 children attending two summer day camps in the Philadelphia, PA area. Associations were found between afternoon PEF (recorded before leaving camp) and same-day O_3 concentrations, and between morning PEF (recorded upon arrival at camp) and previous-day O_3 concentrations. However, the relationship between PEF and O_3 was statistically significant only when a cumulative lag period of 1 to 5 days was considered. Similarly, Naeher et al. (1999), in a sample of 473 nonsmoking women (age 19 to 43 years) living in Vinton, VA, also showed the largest, significant O_3-related decrease in evening PEF with a 5-day cumulative lag exposure.

Another study in southwestern Mexico City analyzed morning and afternoon PEF data collected from 40 school children aged 8 to 11 years (Gold et al., 1999). Subjects provided measurements upon arriving and before departing from school each day. Diminished PEF was associated with 1-day lag O_3 concentrations, but the only statistically significant findings were obtained for PEF regressed on O_3 concentrations with a cumulative 10-day lag period. This may imply a cumulative effect of O_3, or it may reflect confounding by other time-varying factors. These results, however, are in accord with controlled human exposure studies that have shown an attenuated decline in pulmonary function with repeated days of O_3 exposure (see Section 6.6, Repeated Exposure to O_3), and with epidemiologic studies that have assessed lung function over the course of the O_3 season (Brauer et al., 1996; Kinney and Lippmann 2000).

7.2.4 Respiratory Symptoms

Studies published over the past decade represent an improved new body of data on the symptom effects of O_3. Respiratory symptoms are usually measured in the context of acute air pollution field studies using questionnaire forms, or "daily diaries," that are filled out by study subjects, usually without the direct supervision of research staff. Questions address the daily experience of coughing, wheezing, shortness of breath (or difficulty breathing), production of phlegm, and others. While convenient and potentially useful in identifying acute episodes of morbidity, measurements of daily symptoms are prone to a variety of errors. These include misunderstanding of the meaning of symptoms, variability in individual interpretation of symptoms, inability to remember symptoms if not recorded soon after their occurrence, reporting bias if days of high air pollution levels are identifiable by subjects, and the possibility of falsified data. In spite of these potential problems, the ease of data collection has made daily symptom assessment a common feature of field studies. Many of the studies reviewed above for lung function results also included measurements of daily symptoms. Pearce et al. (1998) reports that one advantage in the case of asthma panels is that the population is usually already familiar with the symptom terms such as wheezing and cough. Delfino et al. (1998a) further states that the use of repeated daily symptom diaries has additional advantages of reducing recall bias given the proximity of events and allowing health effects to be modeled with each subject serving as their own control over time. Also, study design can blind the participants from the air pollution

aspect of the study. Careful efforts by study staff can help ensure that the symptom diaries will provide information that is less affected by the potential problems noted.

Similar to studies of lung function, respiratory symptom studies can be divided into two groups, asthma panels or healthy subjects. Asthma panel studies are presented first.

Asthma panels

Most studies examining respiratory symptoms related to O_3 exposure focused on asthmatic children. Among the health outcomes, of particular interest were those associated with asthma, including cough, wheeze, shortness of breath, and increased medication use. Figures 7-3 and 7-4 present the probability density curves for O_3-related cough and medication use among asthmatic children from six studies (Gielen et al., 1997; Jalaludin et al., 2004; Just et al., 2002; Ostro et al., 2001; Romieu et al., 1996, 1997). Only single city/region studies that present odds ratios are included in the figure for consistency. Studies that present change in severity of symptoms, another informative health outcome, are excluded from the figure since this expression differs from indicating simple presence of symptoms. The study by Gent et al. (2003) also is excluded from this figure as odds ratios for cough and mediation use were analyzed for quintiles of O_3 concentrations using the lowest quintile as the reference.

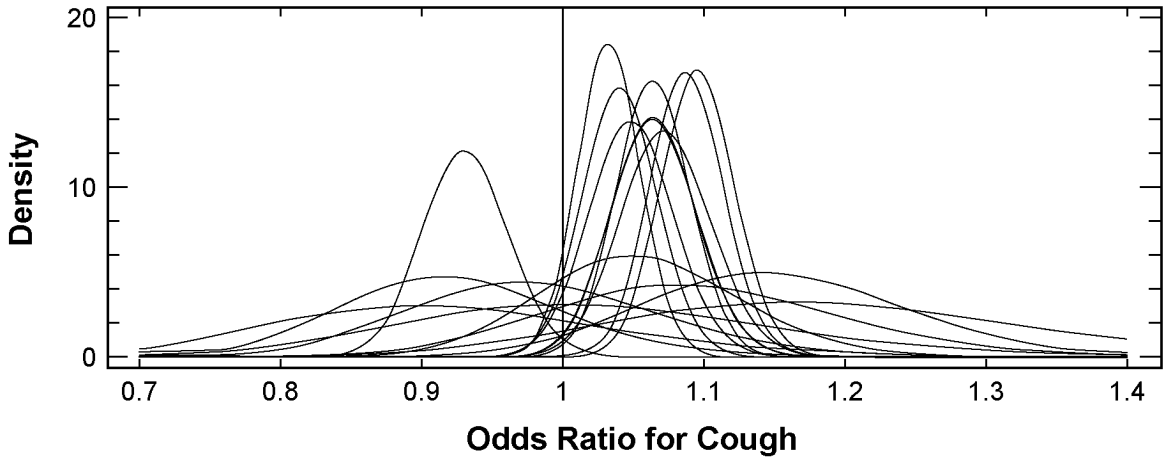


Figure 7-3. Density curves of the odds ratios for the prevalence of cough among asthmatic children. Fifteen odds ratios from six studies (Gielen et al., 1997; Jalaludin et al., 2004; Just et al., 2002; Ostro et al., 2001; Romieu et al., 1996, 1997) are standardized per 40 ppb increase in 1-h max O_3 or equivalent.

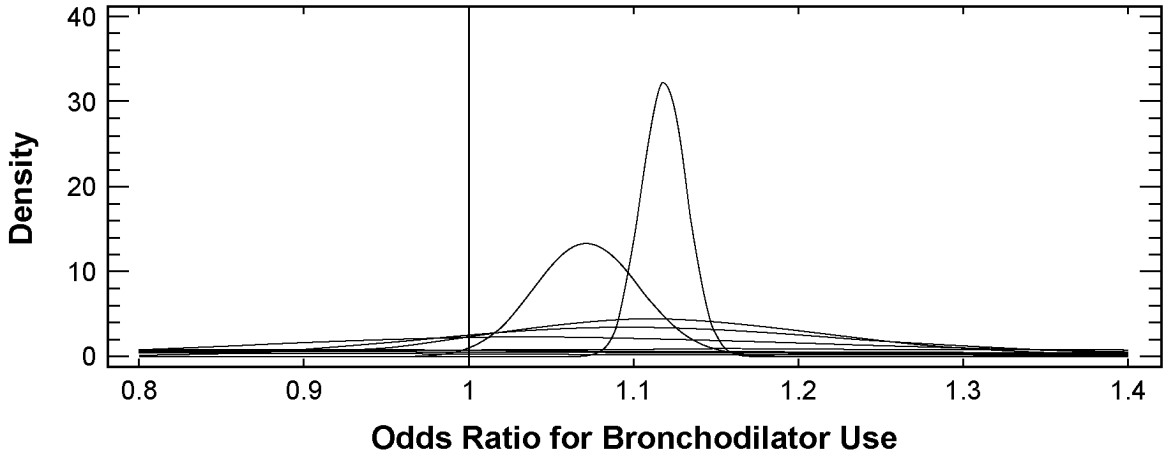


Figure 7-4. Density curves of the odds ratios for the prevalence of extra bronchodilator use among asthmatic children. Nine odds ratios from six studies (Gielen et al., 1997; Jalaludin et al., 2004; Just et al., 2002; Ostro et al., 2001; Romieu et al., 1996, 1997) are standardized per 40 ppb increase in 1-h max O_3 or equivalent.

The various effect estimates for the association between O_3 concentrations and cough are depicted as probability density curves (or density curves) in Figure 7-3. Each density curve represents an individual effect estimate and corresponding standard error. The use of density curves allows one to visualize better the distribution of O_3-related effects on cough. Despite the variability in the individual effect estimates, there is consistency in the O_3 effects as indicated by the considerable overlap in distributions. In general, the majority of the area under the density curves appears to be greater than an odds ratio of one, suggesting a positive association between O_3 concentration and cough among asthmatic children. Figure 7-4 presents the density curves for the various effect estimates for O_3-associated bronchodilator use. Similar to cough, the majority of the area under the density curves in this figure is greater than an odds ratio of one, indicating that O_3 may be associated with increased bronchodilator use in children with mild to severe asthma.

Among the studies reporting results for daily symptoms and asthma medication use, several observed associations with O_3 concentrations that appeared fairly robust (Delfino et al., 2003; Desqueyroux et al., 2002ab; Gent et al., 2003; Hilterman et al., 1998; Just et al., 2002; Mortimer et al., 2000, 2002; Newhouse et al., 2004; Romieu et al., 1996, 1997; Ross et al., 2002; Thurston et al., 1997).

Mortimer et al. (2002) reported morning symptoms in 846 asthmatic children from eight urban areas of the U.S. to be most strongly associated with a cumulative 4-day lag of O_3 concentrations in the NCICAS. The NCICAS used standard protocols which included instructing caretakers of the subjects to record symptoms in the daily diary by observing or asking the child (Mitchell et al., 1997). Symptoms reported included cough, chest tightness, and wheeze. In the analysis pooling data from all eight cities, the odds ratio for the incidence of symptoms was 1.35 (95% CI: 1.04, 1.69) per 30 ppb increase in 8-h avg O_3. Excluding days when 8-h avg O_3 (10 a.m.-6 p.m.) was greater than 80 ppb, the odds ratio was 1.37 (95% CI: 1.02, 1.82) for incidence of morning symptoms. Figure 7-5 presents the density curves of the odds ratios for the incidence of symptoms from the city-stratified analysis and that from the all cities analysis. This figure confirms the regression results that there is a significant increase in odds for incidence of symptoms, as the area under the density curve with an odds ratio greater than one is 99%. The unimodal distribution of the city-stratified summary density curve indicates a lack of significant heterogeneity among the eight cities.

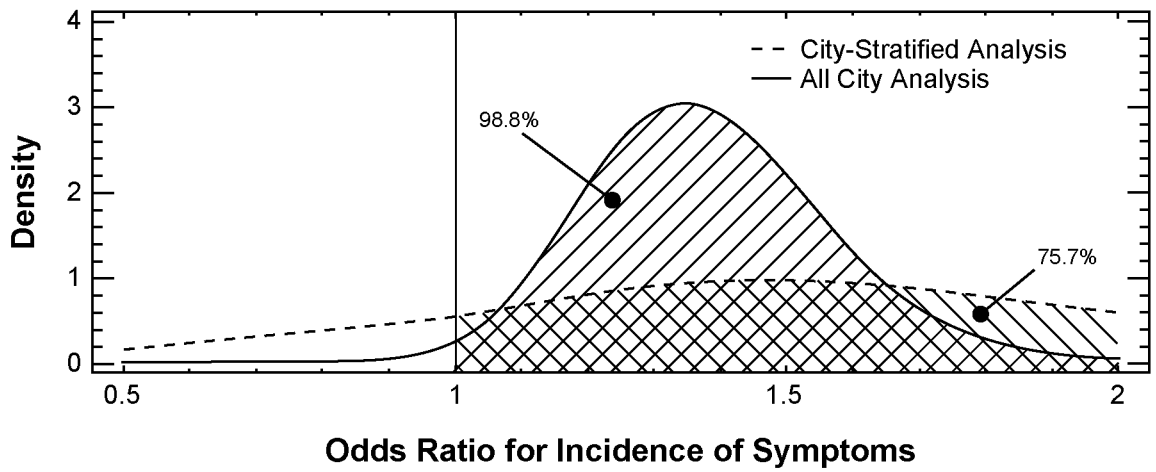


Figure 7-5. Density curves of the odds ratios for the incidence of symptoms per 30 ppb increase in 8-h avg O_3 with a cumulative lag of 1 to 4 days for the individual eight cities and the pooled average of all cities. Note that 99% and 76% of the areas under the curves are greater than one for the pooled cities and individual cities analyses, respectively.

Source: Derived from Mortimer et al. (2002).

Another one of the larger studies was that of Gent and colleagues (2003), where 271 asthmatic children under age 12 and living in southern New England were followed over 6 months (April through September) for daily symptoms. The data were analyzed for two separate groups of subjects, 130 who used maintenance asthma medications during the follow-up period and 141 who did not. The need for regular medication was considered to be a proxy for more severe asthma. Not taking any medication on a regular basis and not needing to use a bronchodilator would suggest the presence of very mild asthma. Significant effects of 1-day lag O_3 were observed on a variety of respiratory symptoms only in the medication user group. Both daily 1-h max and 8-h max O_3 concentrations were similarly related to symptoms such as chest tightness and shortness of breath. Effects of O_3, but not $PM_{2.5}$, remained significant and even increased in magnitude in two-pollutant models. Some of the significant associations were noted at 1-h max O_3 levels below 60 ppb. In contrast, no significant effects were observed among asthmatics not using maintenance medication. In terms of person-days of follow-up, this is one of the larger studies currently available that address symptom outcomes in relation to O_3, and provides supportive evidence for effects of O_3 independent of $PM_{2.5}$.

Some international studies have reported significant symptoms associations with O_3. The incidence of asthma attacks was significantly associated with O_3 concentrations in a group of 60 severe asthmatics (mean age 55 years) followed over a 13-month period in Paris (Desqueyroux et al., 2002a). In a similar study, Desqueyroux et al. (2002b) observed significant O_3-associated exacerbation of symptoms in 39 adult patients (mean age 67 years) with chronic obstructive pulmonary disease (COPD). Interestingly, in contrast to the controlled human studies (see Section 6.3.1, Subjects with COPD), the O_3 effect appeared larger among subjects who smoked and those with more severe COPD. However, the low O_3 concentrations experienced during this study (summer mean 8-h max O_3 of 41 $\mu g/m^3$ or approximately 21 ppb) raise plausibility questions. In a study of 60 nonsmoking asthmatic adults (aged 18 to 55 years) in Bilthoven, the Netherlands, Hilterman and colleagues (1998) reported significant associations between O_3 and daily symptoms of shortness of breath and pain upon deep inspiration. The O_3 associations were stronger than those of PM_{10}, NO_2, SO_2, and black smoke (BS). No differences in response were evident between subgroups of subjects defined on the basis of steroid use or airway hyperresponsiveness. Daily use of bronchodilators or steroid inhalers was not found to be associated with O_3 in this study.

Other studies showed only limited or a lack of evidence for symptom increases associated with O_3 exposure (Avol et al., 1998; Chen et al., 1998; Delfino et al., 1996, 1997a, 1998a; Gielen et al., 1997; Jalaludin et al., 2004; Ostro et al., 2001; Taggart et al., 1996). Ostro et al. (2001) reported no associations between daily symptoms and ambient O_3 concentrations in a cohort of 138 African-American children with asthma followed over 3 months (August to October) in Central Los Angeles and Pasadena, CA. However, the use of extra asthma medication was associated with 1-h max O_3 concentrations at a 1-day lag. Delfino and colleagues (1996) followed 12 asthmatic teens living in San Diego, CA for respiratory symptoms over a two-month period and saw no relationship with central site ambient O_3. Personal O_3 exposures measured with passive diffusion monitors were associated with the composite symptom score and β_2-agonist inhaler use, but the relationship with symptom score disappeared when weekday/weekend differences were controlled in the statistical analysis. Study power was likely compromised by the small sample size. This observation of stronger effects based on personal monitoring is intriguing; it suggests that substantial gains in power may be achieved if exposure misclassification is reduced through the use of personal exposure measurements rather than central site O_3 concentrations. A similar study of 22 asthmatics in Alpine, CA observed no effects of O_3 on symptoms when personal O_3 exposure was used as the exposure metric (Delfino et al., 1997a). However, a later study in the same location involving 24 subjects (Delfino et al., 1998a) did find an association between respiratory symptoms and ambient O_3 exposure, with stronger O_3 effects experienced by asthmatics not on anti-inflammatory medication. In this study, a binary symptom score was used, whereas the earlier study used a linear symptom score of 0 through 6.

In conclusion, the various studies seem to indicate a positive association between O_3 concentrations, and respiratory symptoms and increased medication use in asthmatics. The multicities study by Mortimer et al. (2002) provides an asthmatic population most representative of the U.S., but several single city studies also add to the knowledge base.

Panels of healthy subjects

Fewer studies examined the effect of O_3 on respiratory symptoms in healthy individuals. Neas et al. (1995) reported evening cough was associated with O_3 levels weighted by hours spent outdoors in school children. The study by Linn and colleagues (1996) of 269 school children in

southern California reported no associations between respiratory symptoms and O_3, but subjects were exposed to fairly low O_3 concentrations as determined using personal monitors. Gold et al. (1999) examined symptoms in 40 healthy children in southwest Mexico City. Pollutant exposures were associated with increased production of phlegm in the morning, although the effects of the air pollutants ($PM_{2.5}$, PM_{10}, and O_3) could not be separated in multipollutant models. Hoek and Brunekreef (1995) did not find a consistent association between ambient O_3 levels, and prevalence and incidence of respiratory symptoms in children living in two rural towns in the Netherlands. Collectively, these studies indicate that there is no consistent evidence of an association between O_3 and respiratory symptoms among healthy children.

7.2.5 Acute Airway Inflammation

Acute airway inflammation has been shown to occur among adults exposed to 80 ppb O_3 over 6.6 hours with exercise in controlled chamber studies (Devlin et al., 1991). Kopp and colleagues (1999) attempted to document inflammation of the upper airways in response to summer season O_3 exposures by following a group of 170 school children in two towns in the German Black Forest from March to October of 1994. To assess inflammation, the investigators collected nasal lavage samples at 11 time points spanning the follow-up period. The nasal lavage samples were analyzed for markers of inflammation, including eosinophil cationic protein, albumin, and leukocyte counts. Subjects who were sensitized to inhaled allergens were excluded. When analyzed across the entire follow-up period, no association was detected between upper airway inflammation and O_3 concentrations. More detailed analysis showed that the first significant O_3 episode of the summer was followed by a rise in eosinophil cationic protein levels, however, subsequent and even higher O_3 episodes had no effect. These findings suggest an adaptive response of inflammation in the nasal airways that is consistent with controlled human studies (see Section 6.9, Effects of Inflammation and Host Defense).

Frischer and colleagues (1993) collected nasal lavage samples from 44 school children in Umkirch, Germany the morning after "low" O_3 days (< 140 $\mu g/m^3$ or approximately 72 ppb) and "high" O_3 days (> 180 $\mu g/m^3$ or approximately 93 ppb) to measure levels of biochemical markers of inflammation. The researchers found that higher O_3 levels were significantly associated with increased polymorphonuclear leukocyte counts in all children, and increases in myeloperoxydases and eosinophilic cation proteins among children without symptoms of rhinitis

(n = 30). These results indicated that O_3 was associated with inflammation in the upper airways. Frischer et al. (1997) further investigated whether hydroxyl radical attacks played a role in mediating the O_3-associated inflammatory response of the airways. O*rtho*- and *para*-tyrosine levels were measured in the nasal lavage samples and the *ortho*/*para* radical ratio was used to determine the generation of hydroxyl radicals. Significant increases in the *ortho*/*para* ratio were observed on days following high ambient O_3 levels. However, the *ortho*/*para* ratio was not related to polymorphonuclear leukocyte counts, suggesting that there was no detectable relationship between hydroxyl radical attacks and the inflammatory response seen in these children. Similar to the study by Kopp et al. (1999), the *ortho*/*para* ratio decreased at the end of the summer although O_3 concentrations were still high, providing additional evidence for a possible adaptive response. These findings, however, do not preclude the possibility that other unmeasured effects, including cell damage or lower airway responses, may have occurred with ongoing summer season exposures. In fact, a study of joggers repeatedly exposed to O_3 while exercising over the summer in New York City suggested that cell damage may occur in the absence of ongoing inflammation (Kinney et al., 1996).

In two Mexico City studies by Romieu et al. (1998, 2002), the effect of antioxidant supplements on the association between O_3 and lung function in outdoor workers and asthmatic children was investigated. Romieu and colleagues (1998) observed significant inverse associations between O_3 and lung function parameters, including FVC, FEV_1, and FEF_{25-75} (forced expiratory flow at 25 to 75% of FVC), among outdoor workers who were on the placebo, but not among those taking the antioxidant supplement during the 1st phase of testing. Likewise, O_3 concentrations were associated with declines in lung function among children with moderate to severe asthma who were on the placebo, but no associations were found among those who were taking the vitamin C and E supplement (Romieu et al., 2002). These results indicate that supplementation with antioxidants may modulate the impact of O_3 exposure on the small airways of two potentially susceptible populations, outdoor workers and children with moderate to severe asthma. In a further analysis, genetic factors were found to contribute to the variability between individuals in the effects of O_3 on lung function (Romieu et al., 2004). Individuals with polymorphism of the glutathione S-transferase gene (GSTM1 null genotype) lack glutathione transferase enzyme activity, which plays an important role in protecting cells against oxidative damage. Results from this analysis indicate that asthmatic children with GSTM1 null genotype

were found to be more susceptible to the impact of O_3 exposure on small airways function. Romieu et al. (2004) noted that supplementation with the antioxidant vitamins C and E above the minimum daily requirement might compensate for the genetic susceptibility.

7.2.6 Acute O_3 Exposure and School Absences

The association between school absenteeism and ambient air pollution was assessed in two studies (Chen et al., 2000; Gilliland et al., 2001). In the study by Chen and colleagues (2000), daily school absenteeism was examined in 27,793 students (kindergarten to 6th grade) from 57 elementary school students in Washoe County, NV over a two-year period. One major limitation of this study was that the percent of total daily absences was the outcome of interest, not illness-related absences, as reasons for absences were not noted in all schools. In models adjusting for PM_{10} and CO concentrations, ambient O_3 levels were significantly associated with school absenteeism. With a distributed lag of 1 to 14 days, O_3 concentrations were associated with a 10.41% (95% CI: 2.73, 18.09) excess rate of school absences per 40 ppb increase in 1-h max O_3. PM_{10} and CO concentrations also were significantly associated with school absenteeism, however, the effect estimate for PM_{10} was negative. The inverse relationship between O_3 and PM_{10} may have partially attributed to the negative association observed between PM_{10} and school absenteeism.

Ozone-related school absences also were examined in a study of 1,933 4th grade students from 12 southern California communities participating in the Children's Health Study (Gilliland et al., 2001). Due to its size and comprehensive characterization of health outcomes, this study is especially valuable in assessing the effect of O_3 on illness-related school absenteeism in children. The study spanned a period, January through June 1996, that captured a wide range of exposures while staying mostly below the highest levels observed in the summer season. All school absences that occurred during this period were followed up with phone calls to determine whether they were illness-related. For illness-related absences, further questions assessed whether the illness was respiratory or gastrointestinal, with respiratory symptoms including runny nose/sneeze, sore throat, cough, earache, wheezing, or asthma attacks. Multiple pollutants were measured at a central site in each of the 12 communities. The statistical analysis controlled for temporal cycles, day of week, and temperature, and expressed exposure as a distributed lag out to 30 days. Some concern exists regarding the possibility of residual seasonal

confounding given the six-month time span of the monitoring period. Significant associations were found between the 30-day distributed lag of 8-h avg O_3 (10 a.m.-6 p.m.) and all absence categories. Larger O_3 effects were seen for respiratory causes (147% increase per 30 ppb increase in 8-h avg O_3) than for nonrespiratory causes (61% increase). Among the respiratory absences, larger effects were seen for lower respiratory diseases with wet cough than for upper respiratory diseases. PM_{10} was only associated with upper respiratory disease absences.

Results from Chen et al. (2000) and, more notably, Gilliland et al. (2001) indicate that ambient O_3 concentrations, lagged over two to four weeks, are significantly associated with school absenteeism, particularly respiratory illness-related absences. These two studies on school absenteeism were conducted in communities in Nevada and southern California, however the results are most likely representative of U.S. populations.

7.2.7 Cardiac Physiologic Endpoints

Limited pathophysiologic air pollution studies have examined cardiac physiologic endpoints (Table AX7-2 in Chapter 7 Annex). Several studies examined associations between PM exposures and gaseous pollutants, and various measures of heart beat rhythms in panels of elderly subjects as discussed in the 2004 PM AQCD (Section 8.3.1). Decreased heart rate variability has been identified as a predictor of increased cardiovascular morbidity and mortality. One study examined the increased risk of myocardial infarction and pollutants (Peters et al., 2001). Lack of consistency in the limited studies argued for caution in regards to drawing conclusions on the relationship between cardiovascular outcomes and PM exposure. Among these studies, Gold et al. (2000; reanalysis Gold et al., 2003) and Peters et al. (2000a, 2001) discussed limited evaluation of a potential role for O_3 exposure. In addition, two recent studies provided limited evidence for an association between O_3 concentrations and heart rate variability in primarily elderly populations (Holguín et al., 2003; Park et al., 2004). Two related studies by Rich et al. (2004) and Vedal et al. (2004) examined the relationship between various air pollutants, including O_3, and cardiac arrhythmias using two different study designs, but both did not find any consistent evidence that exposure to air pollution affected the risk of arrhythmias. Two limited controlled human exposure studies with cardiovascular outcomes (Gong et al., 1998a; Superko et al., 1984), described in Chapter 6, Section 6.3.4, provide no supporting data.

The above panel studies with small numbers of subjects had limited ability to adequately test the hypothesis of heart rate variability. A recent large population-based study, the first in this field, examined PM_{10}, O_3, and other gaseous air pollutants, and their potentially adverse effects on cardiac autonomic control (Liao et al., 2004). Liao et al. investigated short-term associations between ambient pollutants and cardiac autonomic control from the 4th cohort examination (1996-1998) of the population-based Atherosclerosis Risk in Communities Study. PM_{10} (24-h avg) and O_3 exposure (8-h avg, 10 a.m.-6 p.m.) one day prior to the randomly allocated examination date were used. They calculated 5-minute heart rate variability indices between 8:30 a.m. and 12:30 p.m. and used logarithmically-transformed data on high-frequency (0.15 to 0.40 Hz) and low-frequency (0.04 to 0.15 Hz) power, standard deviation of normal R-R intervals, and mean heart rate. The effective sample sizes for PM_{10} and O_3 were 4,899 and 5,431, respectively, from three U.S. study centers in North Carolina, Minnesota, and Mississippi. PM_{10} concentrations measured one day prior to the heart rate variability measurements were inversely associated with both frequency and time domain heart rate variability indices. Ambient O_3 concentrations were inversely associated with high-frequency power among whites. Consistently more pronounced associations were suggested between PM_{10} and heart rate variability among persons with a history of hypertension. These findings were cross-sectionally derived from a population-based sample and reflect only the short-term effects of air pollution on heart rate variability. When the regression coefficients for each individual pollutant model were compared, the effects for PM_{10} was considerably larger than the effects for gaseous pollutants such as O_3. While these data are supportive of the hypothesized air pollution-heart rate variability-cardiovascular disease pathway at the population level, replication of these interactions in other studies is needed before any conclusions can be made.

7.2.8 Summary of Field Studies Assessing Acute O_3 Effects

- Results from recent field/panel studies support the evidence from clinical studies that acute O_3 exposure is associated with a significant effect on lung function, as indicated by decrements in FEV_1, FVC, and PEF. The declines in lung function were noted particularly in children and asthmatics.

- Limited evidence suggests that more time spent outdoors, higher levels of exertion, and the related increase in O_3 exposure may potentiate the risk of respiratory effects. In addition to children and asthmatics, adults who work or exercise outdoors may be particularly susceptible to O_3-associated health effects.

- Many new studies have examined the association between O_3 concentrations and a wide variety of respiratory symptoms (e.g., cough, wheeze, production of phlegm, and shortness of breath). Collectively, the results indicate that acute exposure to O_3 is associated with increased respiratory symptoms and increased as-needed medication use in children and asthmatics.

- Additional panel studies investigated the effect of O_3 on other health outcomes, including school absences, and markers of inflammation and oxidative damage. Ozone exposure was associated with significant increases in respiratory-related school absences, as well as increased inflammation and generation of hydroxyl radicals in the upper airways. Use of antioxidant supplements was found to diminish the O_3 effect on lung function.

- Few field studies have examined the association between O_3 and cardiac physiologic outcomes. The current evidence is rather limited but supportive of a potential effect on heart rate variability. Additional studies need to be performed before any conclusions can be made regarding an O_3 effect on cardiovascular outcomes.

7.3 EFFECTS OF OZONE ON DAILY EMERGENCY DEPARTMENT VISITS AND HOSPITAL ADMISSIONS

7.3.1 Summary of Key Findings on Studies of Emergency Department Visits and Hospital Admissions from the 1996 O_3 AQCD

In the 1996 O_3 AQCD, aggregate population time-series studies of O_3-related health effects provided relevant evidence of acute responses, even below a 1-h max O_3 of 0.12 ppm. Emergency room visits and hospital admissions were examined as possible outcomes following exposure to O_3. In the case of emergency room visits, the evidence was limited (Bates et al., 1990; Cody et al., 1992; Weisel et al., 1995; White et al., 1994), but results generally indicated an O_3 effect on morbidity. The strongest and most consistent evidence of O_3 effects, at levels both above and below 0.12 ppm 1-h max O_3, was provided by the multiple studies that had been conducted on summertime daily hospital admissions for respiratory causes in various locales in eastern North America (Bates and Sizto, 1983, 1987, 1989; Burnett et al., 1994; Lipfert and Hammerstrom, 1992; Thurston et al., 1992, 1994). These studies consistently demonstrated that O_3 air pollution was associated with increased hospital admissions, accounting for roughly one to three excess respiratory hospital admissions per million persons with each 100 ppb increase in 1-h max O_3. This association had been shown to remain even after statistically controlling for the possible confounding effects of temperature and copollutants (e.g., H^+, SO_4^{-2}, PM_{10}), as well

as when considering only days with 1-h max O_3 concentrations below 0.12 ppm. Furthermore, these results implied that O_3 air pollution could account for a substantial portion of summertime hospital admissions for respiratory causes on the most polluted days. Overall, the aggregate population time-series studies considered in the 1996 O_3 AQCD provided strong evidence that ambient exposures to O_3 can cause significant exacerbations of preexisting respiratory disease in the general public at concentrations below 0.12 ppm.

7.3.2 Review of Recent Studies of Emergency Department Visits for Respiratory Diseases

Emergency department visits represent an important acute outcome that may be affected by O_3 exposures. Morbidities that result in emergency department visits are closely related to, but are generally less severe than, those that result in unscheduled hospital admissions. In many cases, acute health problems are successfully treated in the emergency department; a subset of more severe cases that present initially to the emergency department may require admission to the hospital.

Several studies have been published in the past decade examining the temporal associations between O_3 exposures and emergency department visits for respiratory diseases (Table AX7-3 in Chapter 7 Annex). Total respiratory causes for emergency room visits may include asthma, pneumonia, bronchitis, emphysema, other upper and lower respiratory infections such as influenza, and a few other minor categories. Asthma visits typically dominate the daily incidence counts. Chronic bronchitis and emphysema often are combined to define COPD, which is a prominent diagnosis among older adults with lung disease. Figure 7-6 presents % changes in emergency department visits for asthma, with results expressed in standardized increments. Results from all lags presented are included in the figure. Weisel et al. (2002) was excluded as relative risks were not presented and could not be estimated. Among the U.S. studies, there was one multicity study which examined three cities in Ohio (Jaffe et al., 2003). Several presented Atlanta, GA data. In general, O_3 effect estimates from summer only analyses tended to be positive and larger compared to results from cool season or all year analyses.

Among studies with adequate controls for seasonal patterns, many reported at least one significant positive association involving O_3. These studies examined emergency department visits for total respiratory complaints (Delfino et al., 1997b, 1998b; Herñandez-Gardûno et al.,

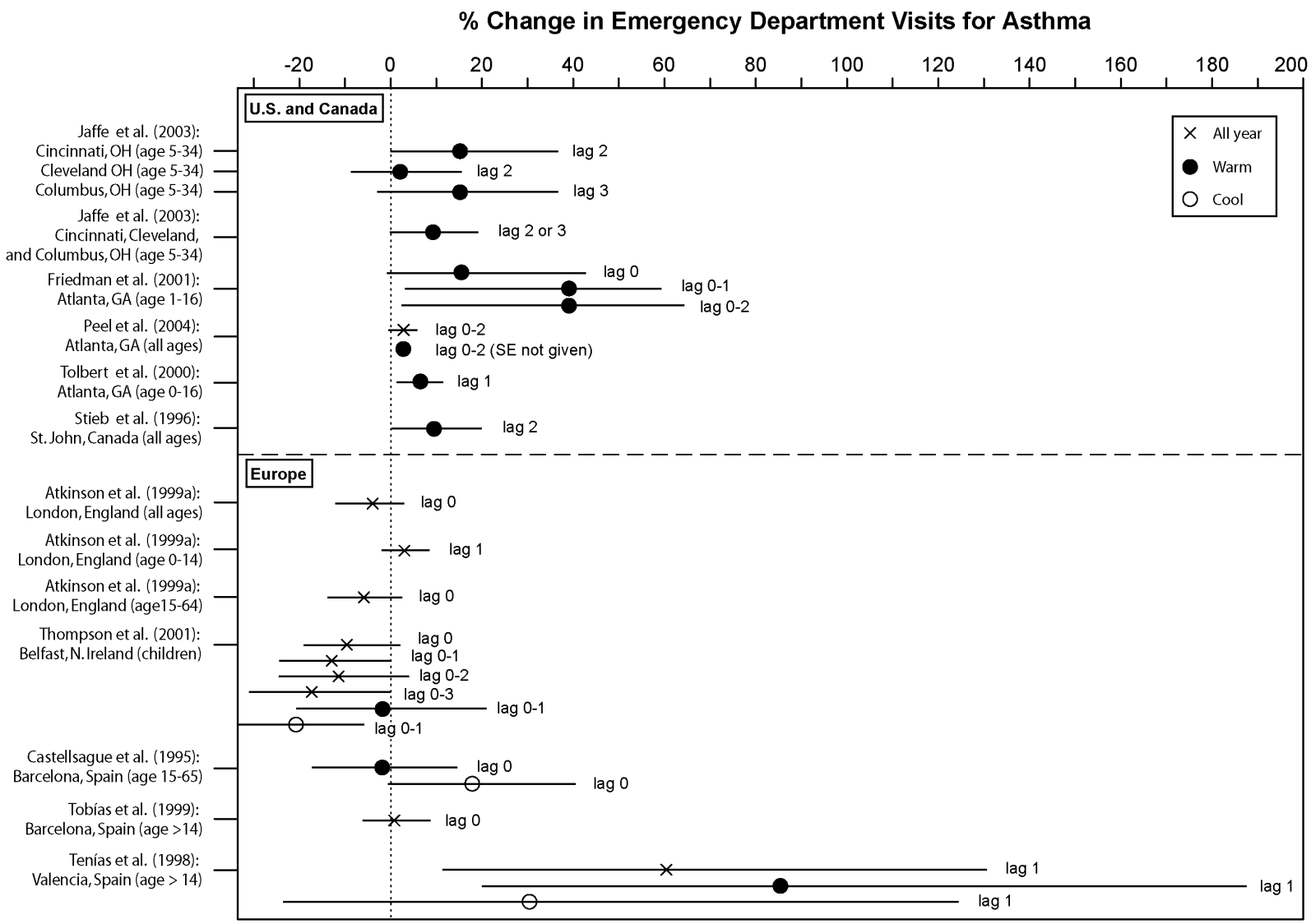


Figure 7-6. Ozone-associated % change (95% CI) in emergency department visits for asthma per 40 ppb increase in 1-h max O_3 or equivalent.

1997; Ilabaca et al., 1999; Jones et al., 1995; Lin et al., 1999), asthma (Friedman et al., 2001; Jaffe et al., 2003; Stieb et al., 1996; Tenías et al., 1998; Tobías et al., 1999; Tolbert et al., 2000; Weisel et al., 2002), and COPD (Tenías et al., 2002).

One recent study examined emergency department visits for total and cause-specific respiratory diseases in Atlanta, GA over an 8-year period (Peel et al., 2004). A distributed lag of 0 to 2 days was specified *a priori*. Ozone concentrations were significantly associated with emergency department visits for total respiratory diseases and upper respiratory infections in all ages. A marginally significant association was observed with asthma visits (2.6% excess risk per 30 ppb increase in 8-h max O_3), which became stronger when analysis was restricted to the warm months (3.1% excess risk). In multipollutant models adjusting for PM_{10}, NO_2 and CO, O_3 was the only pollutant that remained significantly associated with upper respiratory infections. Another large asthma emergency department study was carried out during the months of May through September from 1984 to 1992 in St. John, New Brunswick, Canada (Stieb et al., 1996). Effects were examined separately among children aged less than 15 years and in persons aged 15 years and older. A significant effect of O_3 on emergency department visits was reported among persons 15 years and older. There was evidence of a threshold somewhere in the range below a 1-h max O_3 of 75 ppb. A study in Valencia, Spain from 1994 to 1995 observed that emergency room visits for asthma among persons over 14 years old were robustly associated with relatively low O_3 levels (median 1-h max O_3 of 62.8 $\mu g/m^3$ or approximately 32.4 ppb) (Tenías et al., 1998). The excess risk of asthma emergency room visits was larger in the warm season (May to October), 85% excess risk per 40 ppb increase in 1-h max O_3, compared to the cool season (November-April), 31% excess risk (Tenías et al., 1998).

Among the studies that observed a statistically significant association between O_3 and emergency department visits for respiratory outcomes, O_3 effects were found to be robust to adjustment for PM_{10}, NO_2, SO_2, and black smoke (Lin et al., 1999; Peel et al., 2004; Tenías et al., 1998). One study by Tolbert and colleagues (2000) observed that the significant univariate effects of both O_3 and PM_{10} on pediatric asthma emergency department visits in Atlanta, GA became non-significant in two-pollutant regressions, reflecting the high correlation between the two pollutants ($r = 0.75$).

For several other "positive" studies with total respiratory and asthma outcomes, inconsistencies confound an interpretation of likely causal effects. For example, in a Montreal,

Canada study, O_3 effects on total respiratory emergency department visits were seen in a short data series from the summer of 1993 but not in a similar data series from the summer of 1992 (Delfino et al., 1997b). The significant 1993 results were seen only for persons older than 64 years, in spite of greater asthma prevalence among children. A very similar analysis of two additional summers (1989 and 1990) revealed an O_3 association only for 1989 and again only in persons over 64 years old (Delfino et al., 1998b). An analysis of data on respiratory emergency department visits from June to August of 1990 in Baton Rouge, LA reported O_3 effects in adults, but not in children or among the elderly (Jones et al., 1995).

Tobías and colleagues (1999) showed that regression results for asthma emergency department visits could be quite sensitive to methods used to control for asthma epidemics. Ozone was associated with the outcome variable in only one of eight models tested. An Atlanta, GA study by Zhu et al. (2003) examined asthma emergency department visits in children during three summers using Bayesian hierarchical modeling to address model variability. Data was analyzed at the zip code level to account for spatially misaligned longitudinal data. Results indicated a positive, but nonsignificant relationship between O_3 and emergency room visits for asthma.

Other studies also reported nonsignificant findings for O_3 (Atkinson et al., 1999a; Castellsague et al., 1995; Chew et al., 1999). One study by Thompson and colleagues (2001) in Belfast, Northern Ireland observed a significant 21% decrease in risk of childhood asthma admissions per 20 ppb increase in 24-h avg O_3 in the cold season (November-April). After adjusting for benzene levels, O_3 was no longer associated with asthma emergency department visits. The inverse relationship of O_3 with benzene concentrations ($r = -0.65$), and perhaps with other pollutants, might have produced the apparent protective effect of O_3. No significant O_3 effect was found in the warm season (May-October). The O_3 levels were low in both seasons, with a mean 24-h avg O_3 concentration of 18.7 ppb in the warm season and 17.1 ppb in the cold season. Atkinson et al. (1999a) in London, England also did not find an association between O_3 and emergency department visits at a mean 8-h max O_3 concentration of 17.5 ppb. Several other emergency department studies looking at O_3 are more difficult to interpret due to inadequate control for seasonal patterns, very low O_3 levels, or because no quantitative results were shown for O_3 (Buchdahl et al., 1996, 2000; Garty et al., 1998; Holmén et al., 1997; Lierl and Hornung, 2003; Lipsett et al., 1997; Nutman et al., 1998).

Although several studies found a significant association between O_3 concentrations and emergency department visits for respiratory causes, some inconsistencies were observed. The inconsistencies may be attributable, at least partially, to differences in study design and model specifications among the various studies. For example, ambient O_3 concentrations, length of the study period, and statistical methods used to control confounding by seasonal patterns and copollutants appear to affect the observed O_3 effect on emergency department visits. In general, an excess risk of emergency department visits was observed during the summer season when O_3 concentrations were higher.

7.3.3 Studies of Hospital Admissions for Respiratory Diseases

Hospital admissions represent a medical response to a serious degree of morbidity for a particular disease. Scheduled hospitalizations are planned in advance when a particular clinical treatment is needed. However, unscheduled admissions are ones that occur in response to unanticipated disease exacerbations and are more likely to be affected by environmental factors, such as air pollution. As such, the hospital admissions studies reviewed here focused specifically on unscheduled admissions. Study details and results from hospital admissions studies published over the past decade are summarized in Table AX7-4 (in the Chapter 7 Annex). As a group, these hospitalization studies tend to be larger in terms of geographic and temporal coverage, and indicate results that are generally more consistent than those reviewed above for emergency department visits. The following aspects of these studies should be considered in comparing results: (1) difference in type of respiratory diseases for hospital admission; (2) analysis by season versus all year; (3) O_3 only versus multipollutant models; (4) age of study population; (5) number of exposure lag days; (6) single-city versus multicity studies; (7) mean level of O_3 during study; (8) length of study (e.g., < 5 years versus > 5 years); and (9) type of study (e.g., case-crossover versus time-series).

Figures 7-7 through 7-9 present risk estimates from all total respiratory hospital admission studies. Burnett et al. (1995), which did not present quantitative results for O_3, and Yang et al. (2003), which only presented odd ratios, were excluded from the figure. In cases where multiple lags were presented, the multiday lag was selected to represent the cumulative effect from all days examined. For Luginaah et al. (2004), cumulative lags are not analyzed, thus the effect estimates from a 1-day lag are included in this figure. For studies that presented risk estimates

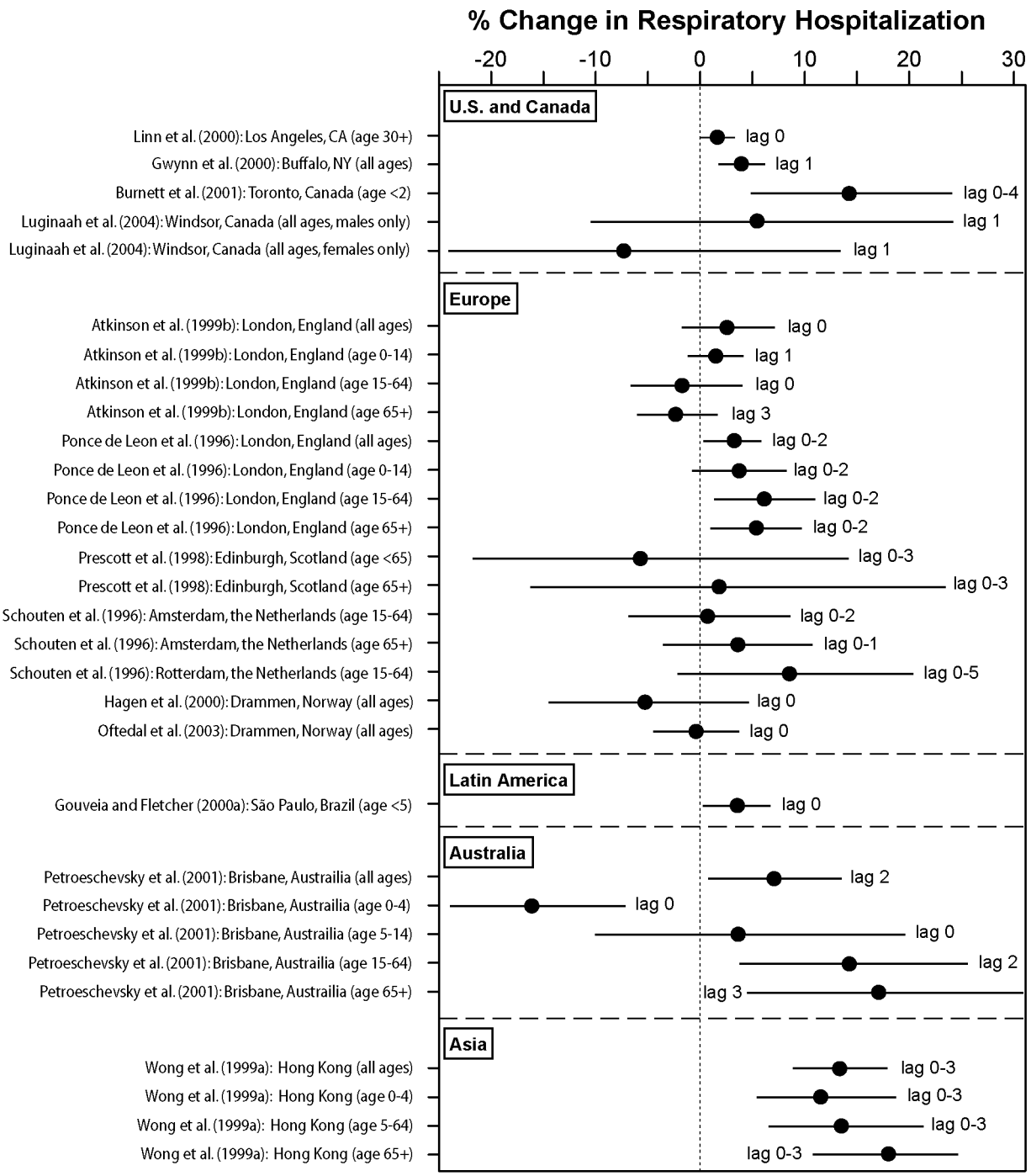


Figure 7-7. Ozone-associated % change (95% CI) in total respiratory hospitalizations <u>for all year analyses</u> per 40 ppb increase in 1-h max O_3 or equivalent.

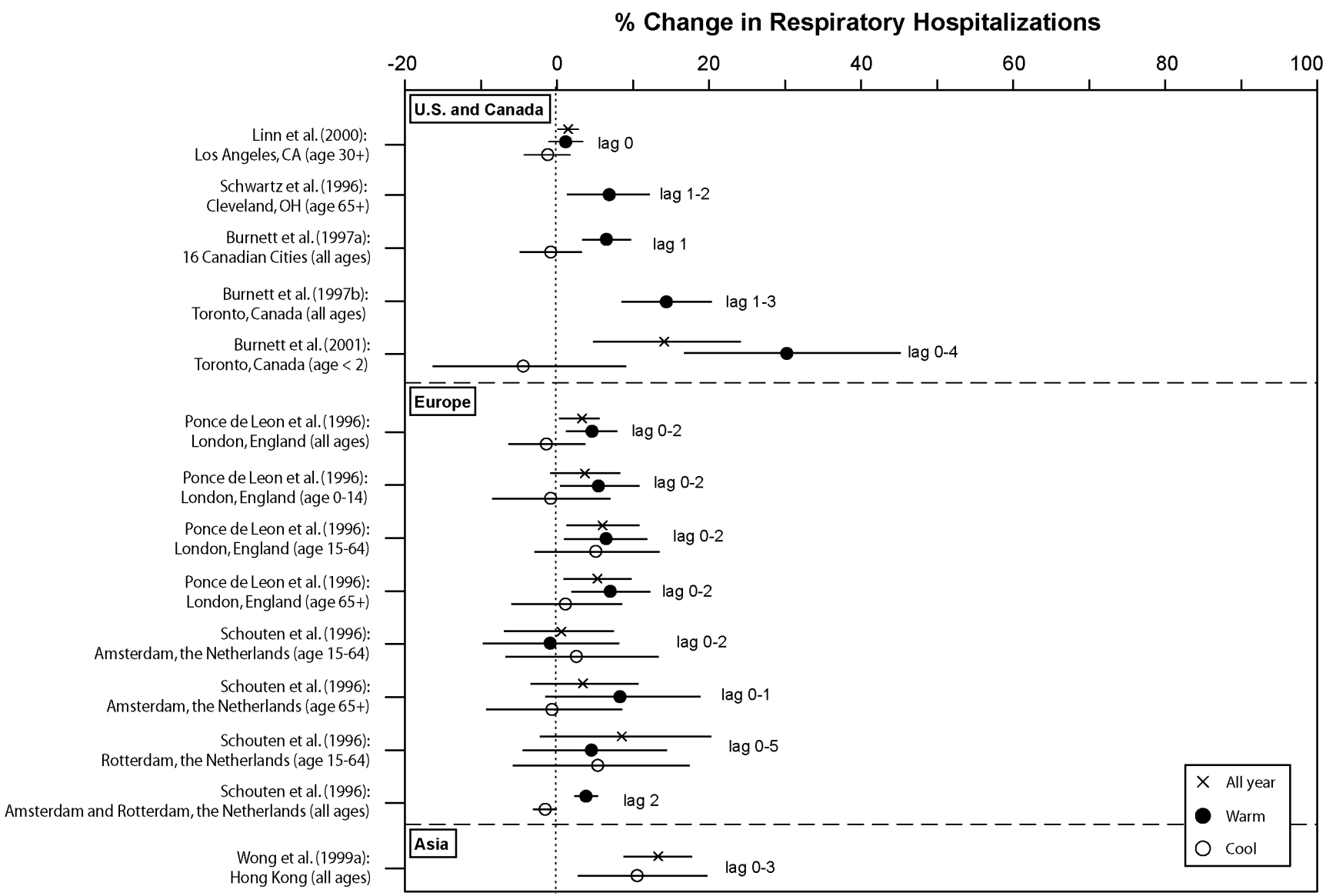


Figure 7-8. Ozone-associated % change (95% CI) in total respiratory hospitalizations by season per 40 ppb increase in 1-h max O_3 or equivalent.

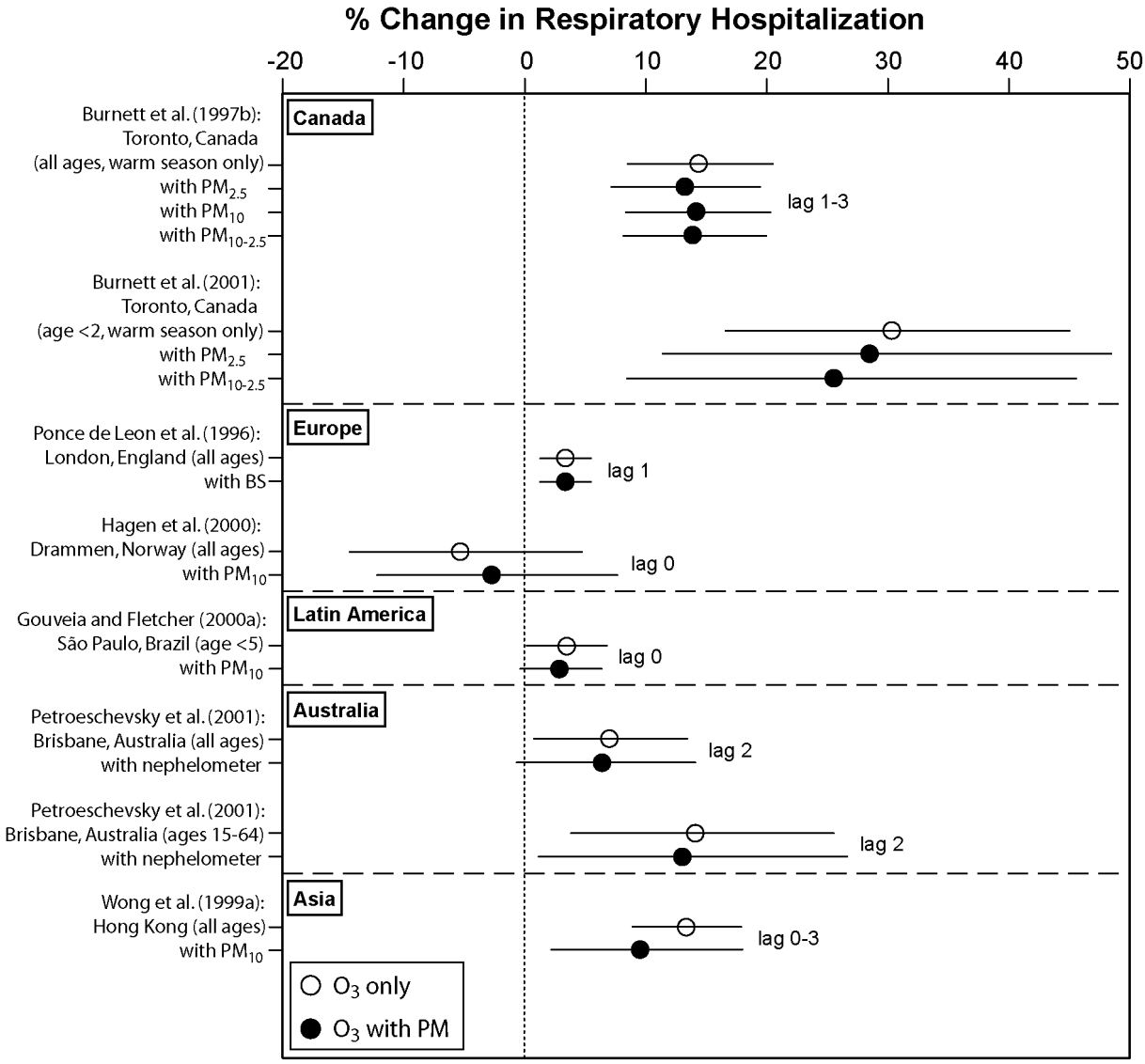


Figure 7-9. Ozone-associated % change (95% CI) in total respiratory hospitalizations with adjustment for PM indices per 40 ppb increase in 1-h max O_3 or equivalent. Analyses performed using all year data unless noted otherwise.

from all four seasons, only the summer and winter estimates are presented. Figure 7-7 plots the relative risk estimates and 95% CIs from 13 studies that analyzed all year data. The preponderance of positive risk estimates, with some that are statistically significant, is readily apparent. The impact of seasonal and multipollutant analyses were further examined in Figures 7-8 and 7-9. In Figure 7-8, it appears that the warm season estimates, collectively, tend to be

larger, positive values compared to all year and cool season estimates. Most of the negative estimates were from analyses using cool season data only, which might reflect the inverse correlation between O_3 and copollutants, namely PM, during that season. Figure 7-9 compares the risk estimates from models with and without adjustment for PM indices. This figure indicates that O_3 risk estimates are fairly robust to PM adjustment in the all year and warm season only data. None of the studies examined PM-adjusted O_3 risk estimates in cool season only data.

The most robust and informative results on the effects of O_3 on respiratory hospital admissions are those from studies carried out using a consistent analytical methodology across a broad geographic area (Anderson et al., 1997; Burnett et al., 1995, 1997a). These studies have all reported a significant O_3 effect on respiratory hospital admissions. The largest such study to-date was carried out using data on all-age respiratory hospital admissions from 16 Canadian cities with populations exceeding 100,000 covering the period 1981 to 1991 (Burnett et al., 1997a). In addition to O_3, the authors evaluated health effects of SO_2, NO_2, CO, and coefficient of haze (a surrogate for black carbon particle concentrations). Pooling the 16 cities, a significant positive association was observed between respiratory hospital admissions and the 1-day lag O_3 concentration in the spring (5.6% excess risk per 40 ppb increase in 1-h max O_3) and summer (6.7%). The results for fall were also positive, though of smaller magnitude (3.8%). There was no evidence for an O_3 effect in the winter season (−0.8%). Control outcomes related to blood, nervous system, digestive system, and genitourinary system disorders were not associated with O_3. In a previous study focused mainly on evaluating health impacts of sulfate particles, Burnett and colleagues (1995) reported results from a time-series analysis of all-age respiratory hospital admissions to 168 hospitals in Ontario, Canada over the 6-year period 1983 to 1988. The outcome data were prefiltered to remove seasonal variations using a weighted 19-day moving average. The authors reported that O_3 was associated with respiratory hospital admissions; however no quantitative results for O_3 were presented.

Results from an analysis of five European cities indicated strong and consistent O_3 effects on unscheduled hospital admissions for COPD (Anderson et al., 1997). The five cities examined – London, Paris, Amsterdam, Rotterdam, and Barcelona – were among those included in the multicity APHEA (Air Pollution on Health: European Approach) study. The number of years of available data varied from 5 to 13 years among the cities. In addition to O_3, the study considered

health impacts of BS, TSP, NO_2, and SO_2. City-specific effect estimates were pooled across cities using weighted means. Significant effects were seen for O_3, BS, TSP, and NO_2. Ozone effects were statistically significant in full year analyses and were larger in the warm season (April-September), 4.6% excess risk per 40 ppb increase in 1-h max O_3, compared to the cool season (October-March), 1.5% excess risk. The authors reported that among all pollutants examined, the most consistent and significant findings were for O_3. In addition, there was no significant heterogeneity in O_3 effects among the cities. No two-pollutant model results were reported.

Several additional studies carried out in one or two cities over a span of five or more years provided substantial additional evidence regarding O_3 effects on respiratory hospital admissions (Anderson et al., 1998; Burnett et al., 1999, 2001; Moolgavkar et al.,1997; Petroeschevsky et al., 2001; Ponce de Leon et al., 1996; Sheppard et al., 1999 [reanalysis Sheppard, 2003]; Yang et al., 2003). Two separate analyses of a large dataset from Toronto, Canada spanning the years 1980 to 1994 reported significant O_3 effects on respiratory hospitalizations for all ages (Burnett et al., 1999) and for persons under the age of 2 years (Burnett et al., 2001). Analysis was performed using Poisson GAM (default convergence criteria) with a nonparametric LOESS prefilter applied to the pollution and hospitalization data. Both studies demonstrated that O_3 effects were robust when PM measures were added to the regression, whereas PM effects from univariate regressions were markedly attenuated when O_3 was added to the regression. These results imply more robust associations with respiratory hospitalizations for O_3 than PM.

Moolgavkar and colleagues (1997) reported significant and robust O_3 effects on respiratory hospital admissions in adults 65 years and older in Minneapolis and St. Paul, MN, but not in Birmingham, AL. The absence of effects in the southern city may reflect less penetration of O_3 into the indoor environment due to greater use of air conditioning, and thus less correlation between central site O_3 monitoring and actual exposures of the urban populace. In Brisbane, Australia during 1987 to 1994, significant O_3 effects on all-age and age-stratified asthma and total respiratory hospital admissions were observed (Petroeschevsky et al., 2001). The O_3 effects were robust to inclusion of PM (based on light scattering) and SO_2 in copollutant regression models. Effect sizes appeared consistent in the warm and cool seasons, possibly reflecting the relatively small degree of seasonal variation in O_3 levels observed in Brisbane.

Less consistent effects of O_3 were seen in other respiratory hospitalization studies (Schouten et al., 1996; Lin et al., 2003; Lin et al., 2004; Morgan et al., 1998a; Oftedal et al., 2003). In a study conducted in Amsterdam and Rotterdam, the Netherlands, significant associations were observed, however results were difficult to interpret due to the large number of statistical tests performed (Schouten et al., 1996). Using a different analytical approach (case-crossover analysis), Lin and colleagues (2003) found no evidence for O_3 effects on asthma admissions in 6- to 12-year-olds over the period 1981 to 1993 in Toronto, Canada. In a California study by Niedell (2004), a negative association was observed between hospitalizations for asthma and naturally occurring seasonal variations in O_3 within zip codes in children aged 0 to 18 years. However, the O_3 effect was found to be influenced by socioeconomic status. Among children of low socioeconomic status, O_3 generally was associated with increased hospitalizations, with statistical significance reached in certain age groups. Niedell further stated that avoidance behavior on high O_3 days may have attributed to the negative relationship observed in children of higher socioeconomic status.

Another set of studies have examined associations between O_3 and respiratory hospitalizations in single cities over shorter (< 5 years) time spans. Positive and significant O_3 effects were reported in Cleveland, OH (Schwartz et al., 1996); Northern New Jersey (Weisel et al., 2002); Toronto, Canada (Burnett et al., 1997b); Helsinki, Finland (Pönkä and Virtanen, 1996); São Paulo, Brazil (Gouveia and Fletcher, 2000a); and Hong Kong (Wong et al., 1999a). The Helsinki study reported significant effects of O_3 on both asthma and on digestive disorders in a setting of very low O_3 concentrations (Pönkä and Virtanen, 1996), which raises questions of plausibility.

No significant association with O_3 was seen in studies from Los Angeles, CA (Linn et al., 2000; Mann et al., 2002; Nauenberg and Basu, 1999); Vancouver, Canada (Lin et al., 2004); London, England (Atkinson et al., 1999b); Edinburgh, Scotland (Prescott et al., 1998); and Drammen, Norway (Hagen et al., 2000). Several of the studies reporting non-significant O_3 effects were carried out in locations with low O_3 levels, suggestive of a nonlinear exposure-response relationship (Lin et al., 2004; Prescott et al., 1998). The non-significant findings in the South Coast air basin, CA area are surprising given the elevated O_3 concentrations observed there (Mann et al., 2002). Inadequate control of seasonal confounding may underlie some of the

non-significant and negative findings. An additional factor likely contributing to the variability of results is the relatively small sample sizes included in some of these studies.

In conclusion, while some inconsistencies are noted across studies, the evidence supports the findings of significant and robust effects of O_3 on various respiratory disease hospitalization outcomes. Large multicity studies, as well as many studies from individual cities have reported significant O_3 associations with total respiratory hospitalizations, asthma, and COPD, especially in studies analyzing the O_3 effect during the summer or warm season.

7.3.4 Association of O_3 with Hospital Admissions for Cardiovascular Disease

Among the subset of hospital admissions studies that have examined associations of O_3 with cardiovascular outcomes, most have found no consistent positive associations (Ballester et al., 2001; Burnett et al., 1995, 1999; Linn et al., 2000; Mann et al., 2002; Petroeschevsky et al., 2001; Prescott et al., 1998). The exceptions are one study in Toronto, Canada, which reported robust associations with both total respiratory and cardiovascular hospital admissions (Burnett et al., 1997b), and one in Hong Kong, in which circulatory, ischemic heart, and heart failure were all significantly associated with O_3 in the cool but not the warm season (Wong et al., 1999b). In the Hong Kong study, O_3 concentrations were similar in both seasons, with warm season levels slightly lower, mean 31.2 $\mu g/m^3$, compared to the cool season, mean 34.8 $\mu g/m^3$. The authors speculated that differing activity patterns and home ventilation factors may have contributed to the seasonal differences in O_3 effects. Weather in Hong Kong is mild throughout the year, but less humid and cloudy in the cool season. Thus, during the cool season people are more likely to open windows or stay outdoors, resulting in higher personal exposures even with similar ambient concentrations. Based on this small set of studies, current evidence does not support a conclusion that O_3 has independent effects on cardiovascular hospitalizations.

7.3.5 Summary of Acute O_3 Effects on Daily Emergency Department Visits and Hospital Admissions

- The vast majority of hospitalization studies conducted over the past decade have looked at effects of O_3 on either total respiratory diseases and/or asthma. Significant associations with O_3 were observed with both outcomes in many cases. Studies of emergency department visits for respiratory conditions also reported significant O_3 effects, but the results tend to be less consistent across studies.

- Many of the daily emergency department visits and hospitalization studies analyzed O_3 risk estimates using year-round data. Given the strong seasonal variations in O_3 concentrations and the changing relationship between O_3 and other copollutants by seasons, inadequate adjustment for seasonal effects might have masked or underestimated the association between O_3 and the respiratory disease outcomes. Season stratified analyses typically yield more reliable O_3 effect estimates.

- Numerous studies have reported O_3 effects while controlling for copollutants, including PM, in the analytical model. The evidence is supportive of independent O_3 effects on respiratory admissions and emergency department visits. In most studies, O_3 effects have been reported to be at least as robust as PM, and in some cases more so.

- A subset of hospital admission studies examined the effect of O_3 on cardiovascular outcomes. The limited evidence is inconclusive regarding the association between O_3 exposure and cardiovascular hospitalizations.

7.4 ACUTE EFFECTS OF OZONE ON MORTALITY

7.4.1 Summary of Key Findings on Acute Effects of O_3 on Mortality From the 1996 O_3 AQCD

A limited number of studies examined O_3-mortality associations at the time of the previous O_3 AQCD, most of which were from the 1950s and 1960s. The 1996 O_3 AQCD considered these historical studies to be flawed because of either inadequate adjustment for seasonal trend or temperature, or because of the use of questionable exposure indices. There were only a few time-series studies that examined O_3-mortality associations between the 1980s and mid-1990s. These studies used more sophisticated approaches in addressing seasonal confounding and weather models. One of these studies (Shumway et al., 1988) focused on the associations with long-term fluctuations in Los Angeles, CA but did not examine short-term associations. A study that reanalyzed the Los Angeles, CA data with a focus on the short-term associations (Kinney and Özkaynak, 1991) did find that, of the PM and gaseous criteria pollutants, O_3 (reported as total oxidants) was most strongly associated with total nonaccidental mortality. Then two studies, one using Detroit, MI data (Schwartz, 1991) and the other using St. Louis, MO and Kingston-Harriman, TN data (Dockery et al., 1992), reported that PM but not O_3 was significantly associated with mortality. However, the 1996 O_3 AQCD discussed that, without sufficient presentation of model diagnosis regarding the relationship between O_3 and the weather

models used, it was difficult to evaluate whether the lack of O_3-mortality associations was possibly due to overspecification of the weather model. In summary, due to the insufficient number of studies that examined O_3-mortality associations and the uncertainties regarding weather model specifications, the 1996 O_3 AQCD was unable to quantitatively assess O_3-mortality excess risk estimates, or even provide qualitative assessment of the likelihood of O_3-mortality associations.

7.4.2 Introduction to Assessment of Current O_3-Mortality Studies

Introductory discussions of the PM mortality effects often cite historical air pollution incidents such as the 1952 London, England smog episode in which thousands of deaths were attributed to the air pollution from coal burning. There is no counterpart "historical episode" for O_3-mortality effects. Instead, the early recognition of the adverse health effects of summer oxidant air pollution, mainly from Los Angeles and other major cities with a high density of automobiles, were based on symptoms such as eye and throat irritations. Thus, the focus of PM epidemiology and that of O_3 epidemiology have been historically different.

As shown in Table AX7-5 in the Chapter 7 Annex, the number of short-term mortality studies that analyzed O_3 has increased markedly since the last publication of the O_3 AQCD in 1996. The increased attention to PM-mortality associations in the early 1990s lead to the increase in studies that also examined O_3, most often as a potential confounder for PM. Although many of these PM studies also reported O_3 estimates, they often lacked specific hypotheses regarding mortality effects of O_3 as the focus of these studies was to examine the PM-mortality effect. This is in contrast to the O_3-morbidity studies, most of which were specifically designed to examine effects of "summer haze" and O_3 (or oxidants) on respiratory and other symptoms, lung functions, and emergency department visits, etc. However, new studies with hypotheses developed specifically for O_3 effects on mortality have become available, such as the large U.S. 95 communities study by Bell et al. (2004), the U.S. 14 cities study by Schwartz (2004), and the 23 European cities study by Gryparis et al. (2004) discussed in the next section.

7.4.3 Single-Pollutant Model O_3-Mortality Risk Estimates

To facilitate a quantitative overview of the O_3-mortality effect estimates and their corresponding uncertainties, the percent excess risks of total nonaccidental mortality calculated using all year data are plotted in Figure 7-10. Studies that only conducted seasonal analyses will be presented in the next section. This figure does not include studies that only examined cause-specific mortality. In studies where multiple lags were presented, the multiday lag was selected to represent the cumulative effect from all days examined. If cumulative lags were not analyzed, the effect estimate from the 0- or 1-day lag was selected for presentation. All effect estimates are from single-pollutant models and include all age groups unless noted otherwise. The majority of the estimates are positive with a few exceptions. Four multicity studies showed positive and significant O_3 effect estimates for all cause (nonaccidental) mortality. An excess mortality risk of 4.5% per 40 ppb increase in 1-h max O_3 was estimated from the four European cities of the APHEA project (Touloumi et al., 1997). The European effect estimate was larger than those from the large U.S. National Morbidity, Mortality and Air Pollution Study (NMMAPS). An excess risk of 1.0% and 0.8% per 20 ppb increase in 24-h avg O_3 was observed from the U.S. 95 communities study (Bell et al., 2004) and U.S. 90 cities study (Samet et al., 2000; reanalysis Dominici et al., 2003), respectively. Similarly, the U.S. 14 cities study by Schwartz (2004) observed an 0.8% excess risk per 40 ppb increase in 1-h max O_3.

Only one multicity study did not observe a statistically significant O_3 effect on mortality. As an extension of the four European cities study, researchers of the APHEA project investigated the effect of O_3 on total, cardiovascular, and respiratory mortality in 23 cities throughout Europe (Gryparis et al., 2004). A cumulative lag of 0 to 1 days was hypothesized *a priori*. A two-stage hierarchical model, which accounted for statistical variance and heterogeneity among cities, was used to estimate the pooled regression coefficients. Due to substantial heterogeneity among cities, random effects regression models were applied. The pooled effect estimate for the 23 European cities was a positive, but nonsignificant value of 0.23% (95% CI: −0.85, 1.95) per 40 ppb increase in 1-h max O_3 for all seasons. The researchers noted that there was a considerable seasonal difference in the O_3 effect on mortality, thus the nonsignificant effect for the all year data might be attributable to inadequate adjustment for confounding by season. This seasonal effect will be discussed further in the next section.

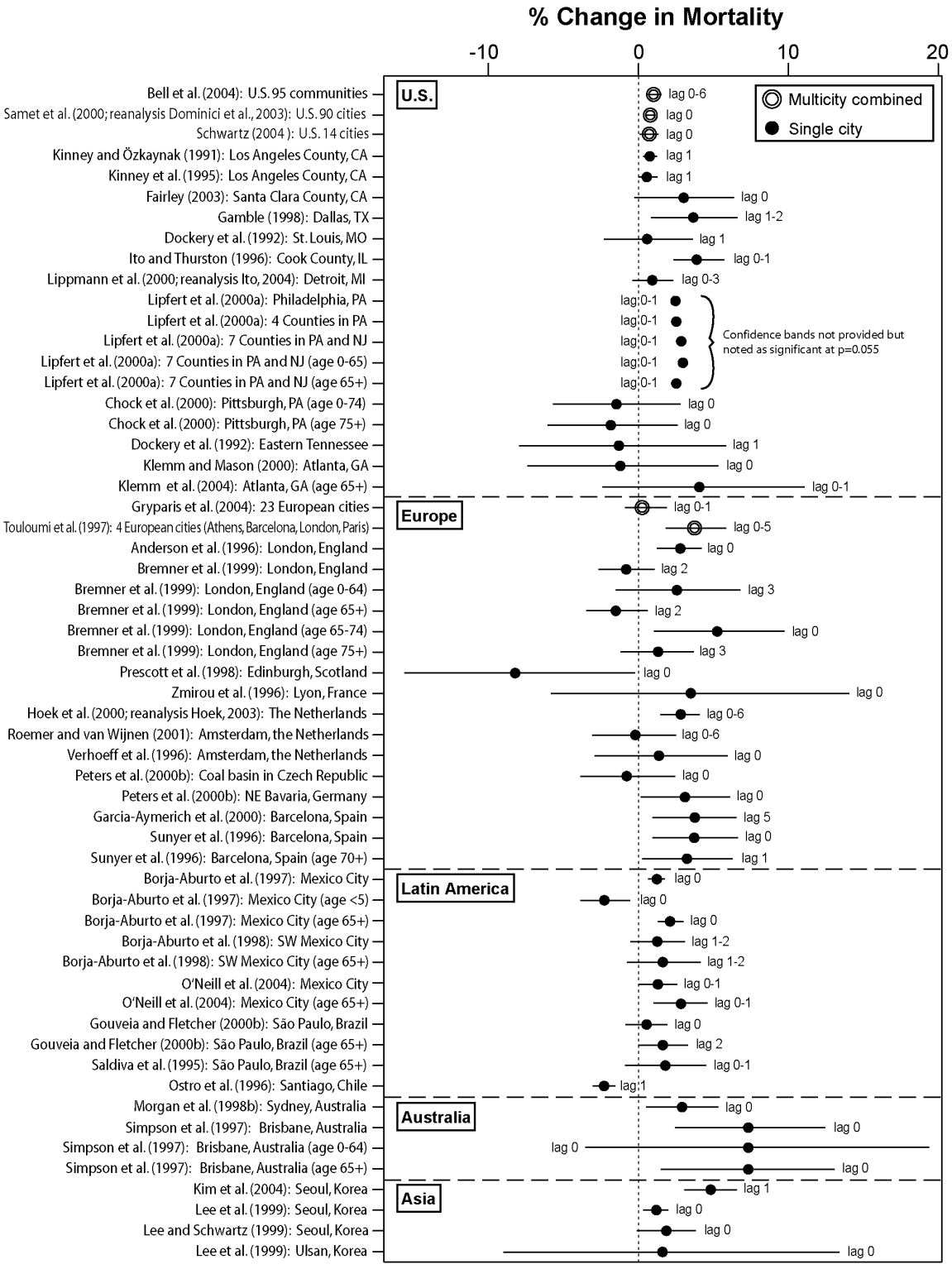


Figure 7-10. All cause (nonaccidental) O_3 excess mortality risk estimates (95% CI) <u>for all year analyses</u> per 40 ppb increase in 1-h max O_3 or equivalent. Analyses include all ages unless otherwise noted.

The U.S. 95 communities study by Bell et al. (2004) extended an earlier analysis (Samet et al., 2000; reanalysis Dominici et al., 2003) on short-term effects of O_3 on total and cardiopulmonary mortality using the NMMAPS data base from 1987 to 2002. The results of this study are discussed in detail here because of the study's emphasis on U.S. data and the inclusion of 95 large communities across the country, making this mortality study most representative of the U.S. population. In addition, this study is one of few that have focused specifically on O_3 hypotheses testing. Within-community results first were calculated using single-day lags of 0, 1, and 2 days, and a 7-day distributed lag in O_3 exposure. A two-stage hierarchical model was used to determine a national average effect estimate. Figure 7-11 presents community-specific and national average O_3 risk estimates for total mortality per 10 ppb increase in 24-h avg O_3 from a constrained 7-day distributed lag model. For total mortality in the single-day lag models, the national estimates of the O_3 effect on mortality were statistically significant for 0-, 1-, and 2-day lags, with the largest effect observed from O_3 concentrations at a 0-day lag. The 7-day distributed lag model estimated the cumulative risk of mortality associated with O_3 concentrations on the same day and six previous days. Results from the constrained distributed lag model indicated that a 10 ppb increase in 24-h avg O_3 in the previous week was associated with a statistically significant increase of 0.52% (95% CI: 0.27, 0.77%) in daily mortality. The mean 24-h avg O_3 concentration was approximately 26 ppb for the 95 communities. Figure 7-11 illustrates the preponderance of cities with a positive yet nonsignificant relationship between O_3 and mortality. However, the national average O_3 mortality estimate, which includes results from all 95 U.S. communities, is positive and statistically significant. These results further support the data presented in Figure 7-10 from nearly 40 studies in locations both in the U.S. and in other countries.

Several studies conducted meta-analyses of O_3-mortality associations (Stieb et al., 2002, 2003; Thurston and Ito, 2001; World Health Organization, 2004). Most of these studies included GAM studies using default convergence criteria except Stieb et al. (2003), which compared effect estimates from GAM-affected studies to non-GAM studies. All of these meta-analyses reported fairly consistent and positive combined estimates, approximately 2% excess total non-accidental mortality per 40 ppb increase in 1-h max O_3. However, most of these studies were not analytical in design in that they did not attempt to examine the source of heterogeneity,

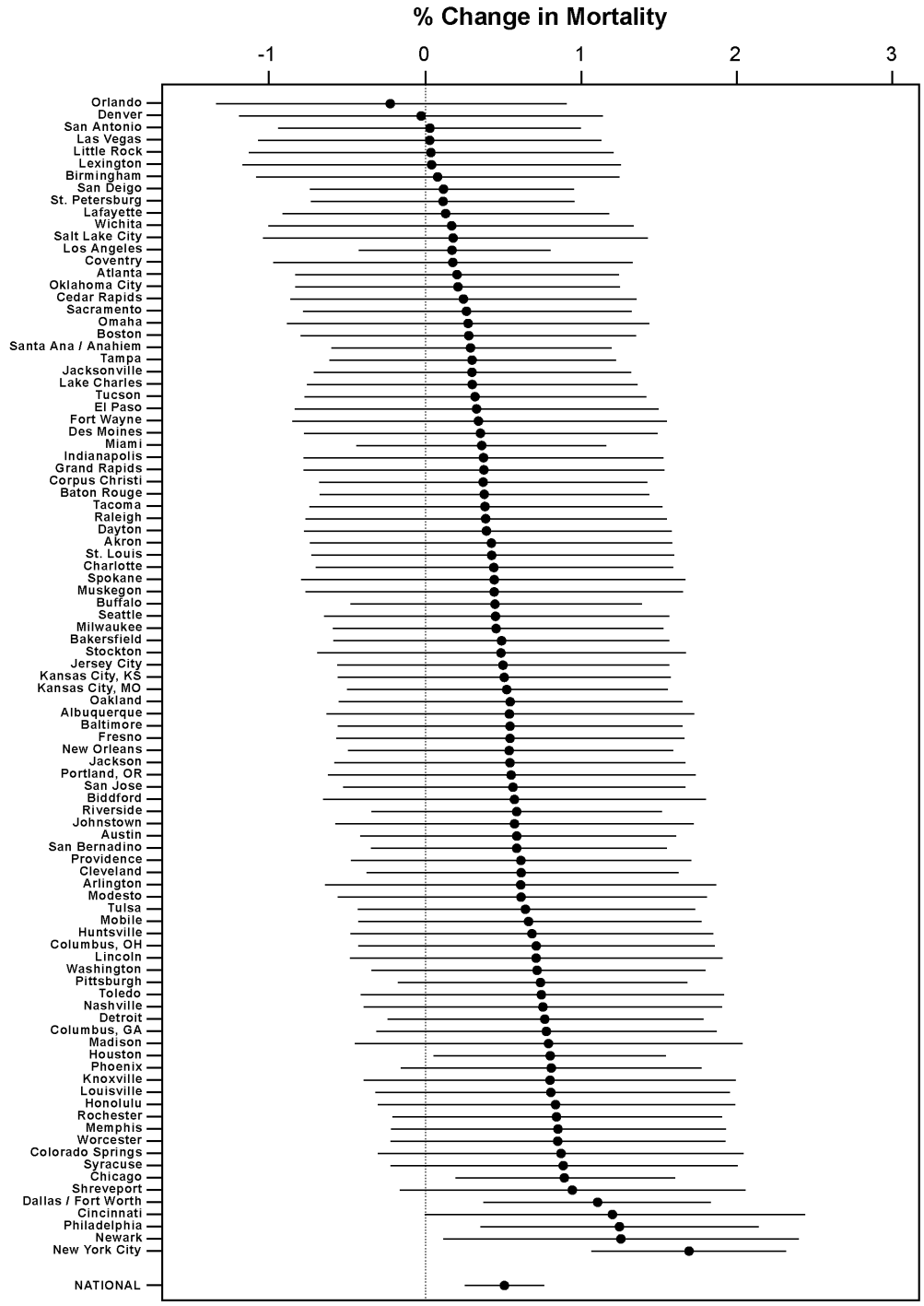


Figure 7-11. Bayesian city-specific and national average estimates for the % change (95% CI) in daily mortality per 10 ppb increase in 24-h avg O_3 in the previous week using a constrained distributed lag model for 95 U.S. communities (NMMAPS), arranged by size of the effect estimate.

Source: Derived from Bell et al. (2004).

although some suggested an influence of weather model specification (Thurston and Ito, 2001) and another reported evidence of publication bias (World Health Organization, 2004) in the past literature. None of these studies addressed the issue of season-specific estimates, and therefore, interpreting these combined estimates requires caution. The estimates from these meta-analyses appear to be larger than the national average estimate of 1.0% excess risk per 20 ppb increase in 24-h avg O_3 from the largest U.S. 95 communities study (Bell et al., 2004). There are a few new meta-analyses and multicity studies currently being conducted specifically to address such issues as season-specific analyses, publication bias, weather model specification, potential confounding by fine particles, distributed lag effects, and the potential influence of air conditioning. These studies are expected to provide new information that will shed light on the outstanding questions.

Collectively, the above studies suggest an excess risk of total nonaccidental mortality associated with acute O_3 exposure. Despite the different analytical approaches and alternative model specifications used in the various studies, overall, the range of estimates were relatively narrow, with the positive estimates from 0 to 7% per 40 ppb increase in 1-h max O_3 or equivalent.

7.4.4 Seasonal Variation in O_3-Mortality Risk Estimates

Since the seasonal cycle of O_3 follows the seasonal cycle of temperature (which is inversely related to the mortality seasonal cycle), inadequate adjustment of temporal trends in the regression model may lead to negative O_3-mortality risk estimates. In addition, as discussed in Section 7.1.3.5, in some cities low-level O_3 during winter may be negatively correlated with PM and other primary pollutants, resulting in negative correlations between O_3 and mortality even in short-term relationships. The confounding effect by season could be substantially reduced by conducting season-stratified analyses.

A fewer number of O_3 mortality studies performed seasonal analyses. Figure 7-12 presents the studies that reported O_3 risk estimates for all cause mortality by season. For those studies that obtained O_3 risk estimates for each of the four seasons, only summer and winter results are shown. The estimates for year-round data analyses, when available, also are shown for comparisons. In all the studies, the O_3 risk estimates are larger during the warm season than the cool season, with the all year estimates generally in between the two seasonal estimates.

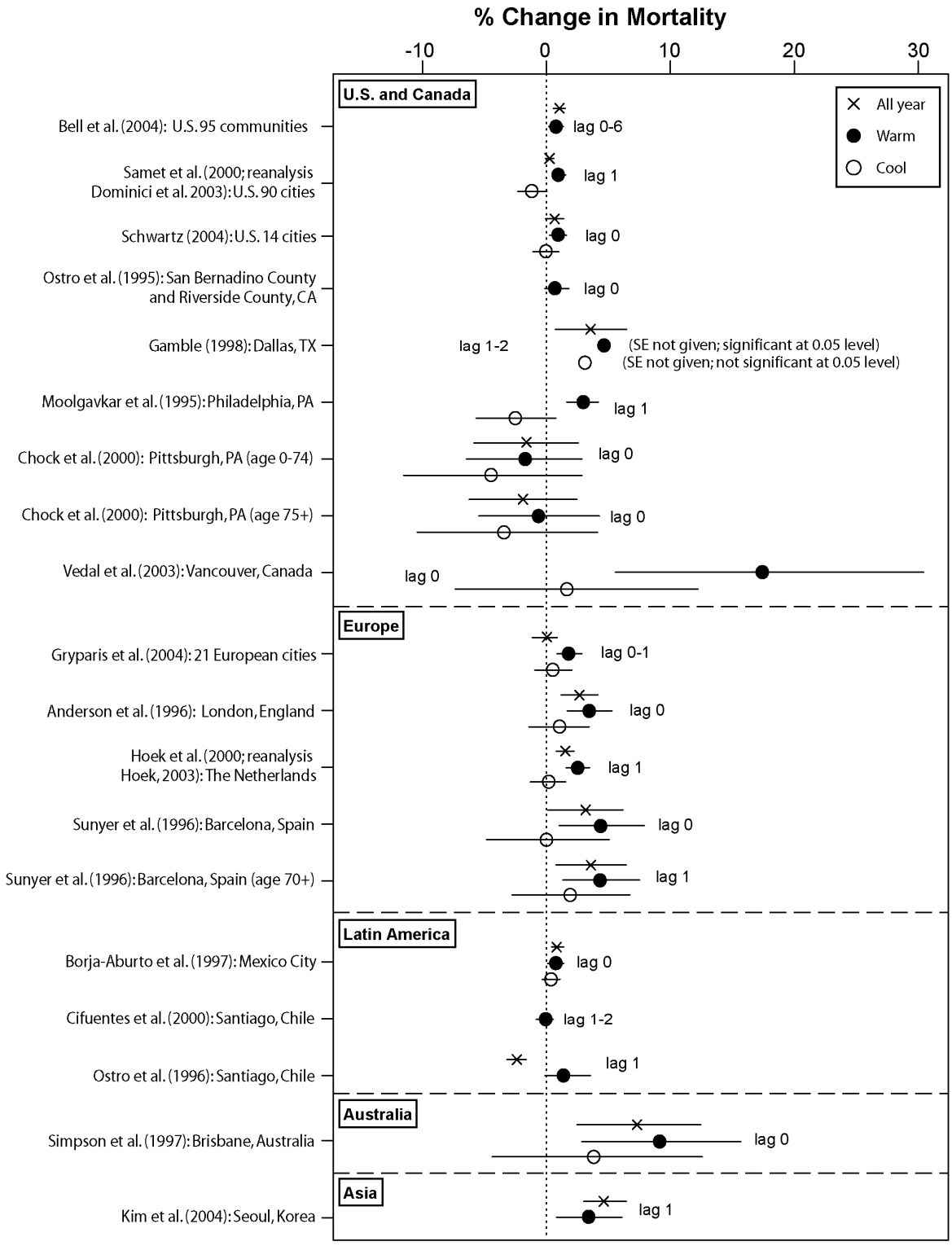


Figure 7-12. All cause (nonaccidental) O_3 excess mortality risk estimates (95% CI) by season per 40 ppb increase in 1-h max O_3 or equivalent. Analyses include all ages unless otherwise noted.

In three U.S. and European multicity studies (Gryparis et al., 2004; Samet et al., 2000 [reanalysis Dominici et al., 2003]; Schwartz, 2004), season-stratified analyses indicated that the O_3-mortality effect estimates were significant and positive in the warm season, with larger effects observed compared to the year-round analyses. The effect estimates from the cool season were notably smaller and less or nonsignificant. In the case of the U.S. 90 cities study, the cool season mortality estimate was negative, which was most likely attributable to the inverse relationship between O_3 and PM in the winter.

In the U.S. 95 communities study by Bell et al. (2004), the warm season (April-October) effect estimate was 0.78% (95% CI: 0.26, 1.30) excess risk per 20 ppb increase in 24-h avg O_3, compared to 1.04% (95% CI: 0.54, 1.55) calculated using all available data. The small difference in the size of the effect estimates between the analysis using all available data and that using warm season only data might be attributable, at least partially, to the varying peak O_3 seasons and the difference in O_3 concentrations by community or region. In addition, the varying seasonal relationship between O_3 and PM by community also might have contributed to the difference, as these mortality effect estimates were not adjusted for confounding by PM.

Studies that conducted analysis by season indicate that O_3 mortality risk estimates are often larger in the warm season compared to the colder season. The larger effects observed in the warm season when O_3 levels are higher are consistent with causal association. The seasonal dependence of O_3-mortality effects complicates interpretation of O_3 risk estimates calculated from year-round data without adequate adjustment of temporal trends.

7.4.5 O_3-Mortality Risk Estimates Adjusting for PM Exposure

As previously mentioned, the confounding between "winter type" pollution (e.g., CO, SO_2, and NO_2) and O_3 is not of great concern because the peaks of these pollutants do not strongly coincide. The main confounders of interest for O_3, especially for the northeast U.S., are "summer haze" type pollutants such as acid aerosols and sulfates. Since very few studies had these chemical measurements, PM (especially $PM_{2.5}$), may serve as surrogates. However, due to the expected high correlation among the constituents of the "summer haze mix," multipollutant models including these pollutants may result in unstable coefficients, and therefore, an interpretation of such results requires some caution.

Figure 7-13 shows the O_3 risk estimates with and without adjustment for PM indices using all year data in studies that conducted two-pollutant analyses. Approximately half of the O_3 risk estimates slightly increased while the other half slightly decreased in value with the inclusion of PM indices in the model. In general, the O_3-mortality risk estimates were robust to adjustment for PM in the models, with the exception of Los Angeles, CA data with PM_{10} (Kinney et al., 1995) and Mexico City data with TSP (Borja-Aburto et al., 1997).

The U.S. 95 communities study by Bell et al. (2004) examined the sensitivity of acute O_3-mortality effects to potential confounding by PM_{10}. Restricting analysis to days when both O_3 and PM_{10} data were available, the community-specific O_3-mortality effect estimates as well as the national average results indicated that O_3 was robust to adjustment for PM_{10} (Bell et al., 2004). One study (Lipfert et al., 2000a) reported O_3 risk estimates with and without sulfate adjustment. Lipfert et al. (2000a) calculated O_3 risk estimates based on mean (45 ppb) less background (not stated) levels of 1-h max O_3 in seven counties in Pennsylvania and New Jersey. The O_3 risk estimate was not substantially affected by the addition of sulfate in the model (3.2% versus 3.0% with sulfate) and remained statistically significant.

Several O_3-mortality studies examined the effect of confounding by PM indices in different seasons (Figure 7-14). In analyses using all year data and warm season only data, O_3 risk estimates were once again fairly robust to adjustment for PM indices, with values showing both slight increases and decreases with the inclusion of PM in the model. In contrast, in the analyses using cool season data only, the O_3 risk estimates all increased with the adjustment of PM indices, although none reached statistical significance. For example, in the European study of 21 cities (two cities that did not have 8-h max O_3 data were excluded from the analysis), the summer O_3-mortality estimate was relatively robust to adjustment for PM_{10}, slightly decreasing from 1.82% (95% CI: 0.99, 3.06) to 1.58% (95% CI: 0.47, 2.88) excess risk per 30 ppb increase in 8-h max O_3 (Gryparis et al., 2004). In contrast, the winter effect estimate increased from 0.70% (95% CI: −0.70, 2.17) to 1.29% (95% CI: −0.46, 3.00) per 30 ppb increase in 8-h max O_3 after adjusting for PM_{10}. These results indicate that the confounding effect by PM may vary by season. Although PM does not appear to significantly confound the association between O_3 and mortality in the analysis of warm season data, during the cool season, the inverse relationship between O_3 and PM_{10} may influence the effect estimate for O_3-related mortality.

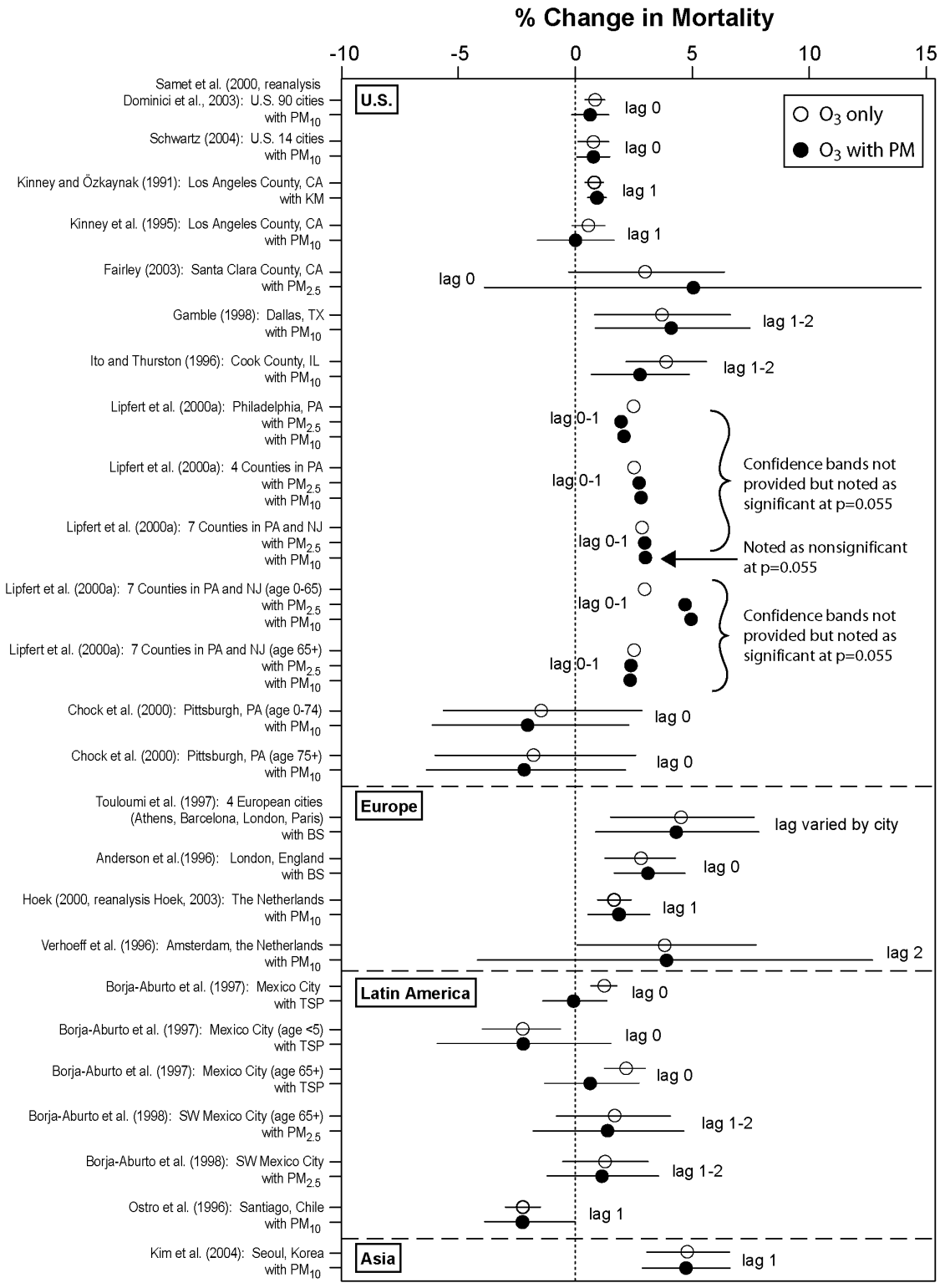


Figure 7-13. All cause (nonaccidental) O_3 excess mortality risk estimates (95% CI) <u>with adjustment for PM indices for all year analyses</u> per 40 ppb increase in 1-h max O_3 or equivalent. Analyses include all ages unless otherwise noted.

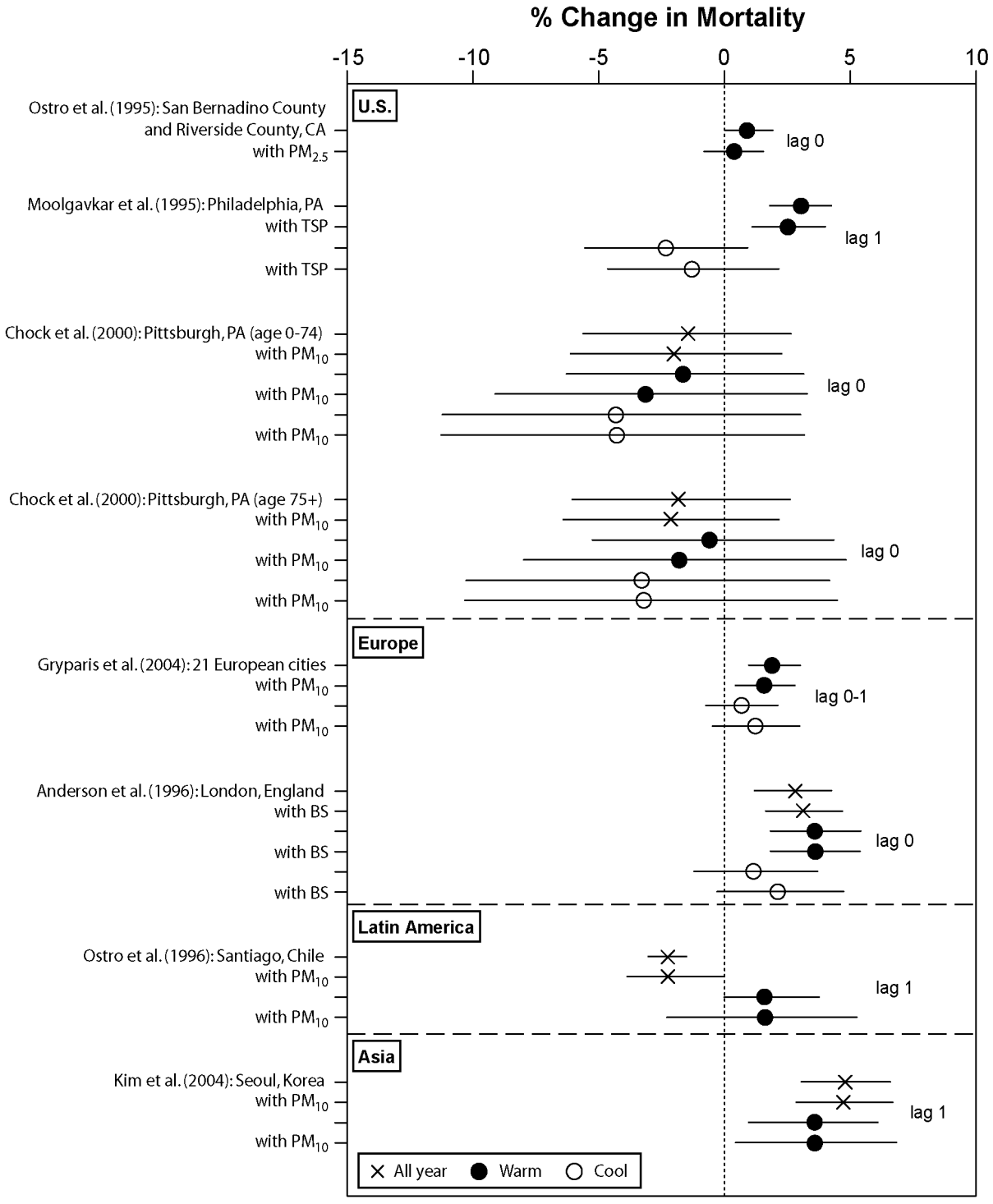


Figure 7-14. All cause (nonaccidental) O_3 excess mortality risk estimates (95% CI) <u>with adjustment for PM indices by season</u> per 40 ppb increase in 1-h max O_3 or equivalent. Analyses include all ages unless otherwise noted.

7.4.6 O_3 Risk Estimates for Specific Causes of Mortality

Many of the time-series mortality studies examined broad underlying causes of mortality, such as cardiovascular and respiratory causes. The U.S. 95 communities study (Bell et al., 2004) analyzed O_3 effect estimates from cardiovascular and respiratory mortality. Significant effects were seen at 0- and 2-day lags with results similar to total mortality. The national average estimate from the constrained distributed lag model was slightly greater for cardiopulmonary deaths, with an excess risk of 1.28% (95% CI: 0.62, 1.97) per 20 ppb increase in 24-h avg O_3 in the preceding week. In a related study, Huang et al. (2004) examined O_3 effects on cardiopulmonary mortality during the summers of 1987 to 1994 in 19 large U.S. cities from the NMMAPS database. In the 7-day distributed lag model, the O_3 effect estimate was 2.52% (95% CI: 0.94, 4.10) excess risk in cardiopulmonary mortality per 20 ppb increase in 24-h avg O_3 (Huang et al., 2004). Several studies observed that the risk estimates for the respiratory category were larger than the cardiovascular and/or total nonaccidental categories (e.g., Anderson et al., 1996; Gouveia and Fletcher, 2000b; Gryparis et al., 2004; Zmirou et al., 1998). In the European 21 multicities study (Gryparis et al., 2004), the warm season effect estimate for respiratory mortality was 6.75% excess risk per 30 ppb increase in 8-h max O_3, compared to 2.70% for cardiovascular mortality and 1.82% for total mortality. In contrast, other studies have found that the risk estimates for the respiratory category were essentially zero or even negative while the risk estimates for total or cardiovascular categories were positive (e.g., Borja-Aburto et al., 1998; Bremner et al., 1999; Lipfert et al., 2000a; Morgan et al., 1998b). These apparent inconsistencies across studies may be due in part to the difference in model specifications, but they may also reflect the lower statistical power associated with the smaller daily counts of the respiratory category (usually accounting for less than 10% of total deaths) compared to the larger daily counts for the cardiovascular category (approximately 40 to 50% of total deaths). Thus, an examination of the differences in risk estimates across specific causes requires a large population and/or a long period of data collection.

The analyses of a 9-year data set for the whole population of the Netherlands (population = 14.8 million) provided O_3 (and other pollutants) risk estimates for more specific causes of mortality, including COPD, pneumonia, and subcategories of cardiovascular causes (Hoek et al., 2000, 2001; reanalysis Hoek, 2003). The excess risk estimate for COPD was small

and not significant (0.8% [95% CI: −2.4, 4.2] per 30 ppb 8-h avg O_3), while the excess risk estimate for pneumonia (5.6% [95% CI: 1.8, 9.5]) was much larger than that for total nonaccidental mortality (1.6% [95% CI: 0.9, 2.4]). The excess risk estimates for some of the cardiovascular subcategories, including heart failure (3.8% [95% CI: 0.5, 7.3]) and thrombosis-related disease (6.0% [95% CI: 1.1, 10.8]), showed greater risk estimates than that for total mortality. However, these elevated relative risks were not specific to O_3. For example, most of the pollutants examined, including PM_{10}, BS, SO_2, NO_2, CO and NO_3^-, were significantly associated with pneumonia. Therefore, it is difficult to make a causal inference specific to O_3 based on these results.

De Leon et al. (2003) examined the role of contributing respiratory causes in the associations between air pollution and nonrespiratory mortality (circulatory and cancer) in New York City during the period of 1985 to 1994. The main finding of this study was that, for the older age group (75+ years), the estimated excess mortality risks for PM_{10} were higher for the nonrespiratory deaths that had contributing respiratory causes, compared to the nonrespiratory deaths without contributing respiratory causes. This pattern was also seen for CO and SO_2, but not for O_3. Therefore, this study did not suggest a role of contributing respiratory causes in the association between O_3 and nonrespiratory causes of deaths.

In summary, these studies examining specific respiratory or cardiovascular causes of death often found risk estimates that were higher than those for the total or broader death cause categories, but their lower statistical power in the smaller subcategories often made it difficult to distinguish the contrasts in estimates.

7.4.7 O_3-Mortality Risk Estimates for Specific Subpopulations

Some studies examined O_3-mortality risk estimates in potentially susceptible subpopulations, such as those with underlying cardiopulmonary disease. Sunyer et al. (2002) examined the associations between air pollution and deaths in a cohort of patients (467 men and 611 women) with severe asthma in Barcelona, Spain during the period of 1986 to 1995. A case-crossover study design was used to estimate excess odds of mortality adjusting for weather and epidemics in three groups: (1) those who had only one asthma emergency department visit; (2) those who had more than one asthma emergency department visit; and (3) those who had more than one asthma and COPD emergency department visit. Those with more than one

asthma emergency department visit showed the strongest associations with the examined air pollutants, with NO_2 being the most significant predictor, followed by O_3. Sunyer et al. (2002) reported a significant association between O_3 and all cause deaths for this group during the warm season, with an odds ratio of 1.90 (95% CI: 1.09, 3.30) per 48 $\mu g/m^3$ increase in 1-h max O_3, compared to an odds ratio of 1.02 (95% CI: 0.73, 1.43) for those with only one asthma emergency department visit and 1.05 (95% CI: 0.73, 1.50) for the group with a concomitant diagnosis of COPD. The magnitude of the effect size estimates reported for patients with more than one asthma emergency department visit was large compared to the total mortality risk estimate (relative risk of 1.03 per 48 $\mu g/m^3$ increase in 1-h max O_3) observed in the related study by Sunyer et al. (1996). In another Barcelona study, Saez et al. (1999) examined asthma mortality death among persons aged 2 to 45 years. Once again, O_3 and NO_2 were the only air pollutants that were significantly associated with asthma mortality death. While the similarity of the patterns of associations between O_3 and NO_2 makes it difficult to speculate on the specific causal role of O_3, the results of these studies suggest that individuals with severe asthma may make up a subpopulation that is sensitive to these pollutants.

Sunyer and Basagna (2001) also performed an analysis of emergency department visits by a cohort with COPD. The results from this study suggested that PM_{10}, but not gases were associated with mortality risks for the COPD cohort. However, a Mexico City study by Téllez-Rojo et al. (2000) observed a significant association between COPD mortality and O_3, along with PM_{10}, among patients living outside a medical unit. For a cumulative 5-day lag, an excess risk of 15.6% (95% CI: 4.0, 28.4) per 1-h max O_3 was observed for COPD mortality.

Goldberg et al. (2003) investigated the association between air pollution and daily mortality with congestive heart failure as the underlying cause of death in patients aged 65 years or more in Montreal, Quebec, Canada during the period of 1984 to 1993. Analysis was stratified into two groups, those whose underlying cause of death was congestive heart failure and those with a diagnosis of congestive heart failure one year before their death. They found no association between daily mortality for congestive heart failure and any pollutants. However, they did find significant associations between daily mortality among those who were classified as having congestive heart failure before death and coefficient of haze, SO_2, and NO_2. Ozone was not significantly associated but showed positive risk estimates for year-round and warm

season data and a negative risk estimate for cool season data. While the 10-year study period for this data was long, the daily mean death counts for the specific subcategory chosen was relatively small (0.7/day for mortality with congestive heart failure as underlying cause of death and 4.0/day for total mortality in patients previously diagnosed with congestive heart failure), limiting the power of the study.

Few studies have examined O_3-mortality effects for specific subpopulations. Among those that investigated the effect of air pollution in populations with underlying cardiopulmonary diseases, associations were not unique to O_3 but were shared with other pollutants. The results from Spain (Saez et al., 1999; Sunyer et al., 2002) suggest that severe asthmatics may be susceptible to the mortality effects associated with NO_2 and O_3.

7.4.8 Summary of Acute O_3 Effects on Mortality

- A substantial body of new data on acute mortality effects of O_3 has emerged since the previous O_3 AQCD. While uncertainties remain in some areas, it can be concluded that robust associations have been identified between various measures of daily O_3 concentrations and increased risk of mortality. The fairly small but consistent associations cannot be readily explained by confounding due to time, weather, nor copollutants.

- The majority of the available O_3-mortality risk estimates were computed using all year data. The results from the studies that conducted analysis by season suggest that the O_3 risk estimates were larger in the warm season. Some of the risk estimates in the cool season were negative, possibly reflecting the negative correlation between low-level O_3 and PM (and other primary pollutants) during that season. Thus, without adequate adjustment for temporal trends, the O_3 risk estimates obtained for year-round data may be misleading and likely underestimate the effects during the warm season.

- Some studies examined specific subcategories of mortality, but most of these studies had limited statistical power to detect associations due to the small daily mortality counts. The large U.S. 95 communities study indicated that there was a slightly greater risk of cardiopulmonary mortality compared to total mortality.

- Few studies examined the effect of O_3 on mortality in subpopulations with underlying cardiopulmonary diseases. Similar to cause-specific mortality, these population-specific studies had limited statistical power to detect associations. The evidence suggests that individuals with severe asthma may be at increased risk of O_3-related mortality, however, similar results were seen with other pollutants.

7.5 CHRONIC EFFECTS OF OZONE

7.5.1 Summary of Key Findings on Studies of Health Effects and Chronic O_3 Exposure from the 1996 O_3 AQCD

The 1996 O_3 AQCD concluded that there was insufficient evidence from the limited number of studies to determine whether long-term ambient O_3 exposures resulted in chronic health effects. However, the aggregate evidence suggested that chronic O_3 exposure, along with other environmental factors, could be responsible for health effects in exposed populations.

7.5.2 Introduction to Morbidity Effects of Chronic O_3 Exposure

Several new longitudinal epidemiologic investigations have yielded information on health effects of long-term O_3 exposures. Epidemiologic interest in investigating long-term effects has been motivated by several considerations. Animal toxicology studies carried out from the late 1980s onward demonstrated that long-term exposures can result in permanent changes in the small airways of the lung, including remodeling of the airway architecture (specifically the distal airways and centriacinar region) and deposition of collagen, as discussed earlier in Chapter 5. These changes result from the damage and repair processes that occur with repeated exposure. Indices of fibrosis also were found to persist after exposure in some of the studies. Collectively, these findings provide a potential pathophysiologic basis for the changes in airway function observed in children in longitudinal studies. Seasonal ambient patterns of exposure may be of greater concern than continuous daily exposure. In the classical study by Tyler et al. (1988), seasonal exposure was associated with greater increases in total lung collagen and pulmonary function changes suggestive of a delay in lung maturation in animals.

Controlled human exposure studies clearly demonstrated acute inflammation in the lung at ambient exposure levels. Epidemiologic studies could examine whether repeated exposures over multiple episode periods and/or multiple years would lead to persistent inflammation and result in damage to the human lung, especially in the small, terminal bronchiolar regions where vulnerability is greatest. However, the challenges to addressing these issues in epidemiology studies are formidable, and as a result there exists relatively limited literature in this area. Long-term O_3 concentrations tend to be correlated with long-term concentrations of other pollutants, making specific attribution difficult. Subtle pulmonary effects require health outcome measures that are sensitive, and must usually be directly collected from individual human subjects, rather

than from administrative data bases. Although these factors make chronic studies difficult and expensive to conduct, efforts must be made to design studies with adequate power to examine the hypothesis being tested. Epidemiologic studies are the only approach to investigate a possible link between chronic exposure to ozone and the occurrence of human health effects.

Here we review studies published from 1996 onward in which health effects were tested in relation to O_3 exposures extending from several weeks to many years (Table AX7-6 in the Chapter 7 Annex). The available literature falls into four general categories: (1) studies examining seasonal changes in lung function and lung function growth as related to O_3 exposures in peak season; (2) studies addressing lung function growth or decline of lung function over several years in relation to long-term O_3 exposures; (3) studies addressing respiratory inflammation in high versus low exposure groups or time periods; and (4) studies addressing longitudinal and cross-sectional associations between long-term O_3 exposures and asthma development and prevalence.

7.5.3 Seasonal O_3 Effects on Lung Function

While it has been well-documented in both chamber and field studies that daily, multihour exposures to O_3 result in transient declines in lung function, much less is known about the effects of repeated exposures to O_3 over extended periods on lung function. Several new studies reported over the past decade have examined lung function changes over seasonal time periods with differing levels of O_3 exposures (Frischer et al., 1999; Horak et al., 2002a,b; Ihorst et al., 2004; Kinney and Lippmann, 2000; Kopp et al., 2000). These seasonal effects of O_3 are examined first in this section. In the next section is a discussion of effects over years, as opposed to over seasons, in addition to multiyear analyses of seasonal studies.

In a large study, Frischer and colleagues collected repeated lung function measurements in 1,150 Austrian school children (mean age 7.8 years) from nine towns that differed in mean O_3 levels. Mean summertime O_3 exposure ranged from 32.4 to 37.3 ppb during the three summers. Lung function was measured in the spring and fall over a three-year period from 1994 to 1996, yielding six measurements per child. The seasonal change in lung function was significantly and inversely associated with seasonal mean O_3 levels. FEV_1 increase was lower by 156.6 mL (-0.029 mL/day/ppb $\times$ 90 days/year $\times$ 3 years $\times$ 20 ppb) for each 20 ppb increase in mean 24-h avg O_3 concentrations over the three summers and 129.6 mL over the three

winters. When analyses were restricted to children who had spent the whole summer period in their community, the changes were greater, with a greater O_3-related reduction of 183.6 mL in FEV_1 growth. Other pollutants (PM_{10}, SO_2, and NO_2) had less consistent associations with lung function growth. Horak et al. (2002a,b) extended the study of Frischer et al. (1999) with an additional year of data and indicated that seasonal mean O_3 was associated with a negative effect on lung function growth, confirming results from the previous three-year study. In an editorial, Tager (1999) stated that the Frischer et al. (1999) data provided the first prospective evidence of an association between exposure to ambient air pollution and alterations in lung growth in children. Tager further noted that the prospective study design represented a substantial improvement over data derived from cross-sectional studies and should be emulated.

Kopp et al. (2000), in a cohort of 797 children in Austria and southwestern Germany, reported lower lung function growth in children exposed to high (44 to 52 ppb O_3) levels of ambient O_3. Children residing in low O_3 (24 to 33 ppb) areas experienced a 43 mL increase in FEV_1 whereas those in high O_3 areas only experienced a 16 mL increase during the summer of 1994. Similar results were found in data from the summer of 1995. In another Austrian study, Ihorst et al. (2004) examined 2,153 children with a median age of 7.6 years and reported summer pulmonary function results revealing a significantly lower FVC and FEV_1 increase associated with higher O_3 exposures in the summer, but not in the winter.

In a pilot study (Kinney and Lippmann, 2000), 72 nonsmoking adults (mean age 20 years) from the 2nd year class of students at the U.S. Military Academy at West Point, NY provided two lung function measurements, one before and one after a five-week long summer training program at four locations. There was a greater decline in FEV_1 among students at the Fort Dix location (78 mL) as compared to students at the other locations (31 mL). Ozone levels at Fort Dix averaged 71 ppb (mean of daily 1-h max O_3) over the summer training period versus mean values of 55 to 62 ppb at the other three locations. In addition to the higher mean O_3 level, Fort Dix had greater peak O_3 values (23 hours > 100 ppb) compared to the other locations (1 hour > 100 ppb). Ambient levels of other pollutants, PM_{10} and SO_2, were relatively low during the study and did not vary across the four sites. Though conclusions are limited by the small size of the study, results are consistent with a seasonal decline in lung function that may be due, in part, to O_3 exposures. Another interesting observation from this study was that a larger decline was observed in subjects with post-summer measurements in the first two weeks after returning from

training compared to those measured in the 3rd and 4th weeks, indicating that O_3-related lung function declines might be reversible.

Collectively, the above studies indicate that seasonal O_3 exposure is associated with declines in lung function growth in children. The study by Kinney and Lippman (2000) provide limited evidence that seasonal O_3 also may affect lung function in adults, though the effect may be somewhat transient.

7.5.4 Chronic O_3 Effects on Lung Function

Lung capacity grows during childhood and adolescence as body size increases, reaches a maximum during the 20s, and then begins to decline steadily and progressively with age. There has long been concern that long-term exposure to air pollution might lead to slower growth in lung capacity, diminished maximally attained capacity, and/or more rapid decline in capacity with age. The concern arises by analogy with cigarette smoking, where it is well-documented that lung function declines more rapidly with age in a dose-dependent manner among adults who smoke cigarettes. Adults who stop smoking return to a normal rate of decline in capacity, although there is no evidence that they regain the capacity previously lost due to smoking (Burchfiel et al., 1995). Because O_3 is a strong respiratory irritant, and is associated with acute lung function declines as well as inflammation and re-structuring of the respiratory airways, it seems plausible that there might be a negative impact of long-term O_3 exposures on lung function. Exposures that affect lung function growth during childhood, in particular, might cause greater long-term risks. Thus, studies of effects on diminished rate of lung function growth in children are especially important.

Several studies published over the past decade have examined the relationship between lung function and long-term O_3 exposure. The most extensive and robust recent study of respiratory effects in relation to long-term air pollution exposures among children has been the Children's Health Study carried out in 12 communities of southern California starting in 1993 (Peters et al., 1999a,b; Gauderman et al., 2000, 2002, 2004a,b). No significant associations were observed between long-term O_3 exposures and self-reports of respiratory symptoms or asthma (Peters et al., 1999a). In the initial report examining the relationship between lung function at enrollment and levels of air pollution in the community, there was evidence that annual mean O_3 levels were associated with decreased FVC, FEV_1, PEF, and FEF_{25-75} (the latter two being

statistically significant) among females but not males (Peters et al., 1999b). Among the 4th graders, a longitudinal analysis of lung function growth over eight years indicated decrements were associated significantly with PM and NO_2, but not with O_3 (Guaderman et al., 2000, 2004a,b). A 2nd cohort of 4th graders were recruited in 1996 and followed over four years (Gauderman et al., 2002). Stratified analyses by time spent outdoors indicated a significant association between decreased PEF growth and O_3 exposure only in children who spent more than 1.3 hours outdoors (Guaderman et al., 2002).

Ihorst et al. (2004) found that there were no associations between lung function growth rate and mean summer O_3 levels for FVC and FEV_1 over a 3.5-year period, in contrast to the significant seasonal effects discussed earlier. Unlike the smaller increase in lung function parameters over the 1st two summers among children in high O_3 areas, a greater increase was observed during the 3rd summer and no difference in increase was observed during the 4th summer. The authors then concluded that medium-term effects on schoolchildren lung growth are possibly present but are not detected over a 3- to 5-year period due to partial reversibility. The study by Frischer et al. (1999) showed results similar to the Ihorst et al. (2004) study. Although a significant O_3-related reduction in lung function growth was observed when three years were analyzed collectively, smaller changes were observed throughout the years. FEV_1 increase was significantly lower by 34.0 mL for each 20 ppb increase in mean 24-h avg O_3 in the 1st year compared to a nonsignificant but greater increase of 7.3 mL in the 3rd year (Frischer et al., 1999). Results from Horak et al. (2002a) indicated that the four-year cumulative reduction in FEV_1 was 151.2 mL with O_3 levels of 20 ppb, which was less than the cumulative estimate of 156.6 mL from the 1st three years, indicating that there was little if any O_3-related changes in lung function growth during the 4th year.

Evidence for a relationship between long-term O_3 exposures and decrements in maximally attained lung function was observed in a nationwide cohort of 520 1st year students at Yale College in New Haven, CT (Galizia and Kinney 1999; Kinney et al., 1998). Each student performed one lung function test in the spring of their 1st year at college. Ozone exposures were estimated by linking 10-year mean summer season 1-h max O_3 levels at the nearest monitoring station to the residential locations reported each year from birth to the time of measurement. Students who had lived four or more years in areas with long-term mean O_3 levels above 80 ppb had significantly lower FEV_1 (−3.07% [95% CI: −0.22, −5.92]) and FEF_{25-75} (−8.11% [95% CI:

−2.32, −13.90]) compared to their classmates with lower long-term O_3 exposures. Stratification by gender indicated that males had much larger effect estimates than females, which might reflect higher outdoor activity levels and corresponding higher O_3 exposure during childhood.

A similar study of 130 1st year college freshmen at the University of California at Berkeley also reported significant effects of O_3 on lung function (Künzli et al., 1997; Tager et al., 1998). Enrollment was limited to students from either the San Francisco or Los Angeles, CA metropolitan areas. After controlling for city of origin, long-term O_3 exposures were associated with declines in FEF_{25-75} and FEF_{75} (forced expiratory flow after 75% of FVC has been exhaled). No effects were seen for PM_{10} and NO_2. Künzli and colleagues noted that significant changes in these mid- and end-expiratory flow measures could be considered early indicators for pathologic changes that might ultimately progress to COPD, as evidenced by animal studies that show that the primary site of O_3 injury in the lung is the centriacinar region (Chapter 5). In another California-based study (Gong et al., 1998b), there was no relationship between long-term changes in lung function (over an approximately 10-year period) and acute responsiveness to O_3 exposure (over a two-hour period in a controlled chamber environment) among persons living in high O_3 communities.

An autopsy pathologic study examining centriacinar region inflammatory disease was part of a discussion of long-term O_3 effects in the animal toxicology studies in Chapter 5. Sherwin et al. (2000) examined subjects for the above pathologic outcome in Miami, FL and Los Angeles, CA residents. A trend towards greater degrees of centriacinar region alterations was observed in the lungs of Los Angeles residents compared to Miami residents, independent of a smoking effect. The results suggest that the greater extent and severity of centriacinar region alterations might be related to the higher O_3 levels in Los Angeles. Beyond the challenge of differentiating the lifetime of exposure for subjects in the two cities, various confounding factors also can impact this study. The pathogenesis of centriacinar region alteration is undoubtedly multifactorial with respiratory infection and adverse environmental influences being two major considerations. In addition, Sherwin et al. (2000) noted that the study was limited due to the relatively small number of cases available. Nonetheless, as observed by Tager (1993), the use of human postmortem specimens is of interest in future epidemiology studies.

7.5.5 Chronic O_3 Exposure and Respiratory Inflammation

As noted in Chapter 6, human chamber studies have demonstrated that brief (2 to 6.6 hours) exposures to O_3 while exercising result in inflammation in the lung, including the alveolar region where gas exchange takes place. This acute effect is potentially important for chronic effects because repeated inflammation can result in the release of substances from inflammatory cells that can damage the sensitive cells lining the lung. Over extended periods, repeated insults of this kind could lead to permanent damage to and re-structuring of the small airways and alveoli. In addition, since inflammation is a fundamental feature of asthma, there is concern that O_3-induced inflammation can exacerbate existing asthma or perhaps promote the development of asthma among genetically pre-disposed individuals. Several studies are discussed next, examining different outcomes related to inflammation.

In a study by Kinney et al. (1996), bronchoalveolar lavage fluids were collected in the summer and winter from a group of 19 adult joggers living and working on an island in New York harbor. The mean 1-h max O_3 for the three-month periods were 58 ppb in the summer and 32 ppb in the winter. PM_{10} and NO_2 concentrations were similar across the two seasons. There was little evidence for acute inflammation in bronchoalveolar lavage fluids collected during the summer as compared to that collected from the same subjects in the winter. However, there was evidence of enhanced cell damage, as measured by lactate dehydrogenase, in the summer lavage fluids. These results indicate that acute inflammatory responses may diminish with repeated exposures over the course of a summer (which have been demonstrated in multiday chamber exposures, Chapter 6, Section 6.9) but cell damage may be ongoing.

Pollution effects in the nose can be viewed as a potential surrogate measure for effects that may occur in the lungs, though doses to nasal tissues are usually higher for a given pollutant concentration. In Chapter 5, morphological effects of O_3 on the upper respiratory tract indicated quantitative changes in the nasal transitional respiratory epithelium. The persistent nature of the O_3-induced mucous cell metaplasia in rats, as discussed in Chapter 5, suggests that O_3 exposure may have the potential to induce similar long-lasting alterations in the airways of humans. A series of interesting studies in Mexico City have demonstrated inflammation and genetic damage to cells in the nasal passages of children chronically exposed to O_3 and other air pollutants (Calderón-Garcidueñas et al., 1995, 1997, 1999). Nasal lavage samples and nasal biopsies from children living in Mexico City were compared to those from children living in a

clean coastal town with no detectable air pollutants. In the first study, urban children (n = 38) from Mexico City were found to have significantly higher polymorphonuclear leukocyte counts and abnormal nasal cytologies compared to nonurban children (n = 28) (Calderón-Garcidueñas et al., 1995). A later study observed that cells collected from the lining of the nose had significantly higher amounts of DNA damage in the urban children in Mexico City (n = 129) versus nonurban children (n = 19) (Calderón-Garcidueñas et al., 1997). Among exposed children, DNA damage was greater with increasing age, suggesting an accumulation of damage over time with ongoing pollution exposures. Another study of 86 urban and 12 nonurban children reported similar findings, in addition to increased levels of specific DNA mutations (Calderón-Garcidueñas et al., 1999). They also noted far higher respiratory symptom prevalence in the urban children. Fortoul et al. (2003) examined DNA strand breaks in nasal epithelial cells from asthmatic and nonasthmatic medical students in Mexico City and noted greater genotoxic damage in asthmatics. These results indicate that asthmatics may have a greater vulnerability for DNA damage, or a decreased ability to repair it, compared to nonasthmatic subjects.

Another outcome of inflammation was examined in a study by Frischer et al. (2001). In this cross-sectional study, urinary eosinophil protein was analyzed as a marker of eosinophil activation in 877 school children living in nine Austrian communities with varying O_3 exposure. The results indicated that O_3 exposure was significantly associated with eosinophil inflammation.

In the Mexico City studies, specific attribution of these adverse respiratory and genotoxic effects to O_3 is difficult given the complex mixture of pollutants present in the ambient air. In particular, the DNA effects seem more plausibly related to other components of urban air, such as semi-volatile organic compounds. However, the inflammatory changes such as increased eosinophil levels observed in the Austrian study would be consistent with known effects of O_3.

7.5.6 Risk of Asthma Development

Recent reports from longitudinal cohort studies have reported associations between the onset of asthma and long-term O_3 exposures (McConnell et al., 2002; McDonnell et al., 1999). Significant associations between new cases of asthma among adult males and long-term O_3 exposure were observed in a cohort of nonsmoking adults in California (Greer et al., 1993;

McDonnell et al., 1999). The Adventist Health and Smog (AHSMOG) study cohort of 3,914 (age 27-87 years, 36% male) was drawn from nonsmoking, non-Hispanic white California seventh day Adventists. Subjects 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 interpolated concentrations of O_3, PM_{10}, 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 (1977-1987), the incidence of new asthma was 2.1% for males and 2.2% for females (Greer et al., 1993). A relative risk of 3.12 per 10 ppb increase in annual mean O_3 was observed in males, compared to a non-significant relative risk of 0.94 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., 1999). For males, the relative risk of developing asthma was 2.27 per 30 ppb increase in 8-h avg O_3 (9 a.m.-5 p.m.). Once again, there was no evidence of an association between O_3 and new-onset asthma in females (relative risk of 0.85). The lack of an association does not necessarily indicate no effect of O_3 on the development of asthma among females. For example, differences in time-activity patterns in females and males may influence relative exposures to O_3, leading to greater misclassification of exposure in females. The consistency of the results in the two studies with different follow-up times and indices of O_3 exposure provides evidence that long-term O_3 exposure may be associated with asthma incidence in adult males. However, as the AHSMOG cohort was drawn from a narrow subject definition, the representativeness of this cohort to the general U.S. population may be limited.

A similar study of incident asthma cases in relation to O_3 among children was carried out in the Children's Health Study (McConnell et al., 2002). Annual surveys of 3,535 initially nonasthmatic children (ages 9 to 16 years at enrollment) enabled identification of new-onset asthma cases through 1998. Communities were stratified by pollution levels, with six high-O_3 communities (mean 1-h max O_3 of 75.4 ppb over four years) and six low-O_3 communities (50.1 ppb). Asthma risk was not higher for residents of the six high-O_3 communities versus residents of the six low-O_3 communities. However, within the high-O_3 communities, asthma risk was 3.3 times greater for children who played three or more sports as compared with children

who played no sports. This association was absent in the low-O_3 communities (relative risk of 0.8). No associations with asthma were seen for PM_{10}, $PM_{2.5}$, NO_2, or inorganic acid vapors. These results suggest effect modification of the impacts of O_3 on asthma risk by physical activity. Playing sports may indicate outdoor activity when O_3 levels are higher and an increased ventilation rate, which may lead to increased O_3 exposure. Replication of these findings in other cohorts would lend greater weight to a causal interpretation.

Recent cross-sectional surveys have 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 (Charpin et al., 1999; Kuo et al., 2002; Ramadour et al., 2000). However, reported O_3 levels were quite low in all cases, with a range of 16 to 27 ppb for 8-h max O_3. In addition, compared to the longitudinal study design, which observes new onset of asthma prospectively, the cross-sectional study design is inherently weaker. Longitudinal studies provide the strongest evidence on the question of asthma development and is the preferred approach for future research.

7.5.7 Respiratory Effects of Chronic O_3 Exposure on Susceptible Populations

Studies on the effect of long-term O_3 exposure on respiratory health has mostly focused on potentially susceptible populations, including children and individuals who exercise outdoors, as discussed in this section. Ozone exposure was associated significantly with declines in lung function or reduced lung function growth, and respiratory inflammation in these susceptible populations.

Other studies have investigated additional symptoms and groups of potentially susceptible individuals. McConnell et al. (1999) examined the association between O_3 levels and the prevalence of chronic lower respiratory tract symptoms in southern California children with asthma (n = 3,676). In this questionnaire-based study, bronchitis, phlegm, and cough were not associated with annual mean O_3 concentrations in children with asthma or wheeze. All other pollutants examined, PM_{10}, $PM_{2.5}$, NO_2, and gaseous acid, was associated with an increase in phlegm, but not cough.

One new study examined a susceptible group not examined before. Goss et al. (2004) investigated the effect of O_3 on pulmonary exacerbations and lung function in individuals with

cystic fibrosis over the age of 6 years (n = 11,484). The study included patients enrolled in the Cystic Fibrosis Foundation National Patient Registry. The registry contained demographic and clinical data collected annually at accredited centers for cystic fibrosis. In 1999 and 2000, the annual mean O_3 concentration 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.

In summary, several studies have identified and investigated potentially susceptible populations. Although effects are not specific to O_3 exposure, the results suggest that O_3 likely contributes to the adverse respiratory health responses observed in these populations.

7.5.8 Mortality Effects of Chronic O_3 Exposure

There is inconsistent and inconclusive evidence for a relationship between long-term O_3 exposure and increased mortality risk (Table AX7-7 in the Chapter 7 Annex). A long-term prospective cohort study (AHSMOG; 1977-1992) of 6,338 nonsmoking, non-Hispanic white subjects living in California found a significant association between long-term O_3 exposure and increased lung cancer risk among males only (Beeson et al., 1998). The relative risk for lung cancer incident among males was 3.56 (95% CI: 1.35, 9.42) per 556 hours/year when O_3 levels exceeded 100 ppb (Beeson et al., 1998). A stronger association was observed in males who never smoked (4.48 [95% CI: 1.25, 16.04]) compared to those who smoked in the past (2.15 [95% CI: 0.42, 10.89]) (Beeson et al., 1998). An expanded study by Abbey et al. (1999) examining mortality effects of long-term O_3 exposure in the same study population confirmed the results of the previous study by Beeson and colleagues. The association between lung cancer mortality and chronic O_3 exposure was significant in males only, with a relative risk of 4.19 (95% CI: 1.81, 9.69) (Abbey et al., 1999). However, the very small numbers of lung cancer deaths (12 for males and 18 for females) raise concerns in regards to the precision of the effect estimate, as evidenced by the wide confidence intervals. No other mortality outcomes were found to be associated with chronic O_3 exposure. A particular strength of this study was the

extensive effort devoted to assessing long-term air pollution exposures, including interpolation to residential and work locations from monitoring sites over time and space. However, the observation of a lung cancer effect but no effect on cardiopulmonary mortality raises concerns. The gender-specific O_3 effects may be partially attributable to the differences in activity and time spent outdoors by gender. The questionnaires indicated that males spent approximately twice as much time outdoors and performed more vigorous outdoor exercises, especially during the summer, compared to the females.

Lipfert et al. (2000b, 2003) reported positive effects on mortality for peak O_3 exposures (95th percentile levels) in the U.S. Veterans Cohort study of approximately 50,000 male 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 defined as follows: 1960-1974; 1975-1981; 1982-1988; and 1989-1996. Three mortality follow-up periods were considered: 1976-1981; 1982-1988; and 1989-1996. In a preliminary screening of regression results, Lipfert et al. (2000b) compared univariate and multivariate models by mean and peak (95th percentile) O_3 concentrations. For mean O_3, a significant negative relationship was reported in the univariate model and a nonsignificant negative relationship was found in the multivariate model. For peak O_3 concentration, the univariate model indicated a nonsignificant positive relationship and the multivariate model resulted in a significant positive relationship. Peak O_3 was used in subsequent analyses. The mean of the peak values ranged from 85 to 140 ppb over the four exposure periods. For concurrent exposure periods, the mortality risk was significant, with a 6.1% excess risk per mean 95th percentile O_3 less estimated background level (not stated). When exposure periods preceding death were considered, the excess mortality risk was nonsignificant (−0.2%). In a further analysis, Lipfert et al. (2003) reported the strongest positive association for concurrent exposure to peak O_3 for the subset with low diastolic blood pressure during the period of 1982-1988. Once again, the O_3 effect was found to be reduced when the exposure (1982-1988) preceded mortality (1989-1996).

No effect of long-term O_3 concentrations on mortality risk was observed in a larger prospective cohort study of approximately 500,000 U.S. adults (Pope et al., 2002). Strong and consistent effects of $PM_{2.5}$ were observed in this study for both lung cancer and cardiopulmonary mortality.

7.5.9 Summary of Chronic O_3 Effects on Morbidity and Mortality

- In the past decade, important new longitudinal studies have examined the chronic effect of O_3 exposure on respiratory health outcomes. Evidence from recent long-term morbidity studies have indicated that chronic exposure to O_3 may be associated with declines in lung function, inflammation, and development of asthma in children and adults. Seasonal decrements or reduced growth in lung function measures have been reported in several studies, however changes appear to be transient. Studies of lung function declines with longer-term or annual data are not as conclusive.

- Few studies have investigated the effect of long-term O_3 exposure on mortality. Uncertainties regarding the exposure period of relevance, and inconsistencies across mortality outcomes and gender raise concerns regarding plausibility. The current evidence is inconclusive for a relationship between chronic O_3 exposure and increased risk of mortality.

7.6 INTERPRETATIVE ASSESSMENT OF THE EVIDENCE IN EPIDEMIOLOGIC STUDIES OF OZONE HEALTH EFFECTS

7.6.1 Introduction

In the 1996 O_3 AQCD, the epidemiology section focused primarily on individual-level camp and exercise studies. These field studies indicated exposure-response relationships between O_3 exposure from the ambient air, and declines in pulmonary function, increases in respiratory symptoms, and exacerbation of asthma, especially in children. Numerous new studies provide additional evidence for evaluating associations between O_3 exposure and the above respiratory health outcomes. The 1996 O_3 AQCD review of aggregate population time-series studies suggested an association between ambient O_3 concentrations and increased hospitalizations. Limited studies examined the O_3-mortality relationship. The current O_3 AQCD further presents results from time-series studies that have addressed previously unresolved issues regarding potential linkages between ambient O_3 concentrations and health outcomes, particularly mortality. Daily time-series studies minimize confounding by population characteristics (e.g., cigarette smoking, diet, occupation) by following the same population from day to day. However, confounders operating over shorter time scales can affect O_3 risk estimates in time-series studies.

In this section, the issues and attendant uncertainties that affect the interpretation of O_3 health effects will be discussed. The consequences of using stationary ambient monitors as an estimate of personal exposure in epidemiology studies will be examined first and a discussion of the temporal relationship between O_3 exposure and the occurrence of health effects will follow. Of particular interest are the issues arising from model specifications in time-series studies to adjust for confounding by temporal factors, meteorological effects, and copollutants. The shape of the concentration-response relationship and heterogeneity of O_3 health effects also will be discussed briefly. All of these issues are of much importance for characterizing and interpreting ambient O_3-health effects associations.

7.6.2 Exposure Assessment

Various methods have been used to assess exposure in air pollution epidemiology studies. Navidi et al. (1999) describes the two methods commonly used to ascertain personal exposure: (1) the microenvironmental (indirect) approach; and (2) the personal sampling (direct) approach. Both methods are associated with measurement error. To determine personal exposure using the microenvironmental approach, the concentrations of the various microenvironments are multiplied by the time spent in each microenvironment. Both the concentration and time component contribute to the measurement error. Although there is no time component to the measurement error in the personal sampling approach, the estimation of exposure using personal monitoring devices do contribute to the error, especially in the case of O_3. The passive badges commonly used for personal sampling of O_3 provide integrated personal exposure information. Their sensitivity to wind velocity, badge placement, and interference with other copollutants may result in measurement error. Results from the error analysis models developed by Navidi et al. (1999) indicated that neither the microenvironmental or personal sampling approach gave reliable health effect estimates when measurement errors were uncorrected. The nondifferential measurement error biased the effect estimates toward zero under the model assumptions. However, if the measurement error was correlated with the health response, a bias away from the null could result. The use of central ambient monitors to estimate exposure also biased the estimates towards the null. Most people spend the majority of their time indoors, where O_3 levels tend to be much lower than outdoor ambient levels. Using ambient concentrations to

determine exposure generally overestimates true personal O_3 exposure, resulting in effect estimates biased towards the null.

7.6.2.1 Relationship between Ambient Concentrations and Personal Exposure to O_3

Several studies have examined the relationship between ambient O_3 concentrations from a central monitoring site and personal O_3 exposure (Avol et al., 1998; Brauer and Brook, 1997; Chang et al., 2000; Delfino et al., 1996; Lee et al., 2004; Liard et al., 1999; Linn et al., 1996; Liu et al., 1995, 1997; O'Neill et al., 2003; Sarnat et al., 2001). In a Baltimore, MD study of older adults, individuals with COPD, and children, 24-h avg ambient O_3 concentrations from a monitoring site were not found to be significantly associated with personal O_3 exposure (Sarnat et al., 2001). The mixed regression effect estimates were $\beta = 0.01$ ($t = 1.21$) and $\beta = 0.00$ ($t = 0.03$), for summer and winter, respectively. A study by O'Neill et al. (2003), in contrast, found a statistically significant association between personal and ambient O_3 concentrations in Mexico City outdoor workers ($\beta = 0.56$, $t = 8.52$). The subjects in the Sarnat et al. (2001) study spent less than 6% of their time outdoors, whereas the personal exposure data from O'Neill et al. (2003) were from subjects who spent the entire measurement period outdoors. A scripted exposure study by Chang et al. (2000) provided supportive evidence for the conflicting results in the Sarnat et al. (2001) and O'Neill et al. (2003) studies. In this study, activities were scripted to simulate activities typical of older adults living in Baltimore, MD. Their activities were derived from the U.S. EPA-sponsored National Human Activity Pattern Survey study (Klepeis, 1999). Chang et al. (2000) compared one-hour personal and ambient O_3 measurements in several microenvironments. There was no correlation between personal and ambient O_3 concentrations in the indoor residence ($r = 0.09$ and $r = 0.05$, for summer and winter, respectively), although a moderate correlation was found in other indoor environments such as restaurants, hospitals, and shopping malls ($r = 0.34$ in summer, $r = 0.46$ in winter). In comparison, the correlation in outdoor environments (near and away from roads) was moderate to high ($0.68 \leq r \leq 0.91$) and statistically significant.

Brauer and Brook (1997) observed that the daily averaged personal O_3 measurements and ambient concentrations were well-correlated after stratifying groups by time spent outdoors. The clinic workers ($n = 25$) spent 9% of their time outdoors (24-hour samples) whereas the farm workers ($n = 15$) spent 100% of their monitored time outdoors (6-14 hour workshift samples).

The personal to ambient O_3 concentration ratios were significantly different for the clinic workers (0.28) and farm workers (0.96). However, the Spearman correlation coefficients were similar in the two groups, 0.60 and 0.64 for the clinic workers and farm workers, respectively. In a Paris, France study by Liard et al. (1999), adults (n = 55) and children (n = 39) wore passive O_3 monitors for 4 consecutive days during three periods. For each period, all adults wore the O_3 monitors over the same 4 days. Likewise, all children wore monitors over the same 4 days for each of the three periods, but on different days from the adults. The ambient O_3 concentrations from the stationary monitoring sites did not explain a high percentage of the variance of personal O_3 exposure (non-significant [value not stated] in adults and 21% in children). However, when personal measurements from all subjects were aggregated for each of the six periods, the 4-day mean personal O_3 exposure was found to be highly correlated with the corresponding mean ambient concentration ($r = 0.83$, $p < 0.05$). Similarly, a study of Los Angeles school children by Linn et al. (1996) found that daily 24-h avg ambient O_3 concentrations from a central site were well-correlated ($r = 0.61$) with daily averaged personal O_3 concentrations (8-10 children/day, 132 total monitoring days).

The low correlation observed between personal O_3 exposures and ambient O_3 concentrations in the study by Sarnat et al. (2001) suggests that O_3 concentrations measured at central ambient monitors do not explain the variance of individual personal exposures. However, in time-series studies, daily averaged personal exposures from the aggregate population is of greater relevance than exposures from specific individuals. Although unresolved issues do remain, the limited evidence indicates that ambient O_3 concentrations from central monitors may serve as valid surrogate measures for aggregate personal O_3 exposures in population time-series studies investigating mortality and hospitalization outcomes.

7.6.2.2 Factors Affecting the Relationship between Ambient Concentrations and Personal Exposures to O_3

In cohort studies investigating acute and chronic morbidity outcomes, O_3 exposure assessment may be improved by accounting for the distance between home and the monitoring site, time-activity patterns (e.g., percentage of time spent outdoors, type of outdoor activity, time of day during outdoor activity), and elements that affect indoor air exchange rates (e.g., ventilation conditions, home characteristics) in conjunction with the ambient O_3 data from stationary monitor sites. Several studies (Geyh et al., 2000; Lee et al., 2004; Liu et al., 1995,

1997) demonstrated that the association between personal O_3 exposure and ambient O_3 concentrations is affected by these factors. A study by Geyh et al. (2000) observed higher indoor and personal O_3 concentrations in a southern California community with 2% air conditioned homes compared to a community with 93% air conditioned homes during the summer (high O_3) months, but showed no difference in O_3 levels during the winter (low O_3) months. Lee et al. (2004) observed that personal O_3 exposure was positively correlated with outdoor time ($r = 0.19$, $p < 0.01$) and negatively correlated with indoor time ($r = -0.17$, $p < 0.01$). Additional factors that affected indoor O_3 levels were air conditioning, window fans, and window opening. The O_3 exposure assessment study by Liu et al. (1995) found that after adjusting for time spent in various indoor and outdoor microenvironments (e.g., car with windows open, car with windows closed, school, work, home, outdoors near home, outdoors other than near home), mean 12-hour ambient O_3 concentrations explained 32% of the variance in personal exposure in the summer.

Other factors, including age, gender, and occupation, also may affect general exposure patterns to O_3 by influencing time-activity patterns. In a southern California study by Avol et al. (1998), boys were found to spend more time outdoors and be more physically active than girls (Avol et al., 1998). Another southern California study found that boys were outdoors 30 minutes longer than girls, and had higher personal O_3 exposure during both high and low O_3 months (Geyh et al., 2000). Outdoors workers also tend to be exposed to higher levels of O_3 (Brauer and Brook, 1997; O'Neill et al., 2003).

The announcement of air quality indices also may influence personal exposures to O_3. Niedell (2004) examined the effect of smog alerts on the relationship between O_3 and hospital admissions for asthma in California children aged 0 to 18 years. Air quality episodes, or smog alerts, are issued in California on days when O_3 concentrations exceed 200 ppb. Smog alerts were found to have a significant negative effect on asthma admissions for children aged 1 to 12 years, providing supportive evidence that avoidance behavior might be present on days of high O_3 concentrations. Avoidance behavior may include staying indoors and exercising less on days when a smog alert is announced, resulting in reduced exposure to O_3. Therefore air quality indices may affect the relationship between ambient and personal O_3 concentrations.

In summary, results indicate that the relationship between ambient and personal O_3 concentrations varies depending on factors such as time spent outdoors, ventilation conditions, personal factors, and air quality indices. The use of questionnaires to obtain information on

personal characteristics, time-activity patterns, and home characteristics may improve further the accuracy of the personal O_3 exposure estimates from ambient O_3 data.

7.6.2.3 Assessing Chronic Exposure to O_3

Several studies examined methods of estimating chronic exposure to O_3. A pilot study (n = 14) by Gonzales et al. (2003) indicated that the use of retrospective questionnaires might be a reliable method for reconstructing past time-activity and location pattern information. The 7-day, 24-h avg O_3 exposures were estimated using data from an ambient monitor (mean 29.5 ppb), and information from prospective diaries and questionnaires completed one year after the monitoring period. The O_3 estimates from both prospective diaries (mean 10.6 ppb) and retrospective questionnaires (mean 11.8 ppb) differed only slightly, although both estimates were greater than the personal exposure measurement (mean 5.7 ppb). A study by Künzli et al. (1997) compared the retrospective assessments of outdoor time-activity patterns using three formats, a questionnaire, a table, and a 24-hour log. College freshmen (n = 44) noted activity patterns at their last residence using two or three methods and then were retested 5-7 days later. The within-subject variance in reporting moderate to heavy activity was 13% for both the activity questionnaire and activity table, and 32% for the 24-hour log. The data from the activity tables also were similar to data published from the California Air Resources Board (CARB) 24-hour recall diary study (Jenkins et al., 1992). Results from the above studies suggest that the use of activity questionnaires or activity tables to determine time-activity patterns may be suitable in large retrospective epidemiologic studies of health effects requiring estimates of chronic exposure to ambient O_3.

7.6.3 O_3 Exposure Indices

Three O_3 indices were used often to indicate daily O_3 exposure: maximum 1-h average (1-h max); maximum 8-h average (8-h max); and 24-h average (24-h avg). The 8-h max O_3 is a frequently used index in newer epidemiologic studies, as it best reflects the current U.S. EPA standard. These O_3 exposure indices are highly correlated as indicated in the following studies. In the 21 European multicities acute mortality study (Gryparis et al., 2004), 1-h max O_3 was found to be highly correlated with 8-h max O_3, with a median correlation coefficient of 0.98 (range: 0.91 - 0.99). Among single city studies, the 1-h max O_3 and 8-h max O_3 also were found

to have correlation coefficients ranging from 0.91 to 0.99 in various cities such as Atlanta, GA (Tolbert et al., 2000; White et al., 1994); southern New England (Gent et al., 2003); Ontario, Canada (Burnett et al., 1994); and Mexico City (Loomis et al., 1996; Romieu et al., 1995). In addition, 1-h max O_3 was found to be highly correlated with 24-h avg O_3, as observed in the Mexico City study by Loomis et al. (1996) ($r = 0.77$) and in the Ontario, Canada study by Burnett et al. (1994) ($r = 0.87$).

All studies discussed in Sections 7.2 to 7.5 were examined for presentation of the three O_3 exposure indices. Several presented the concentration data and correlations among 1-h max, 8-h max, and 24-h avg O_3 ambient measures. Some presented the associated risk estimates of comparable analyses for the three exposure indices. No papers provided an analysis statistically comparing the indices. Summary of the available data is provided below starting with two multicity mortality studies.

In the large U.S. 95 communities study by Bell et al. (2004), increases in O_3-associated daily mortality were estimated using all three O_3 indices. The increments used in this document to standardize expressions of excess risks are 40 ppb for 1-h max O_3, 30 ppb for 8-h max O_3, and 20 ppb for 24-h avg O_3, as discussed in Section 7.1.3.2. For these increments, the effect estimates calculated by Bell et al. (2004) were 1.34%, 1.28%, and 1.04% excess risk in mortality for 1-h max O_3, 8-h max O_3, and 24-avg O_3, respectively. A statistical test examining differences among these risk estimates indicated that there were no significant differences by exposure index. In the European study of 21 cities (of the 23 cities, two did not have 8-h max O_3 data), the O_3-mortality effect estimate for the summer season was slightly smaller for 8-h max O_3, 1.82% excess risk, compared to 1-h max O_3, 2.59% excess risk, but both were statistically significant (Gryparis et al., 2004). Once again, a statistical test between the two risk estimates yielded no significant difference between the indices.

Several single city mortality studies examined multiple O_3 exposure indices (Anderson et al., 1996; Dab et al., 1996; Sunyer et al., 2002; Zmirou et al., 1996; Borja-Aburto et al., 1997). These studies did not differentiate risk estimates by exposure index as the results were considered similar. Hospital admission studies also provided limited data for index comparisons. Schouten et al. (1996) showed the same positive nonsignificant association between total respiratory hospitalizations and O_3 using both 8-h max O_3 and 1-h max O_3 in the summer (4.0% excess risk per standardized increment). For emergency department visits, the

examples of Delfino et al. (1998) and Weisel et al. (2002) provided data not indicative of differences between the indices. A further example, Tolbert et al. (2000) noted an increase in emergency room visits of 4.0% per standard deviation increase (approximately 20 ppb) for both 1-h max O_3 and 8-h max O_3 as being expected since the correlation between the indices was 0.99.

Peak flow asthma panel studies generally only used one index in these studies, thus no comparison data is available. One respiratory symptom study (Gent et al., 2003) did examine both 1-h max O_3 and 8-h max O_3 but noted no differences in the results. Only one FEV_1 panel study examined more than one exposure index. Chen et al. (1999) examined 1-h max O_3 and 24-h avg O_3 and reported at a 1-day lag for children a decrement in FEV_1 of −25.6 mL (−49.1, −2.1) for 1-h max O_3 and −13.6 mL (−33.2, 6.0) for 24-h avg O_3. For 2- and 7-day lags, smaller differences were observed between the two indices. None of these FEV_1 outcomes, including those for a 1-day lag, upon testing were significantly different by index.

Limited information is available to reach conclusions for comparison of the three indices 1-h max O_3, 8-h max O_3, and 24-h avg O_3. In general, for the same distributional increment (e.g., interquartile range), the excess health risk estimates and significance of associations were comparable for all three daily O_3 indices. The similarity in effects for the three exposure indices was consistent for all outcomes. This is expected due to the high correlation among the indices.

The relationship between the various health endpoints and the three O_3 exposure indices, and the associated study designs and analyses are such that the high correlation among the indices presents a significant challenge in distinguishing the most appropriate measure for epidemiologic studies. The commonly used 8-h max O_3 or 8-h avg O_3 index continues to be an appropriate choice as no other exposure index has been demonstrated to offer a better advantage.

7.6.4 Lag Time: Period between O_3 Exposure and Observed Health Effect

The choice of lag days for the relationship between exposure and health effects depends on the hypothesis being tested and the mechanism involved in the expression of the outcome. Effects can occur acutely with exposure on the same or previous day, cumulatively over several days, or after a delayed period of a few days. With knowledge of the mechanism of effect, the choice of lag days can be determined prior to analysis. For example, one can expect cough to occur acutely after exposure with a lag of 0 or 1 day, as O_3 can act as an immediate irritant.

However, an O_3-related inflammatory response may not lead to asthma exacerbation until several days later. An asthmatic may be impacted by O_3 on the first day of exposure, have effects triggered further on the second day, then report to the emergency room for an asthmatic attack three days after exposure. Further, within a population of asthmatics, exacerbation of asthma symptoms may be observed over a period of several days, since each asthmatic has individual aspects of the disease and may be affected by the exposure differently depending on his/her sensitivity and disease severity. Therefore, it may be necessary to examine longer lag periods to fully understand the relationship between O_3 exposure and effect. When the mechanism of the health effect is unknown, investigating the association between outcome and exposure over several days also may be informative.

Most of the O_3 time-series studies investigated a small number of lagged days, typically 0 through 3 days, and/or cumulative lag periods. For outcomes of mortality and hospitalizations, the largest, most significant associations with O_3 concentrations were observed when using short lag periods, in particular a 0-day lag (exposure on same day) and a 1-day lag (exposure on previous day). In the U.S. 95 communities study by Bell et al. (2004), the largest risk estimate for O_3-mortality was obtained with a 0-day lag, followed by diminishing risk estimates with 1-, 2-, and 3-day lags. In a study of 16 Canadian cities by Burnett et al. (1997a), the strongest association between O_3 and respiratory hospitalizations was found at a 1-day lag. Once again, there was a decline in the magnitude and significance of the effect with increasing days lagged for O_3. These results suggest that O_3 has a rapid effect on these respiratory health outcomes.

Less explored is the issue of multiday effects of O_3. When associations are found at multiple lag days, results from a single-day model may underestimate the cumulative effect of O_3 on health outcomes. In the large U.S. 95 communities study (Bell et al., 2004), distributed lag models were used to estimate the effect of O_3 levels on mortality over a lag of 0 to 6 days. Results indicated that when accounting for multiple days, the effect estimates were twice as large as those from single-day analyses, as shown in Figure 7-15. Bell et al. (2004) estimated a cumulative excess risk of 1.04% on daily mortality per 20 ppb increase in 24-h avg O_3 during the previous week, compared to a 0.50% excess risk associated with O_3 exposure on a single day, in this case a 0-day lag. In a related U.S. study of the 19 largest cities by Huang et al. (2004), the O_3 estimate for the summer season was 1.50% excess risk of cardiopulmonary mortality with current-day exposure and 2.52% for a 7-day cumulative lag.

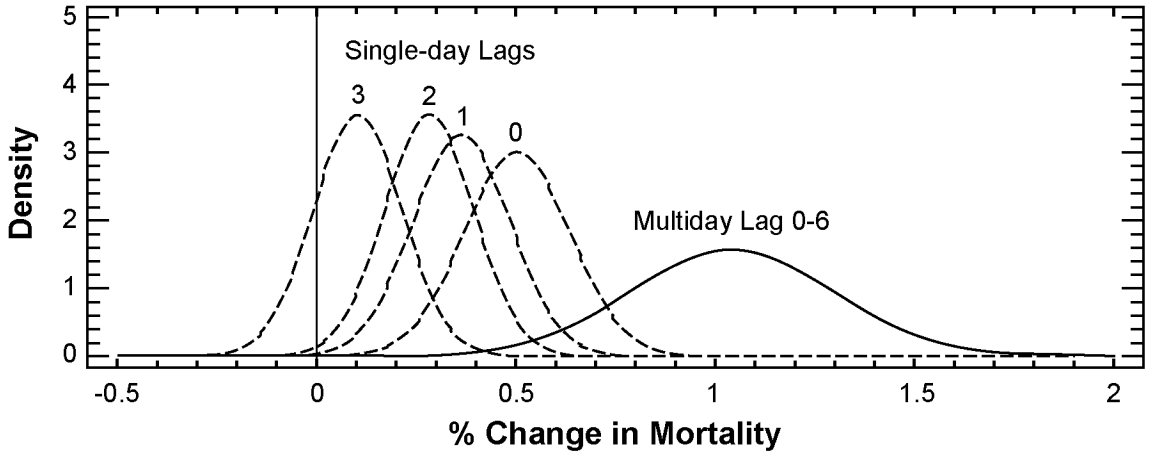


Figure 7-15. Comparison of single-day lags (0-, 1-, 2-, and 3-day) to a cumulative multiday lag (0- to 6-day) for % changes in all cause mortality per 20 ppb increase in 24-h avg O_3 in all ages.

Source: Derived from Bell et al. (2004).

Burnett et al. (2001) investigated the association between respiratory hospitalizations and O_3 in children less than 2 years of age. Lags up to five days were examined after stratifying by season (Figure 7-16). In the summer season, significant associations between O_3 and daily admissions were found in several of the lags, with the largest risk estimate of 12.5% excess risk per 40 ppb increase in 1-h max O_3 at a 1-day lag. In comparison, the O_3-related risk estimate was 30.2% using a cumulative lag period of 5 days. In another study, Anderson et al. (1997) investigated the association between O_3 and daily hospital admissions for COPD in five European cities. Lags up to 5 days were examined, and the largest risk estimates were found using 0- and 1-day lags. Anderson et al. observed a 4.5% excess risk per 40 ppb increase in 1-h max O_3 using a single-day lag compared to a 7.7% excess risk using a 5-day cumulative lag.

Among the field studies, Mortimer et al. (2002) reported O_3-related changes in PEF for single-day lags from 1 to 6 days and a multiday lag period of 5 days. No associations were seen between evening outcome measures and either single-day or multiday exposure lags. Small, nonsignificant morning effects were observed at 1- and 2-day lags. The effect of O_3 on morning outcomes increased over several days, with the strongest association seen using multiday lag

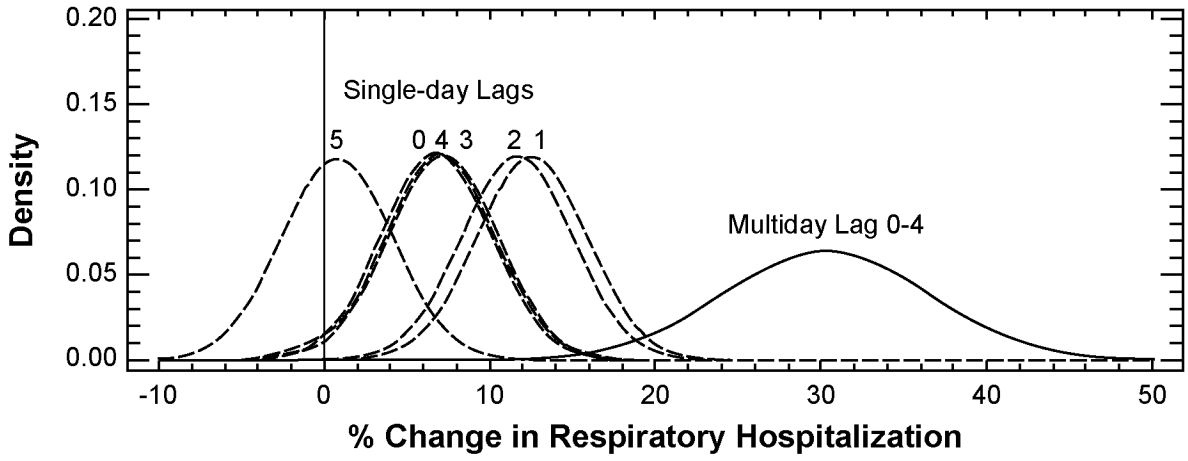


Figure 7-16. Comparison of single-day lags (0-, 1-, 2-, 3-, 4-, and 5-day) to a cumulative multiday lag (0- to 4-day) for % changes in total respiratory hospitalizations per 40 ppb increase in 1-h max O_3 in children less than two years of age.

Source: Derived from Burnett et al. (2001).

periods (Figure 7-17). Unrestricted lag models suggested that the O_3 exposure from 3 to 5 days prior had a greater impact on morning % PEF than more immediate exposures. Results were similar when comparing single- and multiple-day exposure lags on the incidence of respiratory symptoms in the morning (Mortimer et al., 2002).

Weisel et al. (2002) stated that a lag period of 1 to 3 days between exposure to O_3 and hospital admissions or emergency department visits for asthma was plausible because it might take time for the disease to progress to the most serious responses following exposure. In addition, taking medication could delay further the progression of the adverse effect. Mortimer et al. (2002) discussed biological mechanisms for delayed effects on pulmonary function, which included increased bronchial reactivity secondary to airway inflammation associated with irritant exposure. Animal toxicology and human chamber studies (see Chapters 5 and 6) provide further evidence that exposure to O_3 may augment cellular infiltration and cellular activation, enhance release of cytotoxic inflammatory mediators, and alter membrane permeability. Examining longer lag periods allows studies to investigate the cumulative O_3-related effects over several days rather than one day only. The use of longer lag periods also allows for delayed effects at 3 to 6 days to be observed. However, interpretation of

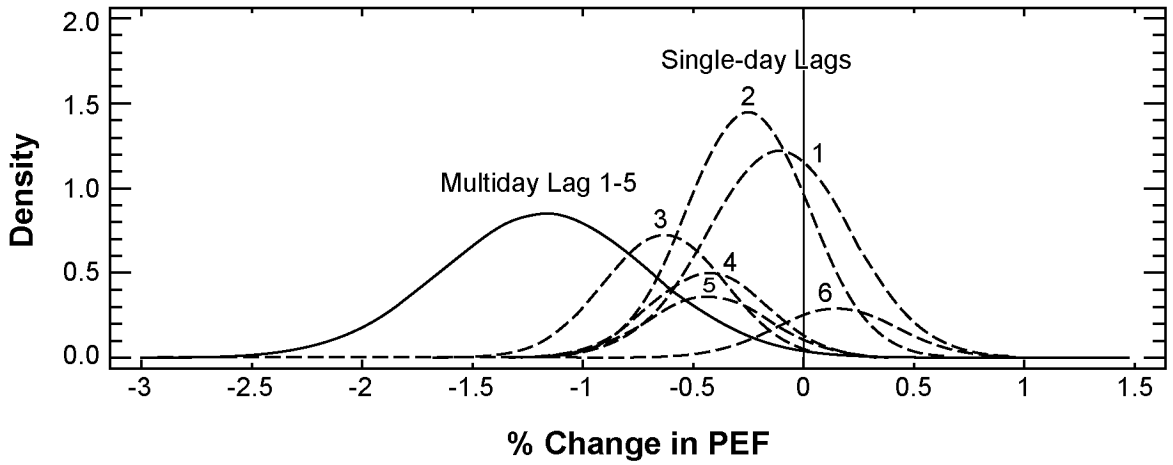


Figure 7-17. Comparison of single-day lags (1-, 2-, 3-, 4-, 5-, and 6-day) to a cumulative multiday lag (1- to 5-day) for % changes in PEF per 30 ppb increase in 8-h avg O_3 in urban children.

Source: Derived from Mortimer et al. (2002).

the results from multiday lags may not be as straightforward as that from single-day lag analyses. Few field studies examined or presented exposure lags of more than 3 days. In a study of asthma symptoms, Delfino et al. (1998) stated that no long-term lag effects were seen, but did not provide the lags examined.

Bias resulting from the selection of lags has not been examined specifically for O_3 effects. However, the issue of lags has been investigated for PM and the results of this analysis are most likely of relevance for O_3. Lumley and Sheppard (2000) performed a simulation study to examine model selection bias in air pollution epidemiology. Sheppard et al. (1999; reanalysis Sheppard, 2003) had investigated the association between asthma hospital admissions and ambient $PM_{2.5}$ concentrations over a eight-year period in Seattle, WA. Note that the results from Lumley and Sheppard (2000) and Sheppard et al. (1999) were based on GAM using default convergence criteria. A negative control analysis, using simulated data with no association between PM exposure and the health outcome, and a positive control analysis, in which a specified non-zero excess risk is added to the simulation, were performed for comparison. The bias from selection of best of seven lags (0 to 6 days) and residual seasonal confounding in the negative control analysis (median log relative risk of 0.0013) was approximately half the log

relative risk estimated from the observed data (0.0027), after adjusting for season and temperature. In the positive control model (true log relative risk of 0.0083), the bias was small (median log relative risk of 0.0080). Results from these simulations indicate that bias from selection of lags may be negligible when the true association is moderately large. However, if the relative risk is relatively small, as in the case of air pollution epidemiology, bias may be of issue. Selection of the largest risk estimate from a series of lags potentially can lead to positive bias towards finding a significant association.

Selection of lag periods should depend on the hypothesis of the study and the potential mechanism of the effect. Bias can result from the reporting of only the largest and most significant risk estimate, as well as the reporting of single-day lag results when significant relationship are found on multiple lag days. Most studies have shown that O_3 has a fairly consistent, immediate effect on respiratory health with the majority finding significant relationships at 0- and 1-day lags, especially for acute mortality and hospitalization outcomes. Some studies indicated a greater cumulative O_3 effect observed over longer lag periods, suggesting that in addition to single-day lags, multiday lags should be investigated to fully capture a delayed O_3 effect on health outcomes. The issue of lags warrants further investigation.

7.6.5 Confounding by Temporal Trends and Meteorologic Effects

The challenge of analyzing acute O_3 effects in time-series studies is to avoid bias due to confounding by daily to seasonal temporal factors. On a seasonal scale, the analysis must remove the influence of the strong seasonal cycles that usually exist in both health outcomes and O_3. On a daily scale, weather factors and other air pollutants also may confound the association of interest. This section discusses the interpretation of effect estimates after adjusting for temporal trends and meteorologic effects.

7.6.5.1 Assessment of O_3 Effects after Adjusting for Temporal Trends and Meteorologic Effects

The relationship between O_3 and health outcomes are significantly affected by temporal trends and meterological factors, namely temperature. Analyses of the association between health outcomes and O_3 concentrations using raw data, therefore, can be misleading. In an analysis of Madrid, Spain data by Díaz et al. (1999), a U-shaped relationship was observed between mortality and O_3 concentrations, with an associated minimum at 35 $\mu g/m^3$

(approximately 18 ppb) for 24-h avg O_3. The negative portion of the slope is likely due to the opposing seasonal cycles in mortality (high in winter) and temperature (low in winter). However, little is discussed about the interpretation of the fitted morbidity and mortality "effects" of temperature. See, for example, Figure 7-18, which shows the fitted nonaccidental mortality as a function of natural spline smoothing of mean temperature in Montreal, Quebec (Goldberg and Burnett, 2003). The positive slope of the temperature-mortality relationship is fitted most tightly in the mild temperature range in which we do not expect mortality effects of temperature. It is possible that temperature has mortality effects in the mild temperature range, however because daily fluctuations of air pollution, especially O_3, are strongly influenced by weather conditions, ascribing the association between temperature and mortality entirely to temperature effects may underestimate the effects of air pollution.

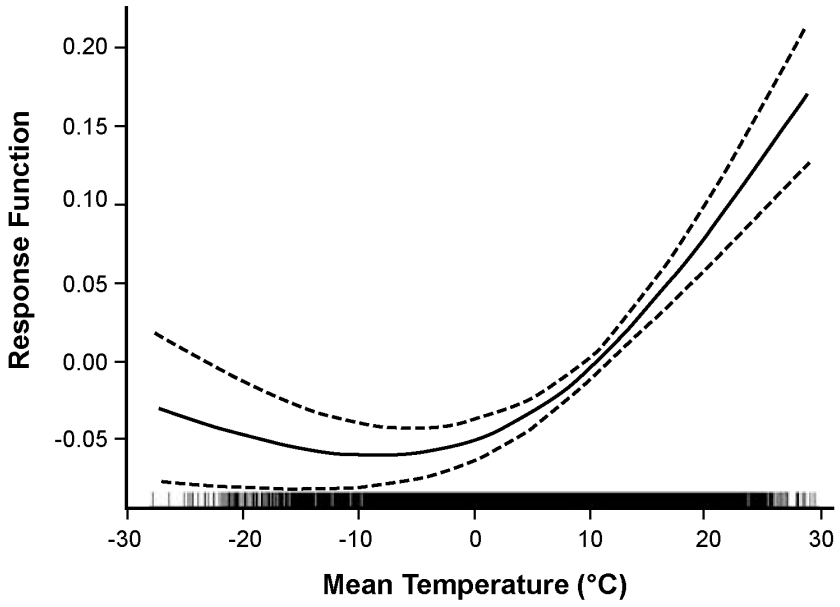


Figure 7-18. Daily nonaccidental mortality in Montreal, Canada as a function of mean temperature, using natural splines with two degrees of freedom.

Source: Goldberg and Burnett (2003).

A 2003 HEI report investigated the impact of the selection of GAM convergence criteria to adjust for temporal trends and weather variables in PM time-series studies (HEI, 2003). These sensitivity analyses included the use of varying degrees of freedom for smoothing terms to adjust for temporal trends in the Poisson regression model. Sensitivity analyses specifically for O_3 effects have not been performed, with the exception of one new study. In the U.S. 95 communities data, Bell et al. (2004) performed a sensitivity analysis of the O_3 excess mortality risk estimates to tripling the degrees of freedom for smoothing terms used to adjust for temporal trends. They found that varying the degrees of freedom from 7 to 21 per year did not significantly affect the O_3-mortality estimates, with effect estimates ranging from 0.82 to 1.08% excess risk per 20 ppb increase in 24-h avg O_3 during the previous week. Using more degrees of freedom in temporal trend fitting (i.e., controlling shorter temporal fluctuations) means ascribing more details of daily health outcomes to unmeasured potential confounders and possibly taking away real weather and air pollution effects. However, results from this large multicity study indicated that O_3 effects were robust to aggressive smoothing of temporary trends.

Temporal cycles in daily hospital admissions or emergency department visits are often considerably more episodic and variable than is usually the case for daily mortality. As a result, smoothing functions that have been developed and tuned for analyses of daily mortality data may not work as well at removing cyclic patterns from morbidity counts. Two methods are commonly used for season adjustment, and an important distinction exists in the manner in which these adjustments are applied in the analysis. The pre-adjustment method involves applying the adjustment to both outcome and air pollution variables prior to the regression analysis. In this case, the regression is done on the residuals following subtraction of smooth functions for each variable. The second method, co-adjustment involves applying the adjustment as part of the regression analysis, by fitting a function of time while simultaneously fitting the regression effect of air pollution and weather factors. These two approaches have been viewed as largely interchangeable. However, the co-adjustment approach may lead to biased air pollution effect estimates in cases where both outcome and pollution variables exhibit strong seasonal cycles. This was demonstrated using a 15-year time-series data of daily hospital admissions for acute respiratory diseases in children under 2 years old (Burnett et al., 2001). Note that this analysis used Poisson GAM with default convergence criteria. Pre-adjustment followed by regression analysis yielded a statistically significant estimate of 14.1% increase in

admissions per 40 ppb increase in 1-h max O_3 using year-round data. However, when the co-adjustment method was applied, there was a statistically significant 7.2% decrease in admissions. The authors suggested that the co-adjustment method allows O_3 to compete with the smoothing variable to explain some of the seasonal variability in the outcome, whereas pre-adjustment eliminates the seasonal variability prior to analysis of O_3 effects. Interestingly, when the authors limited the analysis to the warm season (May-August), both methods yielded similar results (32.3% versus 30.0% increase for co-adjustment and pre-adjustment, respectively) implying that stratification by season can remove a significant amount of the confounding seasonality. This finding may be important to consider in reviewing the acute O_3 mortality and morbidity literature since the vast majority of studies published over the past decade have used the co-adjustment method. However, the use of pre-adjustment versus co-adjustment in time-series studies is an unresolved issue. More empirical research in different locales is needed to evaluate the merits of these two methods as far as O_3 is concerned, and to determine what endpoints may be affected.

An interesting study by Schwartz (2004) examined the sensitivity of the O_3-mortality relationship to methods used to control for temperature. Using a case-crossover analysis, the effect of O_3 on mortality was examined in 14 cities across the U.S. from 1986 to 1993. Control days for an event were selected to be all other days from the same month of the same year. Initially, temperature lagged 0 and 1 day was controlled using nonlinear regression splines with 3 degrees of freedom each. In a comparison analysis, control days were restricted to a subset that was matched on temperature. The effect estimate for all year data was a 0.8% excess risk per 40 ppb increase in 1-h max O_3 in the analysis using nonlinear regression splines, compared to a 0.9% excess risk using temperature matched controls. The effect estimates from the two analyses were not significantly different. Results were similar when restricting analysis to warm season only data.

More sensitivity analysis of O_3 effect estimates to the extent of adjustment for temporal trends and meteorological factors is needed, but perhaps it is equally as important to evaluate the epidemiological adequacy of a given adjustment. For example, do the fitted mortality series sufficiently depict influenza epidemics? Or, when larger degrees of freedom (e.g., 12 degrees of freedom per year) are used, what "unmeasured" confounders, other than weather and pollution, are the investigators trying to adjust? Even in PM studies that conducted sensitivity analyses,

investigators rarely stated assumptions clearly, and not enough discussions were provided as to potential reasons for the sensitivity of results.

Given their relationship to health outcomes and O_3 exposure, adjusting for temporal trends and meteorologic factors is critical to obtain meaningful O_3 effect estimates. While the prevailing analytical approaches fit the data flexibly, the estimated effects of meteorologic variables and their impact on the adjusted O_3 effects are not adequately discussed. More work is needed in this area to reduce the uncertainty involved in the epidemiologic interpretation of O_3 effect estimates.

7.6.5.2 Importance of Season-Specific Estimates of O_3 Health Effects

Analysis of O_3 health effects is further complicated as the relationship of O_3 with temperature and with other pollutants appears to change across seasons. Such relationships can be observed in Figure 7-19 from a study by Moolgavkar et al. (1995). In this study, Moolgavkar et al. examined the relationship between daily mortality and air pollution (TSP, SO_2, and O_3) by season in Philadelphia, PA for the period of 1973 to 1988. During the summer, there was a positive relationship between O_3 and TSP, as well as O_3 and SO_2. In contrast, the relationship between O_3 and TSP, and O_3 and SO_2 inversed during the winter. Note that a greater range of O_3 concentrations was observed during the summer. The analyses indicated that while both TSP and SO_2 showed positive and significant associations with mortality in all four seasons in single-pollutant models, O_3 showed positive and significant associations only in the summer when the mean O_3 concentration was the highest (Figure 7-20). The O_3-mortality association was negative (though not significantly) in the winter when the mean O_3 concentration was low. The addition of TSP or SO_2 in the regression did not attenuate the O_3 effect estimate in the summer, and in the three-pollutant model in the summer, only O_3 remained significant. In another Philadelphia study by Moolgavkar and Luebeck (1996), analyzed using GAM with default convergence criteria but with only one nonparametric smoothing term, O_3 was also positively and significantly associated with mortality in the summer. A negative association between O_3 and mortality was observed during the winter in the single-pollutant model. With all pollutants (TSP, SO_2, NO_2, and O_3) included in the model, the O_3 effect in the summer remained significant. Both studies did not analyze the data for the year-round data set, therefore the relationship between the excess risk estimates for each season and the year-round data could not

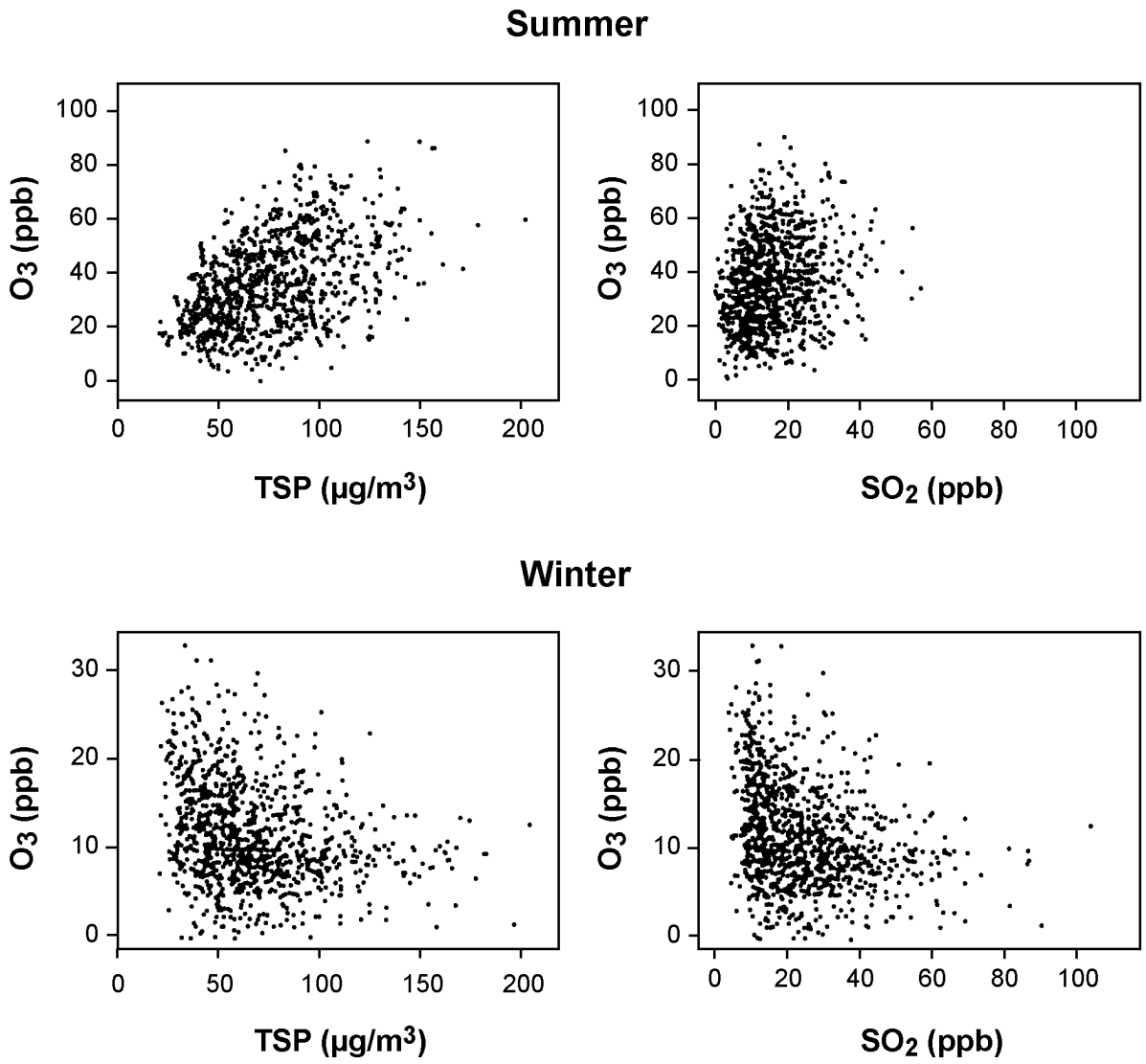


Figure 7-19. Scatterplots of daily levels of O_3 with TSP and SO_2 in Philadelphia, PA by season.

Source: Derived from Moolgavkar et al. (1995).

be compared. The results from these studies, however, suggest that year-round analyses may mask the positive (or negative) associations that may exist in particular seasons.

In the analyses of the U.S. 90 cities data by Samet et al. (2000; reanalysis Dominici et al., 2003), the focus of the study was PM_{10}, but O_3 and other gaseous pollutants also were analyzed in single- and multiple-pollutant models. In the reanalysis (Dominici et al., 2003), the combined

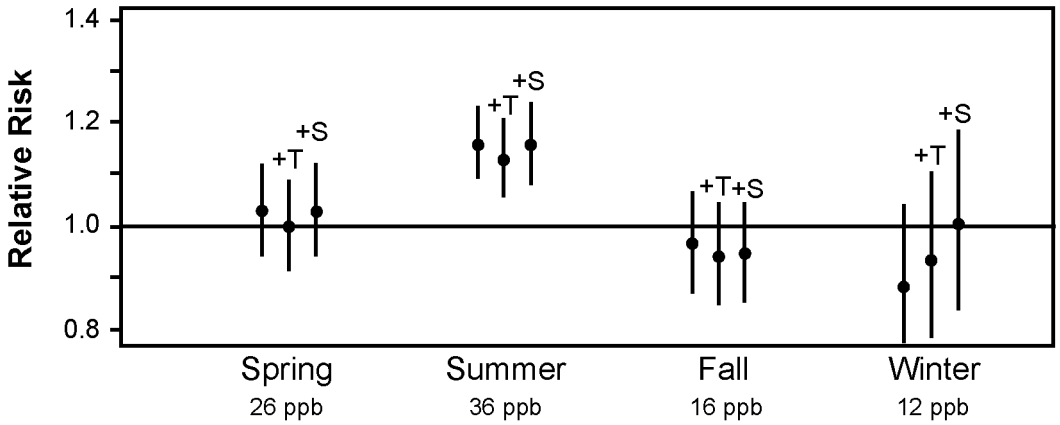


Figure 7-20. Estimated total (nonaccidental) mortality relative risk per 100 ppb increase in 24-h avg O_3 of reach seasonal data set. Within each season, the left-hand estimate is for O_3 alone; the estimate in the middle (+T) is with TSP, the right-hand estimate (+S) is with SO_2 in the model. Seasonal mean O_3 concentrations are noted.

O_3-mortality estimate for all seasons, summer only, and winter only analyses were all statistically significant at a lag of 1 day (see Figure 7-21). However, while an excess risk in mortality was observed for all seasons and summer only analyses, a negative estimate was obtained for the winter only analysis. It should be noted that, Samet et al. and Dominici et al.'s analyses used a weather model specification that is more detailed than other studies in that it had multiple terms for temperature and dewpoint (these two variables are generally highly correlated). Thus, it is possible that the high concurvity of O_3 with these weather covariates may have produced these conflicting results. Another possibility is that, as mentioned previously, the negative correlation between O_3 and PM and other primary pollutants may have produced the apparent negative relationship between O_3 and mortality in the winter (note that PM and mortality were positively associated). In the similar U.S. 95 communities study by Bell et al. (2004), analyses with only winter data were not performed, however, both the all year and summer only analyses indicated statistically significant positive risk estimates (1.04% and 0.78% excess risk per 20 ppb increase in 24-h avg O_3, respectively, using a constrained distributed 7-day lag model).

Anderson et al. (1996) examined the relationship between air pollution (O_3, NO_2, BS, and SO_2) and daily mortality (all cause, cardiovascular, and respiratory) in London, England for the

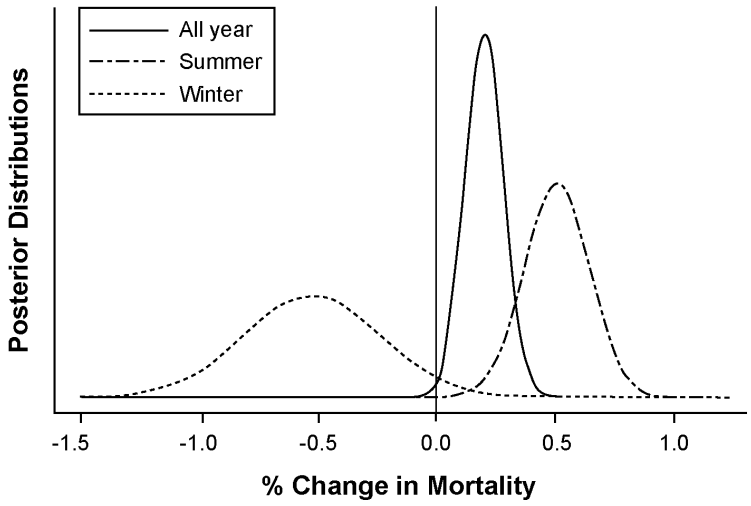


Figure 7-21. Marginal posterior distributions of the national average estimates of O_3 effects on total mortality per 10 ppb increase in 24-h avg O_3 at a 1-day lag for all year, summer (June-August), and winter (December-February) analyses in 90 U.S. cities.

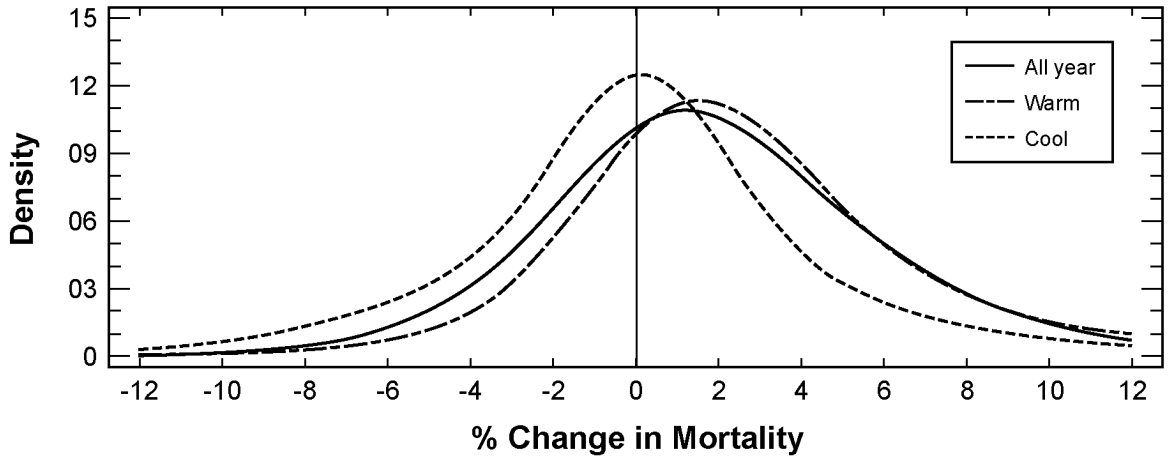
Source: Derived from Dominici et al. (2003).

study period of 1987 to 1992 using a Poisson GLM model. They examined the associations using data from all year, as well as the warm season (April-September) and the cool season (October-March) separately. Their results indicated that the estimated O_3 relative risks were larger in the warm season than in the cool season for all cause mortality. The percent excess risk estimated per 30 ppb increase in 8-h avg O_3 (9 a.m.-5 p.m.) was 3.05% (95% CI: 1.39, 4.7), 4.37% (95% CI: 2.17, 6.62), and 0.96% (95% CI: −1.10, 3.06) for all year, warm season, and cool season, respectively. A similar pattern was seen for cardiovascular mortality, but the estimated risk was negative (not significantly) for the cool season. For respiratory mortality, the estimated excess risks were similar between the cool and warm seasons. Many other studies also reported larger excess mortality risks in the warm (or summer) season than in the cool (or winter) season (see Figure 7-12 in Section 7.4.4). These studies showed cool season risk estimates that were either smaller compared to warm season estimates or slightly negative (but not significant). Of the studies that analyzed data by season, only one study in Pittsburgh, PA (Chock et al., 2000) showed negative risk estimates in the summer.

The studies that observed larger (positive) associations between O_3 and mortality in warm seasons are consistent with the expectation that O_3, if harmful, should have a stronger association with health outcomes in the summer when concentrations are higher. However, the negative O_3-mortality associations seen in the winter in the U.S. 90 cities study (Samet et al., 2000; reanalysis Dominici et al., 2003) and Philadelphia, PA data (Moolgavkar et al., 1995) suggest that further examination of this issue is required. Specifically, if the O_3 level in the winter is shown to be negatively associated with factors (e.g., PM) that are positively associated with mortality, then these potentially spurious negative O_3-mortality associations can be explained. Several examples of this phenomenon also exist in morbidity studies investigating the effect of O_3 on daily hospital admissions and emergency department visits (Anderson et al., 1998; Burnett et al., 2001; Prescott et al., 1998; Thompson et al., 2001). A study by Thompson and colleagues (2001) in Belfast, Northern Ireland observed a significant decrease in emergency department visits for childhood asthma in the cold season (November-April), but not in the warm season. Ozone concentrations were found to be inversely related to benzene levels ($r = -0.65$). After adjusting for benzene levels, there was no significant association between O_3 and asthma emergency department visits.

Unlike the time-series studies examining outcomes of mortality, hospital admissions, and emergency department visits, most acute field studies did not perform year-round analyses. These acute field studies that examined the relationship between O_3 and lung function, respiratory symptoms, and inflammation focused primarily on the O_3 effect during the warm season, when O_3 levels were expected to be high.

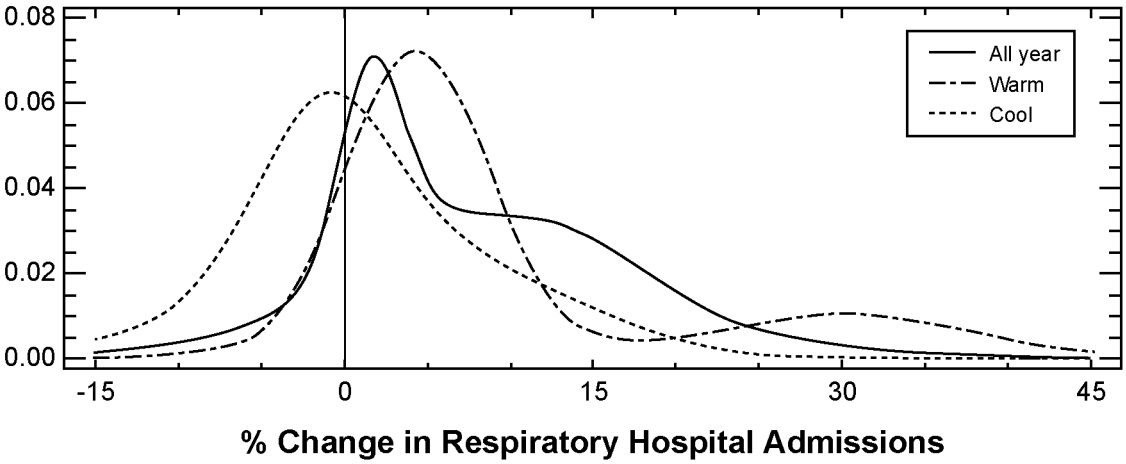
The potential influence of season on O_3 effect estimates was examined using summary density curves. The O_3 effect observed in all year data was compared to effects from warm season and cool season only data (Figures 7-22 and 7-23). Summary probability density curves (or summary density curves) were calculated to review the effect estimates from the various studies. To calculate the summary density curve, the normal distribution function first was determined for each effect estimate and corresponding standard error. Then the individual normal distribution functions were summed together to obtain the pooled normal distribution function. The summary density curve is calculated by taking the derivative of the pooled normal distribution function. Unlike a single normal density curve, the summary density curve is distribution-free and may be multimodal. The summary density curves shown in Figures 7-22



	All year	Warm season	Cool season
% area under the density curve and > 0	84%	89%	73%
Mean (SD) effect estimates	1.7% (4.4%)	2.4% (4.3%)	−0.0% (4.4%)
Mode effect estimates	1.3%	1.6%	0.2%

Figure 7-22. Summary density curves of the % change in all cause mortality for all year data and by season per 40 ppb increase in 1-h max O_3 or equivalent. Effect estimates from 14 studies have been included in the summary density curves (see Figure 7-12 in Section 7.4.4 for the effect estimates).

and 7-23 were smoothed by adding a constant to the standard error of each effect estimate in the calculation of the individual distribution functions. The constant is a default for normal distribution densities and is larger when the number of effect estimates is smaller, as presented by Silverman (1986). Since the normal distribution is unimodal, this constant will oversmooth when the density is multimodal. In Figure 7-22, the summary density curves representing the % all cause (nonaccidental) mortality associated with O_3 concentrations are presented (see Figure 7-12 in Section 7.4.4 for the effect estimates). The summary density curves were calculated using results from 14 studies that reported at least two of the three estimates. This figure indicates that 84% of the area under the density curve has a value greater than zero for all year data compared to 89% for warm season data and 73% for cool season data. Therefore, both all year and warm season data generally indicates a significant, positive O_3 effect. The mean effect



	All year	Warm season	Cool season
% area under the density curve and > 0	84%	88%	53%
Mean (SD) effect estimates	7.7% (8.8%)	9.4% (11.3%)	1.6% (7.3%)
Mode effect estimates	1.9%	4.3%	−0.8%

Figure 7-23. Summary density curves of the % change in total respiratory hospital admissions for all year data and by season per 40 ppb increase in 1-h max O_3 or equivalent. Effect estimates from six studies have been included in the summary density curves (see Figure 7-8 in Section 7.3.3 for the effect estimates).

estimate is a 1.7% excess risk in mortality per 40 ppb increase in 1-h max O_3 using all year data, compared to a slightly larger 2.4% excess risk using warm season data. The cool season only data indicates that there is no excess risk associated with O_3 concentrations.

Similar observations are made when examining the O_3 effect on total respiratory hospital admissions (Figure 7-23). Six studies provided season-specific estimates as well as all year results (see Figure 7-8 in Section 7.3.3 for the effect estimates). Once again, a large % of the area under the summary density curve is greater than zero when using all year and warm season data, 84% and 88%, respectively, compared to cool season data, 53%. The mean O_3 effect estimate also is slightly larger for warm season data only, 9.4% excess risk per 40 ppb increase in 1-h max O_3, compared to all year analyses, 7.7% excess risk. A small O_3 effect (1.6% excess risk) is observed when using cool season data only.

Integrating seasonal influences across the various health outcomes supports the view that O_3 effects are different in the cool and warm seasons, with greater effects observed during the warm season. As this relates to higher O_3 levels produced during the warm season, the larger effects are an appropriate conclusion. Therefore, these results indicate that warm season data should be used to derive quantitative relationships for the effect of O_3 on health outcomes. This conclusion is supported by epidemiologic researchers who focus on warm season data as an *a priori* design for the studies. The results also support a rationale to view the cool season as inappropriate to derive information from in regards to the level of effect. However, studying summer data only when all year data is available weakens the power of the study since less data is analyzed. In addition, increased adverse health outcomes are observed in the winter, some of which may be attributable to O_3. The O_3 effect in the winter time may be masked by the effects of PM due to the negative correlation between these variables (see Section 7.6.6.2 for further discussion). Therefore, analysis of all year data may be improved by adjusting for PM indices in addition to adequate adjustment of meteorological factors and temporal trends. The methods of statistical analysis, in addition to various other factors, need to be considered in the study design stage to choose the proper time period to examine the health effects of O_3. The data presented here may aid in making this choice.

Seasonality influences the relationship between O_3 and health outcomes as it may serve as an indicator for changing meteorologic factors, namely temperature, and copollutant concentrations. Given the potentially significant effect of season, O_3 effect estimates computed for year-round data need to be interpreted with caution. Small or no effects may simply reflect the cancellation of positive associations in the summer and negative associations in the winter, or the presence of confounding due to the strong seasonal character of O_3 concentrations.

7.6.6 Assessment of Confounding by Copollutants

Potential confounding by daily variations in copollutants is another analytical issue to be considered. With respect to copollutants, daily variations in O_3 tend to not correlate highly with most other criteria pollutants (e.g., CO, NO_2, SO_2, PM_{10}), but may be more correlated with secondary fine PM (e.g., $PM_{2.5}$, sulfates) measured during the summer months. Assessing the independent health effects of two pollutants that are somewhat correlated over time is problematic. If high correlations between O_3 and PM or other gaseous pollutants exist in a given

area, then disentangling their relative individual partial contributions to observed health effects associations becomes very difficult. The changing relationship between O_3 and other copollutants also is of issue. In some urban locations, the correlation between PM indices and O_3 is positive in the summer and negative in the winter. This section will further discuss the correlation between O_3 and copollutants and confounding of the O_3 effect by copollutants.

7.6.6.1 Relationship between Personal Exposure to O_3 and Copollutants

To be confounders of the association between O_3 and adverse health effects, copollutants must be associated with both O_3 exposure and the health outcome (Rothman and Greenland, 1998, p. 121). Many studies have shown that copollutants of O_3, namely PM, NO_2, SO_2, and CO, are associated with respiratory and, in some cases, cardiovascular health outcomes. In addition, ambient levels of these copollutants, measured at central monitoring sites, have been found to be highly correlated to ambient O_3 concentrations. However, few studies have examined the association between personal O_3 concentrations and personal exposures to other copollutants. In a scripted exposure study discussed earlier, Chang et al. (2000) examined the relationship between 1-hour personal O_3 and personal $PM_{2.5}$ levels in several microenvironments, including indoors, outdoors, and in vehicles. Chang et al. (2000) did not find a significant correlation between personal O_3 and $PM_{2.5}$ concentrations in any of the microenvironments, even after stratifying the data by season. In a Baltimore, MD study of susceptible populations (older adults, individuals with COPD, and children), Sarnat et al. (2001) found that ambient 24-h avg O_3 concentrations and ambient 24-h avg $PM_{2.5}$ levels were positively correlated ($r = 0.67$, $p < 0.05$) in the summer and negatively correlated ($r = -0.72$, $p < 0.05$) in the winter. However, no relationship was found between 24-h avg personal O_3 and personal $PM_{2.5}$ concentrations. Interestingly, a significant correlation also was observed between ambient O_3 and personal $PM_{2.5}$, with a mixed regression effect estimate of $\beta = 0.28$ ($t = 4.00$) in the summer and $\beta = -0.29$ ($t = -4.68$) in the winter. In contrast to the results from Sarnat et al. (2001), a study by Delfino et al. (2004) did not find a significant correlation between ambient 8-h max O_3 and ambient 24-h avg $PM_{2.5}$ ($r = 0.24$) concentrations during two warm sampling periods, August-October and April-June, in Alpine, CA. Personal PM measurements were taken using a nephelometer which responds mainly to PM in the 0.1 to 10 μm range, with the highest response in the fine PM range. Ambient 8-h max O_3 was not found to be correlated with personal 8-h max

PM (r = 0.03) or personal 24-h avg PM (r = 0.01). Personal O_3 exposure data was not available in this study. Another study by Delfino et al. (1996) found that personal 12-h avg O_3 levels were not associated with ambient $PM_{2.5}$ levels (r = 0.03) in a study of asthmatics (aged 9-16 years old) in San Diego, CA. These studies provide limited evidence for a lack of correlation between personal O_3 levels and personal exposure to PM.

7.6.6.2 Assessment of Confounding Using Multipollutant Regression Models

The multipollutant regression model often is used to determine whether the pollutant-specific effect is robust. However, due to the multicolinearity among O_3 and pollutants, and the changing correlation by seasons, multipollutant regression models may not adjust for potential confounding adequately, especially when using year-round data. Results from the U.S. 90 cities study (Samet et al., 2000; reanalysis Dominici et al., 2003), as depicted in Figure 7-24, indicated that PM_{10} risk estimates were robust to including O_3 and other gaseous pollutants in multipollutant models. In a similar analysis, the effect of copollutants on O_3-mortality risk estimates also were investigated in this dataset. While the addition of PM_{10} in the model did not substantially change the O_3-mortality risk estimate, a slight decline in the O_3 effect was observed (Figure 7-25). In the extended U.S. 95 communities study (Bell et al., 2004), the city-specific O_3-mortality effects were found to be robust to the adjustment for PM_{10}, as indicated by the nearly 1:1 ratio between estimates with and without PM_{10} adjustment shown in Figure 7-26. These results indicated that PM_{10} did not confound the association between O_3 and mortality in this large study. Limited data was available to examine the potential confounding effect of $PM_{2.5}$ on the O_3-mortality relationship. A weighted second-stage linear regression indicated that there was no association between long-term $PM_{2.5}$ average and the community-specific O_3-mortality effect estimate. Several other mortality and morbidity studies have investigated confounding of O_3 risk estimates using multipollutant models with year-round data, and most have reported that O_3 effects were robust to adjustment for copollutants (see Figures 7-9 and 7-13 in Sections 7.3.3 and 7.4.5, respectively).

Since the pollutant most correlated with O_3 in the summer is sulfate (which is in the fine particle size range), especially in the eastern U.S., the potential confounder of main interest for O_3 is $PM_{2.5}$ and sulfate in the summer. However, the results from two-pollutant regression models with O_3 and sulfate (or $PM_{2.5}$) should be interpreted with caution because both of these

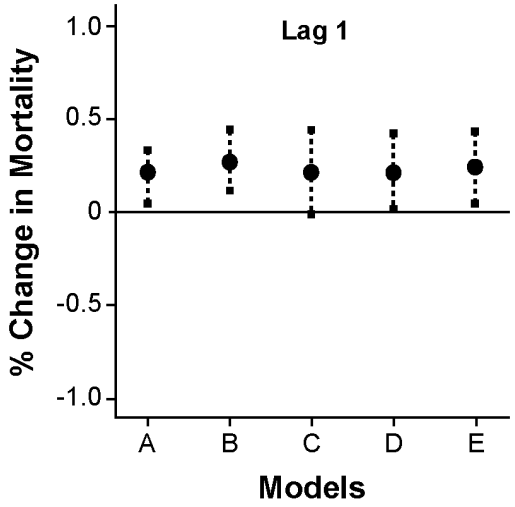


Figure 7-24. Posterior means and 95% posterior intervals of the national average estimate of PM_{10} effects on total mortality from non-external causes per 10 µg/m^3 increase in 24-h avg PM_{10} at a 1-day lag within sets of 90 U.S. cities with pollutant data available. Models A = PM_{10} only; B = PM_{10} + O_3; C = PM_{10} + O_3 + NO_2; D = PM_{10} + O_3 + SO_2; E = PM_{10} + O_3 + CO.

Source: Derived from Dominici et al. (2003).

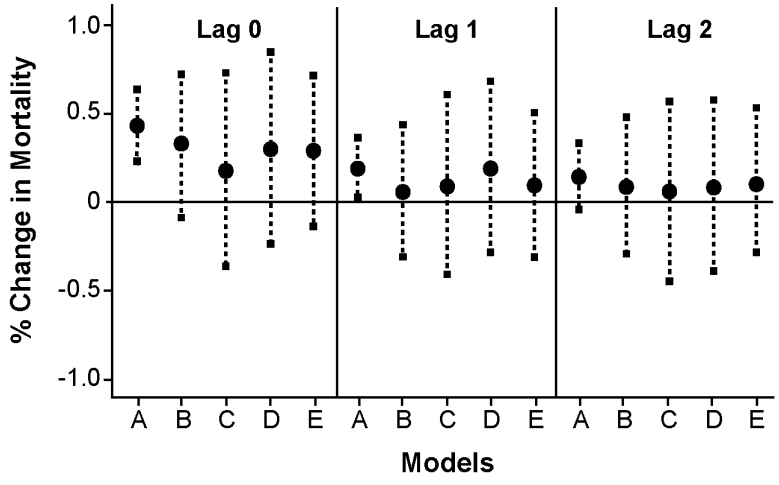


Figure 7-25. Posterior means and 95% posterior intervals of the national average estimate of O_3 effects on total mortality from non-external causes per 10 ppb increase in 24-h avg O_3 at 0-, 1-, and 2-day lags within sets of 90 U.S. cities with pollutant data available. Models A = O_3 only; B = O_3 + PM_{10}; C = O_3 + PM_{10} + NO_2; D = O_3 +PM_{10} +SO_2; E = O_3 + PM_{10} + CO.

Source: Derived from Dominici at al. (2003).

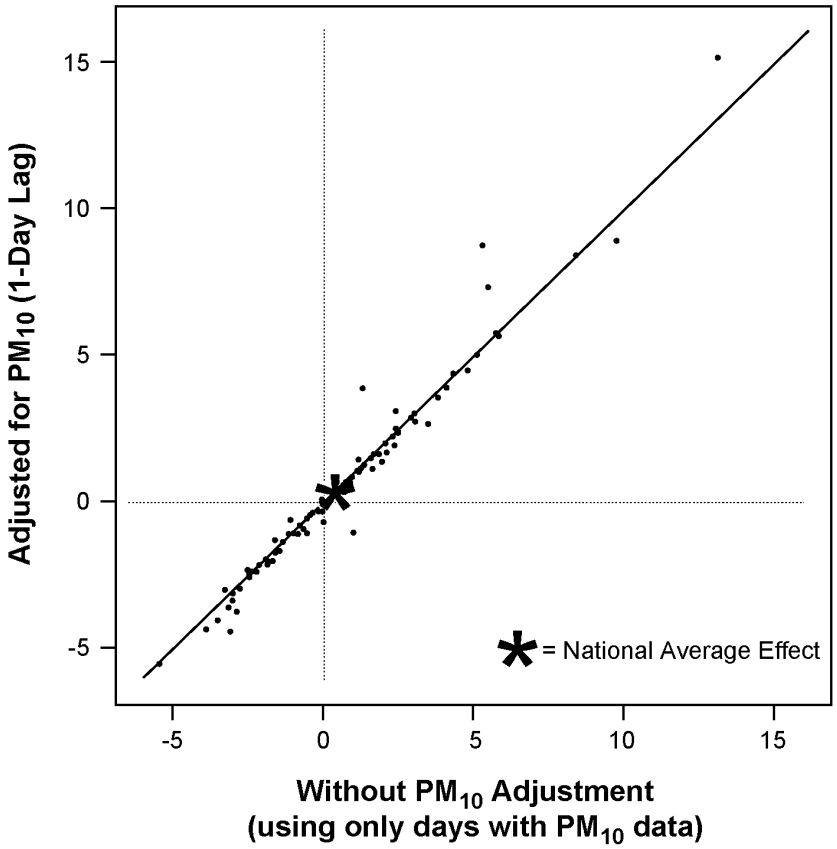


Figure 7-26. Maximum likelihood estimates of O_3-mortality for 95 U.S. communities, determined using a constrained distributed lag model for lags 0 through 6 days. Same dataset was used for O_3 estimates with and without adjustment for PM_{10}.

Source: Derived from Bell et al. (2004).

pollutants are formed under the same atmospheric condition and are both part of the "summer haze" pollution mix. A simple two-pollutant regression model does not address their possible synergistic effects, and the high correlation between the two pollutants may lead to unstable and possibly misleading results. In any case, most studies that analyzed O_3 with PM indices did not have $PM_{2.5}$ data and very few examined sulfate data. The studies that did have $PM_{2.5}$ data, including Santa Clara County, CA (Fairley, 1999; reanalysis Fairley, 2003), Philadelphia, PA (Lipfert et al., 2000a), and Detroit, MI (Lippmann et al., 2000; reanalysis Ito, 2003), examined copollutant models for year-round data only, but O_3 mortality risk estimates were not

substantially affected by the addition of $PM_{2.5}$. A mortality study by Lipfert et al. (2000a) also found that O_3 risk estimates were not affected by the addition of sulfate. Amongst the morbidity studies, the two summertime studies in Toronto, Canada by Burnett et al. (1997b, 2001) found that the O_3 effect was only slightly attenuated after including $PM_{2.5}$ in the model. In one of these studies (Burnett et al., 1997b), the effect of O_3 also was adjusted for sulfate. With the addition of sulfate in the model, the risk estimate for O_3 remained relatively stable, from an 11% excess risk to a 9% excess risk per 20 ppb increase in 12-h avg O_3 at a 1-day lag.

Other studies have estimated O_3 health risks with copollutants in the model by season. Amongst the mortality studies (see Figure 7-14 in Section 7.4.5), the O_3 risk estimates in the warm season were mostly positive and significant, with the exception of the Pittsburgh, PA analysis by Chock et al. (2000). Adjusting for copollutants, in particular PM indices, did not substantially change the O_3-mortality effect estimates, with both slight reductions and increases observed in the adjusted estimates. In the analysis using cool season data only, the O_3 effect estimates were generally negative, but none were statistically significant. In contrast to the analyses of warm season data, the O_3 risk estimates all increased slightly with the adjustment of PM indices. The inverse relationship between O_3 and PM during the cool season most likely influenced the O_3-mortality effect estimates in the single-pollutant models. Thus, the confounding effect by PM indices appears to vary by season, most likely due to the changing relationship between O_3 and PM by season. These results indicate that although PM does not seem to influence significantly the association between O_3 and mortality during the warm season, PM may be a confounder of the O_3-mortality relationship in the cool season.

A study of respiratory hospitalizations in 16 Canadian cites by Burnett et al. (1997a) also stratified O_3 risk estimates by season. A preliminary analysis studying O_3 effects found that a positive association between O_3 and respiratory hospitalizations was observed in the spring, summer, and fall, but not in the winter. In an analysis restricted to warmer months (April-December), the pooled O_3 risk estimates for all cities were significant but attenuated with the addition of copollutants and dewpoint temperature into the model. Of the 16 Canadian cities, Montreal and Vancouver showed no association between O_3 and respiratory hospitalizations after adjusting for dewpoint temperature. After the exclusion of these two cities, the O_3 risk estimates were found to be robust with the addition of copollutants in the models. Two additional respiratory hospitalization studies in the metropolitan Toronto, Canada area by

Burnett et al. (1997b, 2001) also observed consistent O_3 risk estimates with the inclusion of copollutants. The analyses in both studies were restricted to warm months (May-September).

In field studies, power to assess independent O_3 effects may be limited by small sample sizes and short follow-up times. Among the field studies, the O_3 effect also was found to be robust to the addition of copollutants in multipollutant models, with a few exceptions. For example, the effect of O_3 on PEF was not robust to adjustments for $PM_{2.5}$ and sulfate, in studies by Romieu et al. (1996) and Neas et al. (1999). In general, however, O_3 effects on respiratory symptoms (Romieu et al., 1996), lung function parameters (Brauer et al., 1996, Gold et al., 1999), and asthma medication use (Gent et al., 2003) were robust to inclusion of $PM_{2.5}$. Often, the effects for O_3 were found to be stronger than those for PM.

Multipollutant regression analyses indicated that O_3 risk estimates were not sensitive to the inclusion of copollutants, including $PM_{2.5}$ and sulfate, in both year-round and warm season data. These results suggest that the effect of O_3 on respiratory health outcomes appears to be robust and independent of the effects of other copollutants. However, there is concern as to whether analysis of the O_3 effect on health outcomes is confounded by PM indices in the cool season. In addition, uncertainty remains as to the use of multipollutant regression models to assess the independent health effects of pollutants that are correlated.

7.6.7 Issues of Model Uncertainty and Multiple Hypothesis Testing

Epidemiologic studies that investigated the association between O_3 and various health outcomes often found a significant effect. A major concern is whether these significant associations are an artifact of model selection resulting from multiple hypothesis testing. Testing multiple hypotheses may, at times, be appropriate. For example, developing several hypotheses *a priori* allows researchers to explore more thoroughly potential mechanisms for an O_3-related health effect. Sensitivity analyses, which are critical for model validation, also use multiple hypothesis testing. The basic issue with multiple hypothesis testing is that an extremely large number of models are possible, any of which may turn out to give the best statistical "fit" of a given set of data. Including all potentially confounding variables into the model is not practical as this may result in overfitting the model and inflated standard errors. On the other hand, selection of one "best" model ignores the uncertainty involved in model selection and leads to an underestimation of the error. Akaike Information Criterion and Bayes Information

Criterion are some of the statistical methods used to assist in model variable selection. Recent attention has focused on Bayesian model averaging as an efficient method to incorporate model uncertainty into decision-making.

A few authors have applied Bayesian model averaging to study the effect of air pollution on mortality. Clyde et al. (2000) and Clyde (2000) used Bayesian model averaging to analyze the relationship between mortality and PM concentrations from Phoenix, AZ and Birmingham, AL, respectively. In addition to the uncertainty of effect estimation, Bayesian model averaging incorporated uncertainty regarding the choice of confounding variables, pollutants, and lags. In the Phoenix, AZ study, Clyde et al. (2000) did not observe a $PM_{2.5}$ effect on mortality, but did find that coarse PM ($PM_{10-2.5}$) was significantly associated with increased mortality. In a reanalysis of the Birmingham, AL study (original analysis Schwartz, 1993), Clyde (2000) observed that the PM_{10} effect originally estimated by Schwartz was plausible but Bayesian model averaging results supported a smaller risk estimate. However, Clyde (2000) noted that her analysis of the Birmingham data did not take into consideration factors that might bias the estimated effect toward the null. For example, measurement error in the exposure variables were not considered. In addition, the Poisson model (similar to many other regression models) assumed that all individuals in a population had equal risks, including potentially susceptible populations such as those with respiratory illnesses and outdoor workers.

Only one study using Bayesian model averaging reported a coefficient for O_3-related mortality. Koop and Tole (2004) used Bayesian model averaging to analyze the effect of various air pollutants, including O_3, SO_2, CO, NO, NO_2, $PM_{10-2.5}$, and $PM_{2.5}$, on mortality in Toronto, Canada. Current values and up to 3-day lags were considered. In addition, a comprehensive set of meteorological variables were included in the models. The 50+ explanatory variables required the fitting of an enormous number of potential models. Although the point estimates for all pollutants were positive, very small effects were found. Sixty-six percent of the PM data used to calculate these effect estimates were imputed. Ozone data was collected daily, eliminating the need for imputation. For O_3, the cumulative effect on non-accidental deaths was an excess of 0.054 deaths (posterior SD 0.159) per one standard deviation (9.15 ppb, 24-h avg O_3) increase in O_3 levels. The most probable O_3 model estimated the same-day effect of O_3 on mortality to be a statistically significant 0.526 (posterior SD 0.176) excess deaths. However, this most probably model received only 0.23% of the probability. Koop and Tole concluded that the

standard error of the cumulative effect was much too large to base policy advice. However, in the context of the many interaction terms, meteorological variables, smoothing surfaces, and the relatively loose posterior distribution, it is likely that Koop and Tole have overestimated the variance of their pollution coefficients.

Model diagnostics may be a way to reduce model uncertainty (George, 1999 in comments to Hoeting et al., 1999). However, Hoeting et al. state that model diagnostics is often based upon methods that use multiple testing. They believe that diagnostics should be used first to suggest better models and Bayesian model averaging should be used later to compare all models. The models considered should have "appreciable likelihood" or be excluded from Bayesian model averaging (George, 1999). A problem with Bayesian model averaging occurs when variables are highly correlated. When this occurs, the estimated posterior effects may be diluted, resulting in smaller coefficients (George, 1999). George believed that the issue of dilution could be addressed by altering the prior probabilities used. In their reply to George, Hoeting et al. stated that dilution is a problem when the highly correlated variables act by the same mechanism and may serve as surrogates for the same variable, which may be the case for air pollutants.

While Bayesian model averaging can theoretically be used to take into account uncertainty, claims of causality based on observational studies may be highly sensitive to the choice of prior distributions and class of models under consideration (Clyde et al., 2000). Additional research in this area may provide new and interesting insights into the issues of model uncertainty and multiple hypothesis testing.

7.6.8 Concentration-Response Function and Threshold

An important consideration in determining whether a safe level of O_3 can be identified is whether the concentration-response relationship is linear across the full concentration range or instead shows evidence of a threshold. Of particular interest is the shape of the concentration-response curve in the vicinity of the current 8-h NAAQS for O_3 of 80 ppb. The O_3 concentration-response relationship has been explored in several studies.

To examine the shape of the concentration-response relationship between O_3 and mortality, Gryparis et al. (2004) used meta-smoothing to combine smooth curves across the 23 European cities in a hierarchical model. For the summer period, the estimated concentration-response

curve did not appear to deviate significantly from linearity within the range of O_3 concentrations commonly observed in European cities.

In the U.S. 95 communities study (Bell et al., 2004), effect estimates calculated using only days with 24-h avg O_3 levels less than 60 ppb were compared to those using all data. At a lag of 1 day, O_3 was associated with an excess risk of 0.36% per 20 ppb increase in 24-h avg O_3 using data from all days and only a slightly smaller risk of 0.30% when data was limited to days less than 60 ppb. These results suggest that if there is a threshold, it is present at 24-h avg O_3 levels below 60 ppb. Fairley (2003) reanalyzed the Santa Clara County mortality data using GAM with stringent convergence criteria and examined a new exposure index for O_3. He noted O_3 concentrations exceeding 60 ppb each hour and calculated a daily sum of these exceedances. Fairley's index incorporates measures of concentration and exposure duration. This type of index is called a linear time-integrated concentration, also known as dosage. The O_3 index with the 60 ppb threshold level was found to be significantly associated with mortality in single-pollutant models as well as in multi-pollutant models. Two other threshold levels were examined, 40 ppb and 80 ppb. Both produced statistically significant results in single-pollutant models. These results indicate that the threshold for O_3-mortality effects is less than 40 ppb. The implication for thresholds in terms of the three standard indices (i.e., 1-h max, 8-h max, and 24-h avg) is unclear, but there may be an empirical relationship.

Vedal et al. (2003) observed that the annual mean 1-h max O_3 concentration of 27.3 ppb in Vancouver, Canada, was lower than that in any of the 90 NMMAPS cities (Samet et al., 2000), thus a study in this city may be able to better focus on the shape of the concentration-response curve at lower levels. In this Vancouver study, a statistically significant O_3 effect was observed on total mortality at a 0-day lag during the summer. Statistically significant effects on respiratory mortality at a 2-day lag and marginally significant effects on cardiovascular mortality at a 0-day lag also were observed for O_3 in the summer. The O_3 effect on mortality was found to be robust in two-pollutant models. Vedal et al. (2003) questioned if O_3, other gaseous pollutants, and PM were acting as surrogate markers of pollutant sources that contain more toxic compounds, as the low measured concentrations were unlikely in their opinion to cause the observed effects. They further stated that measurement error and interference by meteorological factors might have contributed to the inability to detect a threshold. Vedal et al. (2003) concluded that O_3 concentrations were associated with adverse effects on mortality even at low

levels. Although this study supports the argument that there is no threshold concentrations below which adverse effects cannot be detected, the results must be interpreted with caution as concerns remain.

Kim et al. (2004) investigated the presence of a threshold in O_3-mortality effects in Seoul, Korea by analyzing data using a log linear GAM (linear model), a cubic natural spline model (nonlinear model), and a B-mode splined model (threshold model). Models were stratified by season and adjusted for PM_{10}, long-term time trend, and meteorological variables. An estimated threshold value of 47 ppb was observed for 1-h max O_3. None of the other pollutants examined, including PM_{10}, SO_2, NO_2, and CO, had a nonlinear association with mortality. Using summer data only, the B-spline model resulted in an excess mortality risk of 7.1% (95% CI: 3.1, 11.2) per 40 ppb increase in 1-h max O_3, compared to an excess risk of 3.6% (95% CI, 0.5, 6.8) calculated using the log linear model. If a threshold truly exists, results from the Kim et al. (2004) study suggest that the use of log-linear models may underestimate the O_3 effect on mortality at levels above the threshold.

In London, England data (Anderson et al., 1996), an adjusted O_3-mortality plot indicated a possible threshold level around 50 ppb for 8-h avg O_3. A study by Simpson et al. (1997) in Brisbane, Australia observed a significant excess risk in mortality only in the highest quintile of O_3 exposure, which had a mean concentration of 42 ppb for 1-h max O_3. One study by Lipfert et al. (2000b) examined the presence of a threshold in the effect of chronic O_3 exposure on mortality in U.S. veterans. A simple concentration-response plot comparing the risk estimate in the upper two tertiles to that from the lowest tertile seemed to indicate a threshold level of approximately 140 ppb of 1-h max O_3 during the period 1975 to 1981 for both concurrent mortality (1975-1981) and delayed mortality (1982-1988).

Among several studies with morbidity outcomes, examination of the shape of the concentration-response function indicated evidence of an effect threshold. In a study of all-age respiratory hospital admissions in Toronto, Canada, effects of O_3 appeared to become apparent only above approximately 30 ppb daily 1-h max O_3 (Burnett et al., 1997b). In London, England, Ponce de Leon et al. (1996) observed an indication of a threshold in the O_3 effect on hospitalizations at 40 to 50 ppb for 8-h max O_3 and 50 to 60 ppb for 1-h max O_3. In a study of emergency department visits for asthma in St. John, Canada, effects observed in the over 15 years age group were apparent only when data above the 95th percentile (75 ppb daily 1-h max

O_3) were included (Stieb et al., 1996). However, other morbidity studies observed a monotonic increase in the concentration-response function, suggesting that there was no threshold in O_3 effects on hospitalizations and emergency department visits (Burnett et al., 1997a; Jaffe et al., 2003; Petroeschevsky et al., 2001; Tenías et al., 1998).

In a field study by Mortimer et al. (2002), the association of ambient O_3 levels with PEF and asthma symptoms was investigated in eight urban cities in the U.S. The mean 8-h avg O_3 was 48 ppb, with less than 5% of days exceeding 80 ppb. Analysis performed using all data indicated that a 15 ppb change in 8-h avg O_3 was associated with statistically significant decrements in PEF (−0.59% [95% CI: −1.05, −0.13]) and increased incidence of respiratory symptoms (odds ratio of 1.16 [95% CI: 1.02, 1.30]) over multiday lag periods. When data was restricted to days when ambient O_3 concentrations were less than 80 ppb, the O_3 effects persisted, with a significant PEF decline (−0.70% [95% CI: −1.29, −0.12]) and incidence of morning symptoms (odds ratio of 1.17 [95% CI: 1.01, 1.35]). A study by Chen et al. (1999) also found that there was no clear threshold in the O_3 effect on FEV_1 and FVC in Taiwanese school children.

Note that adjusting for seasonal cycles does not address the issue of the changing relationship between O_3 concentrations and personal exposure across seasons. The ambient O_3 levels are lower in the cold season, but people are likely to be exposed to even lower levels of O_3 in cold seasons due to the shorter time spent outdoors and the longer time spent indoors with closed windows. This is in contrast to what occurs with fine particles, which can effectively penetrate the indoors. Thus, a more "accurate" concentration-response relationship may need to be examined in a summer-only data set (which may suffer low data density in the low concentration range). Even for summer data, however, an interpretation of the relationship is not straightforward because of the possible influence of the use of air conditioning (an effective remover of O_3). Greater use of air conditioning would be expected on hot days when the O_3 level is higher, but the use of air conditioning may also vary from city to city and across social class within a city. These complications make it difficult to examine the existence of a threshold of O_3 health effects in the observational data.

Limited studies have examined the issue of thresholds in O_3 health effects studies. Some studies have found a low level threshold while others have found no threshold in O_3 effects. An absence of a detectable threshold in population studies does not indicate an absence of

individual thresholds. For a further discussion on thresholds in air pollutant health effects, see Section 8.4.7 in the 2004 PM AQCD. While no conclusion can be made regarding the threshold issue, the limited evidence shows that the possible threshold level may be well below the current standards. The distribution of thresholds, particularly around the NAAQS value of 80 ppb for 8-h max O_3, needs to be further investigated.

7.6.9 Spatial Variability in O_3 Effect

As described in Chapter 3 of this AQCD, O_3 concentrations tend to be more spatially variable than $PM_{2.5}$ concentrations in urban areas. In addition, relative personal exposures to O_3 likely vary by region. This spatial variability in O_3 concentrations and personal exposures may contribute to the heterogeneity in observed O_3 health effects. More than 80% of the O_3-mortality estimates from the various studies conducted in North America, South America, Europe, and Australia were between 0 and 7% excess risk per 40 ppb increase in 1-h max O_3 using year-round data. In general, the O_3-mortality estimates were greater when using summer only data compared to year-round data. Though not all statistically significant, most of the O_3-mortality estimates were greater than zero, indicating a positive relationship between O_3 exposure and mortality. The O_3 risk estimates from the numerous hospitalization and emergency department visit studies were generally larger in magnitude and more variable from study to study compared to the mortality studies. These differences in the O_3 effect estimates may be attributable to the greater variability in the outcome measure, such as more subcategories of outcome and varying degrees of severity, in hospitalization studies compared to mortality studies.

As differences in study design, population, and data analysis may affect risk estimates, studies that were conducted in multiple cities using standardized methods were further examined to investigate the spatial heterogeneity of O_3 effects. Bell et al. (2004) conducted a time-series analysis of O_3 and mortality in 95 U.S. communities from 1987 to 2000. A 10 ppb increase in O_3 in the previous week was associated with a statistically significant increase of 0.52% excess risk of mortality in the pooled analysis of 95 communities. Although some heterogeneity was observed among the communities (previously shown in Figure 7-11 of Section 7.4.3), the range of the community-specific effect estimates were fairly narrow. Of the 95 U.S. communities, 93 had positive O_3-mortality risk estimates. Only 5 had risk estimates greater than 1% per 10 ppb

increase in 24-h avg O_3 during the previous week, with all communities indicating an excess mortality risk less than 2%.

Greater heterogeneity was observed in the European study of 23 cities in 14 countries (Gryparis et al., 2004). In the year-round analyses, only 8 of the 23 cities had positive O_3-mortality effect estimates. However, in the analyses using summer data only, the risk estimates were positive in 19 of the 23 cities, with a range of 0.8 to 8% excess risk per 40 ppb increase in 1-h max O_3. The heterogeneity may be attributable to the considerable variability among countries in factors that may influence the relationship between ambient O_3 concentrations and personal exposure to O_3, such as climate, use of air conditioning, personal activity patterns, and socioeconomic factors. In addition, the variability in the concentration and composition of co-existing pollutants by cities or countries may contribute to the heterogeneity in the O_3-mortality effects. For example, concentrations of NO_2 may vary widely by region, depending on the differences in traffic density.

Among the hospitalization studies, Burnett et al. (1997a) conducted the largest multicity study of 16 Canadian cities. The mean daily 1-h max O_3 was 31 ppb in the 16 cities. The pooled O_3 estimate was 5.6% (95% CI: 3.4, 7.9) excess risk in respiratory hospitalization per 40 ppb increase in 1-h max O_3 using warm season data (April to December). The risk estimates were fairly homogenous across the 16 Canadian cities, ranging from 3.1% for Vancouver to 7.7% for Quebec City.

Anderson et al. (1997) investigated the association between O_3 and hospital admissions for COPD in five European cities, London, Paris, Amsterdam, Rotterdam, and Barcelona. The pooled risk estimate was 5.0%, 4.7%, and 3.5% excess per 30 ppb increase in 8-h max O_3 for year-round, warm season, and cool season data, respectively. Results from the APHEA study showed similar variability to that from the Burnett et al. (1997a) study. The year-round excess risk estimates were lower in the two Dutch cities, 3.0%, compared to that in Paris, 9.8%. In general, however, there was no significant evidence of heterogeneity in the O_3 effects among the five European cities.

Among the field studies, various respiratory health outcomes were examined, including PEF, spirometric parameters, respiratory symptoms, and medication use. Only one field study investigated the O_3 effect in several locations (Mortimer et al., 2002). Mortimer et al. (2002) investigated the association between ambient O_3 concentrations, and PEF and asthma symptoms

in asthmatic children living in eight urban cities in the U.S. - St. Louis, MO; Chicago, IL; Detroit, MI; Cleveland, OH; Washington, DC; Baltimore, MD; East Harlem, NY; and Bronx, NY. In the analysis pooling data from all eight cities, a 15 ppb increase in 8-h avg O_3 was associated with a significant decrement of −0.59% in morning PEF for a 5-day cumulative lag period. The % changes in PEF were negative in all cities except for Baltimore, 0.24%. Among the other seven cities, the % changes in PEF were quite homogenous, with values ranging from −0.54% for Washington, DC to −0.86% for St. Louis. A 15 ppb increase in 8-h avg O_3 also was associated with an increased incidence of morning symptoms in the pooled analysis (odds ratio of 1.16 for a 4-day cumulative lag period). In all cities except for St. Louis, there was an increase in the incidence of morning symptoms. The odds ratios for incidence of morning symptoms varied more by city compared to the PEF measurements, ranging from 1.09 for Chicago to 1.72 for Detroit. The greater variance in incidence of symptoms may indicate the lack of standardization in the use of symptoms as a health outcome measure.

Most of the multicity studies found consistent O_3 effect estimates for mortality, hospitalizations, and other respiratory health outcomes. The slight heterogeneity of O_3 effects may be partially attributable to the use of centrally located ambient monitors to assess exposure. There may be differences in relative personal exposures to O_3 due to varying factors, namely use of air conditioning and activity patterns, that affect the relationship between personal exposure and ambient concentrations. The variability in the concentration and composition of copollutants present also may contribute to the heterogeneity of the effect of O_3 on health outcomes as confounding by copollutants may vary by region.

7.6.10 Health Effects of O_3 in Susceptible Populations

In this section, the effects of O_3 on morbidity and mortality in potentially susceptible populations will be examined. In epidemiology studies of O_3 health effects, the most widely studied subpopulation was asthmatics. Also of interest were the observed health effects of O_3 on different age groups, particularly children and the elderly. This section begins with a discussion of the O_3-related health effects in asthmatics.

7.6.10.1 Health Effects Associated with Ambient O_3 Exposure in Asthmatics

Epidemiological studies of health effects from acute O_3 exposure in asthmatics have examined a range of outcomes: pulmonary function, respiratory symptoms, inflammation, emergency room visits, hospital admissions, and mortality. Chronic O_3 exposures have been associated with similar outcomes, with the exception of emergency room visits and hospitalizations. Both are discussed in the earlier text. This subsection draws together this information to examine whether the evidence indicates that O_3 exposure impacts asthmatics.

In Germany and Mexico City, O_3 exposure was associated with a decline in FEV_1 in asthmatic adults and children (Höppe et al., 1995a; Romieu et al., 2002). Change in FEV_1 also was examined in a group of asthmatic hikers in Mount Washington, NH (Korrick et al., 1998). Compared to the healthy subjects, the asthmatic subjects experienced a four-fold greater decline in FEV_1 with the same exposure to O_3. The results from the hiker study are consistent with those observed in controlled human exposure studies (discussed in Chapter 6), which also indicate significantly greater decrements in FEV_1 among mild asthmatics versus nonasthmatic subjects with heavy intermittent exercise.

PEF was examined in panels of asthmatics in several field studies (see Figures 7-1a and 7-1b). Collectively, all the studies indicated decrements of morning peak flow but most of the estimates were not statistically significant. One multicity study of eight urban areas in the U.S. observed reductions in morning PEF that were not significant in each individual city (Mortimer et al., 2002). However, the analysis combining data from all eight cities indicated a statistically significant change with a cumulative lag of 1 to 5 days. Further analysis showed that the incidence of $\geq$ 10% decline in morning PEF was statistically significant, which was discussed by the author as an indication that O_3 exposure may be associated with clinically significant changes in PEF in asthmatic children. The study examined 846 asthmatic children, the largest asthma panel study reported.

Respiratory symptom increases in asthma panels were examined in several field studies, some of which also examined PEF as discussed above. The health indicators examined varied among these studies and the analyses results were both negative and positive with a few being statistically significant. Collectively, they are suggestive of a potential effect on respiratory symptoms but the evidence in the available studies is not strong. Two U.S. studies examining larger panels may be better studies from which to draw inferences as the large sample size

provides greater power to examine the effect of O_3 on respiratory symptoms. The eight U.S. urban cities study mentioned above reported morning symptoms in the 846 asthmatic children to be most strongly associated with a 4-day cumulative lag period of O_3 concentrations (Mortimer et al., 2002). A New England study examined 271 asthmatic children and observed a significant O_3 effect on a variety of respiratory symptoms at a lag of 1 day among the 130 subjects who used maintenance asthma medications (Gent et al., 2003).

Few epidemiological studies have examined airway inflammation in asthmatics. A Mexico City study indicated that supplementation with antioxidants may modulate the impact of O_3 exposure on the small airways of children with moderate to severe asthma (Romieu et al., 2002). A related study indicated that asthmatic children with GSTM1 null genotype were found to be more susceptible to the impact of O_3 exposure on small airways (Romieu et al, 2004). An additional study in Mexico City examined DNA strand breaks in nasal epithelial cells in asthmatic and nonasthmatics medical students and noted greater genotoxic damage in asthmatics (Fortoul et al., 2003).

Emergency department visits for asthmatics have been examined in several studies and range from negative to positive results with limited analyses providing significant results (see Figure 7-6 in Section 7.3.2). Examination of the studies indicated that seasonal summer studies tended to yield positive outcomes, as expected based on earlier discussions. Two studies in Atlanta, GA (Tolbert et al., 2000) and Valencia, Spain (Tenías et al., 1998) indicated significant, positive effects in warm season analyses. Further, a Canadian study, one of the larger studies conducted in the summer season, reported a large significant increase in asthma emergency department visits when the daily 1-h max O_3 concentration exceeded 75 ppb (Stieb et al., 1996). A three-city study in Ohio also indicated a positive result during the summer (Jaffe et al., 2003). Other studies of mostly year-long data tended to produce nonsignificant results, which in some cases were negative (Atkinson et al., 1999a; Castellsague et al., 1995; Thompson et al., 2001; Tobías et al., 1999).

Hospital admission studies that specifically examined asthmatics were fewer in number than those that examined total respiratory diseases. Significant effects were noted in all age groups in studies conducted in Seattle, WA (Sheppard et al., 2003), New Jersey (Weisel et al., 2002), Toronto, Canada (Burnett et al., 1999), London, England (Anderson et al., 1998), Brisbane, Australia (Petroeschevsky et al., 2001), and Hong Kong (Wong et al., 1999a).

However, several other studies, mostly examining the effect on asthmatic children, did not observe a significant relationship (Gouveia and Fletcher, 2000a; Lin et al., 2003; Morgan et al., 1998; Nauenberg and Basu, 1999; Schouten et al., 1996).

Acute mortality related to asthma was examined in Barcelona, Spain (Saez et al., 1999; Sunyer et al., 2002). Severe asthmatics with more than one asthma emergency visit showed the strongest mortality associations with air pollutants, NO_2 being the most significant predictor followed by O_3 (Sunyer et al., 2002).

Recent reports from longitudinal cohort studies in California have reported associations between the onset of asthma and long-term O_3 exposures (Greer et al., 1993; McConnell et al., 2002; McDonnell et al., 1999). Significant associations were seen in males but not females (Greer et al., 1993; McDonnell et al., 1999). In six high O_3 communities, asthma risk was elevated for children who played three or more sports as compared with children who played no sports (McConnell et al., 2002). Playing sports may indicate outdoor activity and an increased ventilation rate which may lead to increased exposure. These outcomes would benefit from replication in other cohorts in regards to indicating weight of a causal interpretation.

A few studies provide limited discussion of concentration-response functions and thresholds. In the eight urban areas U.S. study, the odds ratios for incidence of $\geq$ 10% decline in morning PEF and incidence of morning symptoms when excluding days with 8-h avg O_3 greater than 80 ppb were nearly identical to those including data from all days (Mortimer et al., 2002) In the New England asthma panel study (Gent et al., 2003), some of the significant associations for symptoms occurred at 1-h max O_3 levels below 60 ppb. In the St. John, Canada study (Stieb et al., 2003), a significant effect of O_3 on emergency department visits was reported with evidence of a threshold somewhere in the range below a 1-h max O_3 of 75 ppb in the 15 years and over age group.

Overall, asthma subjects have been examined across most health endpoints of interest. The results reported in these studies range from negative to positive estimates with some indicating a significant positive excess risk associated with O_3. While no endpoint in itself seems to indicate an unquestionable demonstration of an association, studies with adequate sample size and understandable power consistently provide strong positive and significant results, especially during the summer months when higher O_3 levels occur. This view is strengthened as positive results are obtained cohesively across the varied outcomes. Therefore, based on the evidence it

seems prudent to consider asthmatics as a potentially susceptible group that requires protection from O_3 exposures.

A study by Niedell (2004) examined the relationship between air pollutants and asthma hospitalizations in California. The most recent EPA O_3 report (U.S. Environmental Protection Agency, 2004b) indicated that O_3 levels in the pacific southwest region had decreased by 9% from 1990 to 2003. This downward trend in O_3 levels was mostly influenced by the improvements in Los Angeles and other southern California metropolitan areas. As shown in Figure AX3-60 of the Chapter 3 Annex, O_3 concentrations decreased by over 30% in Los Angeles from 1992 to 1998. Results from this study noted declines in levels of air pollutants since 1992 and decreased asthma admissions in 1998 for children aged 1 to 18 years ranging from 5 to 14%, depending on the age group. The greatest decline (> 10%) in air pollution-related asthma admissions was observed among 3 to 12 year old children. Although this benefit analysis was not specific to O_3, it provides evidence of decreased morbidity resulting from reduced air pollutant concentrations, including O_3. Many studies have reported short-term associations between O_3 and morbidity outcomes, yet a largely unaddressed question remains as to the extent to which reductions in ambient O_3 actually lead to reductions in adverse health outcomes attributable to O_3. This question is not only important in terms of "accountability" from the regulatory point of view, but it is also a scientific question that challenges the predictive validity of statistical models and their underlying assumptions used thus far to estimate excess health effects due to ambient O_3.

7.6.10.2 Age-Related Differences in O_3 Effects

Several mortality studies have investigated age-related differences in O_3 effects. Among the studies that observed a significant association between O_3 and mortality, a comparison of all age or younger age (≤ 65 years of age) O_3-mortality risk estimates to that of the elderly population (> 65 years) indicates that, in general, the elderly population is more susceptible to O_3 effects (Borja-Aburto et al. 1997; Bremner et al., 1999; Gouveia and Fletcher 2000b; O'Neill et al., 2004; Simpson et al., 1997; Sartor et al., 1995; Sunyer et al., 2002). For example, a study by Gouveia and Fletcher (2000b) examined the O_3-mortality effect by age in São Paulo, Brazil. There were 151,756 deaths for all non-violent causes over the period of 1991 to 1993, of which 49% occurred in the elderly. Among all ages, O_3 was associated with a non-significant 0.6%

(95% CI: −0.8, 2.0) excess risk in all cause mortality per 40 ppb increase in 1-h max O_3. In comparison, in the elderly population, the O_3-mortality risk estimate was nearly three-fold greater, 1.7% (95% CI: 0.0, 3.3). Similarly, a Mexico City study found that O_3-mortality risk estimates were 1.3% and 2.8% per 20 ppb increase in 24-h avg O_3 concentration in all ages and the elderly, respectively (O'Neill et al., 2004).

The large U.S. 95 communities study (Bell et al., 2004) did not find evidence of significant heterogeneity in risk across three age groups, < 65 years, 65 to 74 years, and ≥ 75 years of age. Effect estimates were only slightly higher for those 65 to 74 years, 1.40% excess risk per 20 ppb increase in 24-h avg O_3, compared to individuals less than 65 years and 75 years or greater, 1.00% and 1.04%, respectively. However, Bell et al. (2004) noted that despite similar effect estimates, the absolute effect of O_3 is substantially greater in the elderly population due to the higher underlying mortality rates, which leads to a larger number of extra deaths for the elderly compared to the general population.

Few mortality studies examined another potentially susceptible age group, young children under the age of 5 years. The results were mixed, with one Mexico City study showing a lower risk of O_3-related all cause mortality in young children compared to all ages and the elderly (Borja-Aburto et al., 1997) and another study showing a greater risk in respiratory mortality in young children compared to the elderly (Gouveia and Fletcher, 2000b). It should be noted that approximately 10% of mortality occurred in young children, thus the statistical power to study the O_3 effect in this age group was limited.

With respect to age-specificity of associations between O_3 and acute respiratory hospitalizations or emergency department visits, no clear pattern emerges from recent studies. Significant associations have been reported for all ages (Anderson et al., 1997; Burnett et al., 1995, 1997b, 1999; Weisel et al., 2002), adults or elderly (Burnett et al., 1997a; Delfino et al., 1997, 1998; Moolgavkar et al., 1997; Schwartz et al., 1996; Yang et al., 2003), and children (Burnett et al., 2001; Gouveia and Fletcher, 2000a; Lin et al., 1999; Pönkä and Virtanen, 1996; Tolbert et al., 2000; Yang et al., 2003). Interestingly, studies that have examined effects in multiple age strata often have seen effects only in non-pediatric strata (Delfino et al., 1997, 1998; Stieb et al., 1996; Jones et al., 1995). Several studies that focused on children did not report significant O_3 effects, though in some cases these studies are limited by small size, inadequate control of seasonal patterns, or very low O_3 levels (Lierl and Hornung, 2003; Lin

et al., 2003; Thompson et al., 2001). If O_3 is causally related to exacerbations of respiratory diseases leading to hospital usage, one would expect to see effects most prominently among children, for whom asthma is most prevalent and exposures may be greater.

Many of the field studies focused on the effect of O_3 on the respiratory health of school children, however, none have compared the results from children to that in other age groups. In general, children experienced significant decrements in pulmonary function parameters, including PEF, FEV_1, and FVC (Castillejos et al., 1995; Chen et al., 1999; Gielen et al., 1997; Gold et al., 1999; Jalaludin et al., 2000; Mortimer et al., 2002; Romieu et al., 1996; Thurston et al., 1997), and some experienced increases in respiratory symptoms (Delfino et al., 2003; Gold et al., 1999; Neas et al., 1995; Romieu et al., 1996, 1997; Thurston et al., 1997) and asthma medication use (Delfino et al., 1996; Just et al., 2002; Ostro et al., 2001). These respiratory heath effects were observed in both healthy and asthmatic children.

Collectively, there is supporting evidence of age-related differences in susceptibility to O_3 health effects. The elderly population (> 65 years of age) appear to be at increased risk of O_3-related mortality and hospitalizations, and children (< 18 years of age) experience other potentially adverse respiratory health outcomes with increased O_3 exposure.

7.6.11 Summary of Key Findings and Conclusions Derived From O_3 Epidemiologic Studies

In the previous 1996 O_3 AQCD, there was considerable evidence of O_3-related respiratory health effects from individual-level camp and exercise studies, as well as some consistent evidence from time-series studies of emergency room visits and hospitalizations. Since the 1996 document, more field studies have been conducted, with some emphasis on additional outcome markers such as respiratory symptoms and asthma medication use. Another significant addition to the current O_3 AQCD is the substantial number of short-term O_3 mortality studies, which is in part due to the increase in the number of studies that examined PM-mortality associations. Considering the wide variability in possible study designs and statistical model specification choices, the reported O_3 risk estimates for the various health outcomes are in reasonably good agreement. In the case of O_3-mortality time-series studies, combinations of choices in model specifications (the number of weather terms and degrees of freedom for smoothing of mortality-temporal trends) alone may explain the extent of the difference in O_3 risk estimates across

studies. As use of time-series studies to investigate air pollution effects has become more common, there has been a great effort to evaluate the issues surrounding these studies.

In this section, conclusions regarding O_3 health effects from the epidemiologic evidence and the issues that may affect the interpretation of the effect estimates are briefly summarized. A more integrative synthesis of all relevant information will be presented in Chapter 8 of this AQCD.

(1) Field/panel studies of acute O_3 effects. Results from recent field/panel studies continue to confirm that short-term O_3 exposure is associated with acute decrements in lung function, increased respiratory symptoms, and increased medication use, particularly in children and asthmatics. Taken together with the evidence from controlled human exposure studies, O_3 is likely causally related to the various respiratory health outcomes.

(2) O_3 effects on emergency department visits and hospitalizations. Large multicity studies, as well as many studies from individual cities have reported a significant O_3 effect on total respiratory, asthma, and COPD hospital visits and admissions. Studies using year-round data noted some inconsistencies in the O_3 effect on daily emergency department visits and hospitalizations. However, studies with data restricted to the summer or warm season, in general, indicated positive and robust associations between ambient O_3 concentrations and respiratory morbidity.

(3) Acute O_3 effects on mortality. The majority of the studies suggest an elevated risk of mortality associated with acute exposure to O_3, especially in the summer or warm season when O_3 levels are expected to be high. However, as the magnitude of the O_3-mortality risk estimates are generally small, bias due to the uncertainties regarding model specification and adjustment for confounding may be of concern.

(4) Chronic O_3 effects on morbidity and mortality. Few studies have investigated the effect of chronic O_3 exposure on morbidity and mortality. The strongest evidence is for the association between O_3 exposure and seasonal decrements or reduced growth in lung function measures in adults and children. Less conclusive are longitudinal studies investigating the association of chronic O_3 exposure on yearly lung function, asthma incidence, and respiratory symptoms. Chronic O_3-mortality studies observed inconsistencies across exposure periods, cause-specific mortality outcomes, and gender. Based on the current evidence, the chronic effect of O_3 exposure on morbidity and mortality outcomes is still inconclusive.

(5) Exposure assessement. Exposure misclassification may result from the use of stationary ambient monitors to determine exposure in population studies. Although central ambient monitors do not explain the variance of individual personal exposures, significant correlations are found between aggregate personal O_3 measurements and O_3 concentrations from ambient monitors. A simulation study indicated that the use of ambient monitor data will tend to bias effect estimates towards the null.

(6) O_3 exposure indices. The three most commonly used daily O_3 exposure indices, 1-h max O_3, 8-max O_3, and 24-h avg O_3, were found to be highly correlated in studies conducted in various regions. In addition, the effect estimates and significance of associations across all health outcomes were comparable when using the same distributional increment for all three indices. The commonly used 8-h max O_3 index, which is also reflective of the new 8-h NAAQS for O_3, continues to be an appropriate choice.

(7) Selection of exposure lag structure. Most studies did not hypothesize *a priori* the temporal relationship between O_3 exposure and the occurrence of health effects. Bias can result from the selection of the largest, most significant effect estimates. However, the majority of the studies found an immediate O_3 effect, with health effects having the strongest associations with exposure on the same day and/or previous day. Some studies found greater cumulative effects of O_3 over longer lag periods, indicating that multiday lags also should be investigated.

(8) Sensitivity to model specifications for temporal trends. Ozone effect estimates that were reported in studies whose main focus was PM often were calculated using the same model specifications as PM. The sensitivity of the O_3 risk estimates to alternative model specifications has not been throughly investigated. Uncertainty remains regarding the extent of confounding on estimates of O_3 health risks, however limited evidence indicates that O_3 effects were robust to various model specifications for temporal trend adjustment.

(9) Influence of seasonal trends. An evaluation of the confounding effects of meteorologic factors and copollutants on O_3 risk estimates is complicated by their changing relationship with O_3 across seasons. Mortality and morbidity effect estimates calculated using all year or cool season data are generally smaller than those from warm season only data. In locations where seasonal variability may be considerable, efforts should be made to determine season-specific risk estimates.

(10) Confounding by copollutants. Multipollutant models most often are used to adjust for confounding by copollutants. Results from these analyses indicate that copollutants generally do not appear to confound the association between O_3 and acute health effects. However, due to the varying concurvity across pollutants, multipollutant models may not be adequate to determine the independent effects of individual pollutants. Given the limitations, results generally suggest that the inclusion of copollutants into the models do not substantially affect O_3 risk estimates.

(11) Model uncertainty and multiple testing. Various statistical methods have been used to assist model selection. While Bayesian model averaging is a useful tool that incorporates model uncertainty into the effect estimates, its use may be limited due to the large number of variables typically considered in air pollution health effects and the high degree of correlation between the various air pollutants.

(12) Concentration-response function. Supporting evidence for an effect threshold is provided by the numerous studies where O_3 effects are seen only in the warm months when O_3 levels are higher and more variable. However, in the few mortality and morbidity studies that have specifically examined the O_3 concentration-response relationship, there is conflicting evidence regarding the presence of an effect threshold. Lack of evidence for a population-level threshold does not preclude the existence of individual thresholds.

(13) Spatial variability in O_3 effects. Consistent O_3 effect estimates were observed overall for mortality, hospitalizations, and other respiratory health outcomes in multicity studies, indicating little heterogeneity of O_3 effects by location. The slight heterogeneity observed may be partially attributable to the differences in relative personal exposure to O_3 and the varying concentration and composition of copollutants present by region.

(14) O_3 health effects in asthmatics. The effect of O_3 on asthmatics has been examined widely in both time-series studies and field panel studies. Across various respiratory health outcomes, results were consistently positive and, at times, statistically significant, indicating that asthmatics may be a potentially susceptible population that requires protection from O_3 exposures.

(15) Age-related differences in O_3 health effects. Supporting evidence exists for heterogeneity in the effects of O_3 by age. The elderly population (> 65 years of age) appear to be at greater risk of O_3-related mortality and hospitalizations compared to all age or younger populations. In addition, negative respiratory health outcomes were associated with O_3 exposure in children (< 18 years of age).

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CHAPTER 7 ANNEX

EPIDEMIOLOGICAL STUDIES OF HUMAN HEALTH EFFECTS ASSOCIATED WITH AMBIENT OZONE EXPOSURE

Table AX7-1. Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
United States					
Mortimer et al. (2002) Eight urban areas in the U.S.: St. Louis, MO; Chicago, IL; Detroit, MI; Cleveland, OH; Washington, DC; Baltimore, MD; East Harlem, NY; Bronx, NY Jun-Aug 1993	Examined 846 asthmatic children aged 4-9 years for O_3 exposure effects on PEF and morning symptoms using linear mixed effect models and GEE.	8-h avg O_3 (10 a.m.-6 p.m.): 44 ppb	PM_{10}, NO_2, SO_2	No associations were seen between single or multiday O_3 measures and any evening outcome measure. The effects of O_3 on morning outcomes increased over several days with the strongest associations seen for multiday lags. Joint modeling of O_3 with NO_2 or SO_2 resulted in slightly reduced estimates for each pollutant.	8-h avg O_3 (per 15 ppb): % change in morning PEF: Lag 1-5: All areas: –0.59% (–1.05, –0.13) St. Louis: –0.86% (–2.10, 0.38) Chicago: –0.62% (–2.41, 1.16) Detroit: –0.75% (–2.36, 0.86) Cleveland: –0.62% (–2.23, 0.99) Washington, DC: –0.54% (–2.02, 0.93) Baltimore: 0.24% (–0.95, 1.43) East Harlem: –0.73% (–1.63, 0.17) Bronx: –0.69% (–1.54, 0.15) Odds ratios: Morning symptoms: Lag 1-4: All areas: 1.16 (1.02, 1.30) St. Louis: 0.82 (0.59, 1.14) Chicago: 1.09 (0.69, 1.72) Detroit: 1.72 (1.12, 2.64) Cleveland: 1.20 (0.81, 1.79) Washington, DC: 1.11 (0.72, 1.72) Baltimore: 1.19 (0.89, 1.60) East Harlem: 1.22 (0.97, 1.53) Bronx: 1.23 (0.98, 1.54)

Table AX7-1 (cont'd). Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
United States (cont'd)					
Mortimer et al. (2000) Eight urban areas in the U.S.: St. Louis, MO; Chicago, IL; Detroit, MI; Cleveland, OH; Washington, DC; Baltimore, MD; East Harlem, NY; Bronx, NY Jun-Aug 1993	A cohort of 846 asthmatic children aged 4-9 years examined for effects of summer O_3 exposure on PEF and morning symptoms. Two subgroups were compared: (1) low birth weight or premature and (2) normal birth weight or full-term. Analysis using GEE and linear mixed models.	8-h avg O_3 (10 a.m.-6 p.m.): 48 ppb	None	Low birth weight and premature asthmatic children had greater declines in PEF and higher incidence of morning symptoms than normal birth weight and full-term asthmatic children.	8-h avg O_3 (per 15 ppb): % change in morning PEF: Low birth weight: Lag 1-5: −1.83% (−2.65, −1.01) Normal birth weight: Lag 1-5: −0.30% (−0.79, 0.19) Interaction term for birth weight, $p < 0.05$ Odds ratios: Morning symptoms: Low birth weight: Lag 1-4: 1.42 (1.10, 1.82) Normal birth weight: Lag 1-4: 1.09 (0.95, 1.24) Interaction term for birth weight, $p < 0.05$
Avol et al. (1998) Southern California communities Spring-summer 1994	Three panels of children (age 10-12 years): (1) asthmatic (n = 53); (2) wheezy (n = 54); and (3) healthy (n = 103). Examined for symptoms, medication use, outdoor time, physical activity, and pulmonary function measures in relation to O_3 exposure, via logistic regression and GLM.	Stratified analysis of low and high 24-h avg O_3: Fixed site O_3: Low: < 100 ppb High: > 100 ppb Personal O_3: Low: ≤ 15.6 ppb High: ≥ 32.4 ppb	None	The three groups responded similarly. Few pulmonary function or symptom associations. Asthmatic children had the most trouble breathing, the most wheezing, and the most inhaler use on high O_3 days in the spring. Ozone levels were considered too low during the period of the study. Noncompliance by subjects may have been a problem. Other analysis methods may have been more appropriate.	Multiple endpoints analyzed. Few consistent or statistically significant responses to O_3 exposure reported.

Table AX7-1 (cont'd). Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
United States (cont'd)					
Gilliland et al. (2001) 12 Southern California communities Jan-Jun 1996	1,933 4th grade children (age 9-10 years) followed for school absences. Each absence classified as illness-related or not. Among former, classified into respiratory or gastrointestinal. Respiratory absences further classified into upper or lower. Pollution measured in central site in each town. Analysis of distributed lag effects controlling for time, day of week, and temperature in a Poisson model.	8-h avg O_3 (10 a.m.-6 p.m.): Levels not reported.	PM_{10}, NO_2	O_3 strongly associated with illness-related and respiratory absences. PM_{10} only associated with upper respiratory absences. Long distributed lag effects for O_3 raise questions about adequacy of control for seasonal changes.	8-h avg O_3 (per 20 ppb): % change in absences: All illness: 62.9% (18.4, 124.1) Nonrespiratory illnesses: 37.3% (5.7, 78.3) Respiratory illnesses: 82.9% (3.9, 222.0) Upper respiratory: 45.1% (21.3, 73.7) Lower respiratory with wet cough: 173.9% (91.3, 292.3)
Linn et al. (1996) Three towns in California: Rubidoux, Upland, Torrance Fall-spring 1992-1993 and 1993-1994	269 school children (age unspecified), each followed for morning/afternoon lung function and symptoms for one week in fall, winter, and spring over 2 school years. Personal exposure monitoring in a subset. Analyzed afternoon symptoms versus same day pollution and morning symptoms versus 1-day lag pollution.	24-h avg O_3: Personal: 5 ppb SD 3 Central site: 23 ppb SD 12	$PM_{2.5}$, NO_2	Central site O_3 correlated with personal exposures, r = 0.61. Ozone effects observed on lung function but only significant for FEV_1 in one analysis. No effects on symptoms. Ozone effects were not robust to NO_2 or $PM_{2.5}$. Power may have been limited by short followup within seasons (limiting both person-days and variability in exposures).	Change in lung function (per ppb): FEV_1 next morning: −0.26 mL (SE 0.25), p = 0.30 FEV_1 afternoon: −0.18 mL (SE 0.26), p = 0.49 FEV_1 crossday difference: −0.58 mL (SE 0.23), p = 0.01 FVC next morning: −0.21 mL (SE 0.22), p = 0.34 FVC afternoon: −0.20 mL (SE 0.29), p = 0.48 FVC crossday difference: −0.25 mL (SE 0.25), p = 0.32

Table AX7-1 (cont'd). Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
United States (cont'd)					
Ostro et al. (2001) Central Los Angeles and Pasadena, CA Aug-Oct 1993	138 African-American children aged 8-13 years with doctor diagnosed asthma requiring medication in past year followed for daily respiratory symptoms and medication use. Lags of 0 to 3 days examined.	1-h max O_3: Los Angeles: 59.5 ppb SD 31.4 Pasadena: 95.8 ppb SD 49.0	PM_{10}, NO_2, pollen, mold	Correlation between PM_{10} and O_3 was r = 0.35. Significant O_3 effect seen for extra medication use (above normal use). No O_3 effect on symptoms in expected direction observed. Inverse association seen for cough. PM_{10} effects seen at a lag of 3 days. Time factors not explicitly controlled in analysis; may have led to confounding of O_3 effects.	1-h max O_3 (per 40 ppb): Odds ratios: Extra medication use: Lag 1: 1.15 (1.12, 1.19) Respiratory symptoms: Shortness of breath: Lag 3: 1.01 (0.92, 1.10) Wheeze: Lag 3: 0.94 (0.88, 1.00) Cough: Lag 3: 0.93 (0.87, 0.99)
Delfino et al. (2003) Los Angeles, CA Nov 1999-Jan 2000	A panel study of 22 Hispanic children with asthma aged 10-16 years. Filled out symptom diaries in relation to pollutant levels. Analysis using GEE model.	1-h max O_3: 25.4 ppb SD 9.6	NO_2, SO_2, CO, volatile organic compounds, PM_{10}	Support the view that air toxics in the pollutant mix from traffic may have adverse effects on asthma in children.	1-h max O_3 (per 14.0 ppb): Odds ratio: Symptoms interfering with daily activities: Lag 0: 1.99 (1.06, 3.72)
Delfino et al. (1997a) Alpine, CA May-Aug1994	22 asthmatics aged 9-46 years followed for respiratory symptoms, morning-afternoon PEF, and β_2 agonist inhaler use. Personal O_3 measured for 12 hours/day using passive monitors. GLM mixed model.	Ambient: 12-h avg O_3 (8 a.m.-8 p.m.): 64 ppb SD 17 Personal: 12-h avg O_3: (8 a.m.-8 p.m.) 18 ppb SD 14	PM_{10}, pollen, fungi	No O_3 effects observed.	No quantitative results for O_3.

Table AX7-1 (cont'd). Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
United States (cont'd)					
Delfino et al. (1998a) Alpine, CA Aug-Oct 1995	A panel of 24 asthmatics aged 9-17 years followed for daily symptoms. Analysis using GEE model.	1-h max O_3: 90 ppb SD 18	PM_{10}	Asthma symptoms were significantly associated with both ambient O_3 and PM_{10} in single-pollutant models. Ozone effects generally robust to PM_{10}. Current day O_3 effects strongest in asthmatics not on anti-inflammatory medication. Effects of O_3 and PM_{10} were largely independent. The largest effects for PM_{10} were seen for a 5-day distributed lag. For O_3 effects, there were no lag day effects; current day results showed the greatest effect.	1-h max O_3 (per 58 ppb): Odds ratios: O_3 only model: Lag 0: 1.54 (1.02, 2.33) O_3 with PM_{10} model: Lag 0: 1.46 (0.93, 2.29)
Delfino et al. (2004) Alpine, CA Aug-Oct 1999, Apr-Jun 2000	19 asthmatic children (age 9-17 years) followed daily for 2 weeks to determine relationship between air pollutants, namely PM, and FEV_1. Linear mixed model used for analysis.	8-h max O_3: 62.8 ppb SD 15.1 IQR 22.0	$PM_{2.5}$, PM_{10}, NO_2	Significant declines in FEV_1 associated with various PM indices (personal, indoor home, etc.), but not ambient O_3 levels.	No quantitative results for O_3.
Delfino et al. (1996) San Diego, CA Sep-Oct 1993	12 well-characterized moderate asthmatics aged 9-16 years (7 males, 5 females) followed over 6 weeks for medication use and respiratory symptoms. Allergy measured at baseline with skin prick tests. Personal O_3 measured with passive badge. Analysis with GLM mixed model.	Ambient: 1-h max O_3: 68 ppb SD 30 Ambient: 12-h avg O_3: 43 ppb SD 17 Personal: 12-h avg O_3: 11.6 ppb SD 11.2	$PM_{2.5}$, SO_4^{2-}, H^+, HNO_3, pollen, fungal spores	No effect of ambient O_3 on symptom score. Personal O_3 significant for symptoms, but effect disappeared when confounding day of week effect was controlled with weekend dummy variable. β_2 inhaler used among 7 subjects was significantly related to personal O_3. Results of this small study suggest the value of personal exposure data in providing more accurate estimates of exposures. However, nearly 50% of personal O_3 measurements were below limits of detection, diminishing value of these data. Pollen and fine particulate (low levels) were not associated with any of the outcomes.	Change in β_2-agonist inhaler use (per ppb personal O_3): 0.0152 puffs/day (SE 0.0075), p = 0.04

Table AX7-1 (cont'd). Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
United States (cont'd)					
Chen et al. (2000) Washoe County, NV 1996-1998	School absenteeism examined among 27,793 students (kindergarten to 6th grade) from 57 elementary schools. First-order autoregression models used to assess relationship between O_3 and school absenteeism after adjusting for weather, day of week, month, holidays, and time trends. Ozone levels from the current day, and cumulative lags of 1-14 days, 1-21 days, and 1-28 days examined.	1-h max O_3: 37.45 ppb SD 13.37	PM_{10}, CO	Multipollutant models were examined. Ozone concentrations in the preceding 14 days were significantly associated with school absenteeism for students in grades 1 through 6, but not those in kindergarten. Both PM_{10} and CO concentrations on the concurrent day were associated with school absenteeism, but the estimate for PM_{10} was a negative value.	1-h max O_3 (per 50 ppb): Total absence rate: O_3 with PM_{10} and CO model: Lag 1-14: 3.79% (1.04, 6.55)
Newhouse et al. (2004) Tulsa, OK Sep-Oct 2000	24 subjects aged 9-64 years with physician diagnosis of asthma. Performed PEF twice daily (morning and afternoon), and reported daily respiratory symptoms and medication use. Forward stepwise multiple regression models and Pearson correlation analyses.	24-h avg O_3: 30 ppb Range 10-70	$PM_{2.5}$, CO, SO_2, pollen, fungal spores	Among ambient air pollutants, O_3 seemed to be most significant factor. Morning PEF values significantly associated with average and maximum O_3 levels on the previous day. Individual symptoms, including wheezing, headache, and fatigue, also significantly related to average and maximum daily O_3. Multiple regression analyses produced complex models with different predictor variables for each symptom.	Pearson correlation coefficient: Morning PEF: Mean O_3 levels: Lag 1: -0.274, $p < 0.05$ Maximum O_3 levels: Lag 1: -0.289, $p < 0.05$

Table AX7-1 (cont'd). Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
United States (cont'd)					
Ross et al. (2002) East Moline, IL and nearby communities May-Oct 1994	59 asthmatics aged 5-49 years recruited. 19 lost to follow-up, yielding study population of 40. Assessment of PEF and respiratory symptoms. Analytical methods unclear in terms of control for time factors.	8-h max O_3: 41.5 ppb SD 14.2 IQR 20	PM_{10}, SO_2, NO_2, pollen, fungi	Saw significant associations between O_3 and both PEF declines and symptom increases. Most but not all effects remained after controlling for temperature, pollen and fungi. The O_3 effect on morning PEF disappeared after adjusting for temperature. No PM_{10} effects observed.	8-h max O_3 (per 20 ppb): Change in PEF (L/min): Morning: Lag 0-1: −2.29 (−4.26, −0.33) Afternoon: Lag 0: −2.58 (−4.26, −0.89) Symptom score (on scale of 0-3): Morning: Lag 1-3: 0.08 (0.03, 0.13) Afternoon: Lag 1-3: 0.08 (0.04, 0.12)
Neas et al. (1995) Uniontown, PA Summer 1990	83 4th and 5th grade children reported twice daily PEF and the presence of cold, cough, or wheeze. Relationship to pollutants was analyzed by an autoregressive linear regression model/GEE. The number of hours each child spent outdoors during the preceding 12-h period was evaluated.	12-h avg O_3: Daytime (8 a.m.-8 p.m.): 50.0 ppb Overnight (8 p.m.-8 a.m.): 24.5 ppb	SO_2, PM_{10}, H^+	Evening cough was associated with O_3 levels weighted by hours spent outdoors during the prior 12 hours. A decrease in PEF was associated with O_3 levels weighted by hours spent outdoors. When particle-strong acidity was added to the model, the decrement was decreased and no longer significant.	12-h avg O_3 (per 30 ppb increment weighted by proportion of time spent outdoors during prior 12 hours): Evening PEF: −2.79 L/min (−6.7, −1.1) Odds ratio: Evening cough: 2.20 (1.02, 4.75)
Neas et al. (1999) Philadelphia, PA Jul-Sep 1993	156 children aged 6-11 years at two summer camps followed for twice-daily PEF. Analysis using mixed effects models adjusting for autocorrelated errors.	Daytime 12-h avg O_3 (9 a.m-9 p.m.): SW camp: 57.5 ppb IQR 19.8 NE camp: 55.9 ppb IQR 21.9	H^+, SO_4^{2-}, $PM_{2.5}$, PM_{10}, $PM_{10-2.5}$	Some O_3 effects detected as well as PM effects. Similar O_3-related decrements observed in both morning and afternoon PEF. Ozone effects not robust to SO_4^{2-} in two-pollutant models, whereas SO_4^{2-} effects relatively robust to O_3.	12-h avg O_3 (per 20 ppb): Morning and evening PEF: O_3 only models: Lag 0: −1.38 L/min (−2.81, 0.04) Lag 1-5: −2.58 L/min (−4.81, −0.35) O_3 with SO_4^{2-} model: Lag not specified: −0.04 L/min

Table AX7-1 (cont'd). Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
United States (cont'd)					
Gent et al. (2003) Southern New England Apr-Sep 2001	271 children (age < 12 years) with active, doctor-diagnosed asthma followed over 183 days for respiratory symptoms. For analysis, cohort split into two groups: 130 who used maintenance medication during follow-up and 141 who did not, on assumption that medication users had more severe asthma. Logistic regression analyses performed.	1-h max O_3: 58.6 ppb SD 19.0 8-h max O_3: 51.3 ppb SD 15.5	$PM_{2.5}$	Correlation between 1-h max O_3 and daily $PM_{2.5}$ was 0.77 during this warm-season study. Large numbers of statistical tests performed. Significant associations between symptoms and O_3 seen only in medication users, a subgroup considered to be more sensitive. $PM_{2.5}$ significant for some symptoms, but not in two-pollutant models. Ozone effects generally robust to $PM_{2.5}$.	1-h max O_3 (per 50 ppb): Odds ratios: Regular medication users (n = 130): Chest tightness: O_3 only model: Lag 1: 1.26 (1.00, 1.48) O_3 with $PM_{2.5}$ model: Lag 1: 1.42 (1.14, 1.78) Shortness of breath: O_3 only model: Lag 1: 1.22 (1.02, 1.45)
Korrick et al. (1998) Mount Washington, NH Summers 1991, 1992	Evaluated the acute effects of ambient O_3 on pulmonary function of exercising adults. 530 hikers (age 15-64 years) were examined. Analysis using a general linear regression model.	Mean O_3 per hour of hiking: 40 ppb Range 21-74	$PM_{2.5}$, smoke, acidity	With prolonged outdoor exercise low-level exposures to O_3 were associated with significant effects on pulmonary function. Hikers with asthma had a 4-fold greater responsiveness to exposure to O_3.	% change in lung function (per 50 ppb O_3): FEV_1: −2.6% (−4.7, −0.4) FVC: −2.2% (−3.5, −0.8)
Thurston et al. (1997) Connecticut River Valley, CT June 1991, 1992, 1993	Children (age 7-13 years) with moderate-to-severe asthma followed for medication use, lung function, and medical symptoms at a summer asthma camp for one week in 1991 (n = 52), 1992 (n = 58), and 1993 (n = 56). Analysis was conducted using both Poisson modeling and GLM.	1-h max O_3: 1991: 114.0 ppb 1992: 52.2 ppb 1993: 84.6 ppb 1991-1993: 83.6 ppb	H^+, SO_4^{2-}	O_3 was most consistently associated with acute asthma exacerbation, chest symptoms, and lung function decrements. Pollen was poorly associated with any adverse effect. Consistent results were obtained between the aggregate and individual analyses.	1-h max O_3 (per 83.6 ppb): Relative risks: β_2-agonist use: 1.46, $p < 0.05$ Chest symptoms: 1.50, $p < 0.05$ Change in PEF (per ppb): −0.096 L/min, $p < 0.05$

Table AX7-1 (cont'd). Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
United States (cont'd)					
Naeher et al. (1999) Vinton, VA Summers 1995, 1996	Relationship between O_3 and daily change in PEF studied in a sample of 473 nonsmoking women aged 19-43 years who recently delivered babies. PEF performed twice daily for a 2-week period. Mixed linear random coefficient model.	8-h max O_3: 53.69 ppb Range 17.00-87.63 24-h avg O_3: 34.87 ppb Range 8.74-56.63	$PM_{2.5}$, PM_{10}, SO_4^{2-}, H^+	O_3 was the only exposure related to evening PEF with 5-day cumulative lag exposure showing the greatest effect.	24-h avg O_3 (per 30 ppb): Evening PEF: Lag 1-5: −7.65 L/min (−13.0, −2.25)
Canada					
Brauer et al. (1996) Fraser Valley, British Columbia, Canada Jun-Aug 1993	58 berry pickers aged 10-69 years had lung function measured before and after a series of outdoor work shifts (average duration = 11 hours) over 59 days. Analysis using pooled regression with subject-specific intercepts, with and without temperature control.	1-h max O_3: 40.3 ppb SD 15.2 Work shift O_3: 26.0 ppb SD 11.8	$PM_{2.5}$, SO_4^{2-}, NO_3^-, NH_4^+, H^+	End shift FEV_1 and FVC significantly diminished in relation to O_3 levels. $PM_{2.5}$ also related to lung function declines, but O_3 remained significant in 2-pollutant models. Next morning lung function remained diminished following high O_3 days. Ozone effects still evident at or below 40 ppb. There was an overall decline of lung function of roughly 10% over course of study, suggesting subchronic effect. Levels of other pollutants low during study.	Change in lung function (per ppb 1-h max O_3): Endshift lung function: FEV_1: −3.8 mL (SE 0.4) FVC: −5.4 mL (SE 0.6) Next morning function: FEV_1: −4.5 mL (SE 0.6) FVC: −5.2 mL (SE 0.7)

Table AX7-1 (cont'd). Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
Canada (cont'd)					
Brauer and Brook (1997) Fraser Valley, British Columbia, Canada Jun-Aug 1993	Additional analysis of Brauer et al., 1996 with personal exposure presented for three groups, stratified by time spent outdoors. Group 1: 25 individuals who spent most of the day indoors. Group 2: 25 individuals who spent much of the day indoors, but still spent several daylight hours outdoors. Group 3: 15 individuals who spent the entire work day outdoors.	1-h max O_3: Ambient: 40 ppb SD 15 Range 13-84	$PM_{2.5}$, SO_4^{2-}, NO_3^-, NH_4^+, H^+	Group 1: 9.0% sampling time (24-h) outdoors. Personal to ambient O_3 ratio was 0.28. Group 2: 25.8% sampling time (24-h) outdoors. Personal to ambient O_3 ratio was 0.48. Group 3: 100% sampling time (11-h workshift) outdoors. Personal to ambient O_3 ratio was 0.96. One of the first direct demonstrations that magnitude of personal exposure to O_3 is related to amount of time spent outdoors. Further showed that, on average, outdoor fixed O_3 monitors were representative of day-to-day changes in O_3 exposure experienced by the study population.	Same outcomes as reported in Brauer et al., 1996.
Europe					
Scarlett et al. (1996) Surrey, England Jun-Jul 1994	Examined 154 children aged 7 years in a primary school next to a major motorway for O_3 exposure effects on $PEF_{0.75}$, FVC, and FEV_1 using autoregression for % change in function.	8-h max O_3: 50.7 ppb SD 24.48	PM_{10}, NO_2, pollen	No significant association was seen between pulmonary function measures and O_3 levels. No pollen effects.	Change in lung function (per ppb O_3 weighted by inverse of variance): $FEV_{0.75}$: Lag 1: 0.01 mL (−0.12, 0.13) FVC: Lag 1: 0.07 mL (−0.09, 0.23) $FEV_{0.75}$/FVC: Lag 1: −0.1% (−5.1, 4.8)

Table AX7-1 (cont'd). Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
Europe (cont'd)					
Taggart et al. (1996) Runcorn and Widnes in NW England Jul-Sep 1993	Investigated the relationship of asthmatic bronchial hyperresponsiveness and pulmonary function to ambient levels of summertime air pollution among 38 adult nonsmoking asthmatics (age 18-70 years) using log-linear models. Analysis limited to investigation of within subject variance of the dependent variables.	1-h avg O_3: Maximum 61 µg/m^3 24-h avg O_3: Maximum 24.5 µg/m^3	SO_2, NO_2, smoke	No association found for O_3. Changes in bronchial hyperresponsiveness were found to correlate significantly with change in the levels of 24-h mean SO_2, NO_2, and smoke.	24-h avg O_3 (per 10 µg/m^3): % change in bronchial hyperresponsiveness: Lag 1: 0.3% (−16.6, 20.6) Lag 2: 2.6% (−22.1, 34.9)
Desqueyroux et al. (2002a) Paris, France Nov 1995-Nov 1996	60 severe asthmatics (mean age 55 years) were monitored by their physicians for asthma attacks. Asthma attacks were based on medical data collected by a pulmonary physician at time of clinical examination. Analysis using GEE.	8-h avg O_3 (10 a.m.-6 p.m.): Summer: 41 µg/m^3 SD 18 Winter: 11 µg/m^3 SD 10	PM_{10}	Significant associations between PM_{10}, O_3, and incident asthma attacks were found. Low O_3 levels raise plausibility concerns.	8-h avg O_3 (per 10 µg/m^3): Odds ratio: Lag 2: 1.20 (1.03, 1.41)
Desqueyroux et al. (2002b) Paris, France Oct 1995-Nov 1996	39 adult patients with severe COPD (mean age 67 years) followed over 14 months by physicians for exacerbations. Logistic regression with GEE, examining exposure lags of 0 to 5 days.	8-h avg O_3 (10 a.m.-6 p.m.): Summer: 41 µg/m^3 SD 18 Winter: 11 µg/m^3 SD 10	PM_{10}, SO_2, NO_2	50 COPD exacerbations observed over follow-up period. 1-, 2-, and 3-day lag O_3 significantly related to exacerbations. No other pollutants significant. Low O_3 levels raise plausibility and confounding concerns.	8-h avg O_3 (per 10 µg/m^3): Odds ratio: Lag 1: 1.56 (1.05, 2.32) Effects appeared larger among smokers and those with worse gas exchange lung function.

Table AX7-1 (cont'd). Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
Europe (cont'd)					
Just et al. (2002) Paris, France Apr-Jun 1996	82 medically diagnosed asthmatic children (mean age 10.9 years) followed for O_3 exposure and PEF, asthmatic attacks, cough, supplementary use of β_2-agonists, and symptoms of airway irritation. Analysis by GEE.	24-h avg O_3: 58.9 µg/m^3 SD 24.5 Range 10.0-121.0	PM_{10}, NO_2	In asthmatic children, O_3 exposure was related to the occurrence of asthma attacks and additional bronchodilator use. O_3 was the only pollutant associated with changes in lung function, as shown by an increase in PEF variability and decrease in PEF.	24-h avg O_3 (per 10 µg/m^3): % change in daily PEF variability: Lag 0-2: 2.6%, p = 0.05 Odds ratio: Supplementary use of β_2-agonist on days on which no steroids were used: Lag 0: 1.41 (1.05, 1.89)
Lagerkvist et al. (2004) Brussels, Belgium May 2002	57 children (mean age 10.8 years) stratified by swimming pool attendance. Pulmonary function test performed and Clara cell protein levels measured in blood before and after light exercise outdoors for two hours. Analysis using student's t-test and Pearson correlation test. For dose calculations, O_3 levels indoors assumed to be 50% of the mean outdoor O_3 concentration.	Daytime outdoor O_3: Range 77-116 µg/m^3 Exposure dose: Range 352-914 µg/m^3·hour	None	Ozone levels did not have any adverse effect on FEV_1 after 2 hours of outdoor exercise. In addition, no significant differences were observed between Clara cell protein levels before and after exercise. A marginally significant positive correlation between ambient O_3 dose and Clara cell protein levels observed among the nonswimmers, indicating increased antioxidant activity following O_3 exposure in this group. The lack of a clear relationship between Clara cell protein levels and O_3 dose may be attributable to the short period of time between measurements and diurnal variability of the protein levels.	Pearson correlation: O_3 exposure dose and Clara cell protein levels in serum: All subjects (n = 54): r = 0.17, p = 0.21 Nonswimmers (n = 33): r = 0.34, p = 0.06 Swimmers (n = 21): r = −0.08, p = 0.74

Table AX7-1 (cont'd). Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
Europe (cont'd)					
Frischer et al. (1993) Umkirch, Germany May-Oct 1991	Nasal lavage repeatedly performed on 44 school children (age 9-11 years) according to protocol published by Koren et al. (1990). Samples collected morning after "low" and "high" O_3 days. Nasal lavage samples analyzed for polymorphonuclear leukocyte counts, albumin, tryptase, eosinophil cationic protein, and myeloperoxidase. Analysis using individual regression methods.	Stratified analysis of half hour avg O_3 at 3 p.m.: Low: < 140 µg/m^3 High: > 180 µg/m^3	None	Significant higher polymorphonuclear leukocyte counts after high O_3 days. In children without symptoms of rhinitis, significantly elevated myeloperoxydase and eosinophil cationic protein concentrations detected. Results suggest that ambient O_3 produces an inflammatory response in the upper airways of healthy children.	Children without symptoms of rhinitis (n = 30): Myeloperoxydase: Low O_3: median 77.39 µg/L High O_3: median 138.60 µg/L $p < 0.05$; Wilcoxon sign rank test Eosinophilic cationic protein: Low O_3: median 3.49 µg/L High O_3: median 5.39 µg/L $p < 0.05$; Wilcoxon sign rank test
Frischer et al. (1997) Umkirch, Germany May-Oct 1991	Examined 44 school children aged 9-11 years for ratio of *ortho*-tyrosine to *para*-tyrosine in nasal lavage as a marker of hydroxyl radical attack. Nasal lavage performed according to protocol published by Koren et al. (1990). Concomitant lung function tests performed. Analysis using individual regression methods.	Stratified analysis of ½-h avg O_3 at 3 p.m.: Low: < 140 µg/m^3 High: > 180 µg/m^3	None	Ambient O_3 was associated with the generation of hydroxyl radicals in the upper airways of healthy children and significant lung function decrements. However, the *ortho/para* ratio was not related to polymorphonuclear leukocyte counts. Passive smoking was not related to outcomes.	FEV_1 (% predicted): Low: 105.4 (SD 15.6) High: 103.9 (SD 15.0) Δ: 1.5, $p = 0.031$ *Ortho*/*para* ratio: Low: 0.02 (SD 0.07) High: 0.18 (SD 0.16) Δ: 0.17, $p = 0.0001$

Table AX7-1 (cont'd). Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
Europe (cont'd)					
Höppe et al. (1995a,b) Munich, Germany Apr-Sep 1992-1994	Five study groups (age 12-95 years): (1) senior citizens (n = 41); (2) juvenile asthmatics (n = 43); (3) forestry workers (n = 41); (4) athletes (n = 43); and (5) clerks (n = 40) as a control group. Examined for lung function (FVC, FEV_1, PEF) and questions on irritated airways. Each subject tested 8 days, 4 days with elevated or high O_3 and 4 days with low O_3. Analysis using Wilcoxon matched pairs signed rank test and linear regression.	½-h max O_3 (1 p.m.-4 p.m.): Seniors: High: 70 ppb Low: 31 ppb Asthmatics: High: 74 ppb Low: 34 ppb Forestry workers: High: 64 ppb Low: 32 ppb Athletes: High: 71 ppb Low: 28 ppb Clerks: High: 68 ppb Low: 15 ppb	None	No indication that senior citizens represent a risk group in this study. Senior citizens had the lowest ventilation rate (mean 10 L/min). Athletes and clerks experienced significant decrements in lung function parameters. Well-medicated juvenile asthmatics have a trend towards large pulmonary decrements. Forestry workers were exposed to motor tool exhaust, which might be a potential promoting factor.	½-h max O_3 (per 100 ppb): Change in lung function: Seniors: FEV_1: 0.034 L (SD 0.101) PEF: 0.006 L/s (SD 0.578) Asthmatics: FEV_1: −0.210 L (SD 0.281) PEF: −0.712 L/s (SD 0.134)* Forestry workers: FEV_1: −0.140 L (SD 0.156) PEF: −1.154 L/s (SD 0.885)* Athletes: FEV_1: −0.152 L (SD 0.136)* PEF: −0.622 L/s (SD 0.589)* Clerks: FEV_1: −0.158 L (SD 0.114)* PEF: −0.520 L/s (SD 0.486)* *$p < 0.05$
Kopp et al. (1999) Two towns in Black Forest, Germany Mar-Oct 1994	170 school children (median age 9.1 years) followed over 11 time points with nasal lavage sampling. Subjects were not sensitive to inhaled allergens. Nasal lavage samples analyzed for eosinophil cationic protein, albumen, and leukocytes. Analysis using GEE.	½-h max O_3: Villingen: 64 µg/m^3 5%-95% 1-140 Freudenstadt: 105 µg/m^3 5%-95% 45-179	PM_{10}, NO_2, SO_2, TSP	Eosinophil cationic protein and leukocyte levels peaked soon after first major O_3 episode of summer, but did not show response to later, even higher, O_3 episodes. These observations are consistent with an adaptive response in terms of nasal inflammation.	Change in log eosinophil cationic protein concentration (per µg/m^3 O_3): Early summer: 0.97 (0.03, 1.92) Late summer: −0.43 (−1.34, 0.47)

Table AX7-1 (cont'd). Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
Europe (cont'd)					
Ulmer et al. (1997) Freudenstadt and Villingen, Germany Mar-Oct 1994	135 children aged 8-11 years in two towns were evaluated. Pulmonary function was associated with the highest O_3 concentration in the previous 24 hours. An initial cross-sectional analysis was followed by a longitudinal analysis using GEE with the data at four time periods (Apr, Jun, Aug, Sep).	½-h max O_3: Freudenstadt: Median 50.6 ppb 90th% interval 22.5-89.7 Villingen: Median 32.1 ppb 90th% interval 0.5-70.1	None	In the cross-sectional analysis, a significant negative association between O_3 exposure and FVC was only shown at the June testing. For FEV_1, no significant associations were detected. In contrast, the longitudinal analysis obtained a statistically significant negative correlation between O_3 exposure, and FVC and FEV_1 for the subpopulation living in the town with higher O_3 levels, Freudenstadt. The associations were more pronounced in males than females.	Change in lung function (per $\mu g/m^3$ ½-h max O_3): FEV_1: Freudenstadt: −1.13 mL, p = 0.002 Villingen: −0.19 mL, p = 0.62 FVC: Freudenstadt: −1.23 mL, p = 0.002 Villingen: 0.02 mL, p = 0.96
Cuijpers et al. (1994) Maastricht, the Netherlands Nov-Dec 1990 (baseline), Aug 8-16 1991 (smog episode)	During episode, 212 children (age unspecified) randomly chosen from 535 reexamined for lung function and symptoms. Corrected baseline lung function compared by paired t-test. Difference in prevalence of respiratory symptoms examined.	Baseline: 8-h avg O_3: Range 2-56 $\mu g/m^3$ Smog episode: 1-h max O_3: Exceeded 160 $\mu g/m^3$ on 11 days	PM_{10}, SO_2, NO_2	Small decrements in FEV_1 and FEF_{25-75} were found in the 212 children. However, significant decreases in resistance parameters also were noted. Each day a different group of 30 children were measured. The results of the lung function are contradictory in that spirometry suggest airflow obstruction while impedance measurement suggest otherwise. Respiratory symptoms impacted by low response rate of 122 of 212 children due to summer holidays. No increase was observed.	Change in lung function and impedance between baseline and smog episode: FEV_1: −0.032 L (SD 0.226), $p \leq 0.05$ FEF_{25-75}: −0.086 L/s (SD 0.415), $p \leq 0.01$ Resistence at 8 Hz: −0.47 $cmH_2O/(L/s)$ (SD 1.17), $p \leq 0.05$
Gielen et al. (1997) Amsterdam, the Netherlands Apr-Jul 1995	61 children aged 7-13 years from two special schools for chronically ill children, followed for twice-daily PEF, symptoms, and medication usage. 77% of cohort had doctor-diagnosed asthma.	1-h max O_3: 77.3 $\mu g/m^3$ SD 15.7 8-h max O_3: 67.0 $\mu g/m^3$ SD 14.9	PM_{10}, BS, pollen	Morning PEF significantly associated with 8-h max O_3 at a lag of 2 days. BS also associated with PEF. Among 14 symptom models tested, only one yielded a significant O_3 finding (for upper respiratory symptoms). PM_{10} and BS, but not O_3, were related to β_2-agonist inhaler use.	8-h max O_3 (per 83.2 $\mu g/m^3$): % change in PEF: Morning: Lag 2: −1.86% (−3.58, −0.14) Afternoon: Lag 2: −1.88% (−3.94, 0.18)

Table AX7-1 (cont'd). Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
Europe (cont'd)					
Hilterman et al. (1998) Bilthoven, the Netherlands Jul-Oct 1995	60 adult nonsmoking intermittent to severe asthmatics (age 18-55 years) followed over 96 days. Measured morning and afternoon PEF, respiratory symptoms, and medication use. Analysis controlled for time trends, aeroallergens, environmental tobacco smoke exposures, day of week, temperature. Lags of 0 to 2 days examined.	8-h max O_3: 80.1 µg/m^3 Range 6-94	PM_{10}, NO_2, SO_2, BS	O_3 had strongest association with symptoms of any pollutant analyzed. PEF lower with O_3 but not statistically significant. No effect on medication use. No effect modification by steroid use or hyperresponsiveness.	8-h max O_3 (per 100 µg/m^3): Odds ratios: Respiratory symptoms: Shortness of breath: Lag 0: 1.18 (1.02, 1.36) Sleep disturbed by breathing: Lag 0: 1.14 (0.90, 1.45) Pain on deep inspiration: Lag 0: 1.44 (1.10, 1.88) Cough of phlegm: Lag 0: 0.94 (0.83, 1.07) Bronchodilator use: Lag 0: 1.05 (0.94, 1.19)
Hoek and Brunekreef (1995) Deurne and Enkhuizen, the Netherlands Mar-Jul 1989	The occurrence of acute respiratory symptoms investigated in children aged 7-11 years (Deurne n = 241; Enkhuizen n = 59). Symptoms included cough, shortness of breath, upper and lower respiratory symptoms, throat and eye irritation, headache and nausea. Ozone-related symptom prevalence and incidence were examined. Lags of 0 and 1 day, and mean O_3 concentration from previous week were investigated. Analyses using 1st-order autoregressive models and logistic regression models.	1-h max O_3: Deurne: 57 ppb SD 20 Range 22-107 Enkhuizen: 59ppb SD 14 Range 14-114	PM_{10}, NO_2, SO_2	No consistent association between ambient O_3 concentrations and the prevalence or incidence of symptoms in either city. The one significant positive coefficient in Enkhuizen for prevalence of upper respiratory symptoms was not confirmed by the Deurne results. No associations of daily symptom prevalence or incidence found with any of the other copollutants examined.	1-h max O_3 (per 50 ppb): Prevalence of symptoms: Deurne: Any respiratory symptom: Lag 0: −0.06 (SE 0.04) Cough: Lag 0: −0.07 (SE 0.07) Upper respiratory symptoms: Lag 0: −0.06 (SE 0.05) Enkhuizen: Any respiratory symptom: Lag 0: 0.12 (SE 0.07) Cough: Lag 0: −0.07 (SE 0.18) Upper respiratory symptoms: Lag 0: 0.18 (SE 0.09)* *$p < 0.05$

Table AX7-1 (cont'd). Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
Latin America					
Castillejos et al. (1995) SW Mexico City Aug 1990-Oct 1991	Children aged 7½-11 years (22 males, 18 females) tested up to 8 times for FEV_1 and FVC, before and after exercise. Target minute ventilation was 35 L/min/m^2. Analysis using GEE models.	1-h max O_3: 112.3 ppb Range 0-365 5th quintile mean 229.1 ppb	PM_{10}	The mean % decrements in lung function were significantly greater than zero only in the fifth quintile of O_3 exposure (183-365 ppb).	% change with exercise in 5th quintile of O_3 exposure (183-365 ppb): FEV_1: −2.85% (−4.40, −1.31) FVC: −1.43% (−2.81, −0.06)
Gold et al. (1999) SW Mexico City 1991	40 school children aged 8-11 years in polluted community followed for twice-daily PEF and respiratory symptoms. PEF deviations in morning/afternoon from child-specific means analyzed in relation to pollution, temperature, season, and time trend. Morning symptoms analyzed by Poission regression.	24-h avg O_3: 52.0 ppb IQR 25	$PM_{2.5}$, PM_{10}	Reported significant declines in PEF in relation to 24-h avg O_3 levels. Effects did not vary by baseline symptom history. Lags chosen to maximize effects and varied by outcome. Ozone generally robust to $PM_{2.5}$. Morning phlegm significantly related to 24-h avg O_3 at a 1-day lag.	24-h avg O_3 (per 25 ppb): % change in PEF: Morning: Lag 1-10: −3.8% (−5.8, −1.8) Afternoon: Lag 0-9: −4.6% (−7.0, −2.1) % change in phlegm: Morning: Lag 1: 1.1% (1.0, 1.3)

Table AX7-1 (cont'd). Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
Latin America (cont'd)					
Romieu et al. (1996) N Mexico City Apr-Jul 1991, Nov 1991-Feb 1992	71 mildly asthmatic children aged 5-13 years followed for PEF and respiratory symptoms. Lower respiratory symptoms included cough, phlegm, wheeze and/or difficulty breathing.	1-h max O_3: 190 ppb SD 80	$PM_{2.5}$, PM_{10}, NO_2, SO_2	O_3 effects observed on both PEF and symptoms. Symptom, but not PEF, effects robust to PM_{10} in two-pollutant models. Symptoms related to O_3 included cough and difficulty breathing.	1-h max O_3 (per 50 ppb): Change in PEF (L/min): Morning: Lag 0: −2.44 (−4.40, −0.49) Lag 1: −0.23 (−0.41, 1.62) Lag 2: −1.49 (−3.80, 0.80) Afternoon: Lag 0: −0.56 (−2.70, 1.60) Lag 1: −1.27 (−3.20, 0.62) Lag 2: −1.92 (−4.50, 0.66) Odds ratios: Lower respiratory symptoms: Lag 0: 1.09 (1.03, 1.15) Lag 1: 1.10 (1.04, 1.17) Lag 2: 1.04 (0.97, 1.12)
Romieu et al. (1997) SW Mexico City Apr-Jul 1991, Nov 1991-Feb 1992	Same period as Romieu et al., 1996, but in different section of city. 65 mildly asthmatic children aged 5-13 years followed for twice-daily PEF, and respiratory symptoms. Up to 2 months follow-up per child. Analysis included temperature and looked at 0- to 2-day lags. No time controls. Lower respiratory symptoms included cough, phlegm, wheeze and/or difficulty breathing.	1-h max O_3: 196 ppb SD 78	PM_{10}	O_3 had significant effects on PEF and symptoms, with largest effects at lags 0 and 1 day. Symptoms related to O_3 included cough and phlegm. Ozone effects stronger than those for PM_{10}.	1-h max O_3 (per 50 ppb): Change in PEF (L/min): Morning: Lag 0: −1.32 (−3.21, 0.57) Lag 1: −0.39 (−2.24, 1.47) Lag 2: −0.97 (−2.94, 0.99) Afternoon: Lag 0: −1.81 (−3.60, −0.01) Lag 1: −2.32 (−4.17, −0.47) Lag 2: −0.21 (−2.44, 2.02) Odds ratios: Lower respiratory symptoms: Lag 0: 1.11 (1.05, 1.19) Lag 1: 1.08 (1.01, 1.15) Lag 2: 1.07 (1.02, 1.13)

Table AX7-1 (cont'd). Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
Latin America (cont'd)					
Romieu et al. (1998) Mexico City Mar-May 1996 (1st phase) Jun-Aug 1996 (2nd phase)	47 street workers aged 18-58 years randomly selected to take a daily supplement (vitamin C, vitamin E, and beta carotene) or placebo during 1st phase of study. Following washout period, the use of supplements and placebos was reversed during 2nd phase. Pulmonary function test performed twice a week at end of workday. Plasma concentrations of beta carotene and α-tocopherol measured. Analysis using GEE models.	1-h max O_3: 55% of days exceeded 110 ppb Workday hourly average during workday prior to pulmonary function test: 67.3 ppb SD 24	PM_{10}, NO_2	During the 1st phase, O_3 levels were significantly associated with declines in lung function parameters. No associations were observed in the daily supplement group. A significant supplement effect was observed. Ozone-related decrements also were observed during the 2nd phase, however the associations were not significant. Supplementation with antioxidants during the 1st phase may have had a residual protective effect on the lung.	1-h max O_3 (per 10 ppb): Placebo group: 1st phase: FEV_1: Lag 0: −17.9 mL (SE 5.4)* FVC: Lag 0: −14.8 mL (SE 7.1)* 2nd phase: FEV_1: Lag 0: −3.3 mL (SE 6.5) FVC: Lag 0: −0.27 mL (SE 7.8) No significant associations with O_3 observed when taking supplements.
Romieu et al. (2002) Mexico City Oct 1998-Apr 2000	158 asthmatic children aged 6-16 years randomly given a vitamin (C and E) supplement or placebo followed for 12 weeks. Peak flow was measured twice a day and spirometry was performed twice per week in the morning. Double blind study. Plasma concentration of vitamin E levels measured. Analysis using GEE models.	1-h max O_3: 102 ppb SD 47	PM_{10}, NO_2	Ozone levels were significantly correlated with decrements in FEF_{25-75} in the placebo group, but not in the supplement group. When analysis was restricted to children with moderate-to-severe asthma, amplitudes of decrements were larger and significant for FEV_1, FEF_{25-75}, and PEF in the placebo group. Supplementation with antioxidants may modulate the impact of O_3 exposure on the small airways of children with moderate to severe asthma.	1-h max O_3 (per 10 ppb): Children with moderate to severe asthma: Placebo group: O_3 with PM_{10} and NO_2 models: FEV_1: Lag 1: −4.59 mL, p = 0.04 $FEF_{2.5-75}$: Lag 1: −13.32 mL/s, p ≤ 0.01 PEF: Lag 1: −15.01 mL/s, p = 0.04 No association observed in the vitamin supplement group.

Table AX7-1 (cont'd). Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
Latin America (cont'd)					
Romieu et al. (2004) Mexico City Oct 1998-Apr 2000	Additional analysis of Romieu et al., 2002 with data on glutathion S-transferase M1 polymorphism (GSTM1 null genotype) in 158 asthmatic children. Analysis performed using GEE models, stratified by GSTM1 genotype (null versus positive) within the two treatment groups (placebo and antioxidant supplemented).	1-h max O_3: 102 ppb SD 47	None	In the placebo group, O_3 exposure was significantly and inversely associated with $FEF_{2.5-75}$ in children who had the GSTM1 null genotype, with larger effects seen in children with moderate-to-severe asthma. No significant decrements were seen in the GSTM1 positive children. These results provide preliminary evidence that asthmatic children who may be genetically impaired to handle oxidative stress are most susceptible to the effect of O_3 on small airways function.	1-h max O_3 (per 50 ppb): $FEF_{2.5-75}$ in children with moderate to severe asthma: Placebo group: GSTM1 null: Lag 1: −80.8 mL/s, p = 0.002 GSTM1 positive: Lag 1: −34.4 mL/s, p > 0.10 Supplement group: GSTM1 null: Lag 1: −7.3 mL/s, p > 0.10 GSTM1 positive: Lag 1: 2.0 mL/s, p > 0.10
Australia					
Jalaludin et al. (2000) Sydney, Australia Feb-Dec 1994	Three groups of children (mean age 9.6 years): (1) n = 45 with history of wheeze 12 months, positive histamine challenge, and doctor-diagnosed asthma; (2) n = 60 with history of wheeze and doctor-diagnosed asthma; (3) n = 20 with only history of wheeze. Examined for evening PEF and daily O_3 using GEE model and population regression models.	Mean daytime O_3 (6 a.m.-9 p.m.): 12 ppb SD 6.8 Maximum daytime O_3 (6 a.m.-9 p.m.): 26 ppb SD 14.4	PM_{10}, NO_2	A significant negative association was found between daily mean deviation in PEF and same-day mean daytime O_3 concentration after adjusting for copollutants, time trend, meteorological variables, pollen count, and *Alternaria* count. The association was stronger in a subgroup of children with bronchial hyper-reactivity and doctor-diagnosed asthma. In contrast, the same-day maximum O_3 concentration was not statistically associated.	Change in PEF (per ppb mean daytime O_3): All children (n = 125): O_3 only model: −0.9178 (SE 0.4192), p = 0.03 O_3 with PM_{10} model: −0.9195 (SE 0.4199), p = 0.03 O_3 with PM_{10} and NO_2 model: −0.8823 (SE 0.4225), p = 0.04

Table AX7-1 (cont'd). Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
Australia (cont'd)					
Jalaludin et al. (2004) Sydney, Australia Feb-Dec 1994	Same three groups of children as studied in Jalaludin et al., 2000. Examined relationship between O_3 and evening respiratory symptoms (wheeze, dry cough, and wet cough), evening asthma medication use (inhaled β_2-agonist and inhaled corticosteroids), and doctor visits for asthma. Analysis using GEE logistic regression models.	Mean daytime O_3 (6 a.m.-9 p.m.): 12 ppb SD 6.8 Maximum daytime O_3 (6 a.m.-9 p.m.): 26 ppb SD 14.4	PM_{10}, NO_2	No significant O_3 effects observed on evening symptoms, evening asthma medication use, and doctors visits. Also, no differences in the response of children in the three groups. A potential limitation is that the use of evening outcome measures rather than morning values may have obscured the effect of ambient air pollutants. Only consistent relationship was found between mean daytime PM_{10} concentrations and doctor visits for asthma.	Mean daytime O_3 (per 8.3 ppb): Odds ratios: All children (n = 125): Wheeze: Lag 1: 1.00 (0.93, 1.08) Dry cough: Lag 1: 1.03 (0.96, 1.11) Wet cough: Lag 1: 0.97 (0.92, 1.03) Inhaled β_2-agonist use: Lag 1: 1.02 (0.97, 1.07) Inhaled corticosteroid use: Lag 1: 1.02 (0.99, 1.04) Doctor visit for asthma: Lag 1: 1.05 (0.77, 1.43)
Asia					
Chen et al. (1998) Six communities in Taiwan 1994-1995	4,697 school children (age unspecified) from a rural area (Taihsi), urban areas (Keelung and Sanchung), and petrochemical industrial areas (Jenwu, Linyuan, and Toufen) cross-sectionally examined for respiratory symptoms and diseases using parent-completed questionnaires. Multiple logistic regression models used to compare odds of symptoms and diseases in urban or petrochemical areas to the rural area after controlling for potential confounding factors.	24-h avg O_3: Rural area: 52.56 ppb Urban area: Mean range 38.34-41.90 ppb Petrochemical industrial area: Mean range 52.12-60.64 ppb	SO_2, CO, PM_{10}, NO_2	School children in urban communities, but not in petrochemical industrial areas, had significantly more respiratory symptoms and diseases compared to those living in the rural community. However, mean O_3 levels in the urban communities were lower than that of the rural community. No causal relationship could be derived between O_3 and respiratory symptoms and diseases in this cross-sectional study.	Urban areas compared to rural area: Odds ratios: Respiratory symptoms: Morning cough: 1.33 (0.98, 1.80) Day or night cough: 1.67 (1.21, 2.29) Shortness of breath: 1.40 (1.04, 1.91) Wheezing or asthma: 1.68 (1.11, 2.54)

Table AX7-1 (cont'd). Effects of Acute O_3 Exposure on Lung Function and Respiratory Symptoms in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
Asia (cont'd)					
Chen et al. (1999) Three towns in Taiwan: Sanchun, Taihsi, Linyuan May 1995-Jan 1996	Valid lung function data obtained once on each of 895 children (age 8-13 years) in three towns. Examined relation between lung function and pollution concentrations on same day and over previous 1, 2, and 7 days. Multipollutant models examined.	1-h max O_3: Range 19.7-110.3 ppb	SO_2, CO, PM_{10}, NO_2	FEV_1 and FVC significantly associated with 1-day lag O_3. FVC also related to NO_2, SO_2, and CO. No PM_{10} effects observed. Only O_3 remained significant in multipollutant models. No PM_{10} effects. A significant O_3 effect was not evident at O_3 levels below 60 ppb.	Change in lung function: O_3 only models: Lag 1: FEV_1: −0.64 mL/ppb (SE 0.34)* FVC: −0.79 mL/ppb (SE 0.32)* O_3 with NO_2 models: Lag 1: FEV_1: −0.85 mL/ppb (SE 0.34)* FVC: −0.91 mL/ppb (SE 0.37)* *$p < 0.05$

Table AX7-2. Effects of Acute O_3 Exposure on Cardiovascular Outcomes in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
United States					
Liao et al. (2004) Three locations in U.S.: Minneapolis, MN; Jackson, MS; Forsyth County, NC 1996-1998	5,431 cohort members of the Atherosclerosis Risk in Communities study, men and women aged 45-64 years at entry in 1987. Association between O_3 and cardiac autonomic control assessed using 5-minute heart rate variability indices collected over a 4-hour period. Analysis using multivariable linear regression models, adjusting for individual cardiovascular disease risk factors and meteorological factors.	8-h avg O_3 (10 a.m.-6 p.m.): 41 ppb SD 16	PM_{10}, CO, SO_2, NO_2	Significant interaction between O_3 and ethnicity in relation to high-frequency power ($p < 0.05$). Ambient O_3 significantly associated with high-frequency power among whites, but not blacks. No significant O_3 effect on other heart rate variability indices, including low-frequency power and SD of normal R-R intervals. More consistent relationships observed between PM_{10} and heart rate variability indices.	8-h avg O_3 (per 16 ppb): Log-transformed high-frequency power: White race: Lag 1: −0.069 (SE 0.019)* Black race: Lag 1: 0.047 (SE 0.034) Log-transformed high-frequency power: Lag 1: −0.010 (SE 0.016) SD of normal R-R intervals: Lag 1: −0.336 (SE 0.290) *$p < 0.05$
Peters et al. (2000a) Eastern Massachusetts 1995-1997	Records of detected arrhythmias and therapeutic interventions were downloaded from defibrillators implanted in cardiac clinic patients aged 22-85 years (n = 100). Analysis was restricted to defibrillator discharges precipitated by ventricular tachycardias or fibrillation. Data was analyzed by logistic regression models using fixed effect models with individual intercepts.	24-h avg O_3: 18.6 ppb IQR 14.0	$PM_{2.5}$, PM_{10}, BC, CO, NO_2, SO_2	No significant O_3 effects observed for defibrillator discharge interventions. For patients with ten or more interventions, increased arrhythmias were associated significantly with $PM_{2.5}$, CO, and NO_2 at various lag periods, but not O_3.	24-h avg O_3 (per 32 ppb): Odds ratios: Defibrillator discharges: Patients with at least one event: Lag 0: 0.96 (0.47, 1.98) Patients with at least ten events: Lag 0: 1.23 (0.53, 2.87)

Table AX7-2 (cont'd). Effects of Acute O_3 Exposure on Cardiovascular Outcomes in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
United States (cont'd)					
Peters et al. (2001) Greater Boston area, MA Jan 1995-May 1996	Case-crossover study design used to investigate association between air pollution and triggering of myocardial infarction in 772 patients (mean age 61.6 years). For each subject, one case period was matched to three control periods 24 hours apart. Conditional logistic regression used for analysis.	1-h max O_3: 19.8 ppb 24-h avg O_3: 19.9 ppb	$PM_{2.5}$, PM_{10}, $PM_{10-2.5}$, BC, CO, NO_2, SO_2	None of the gaseous pollutants, including O_3, were significantly associated with the triggering of myocardial infarctions. Significant associations reported for $PM_{2.5}$ and PM_{10}.	Odds ratios: Myocardial infarctions: 2-h avg O_3 (per 45 ppb): Lag 1 hour: 1.31 (0.85, 2.03) 24-h avg O_3 (per 30 ppb): Lag 24 hours: 0.94 (0.60, 1.49)
Park et al. (2004) Greater Boston area, MA Nov 2000-Oct 2003	Effect of O_3 on heart rate variability was examined in 497 adult males (mean age 72.7 years). Subjects were monitored during a 4-minute rest period between 8 a.m. and 1 p.m. Ozone levels measured at central site 1 km from study site. Exposure averaging times of 4-hours, 24-hours, and 48-hours investigated. Modifying effects of hypertension, ischemic heart disease, diabetes, and use of cardiac/ antihypertensive medications also examined.	24-h avg O_3: 23.0 ppb SD 13.0	$PM_{2.5}$, particle number concentration, BC, NO_2, SO_2, CO	Of the pollutants examined, only $PM_{2.5}$ and O_3 showed significant associations with heart rate variability outcomes. The 4-hour averaging period was most strongly associated with heart rate variability indices. The O_3 effect was robust in models including $PM_{2.5}$. The associations between O_3 and heart rate variability indices were stronger in subjects with hypertension (n = 335) and ischemic heart disease (n = 142). In addition, calcium-channel blockers significantly influenced the effect of O_3 on low frequency power. Major limitations of this study are the use of a short 4-minute period to monitor heart rate variability and the lack of repeated measurements for each subject.	4-h avg O_3 (per 13 ppb): Change in low frequency power: All subjects: −11.5% (−21.3, −0.4) Subjects with hypertension: −12.6% (−25.0, 1.9) Subjects without hypertension: −5.4% (−21.6, 14.1) Subjects with ischemic heart disease: −25.8% (−41.9, −5.3) Subjects without ischemic heart disease: −4.8% (−16.7, 8.8)

Table AX7-2 (cont'd). Effects of Acute O_3 Exposure on Cardiovascular Outcomes in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
United States (cont'd)					
Gold et al. (2000; reanalysis Gold et al., 2003) Boston, MA Jun-Sep 1997	Repeated measurements of heart rate variability in subjects aged 53-87 years (n = 21, 163 observations). Twenty-five minute protocol included 5 minutes each of rest, standing, exercise outdoors, recovery, and 20 cycles of slow breathing. Ozone levels measured at central site 4.8 miles from study site. Analyses using random effects models and GAM with stringent convergence criteria.	1-h max O_3: 25.7 ppb IQR 23.0	$PM_{2.5}$, NO_2, SO_2	Increased levels of O_3 were associated with reduced r-MSSD (square root of the mean of the squared differences between adjacent normal RR intervals) during the slow breathing period after exercise outdoors. The estimated O_3 effects were similar to those of $PM_{2.5}$. Results suggest that O_3 exposure may decrease vagal tone, leading to reduced heart rate variability.	1-h max O_3 (per 23.0 ppb): Change in r-MSSD: During first rest period: O_3 only model: −3.0 ms (SE 1.9), p = 0.12 During slow breathing period: O_3 only model: −5.8 ms (SE 2.4), p = 0.02 O_3 with $PM_{2.5}$ model: −5.4 ms (SE 2.5), p = 0.03
Canada					
Rich et al. (2004) Vancouver, British Columbia, Canada Feb-Dec 2000	Case-crossover study design used to investigate association between air pollution and cardiac arrhythmia in patients aged 15-85 years (n = 34) with implantable cardioverter defribillators. Controls periods were selected 7 days before and after each case day. Analysis using conditional logistic regression.	1-h max O_3: 27.5 ppb SD 9.7 IQR 13.4	$PM_{2.5}$, PM_{10}, EC, OC, SO_4^{2-}, CO, NO_2, SO_2	No consistent association between any of the air pollutants, including O_3, and implantable cardioverter defribillators discharges. No significant association observed in all year data, however, significant relationship found in winter months at a 3-day lag. Overall, little evidence that air pollutants affect risk of cardiac arrhythmias, however, power was limited to study subtle effects.	No quantitative results for O_3.

Table AX7-2 (cont'd). Effects of Acute O_3 Exposure on Cardiovascular Outcomes in Field Studies

Reference, Study Location and Period	Outcomes and Methods	Mean O_3 Levels	Copollutants Considered	Findings, Interpretation	Effects
Canada (cont'd)					
Vedal et al. (2004) Vancouver, British Columbia, Canada 1997-2000	Retrospective, longitudinal panel study of 50 patients (age 12-77 years) with implantable cardioverter defribillators. Occurrence of cardiac arrhythmia was associated with air pollutants over four-year period. GEE used for analysis.	1-h max O_3: 28.2 ppb SD 10.2 IQR 13.8	PM_{10}, CO, NO_2, SO_2	No consistent association between any of the air pollutants and % change in arrhythmia. Among patients with at least 2 arrhythmia event-days per year, a significant negative relationship between O_3 and arrhythmias was observed at lag 3-day during the summer, but no associations were found during the winter. These results do not provide evidence for an O_3 effect on cardiac arrhythmias in susceptible patients.	No quantitative results for O_3.
Latin America					
Holguín et al. (2003) Mexico City Feb-Apr 2000	Association between O_3 and heart rate variability examined in 34 elderly subjects (mean age 79 years). in a nursing home. Subjects were monitored during a 5-minute rest period between 8 a.m. and 1 p.m. every other day for a 3-month period. A total of 595 observations were collected. Ambient O_3 levels obtained from central site 3 km upwind from study site. Analysis performed using GEE models adjusting for potential confounding factors including age and average heart rate.	1-h max O_3: 149 ppb SD 40	$PM_{2.5}$ (indoor, outdoor, total), NO_2, SO_2, CO	Only $PM_{2.5}$ and O_3 were significantly associated with heart rate variability outcomes. A significant effect of O_3 on heart rate variability was limited to subjects with hypertension (n = 21). In two-pollutant models, the magnitude of the $PM_{2.5}$ effect decreased slightly but remained significant, whereas O_3 was no longer associated with heart rate variability indices.	1-h max O_3 (per 10 ppb): Log_{10} high frequency power/100,000 ms^2: All subjects: −0.010 (−0.022, 0.001) Subjects with hypertension: −0.031 (−0.051, −0.012) Subjects without hypertension: 0.002 (−0.012, 0.016) Log_{10} low frequency power/100,000 ms^2: All subjects: −0.010 (−0.021, 0.001) Subjects with hypertension: −0.029 (−0.046, −0.011) Subjects without hypertension: 0.001 (−0.012, 0.015)

Table AX7-3. Effects of O_3 on Daily Emergency Department Visits

Reference, Study Location and Period	Outcomes and Design	Mean O_3 Levels	Copollutants Considered	Lag Structure Examined	Method, Findings, Interpretation	Effects (Relative Risk and 95% CI)
United States						
Jaffe et al. (2003) Cincinnati, Cleveland, and Columbus, OH Jun-Aug 1991-1996	Daily time series study of emergency department visits for asthma among Medicaid recipients aged 5-34 years.	8-h max O_3: Cincinnati: 60 ppb SD 20 Cleveland: 50 ppb SD 17 Columbus: 57 ppb SD 16	PM_{10}, NO_2, SO_2	1, 2, 3	Poisson regression with control for city, day of week, week, year, minimum temperature, overall trend, and a dispersion parameter. No specific effort to control cycles, but regression residuals were uncorrelated, presumably due to seasonal restriction. Results shown for individual cities and overall. PM_{10} available only every 6th day. Positive relationships between emergency department visits for asthma and 8-h max O_3 levels lagged 2 to 3 days. Results of borderline statistical significance. Other pollutants also related to asthma emergency department visits in single-pollutant models.	8-h max O_3 (per 30 ppb): Cincinnati: Lag 2: 1.16 (1.00, 1.37) Cleveland: Lag 2: 1.03 (0.92, 1.16) Columbus: Lag 3: 1.16 (0.98, 1.37) Three cities: 1.09 (1.00, 1.19)
Jones et al. (1995) Baton Rouge, LA Jun-Aug 1990	Daily emergency department visits for respiratory complaints over a 3-month period in pediatric (age 0-17 years), adult (age 18-60 years), and geriatric (age > 60 years) subgroups.	1-h max O_3: 69.1 ppb SD 28.7 24-h avg O_3: 28.2 ppb SD 11.7	Mold, pollen	Not specified.	Relatively simple statistical approach using ordinary least squares regression to model effects of O_3 by itself and of O_3 along with pollen counts, mold counts, temperature, and relative humidity. No direct control of cycles but authors reported non-significant autocorrelations among model residuals. Data restriction to 3-month period may have reduced any cyclic behavior. Significant associations between respiratory emergency department visits and O_3 observed for adult age group only in multiple regression models.	24-h avg O_3 (per 20 ppb): Pediatric: 0.87 (0.65, 1.09) Adult: 1.20 (1.01, 1.39) Geriatric: 1.27 (0.93, 1.61)

Table AX7-3 (cont'd). Effects of O_3 on Daily Emergency Department Visits

Reference, Study Location and Period	Outcomes and Design	Mean O_3 Levels	Copollutants Considered	Lag Structure Examined	Method, Findings, Interpretation	Effects (Relative Risk and 95% CI)
United States (cont'd)						
Weisel et al. (2002) New Jersey May-Aug 1995	Daily asthma emergency department visits for all ages.	1-h max O_3; 5-h avg O_3 (10 a.m.-3 p.m.); and 8-h avg O_3 (2 p.m.-10 p.m.) analyzed. Levels not reported.	Pollen, spores	0, 1, 2, 3	No control for time, but authors report no autocorrelation, which alleviates concerns about lack of control. Significant O_3 effects reported, even after adjusting for potential confounding by pollen. All three O_3 indices gave essentially same results.	Slope estimate (visits/day/ppb): Excluding data from May 1995 when pollen levels are high: O_3 only model: Lag 0: 0.039, p = 0.049 O_3 with pollen model: Lag 0: 0.040, p = 0.014
Friedman et al. (2001) Atlanta, GA Jul-Aug 1996	Emergency department visits and hospital admissions for asthma in children aged 1-16 years. Outcomes measures during 1996 Summer Olympics were compared to a baseline period of 4 weeks before and after the Olympic Games.	1-h max O_3: Levels not reported.	NO_2, SO_2, CO, PM_{10}, mold	0, 0-1, 0-2	Analysis using Poisson GEE models addressing serial autocorrelation. An overall decrease was observed when comparing the number of visits and hospitalizations during the Olympic Games to the baseline period. However, significant associations between O_3 and asthma events were found during the Olympic Games.	1-h max O_3 (per 50 ppb): Pediatric emergency departments: Lag 0: 1.2 (0.99, 1.56) Lag 0-1: 1.4 (1.04, 1.79) Lag 0-2: 1.4 (1.03, 1.86)
Metzger et al. (2004) Atlanta, GA Jan 1993-Aug 2000	Emergency department visits for total and cause-specific cardiovascular diseases by age groups 19+ years and 65+ years.	8-h max O_3: Median 53.9 ppb 10th % to 90th % range 13.2 - 44.7	NO_2, SO_2, CO, $PM_{2.5}$, PM_{10}, $PM_{10-2.5}$, ultrafine PM count, SO_4^{2-}, H^+, EC, OC, metals, oxygenated hydrocarbons	0-2	Poisson GLM regression used for analysis. *A priori* models specified a lag of 0 to 2 days. Secondary analyses performed to assess alternative pollutant lag structures, seasonal influences, and age effects. Cardiovascular visits were significantly associated with several pollutants, including NO_2, CO, and $PM_{2.5}$, but not O_3.	8-h max O_3 (per 25 ppb): All ages: Total cardiovascular: 1.008 (0.987, 1.030) Dysrhythmia: 1.008 (0.967, 1.051) Congestive heart failure: 0.965 (0.918, 1.014) Ischemic heart disease: 1.019 (0.981, 1.059) Peripheral vascular and cerebrovascular disease: 1.028 (0.985, 1.073)

Table AX7-3 (cont'd). Effects of O_3 on Daily Emergency Department Visits

Reference, Study Location and Period	Outcomes and Design	Mean O_3 Levels	Copollutants Considered	Lag Structure Examined	Method, Findings, Interpretation	Effects (Relative Risk and 95% CI)
United States (cont'd)						
Peel et al. (2004) Atlanta, GA Jan 1993-Aug 2000	Emergency department visits for total and cause-specific respiratory diseases by age groups 0-1, 2-18, 19+, and 65+ years.	8-h max O_3: 55.6 ppb SD 23.8	NO_2, SO_2, CO, $PM_{2.5}$, PM_{10}, $PM_{10-2.5}$, ultrafine PM count, SO_4^{2-}, H^+, EC, OC, metals, oxygenated hydrocarbons	0-2	Poisson GEE and GLM regression used for analysis. *A priori* models specified a lag of 0 to 2 days. Also performed secondary analyses estimating the overall effect of pollution over the previous two weeks. Seasonal analyses indicated stronger associations with asthma in the warm months. Quantitative results not presented for multipollutant, age-specific, and seasonal analyses.	8-h max O_3 (per 25 ppb): All ages: Total respiratory: 1.024 (1.008, 1.039) Upper respiratory infections: 1.027 (1.009, 1.045) Asthma: 1.022 (0.996, 1.049) Pneumonia: 1.015 (0.981, 1.050) COPD: 1.029 (0.977, 1.084)
Tolbert et al. (2000) Atlanta, GA Jun-Aug 1993-1995	Pediatric (aged 0-16 years) asthma emergency department visits over three summers in Atlanta. A unique feature of the study was assignment of O_3 exposures to zip code centroids based on spatial interpolation from nine O_3 monitoring stations.	1-h max O_3: 68.8 ppb SD 21.1 8-h max O_3: 59.3 ppb SD 19.1	PM_{10}, NO_2, mold, pollen	1	*A priori* specification of model, including a lag of 1 day for all pollutants and meteorological variables. Secondary analysis using logistic regression of the probability of daily asthma visits, referenced to total visits (asthma and non-asthma). Significant association with O_3 and PM_{10} in 1-, but not in 2-pollutant models (correlation between O_3 and PM_{10}: r = 0.75). Secondary analysis indicated nonlinearity, with O_3 effects clearly significant only for days ≥ 100 ppb versus days < 50 ppb.	8-h max O_3 (per 20 ppb): Poisson regression: O_3 only model: 1.040 (1.008, 1.074) Logistic regression: O_3 only model: 1.042 (1.017, 1.068) O_3 with PM_{10} model: 1.024 (0.982, 1.069)

Table AX7-3 (cont'd). Effects of O_3 on Daily Emergency Department Visits

Reference, Study Location and Period	Outcomes and Design	Mean O_3 Levels	Copollutants Considered	Lag Structure Examined	Method, Findings, Interpretation	Effects (Relative Risk and 95% CI)
United States (cont'd)						
Zhu et al. (2003) Atlanta, GA Jun-Aug 1993-1995	Asthma emergency department visits in children (age 0-16 years) over three summers in Atlanta.	8-h max O_3: Levels not reported.	None	1	Used Bayseian hierarchical modeling to address model variability and spatial associations. Data was analyzed at the zip code level to account for spatially misaligned longitudinal data. A positive, but nonsignificant relationship between O_3 and emergency room visits for asthma.	8-h max O_3 (per 20 ppb): Posterior median: 1.016 (0.984, 1.049)
Canada						
Delfino et al. (1997b) Montreal, Quebec, Canada Jun-Sep 1992-1993	Daily time series ecologic study of emergency department visits for respiratory complaints within five age strata (< 2, 2-18, 19-34, 35-64, > 64 years).	1-h max O_3: 1992: 33.2 ppb SD 12.6 1993: 36.2 ppb SD 13.8 8-h max O_3: 1992: 28.8 ppb SD 11.3 1993: 30.7 ppb SD 11.5	PM_{10}, $PM_{2.5}$, SO_4^{2-}, H^+	0, 1, 2	Used ordinary least squares, with 19-day weighted moving average pre-filter to control cycles; weather also controlled. Significant effects reported for 1-day lag O_3 in 1993 only for age > 64 years. This O_3 effect reported to be robust in two-pollutant models. Low O_3 levels raise plausibility concerns. Short data series, multiple tests performed, and inconsistent results across years and age groups raise possibility of chance findings.	1993 (age > 64 years): 1-h max O_3 (per 36.2 ppb): Lag 1: 1.214 (1.084, 1.343) 8-h max O_3 (per 30.7 ppb): Lag 1: 1.222 (1.091, 1.354)

Table AX7-3 (cont'd). Effects of O_3 on Daily Emergency Department Visits

Reference, Study Location and Period	Outcomes and Design	Mean O_3 Levels	Copollutants Considered	Lag Structure Examined	Method, Findings, Interpretation	Effects (Relative Risk and 95% CI)
Canada (cont'd)						
Delfino et al. (1998b) Montreal, Quebec, Canada Jun-Aug 1989-1990	Daily time series ecologic study of emergency department visits for respiratory complaints across all ages and within four age strata (<2, 2-34, 35-64, >64 years).	1-h max O_3: 1989: 44.1 ppb SD 18.3 1990: 35.4 ppb SD 12.9 8-h max O_3: 1989: 37.5 ppb SD 15.5 1990: 29.9 ppb SD 11.2	Estimated $PM_{2.5}$	0, 1, 2	Same analytical approach used in Delfino et al., 1997. Results presented only for 1989. Significant effects reported for 1-day lag O_3 in 1989 only for age > 64 years. This O_3 effect reported to be robust in 2-pollutant models.	1989 (age > 64 years): 1-h max O_3 (per 44.1 ppb): Lag 1: 1.187 (0.969, 1.281) 8-h max O_3 (per 37.5 ppb): Lag 1: 1.218 (1.097, 1.338) No significant O_3 effects in other age groups or for 1990.
Stieb et al. (1996) Saint John, New Brunswick, Canada May-Sep 1984-1992	Daily emergency department visits for asthma in all ages, age <15 years and 15+ years.	1-h max O_3: 41.6 ppb Range 0-160 95th % 75	SO_2, NO_2, SO_4^{2-}, TSP	0, 1, 2, 3	Poisson analysis with control of time based on 19-day moving average filter. Also controlled day of week and weather variables. Ozone was only pollutant consistently associated with emergency department visits for asthma, but effect appeared nonlinear, with health impacts evident only above 75 ppb O_3.	1-h max O_3 > 75 ppb: Lag 2: 1.33 (1.10, 1.56)

Table AX7-3 (cont'd). Effects of O_3 on Daily Emergency Department Visits

Reference, Study Location and Period	Outcomes and Design	Mean O_3 Levels	Copollutants Considered	Lag Structure Examined	Method, Findings, Interpretation	Effects (Relative Risk and 95% CI)
Europe						
Atkinson et al. (1999a) London, England 1992-1994	Emergency department visits for respiratory complaints, asthma for all ages and age 0-14, 15-64, and 65+ years.	8-h max O_3: 17.5 ppb SD 11.5	NO_2, SO_2, CO, PM_{10}	0, 1, 2, 3 0-1, 0-2, 0-3	Poisson GLM regression used for analysis. No warm season analysis attempted. PM_{10} positively associated.	8-h max O_3 (per 25.7 ppb): All ages: Total respiratory: Lag 1: 1.017 (0.991, 1.043) Asthma: Lag 1: 1.027 (0.983, 1.072)
Thompson et al. (2001) Belfast, N Ireland 1993-1995	Asthma emergency department admissions in children (age unspecified)	24-h avg O_3: Warm season: 18.7 ppb IQR 9 Cold season: 17.1 ppb IQR 12	PM_{10}, SO_2, NO_2, CO, benzene	0, 0-1, 0-2, 0-3	GLM with sinusoids. Pre-adjustment. Very low O_3 levels in both seasons. No O_3 effect in warm season. Significant inverse O_3 associations in full-year and cold-season models. After adjusting for benzene in model O_3 was no longer negatively associated with asthma visits.	24-h avg O_3 (per 10 ppb): All year: O_3 only model: Lag 0-1: 0.93 (0.87, 1.00) O_3 with benzene model: Lag 0-1: 1.08 (0.97, 1.21) Warm season: O_3 only model: Lag 0-1: 0.99 (0.89, 1.10) Cold season: O_3 only model: Lag 0-1: 0.89 (0.82, 0.97)
Bourcier et al. (2003) Paris, France Jan 1999-Dec 1999	Ophthalmological emergency examination; conjunctivitis and related ocular surface problems.	24-h avg O_3: 35.7 µg/m^3 Range 1-97	PM_{10}, SO_2, NO_2	0, 1, 2, 3	Logistic Regression	Results indicate a strong relation to NO_2 and NO. 24-h avg O_3 (per 69 µg/m^3): Conjunctivitis: Lag 0: 1.13 (0.90, 1.42)

Table AX7-3 (cont'd). Effects of O_3 on Daily Emergency Department Visits

Reference, Study Location and Period	Outcomes and Design	Mean O_3 Levels	Copollutants Considered	Lag Structure Examined	Method, Findings, Interpretation	Effects (Relative Risk and 95% CI)
Europe (cont'd)						
Castellsague et al. (1995) Barcelona, Spain 1985-1989	Daily emergency department visits for asthma in persons aged ≥ 14 years.	1-h max O_3: Summer: 43 ppb IQR 22 Winter: 29 ppb IQR 16	BS, SO_2, NO_2	Not specified.	Poisson regression with year and month dummy variables and extensive control for weather factors (minimum, maximum, mean temperature, relative humidity, dewpoint temperature; continuous and categorical parameterizations)	1-h max O_3 (per 12.7 ppb): Summer: 0.991 (0.939, 1.045) Winter: 1.055 (0.998, 1.116)
Tobías et al. (1999) Barcelona, Spain 1986-1989	Daily asthma emergency department visits. Investigated sensitivity of results to four alternative methods for controlling asthma epidemics.	Levels not reported.	BS, NO_2, SO_2	Not specified.	Poisson analysis using APHEA methodology. Asthma epidemics either not controlled, or controlled with one, six, or individual epidemic dummy variables.	O_3 results were sensitive to method used to control asthma epidemics, with regression coefficients ranging over 5-fold depending on the model. Only 1 of 8 models reported had a significant O_3 effect.
Tenías et al. (1998, 2002) Valencia, Spain 1994-1995	Daily emergency department visits for asthma and COPD in persons aged > 14 years.	1-h max O_3: All year: 62.8 μg/m^3 Warm season: 74.0 μg/m^3 Cool season: 51.4 μg/m^3	BS, NO_2, SO_2, CO	0, 1, 2, 3, 4, 5	Poisson analysis using APHEA methodology. Compared warm and cold season effects. GAM explored in sensitivity analysis. For asthma, both O_3 and NO_2 significant in 1- and 2-pollutant models, and O_3 effect larger in warm season. For COPD, both O_3 and CO significant in both 1- and 2-pollutant models and no difference in O_3 effects by season.	1-h max O_3 (per 10 μg/m^3): Asthma: All year: Lag 1: 1.06 (1.01, 1.11) Warm season: Lag 1: 1.08 (1.02, 1.05) Cold season: Lag 1: 1.04 (0.97, 1.11) COPD: All year: Lag 5: 1.06 (1.02, 1.10)

Table AX7-3 (cont'd). Effects of O_3 on Daily Emergency Department Visits

Reference, Study Location and Period	Outcomes and Design	Mean O_3 Levels	Copollutants Considered	Lag Structure Examined	Method, Findings, Interpretation	Effects (Relative Risk and 95% CI)
Latin America						
Hernández-Garduño et al. (1997) Mexico City May 1992-Jan 1993	Visits to clinics for respiratory diseases in persons aged 1 month to 92 years.	% time exceeding 1-h max O_3 of 120 ppb: 6.1-13.2% by location	SO_2, NO_2, CO	0, 1, 2, 3, 4, 5	GLM with pre-adjustment. Ozone at lags 0 and 5 days significantly associated with daily visits for all ages, age < 14 years, and 15+ years. Neither O_3 nor NO_2 significant in 2-pollutant model.	1-h max O_3 (per maximum less average, value not given) Lag 0: 1.19 (SE 0.08), $p < 0.05$ Lag 5: 1.19 (SE 0.08), $p < 0.05$
Lin et al. (1999) São Paulo, Brazil May 1991-Apr 1993	Daily pediatric (age unspecified) respiratory emergency department visits.	1-h max O_3: 34 ppb SD 22	SO_2, CO, PM_{10}, NO_2	0, 1, 0-1, 0-2, 0-3, 0-4, 0-5	Seasonal control using month dummy variables. Also controlled day of week, temperature. Both O_3 and PM_{10} associated with outcome alone and together.	1-h max O_3 (per 5 ppb): O_3 only model: Lag 0-4: 1.022 (1.016, 1.028) O_3 with PM_{10} model: Lag 0-4: 1.015 (1.009, 1.021)
Ilabaca et al. (1999) Santiago, Chile Feb 1995-Aug 1996	The association between pollutant levels and emergency visits for pneumonia and other respiratory illnesses among children.	O_3 1-h max: Warm season: 66.6 µg/m^3 SD 25.2 Cold season: 27.6 µg/m^3 SD 20.2	PM_{10}, $PM_{2.5}$, SO_2, NO_2	1, 2, 3, 1-7	Poisson regression analysis.	Warm season: 1-h max O_3 (per 30 µg/m^3): Lag 2: 1.019 (1.003, 1.035) Cold season: 1-h max O_3 (per 24 µg/m^3): Lag 2: 0.995 (0.978, 1.011)
Asia						
Chew et al. (1999) Singapore Jan 1990-Dec 1994	Emergency department visits for asthma in persons aged 3-21 years.	1-h max O_3: 23 ppb SD 15	TSP, PM_{10}, SO_2, NO_2	0, 1, 2	Simplistic but probably adequate control for time by including 1-day lagged outcome as covariate. In adjusted models that included covariates, O_3 had no significant effect.	No quantitative results presented for O_3.

Table AX7-4. Effects of O_3 on Daily Hospital Admissions

Reference, Study Location and Period	Outcomes and Design	Mean O_3 Levels	Copollutants Considered	Lag Structure Examined	Method, Findings, Interpretation	Effects (Relative Risk and 95% CI)
United States						
Niedell (2004) California 1992-1998	Emergency department visits for asthma within five age strata (0-1, 1-3, 3-6, 6-12, and 12-18 years).	O_3 (index not specified): 38.9 ppb SD 17.8 Low SES: 40.1 ppb High SES: 38.3 ppb	CO, NO_2, PM_{10}; multipollutant models	Not specified.	Statistical analysis using naturally occurring seasonal variations in pollutant concentrations within zip codes. Methodology not clearly stated. Consistent significant positive effects only observed for CO. Negative O_3 effect observed in all age groups. Number of smog alerts was negatively associated with asthma hospitalizations, indicating avoidance behavior on high O_3 days. Interaction term with indicator variable for low SES was positive in all age groups and statistically significant in age 3-6 years and 12-18 years, after adjusting for number of smog alerts.	Slope estimate (adjusting for number of smog alerts): O_3 with CO, NO_2, and PM_{10} models: Age 3-6 years: −0.038 (SE 0.014) Age 6-12 years: −0.044 (SE 0.013) Age 12-18 years: −0.022 (SE 0.011) O_3 × low SES interaction term: Age 3-6 years: 0.092 (SE 0.026) Age 6-12 years: 0.024 (SE 0.024) Age 12-18 years: 0.042 (SE 0.019)
Mann et al. (2002) South Coast air basin, CA 1988-1995	Ischemic heart disease admissions for age 40+ years.	8-h max O_3: 50.3 ppb SD 30.1 IQR 39.6	PM_{10}, CO, NO_2	0, 1, 2, 3, 4, 5, 0-1, 0-2, 0-3, 0-4	Poisson GAM with cubic B-splines; co-adjustment. No significant O_3 effects observed overall or in warm season. CO and NO_2 significant in full-year analyses.	O_3 coefficients all negative, but no consistent, significant effect.
Linn et al. (2000) Los Angeles, CA 1992-1995	Total respiratory and total cardiovascular admissions for age 30+ years.	24-h avg O_3: Winter: 14 ppb SD 7 Spring: 32 ppb SD 10 Summer: 36 ppb SD 8 Fall: 15 ppb SD 9	PM_{10}, CO, NO_2	0	Poisson GLM; co-adjustment. Only significant O_3 effects observed were inverse associations with total cardiac admission in full-year and winter season, suggesting residual confounding. No significant effects of O_3 on respiratory admissions.	24-h avg O_3 (per 10 ppb): All year: Respiratory: 1.008 (1.000, 1.016) Cardiovascular: 0.993 (0.987, 0.999)

Table AX7-4. Effects of O_3 on Daily Hospital Admissions

Reference, Study Location and Period	Outcomes and Design	Mean O_3 Levels	Copollutants Considered	Lag Structure Examined	Method, Findings, Interpretation	Effects (Relative Risk and 95% CI)
United States (cont'd)						
Nauenberg and Basu (1999) Los Angeles, CA 1991-1994	Unscheduled asthma admissions for all ages.	24-h avg O_3: 19.88 ppb SD 11.13	PM_{10}	0, 0-7	Poisson GLM with pre-adjustment. No significant effects of O_3. No warm season results presented.	24-h avg O_3 (per 20 ppb): All insurance categories: Lag 0: 1.01 (0.93, 1.08)
Sheppard et al. (1999; reanalysis Sheppard, 2003) Seattle, WA 1987-1994	Asthma admissions for age < 65 years.	8-h max O_3: 30.4 IQR 20	$PM_{2.5}$, PM_{10}, $PM_{10-2.5}$, SO_2, CO	1, 2, 3	Poisson GAM, reanalyzed with stringent convergence criteria; poisson GLM. Results stratified by season. Ozone significant predictor of outcome. No 2-pollutant model results reported for O_3.	8-h max O_3 (per 20 ppb): GLM with natural splines: Lag 2: 1.07 (1.01, 1.13)
Moolgavkar et al. (1997) Minneapolis/St. Paul, MN and Birmingham, AL 1986-1991	Total respiratory, pneumonia, and COPD admissions for age > 64 years.	24-h avg O_3: Minnesota: 26.2 ppb IQR 15.3 Alabama: 25.1 ppb IQR 12.7	PM_{10}, SO_2, NO_2	0, 1, 2, 3	Poisson GLM with co-adjustment. Both O_3 and PM_{10} significant in MN; not in AL. Ozone, but not PM_{10}, effects were robust to NO_2 and SO_2.	24-h avg O_3 (per 15 ppb): Minnesota: Total respiratory: Lag 1: 1.060 (1.033, 1.087) Pneumonia: Lag 1: 1.066 (1.034, 1.098) COPD: Lag 0: 1.045 (0.995, 1.067)
Schwartz et al. (1996) Cleveland, OH Apr-Oct 1988-1990	Total respiratory admissions for age 65+ years.	1-h max O_3: 56 ppb IQR 28	PM_{10}, SO_2	1-2	Poisson GLM with sinusoids; co-adjustment. Results available only for warm season. Ozone and PM_{10} both significant predictors of outcome. No 2-pollutant models reported.	1-h max O_3 (per 100 $\mu g/m^3$): 1.09 (1.02, 1.16)

Table AX7-4 (cont'd). Effects of O_3 on Daily Hospital Admissions

Reference, Study Location and Period	Outcomes and Design	Mean O_3 Levels	Copollutants Considered	Lag Structure Examined	Method, Findings, Interpretation	Effects (Relative Risk and 95% CI)
United States (cont'd)						
Weisel et al. (2002) New Jersey May-Aug 1995	Asthma admissions for all ages.	1-h max O_3; 5-h avg O_3 (10 a.m.-3 p.m.); and 8-h avg O_3 (2 p.m.-10 p.m.) analyzed. Levels not reported.	Pollen, spores	0, 1, 2, 3	No control for time, but authors report no autocorrelation, which alleviates concerns about lack of control. Significant O_3 effects reported after adjusting for potential confounding by pollen.	Slope estimate (admissions/day/ppb): 5-h avg O_3 and 8-h avg O_3: O_3 only model: Lag 2: 0.099, p = 0.057 All three O_3 indices: O_3 with pollen model: Lag 2: 0.11, p = 0.033
Canada						
Burnett et al. (1997a) 16 Canadian cities 1981-1991	Total respiratory admissions for all ages, age <65 years and 65+ years.	1-h max O_3: All cities: All year: 31 ppb 95th % 60 Apr-Dec only: 33 ppb 95th % 64	SO_2, NO_2, CO, coefficient of haze	0, 1, 2, 0-1, 0-2, 1-2	Poisson GLM with co-adjustment. Results stratified by season. Significant O_3 effect observed in warm season only. No O_3 effects on control outcomes. Results consistent across cities.	1-h max O_3 (per 30 ppb): All ages: Jan-Mar: Lag 1: 0.994 (0.964, 1.025) Apr-Jun: Lag 1: 1.042 (1.012, 1.073) Jul-Sep: Lag 1: 1.050 (1.026, 1.074) Oct-Dec: Lag 1: 1.028 (0.998, 1.059)
Burnett et al. (1995) 168 Hospitals in Ontario, Canada 1983-1988	Respiratory and cardiovascular admissions for all ages and within age strata. Study focused mainly on testing for sulfate effects.	1-h max O_3: 36.3 ppb	SO_4^{2-}	1	GLM with pre-adjustment of outcome variables. Results stratified by season. Authors report that O_3 associated with respiratory admission in warm season only.	No quantitative results presented for O_3.

Table AX7-4 (cont'd). Effects of O_3 on Daily Hospital Admissions

Reference, Study Location and Period	Outcomes and Design	Mean O_3 Levels	Copollutants Considered	Lag Structure Examined	Method, Findings, Interpretation	Effects (Relative Risk and 95% CI)
Canada (cont'd)						
Burnett et al. (1997b) Toronto, Ontario, Canada Summers 1992-1994	Unscheduled respiratory and cardiovascular admissions for all ages.	1-h max O_3: 41.2 ppb IQR 22	$PM_{2.5}$, PM_{10}, H^+, SO_4^{2-}, SO_2, NO_2, CO, coefficient of haze	0, 1, 2, 3, 4, 2 to 5 multiday periods lagged 1 to 4 days	Poisson GLM with co-adjustment. Results stratified by season. Ozone and coefficient of haze strongest predictors of outcomes. Ozone effects on both outcomes were robust to PM. PM effects were not robust to O_3.	12-h avg O_3 (8 a.m.-8 p.m.) (per 11.5 ppb): Models adjusted for temperature and dewpoint: Respiratory : Lag 1-3: 1.064 (1.039, 1.090) Cardiovascular: Lag 2-4: 1.074 (1.035, 1.115)
Burnett et al. (1999) Toronto, Ontario, Canada 1980-1994	Cause-specific respiratory and cardiovascular admissions for all ages. Cause categories included asthma, COPD, respiratory infections, heart failure, ischemic heart disease, and cerebrovascular disease.	24-h avg O_3: 19.5 ppb IQR 19	Estimated $PM_{2.5}$, PM_{10}, $PM_{10-2.5}$, CO, NO_2, SO_2	0, 1, 2, 0-1, 0-2, 1-2, 1-3, 2-3, 2-4	Poisson GAM with LOESS pre-filter applied to pollution and hospitalization data. Ozone effects seen for respiratory outcomes only. Ozone effect robust to PM; not vice versa. No seasonal stratification.	24-h avg O_3 (per 19.5 ppb): Asthma: Lag 1-3: 1.063 (1.036, 1.091) COPD: Lag 2-4: 1.073 (1.038, 1.107) Respiratory infection: Lag 1-2: 1.044 (1.024, 1.065)
Burnett et al. (2001) Toronto, Ontario, Canada 1980-1994	Acute respiratory disease admissions for age <2 years.	1-h max O_3: 45.2 ppb IQR 25	Estimated $PM_{2.5}$, PM_{10}, $PM_{10-2.5}$, CO, NO_2, SO_2	0, 1, 2, 3, 4, 5, 0-4	Poisson GAM with LOESS pre-filter applied to pollution and hospitalization data. Sensitivity analyses using co-adjustment. Results stratified by season. Ozone effects significant only in summer. Ozone effect robust to PM; not vice versa.	1-h max O_3 (per 45.2 ppb): Summer: O_3 only model: Lag 0-4: 1.348 (1.193, 1.524) O_3 with $PM_{2.5}$ model: Lag 0-4: 1.330 (1.131, 1.565)

Table AX7-4 (cont'd). Effects of O_3 on Daily Hospital Admissions

Reference, Study Location and Period	Outcomes and Design	Mean O_3 Levels	Copollutants Considered	Lag Structure Examined	Method, Findings, Interpretation	Effects (Relative Risk and 95% CI)
Canada (cont'd)						
Lin et al. (2003) Toronto, Ontario, Canada 1981-1993	Asthma admission for age 6-12 years. Case-crossover design.	1-h max O_3: 30 ppb IQR 20	CO, SO_2, NO_2	0, 0-1, 0-2, 0-3, 0-4, 0-5, 0-6	Case-crossover analysis. No O_3 effects observed.	1-h max O_3 (per 20 ppb): Odds ratios: Males: Lag 0: 0.96 (0.88, 1.04) Females: Lag 0: 0.86 (0.78, 1.04)
Luginaah et al. (2004) Windsor, Ontario, Canada Apr 1995-Dec 2000	Respiratory hospital admissions by gender for all ages and age 0-14, 15-64 and 65+ years.	1-h max O_3: 39.3 ppb SD 21.4	NO_2, SO_2, CO, PM_{10}, coefficient of haze, total reduced sulfur compounds	0, 0-1, 0-2	Conducted both time-series analysis using Poisson GAM with natural splines and bidirectional case-crossover analysis using conditional logistic regression models. For case-crossover analysis, control periods selected two weeks before and after the case period. Results were consistent for the time-series and case-crossover analyses. Significant associations were found for all pollutants except O_3 and total reduced sulfur compounds.	1-h max O_3 (per 29 ppb): All ages: Time-series analysis: Males: Lag 0: 1.04 (0.92, 1.17) Females: Lag 0: 0.95 (0.82, 1.10) Case-crossover analysis: Males: Lag 0: 1.06 (0.93, 1.22) Females: Lag 0: 1.01 (0.77, 1.34)
Lin et al. (2004) Vancouver, British Columbia, Canada 1987-1998	Asthma admissions for age 6-12 year.	1-h max O_3: 28.02 ppb SD 11.54 IQR 14.81	CO, SO_2, NO_2	0, 0-1, 0-2, 0-3, 0-4, 0-5, 0-6	Poisson GAM with LOESS (using default convergence criteria). Repeated analysis with natural cubic splines using 1,000 iterations with convergence criteria 10^{-15}. Results were similar for both analyses. NO_2 exposure associated for males in low SES but not high. No association for CO and O_3 in either SES group.	1-h max O_3 (per 14.8 ppb): Males: Low SES: Lag 1: 0.85 (0.76, 0.94) High SES: Lag 1: 0.93 (0.83, 1.04) Females: Low SES: Lag 1: 1.11 (0.97, 1.28) High SES: Lag 1: 0.91 (0.78, 1.05)

Table AX7-4 (cont'd). Effects of O_3 on Daily Hospital Admissions

Reference, Study Location and Period	Outcomes and Design	Mean O_3 Levels	Copollutants Considered	Lag Structure Examined	Method, Findings, Interpretation	Effects (Relative Risk and 95% CI)
Canada (cont'd)						
Yang et al. (2003) Vancouver, British Columbia, Canada Jan 1986-Dec 1998	Daily respiratory admissions in children aged < 3 years and adults aged 65+ years.	24-h avg O_3: 13.41 ppb SD 66.61 IQR 9.74	CO, NO_2, SO_2, coefficient of haze	1, 2, 3, 4, 5	Used bidirectional case-crossover analysis, comparing air pollution on day of admission to levels one week prior and after. SES evaluated. O_3 was positively associated with respiratory hospital admissions among young children and the elderly.	24-h avg O_3 (per 9.74 ppb): Odds ratios: Age < 3 years: Lag 4: 1.22 (1.15, 1.30) Age 65+ years: Lag 4: 1.13 (1.09, 1.18)
Europe						
Anderson et al. (1997) Five European cities: London, Paris, Amsterdam, Rotterdam, Barcelona Study periods vary by city, ranging from 1977-1992	Emergency COPD admissions for all ages. Each city analyzed previously by individual teams. Results combined here via meta-analysis.	Range across five cities: 1-h max O_3 (median): All year: 36-77 µg/m^3 Warm season: 48-91 µg/m^3 Cool season: 20-64 µg/m^3	TSP, SO_2, NO_2, BS	0, 1, 2, 3, 4, 5	Poisson GLM using APHEA methodology. Results stratified by season. Ozone most consistent and significant predictor of admissions. Warm season effect larger.	1-h max O_3 (per 50 µg/m^3): Weighted mean effect across five cities (best lag selected for each city): All year: 1.03 (1.01, 1.05) Warm season: 1.03 (1.01, 1.05) Cool season: 1.01 (0.98, 1.05)
Anderson et al. (1998) London, England 1987-1992	Admissions for asthma in all ages and age 0-14, 15-64, and 65+ years.	8-h max O_3: 15.5 ppb IQR 13 1-h max O_3: 20.6 ppb IQR 16	SO_2, NO_2, BS, pollens	0, 1, 2, 0-1, 0-2	Poisson GLM using APHEA method; co-adjustment. Ozone significantly associated with asthma admissions in the warm season for all ages and for age 15-64 years. Warm season O_3 effect robust in 2-pollutant models. Inverse associations observed in the cool season for some age groups.	8-h max O_3 (per 10 ppb): All ages: Warm season: Lag 1: 1.022 (1.006, 1.038) Cool season: Lag 1: 0.968 (0.946, 0.992)

Table AX7-4 (cont'd). Effects of O_3 on Daily Hospital Admissions

Reference, Study Location and Period	Outcomes and Design	Mean O_3 Levels	Copollutants Considered	Lag Structure Examined	Method, Findings, Interpretation	Effects (Relative Risk and 95% CI)
Europe (cont'd)						
Atkinson et al. (1999b) London, England 1992-1994	Total and cause-specific respiratory and cardiovascular admissions in all ages and in all ages and age 0-14, 15-64, and 65+ years.	8-h max O_3: 17.5 ppb SD 11.5	NO_2, SO_2, CO, PM_{10}, BS	0, 1, 2, 3, 0-1, 0-2, 0-3	Poisson GLM using APHEA methodology. No significant associations seen between O_3 and respiratory admissions. Ozone was positively associated with total cardiovascular admissions in age 65+ years. Seasonal analyses were not conducted.	8-h max O_3 (per 25.7 ppb): All ages: Total respiratory: Lag 1: 1.012 (0.990, 1.035) Total cardiovascular: Lag 2: 1.023 (1.002, 1.046)
Ponce de Leon et al. (1996) London, England Apr 1987-Feb 1992	Total respiratory admissions in several age strata: all ages, 0-14, 15-64, 65+ years.	8-h avg O_3 (9 a.m.-5 p.m.): 15.6 ppb SD 12 IQR 14	BS, SO_2, NO_2	0, 1, 2, 0-1, 0-2, 0-3,	Poisson GLM using APHEA co-adjustment methodology. Ozone significant predictor overall. Effect larger and more significant in warm season. Effect robust to copollutants. Effects varied by age.	All ages: All year: 8-h avg O_3 (per 26 ppb): Lag 1: 1.029 (1.011, 1.048) Warm season: 8-h avg O_3 (per 29 ppb): Lag 1: 1.048 (1.025, 1.073) Cool season: 8-h avg O_3 (per 20 ppb): Lag 1: 0.996 (0.972, 1.021)
Prescott et al. (1998) Edinburgh, Scotland 1992-1995	Total respiratory and cardiovascular admissions for age < 65 years and 65+ years.	24-h avg O_3: 14.5 ppb Range 1-37	BS, PM_{10}, NO_2, SO_2, CO	0, 1, 1-3	Poisson GLM, month dummy variables; co-adjustment. No O_3 or other pollution effects on respiratory admissions. Significant inverse association of O_3 with cardiac admissions in older age group. Very low O_3 concentrations.	24-h avg O_3 (per 10 ppb): Respiratory: Age < 65 years: Lag 1-3: 0.971 (0.885, 1.068) Age 65+ years: Lag 1-3: 1.009 (0.916, 1.111) Cardiovascular: Age < 65 years: Lag 1-3: 1.041 (0.946, 1.144) Age 65+ years: Lag 1-3: 0.941 (0.886, 0.999)

Table AX7-4 (cont'd). Effects of O_3 on Daily Hospital Admissions

Reference, Study Location and Period	Outcomes and Design	Mean O_3 Levels	Copollutants Considered	Lag Structure Examined	Method, Findings, Interpretation	Effects (Relative Risk and 95% CI)
Europe (cont'd)						
Schouten et al. (1996) Amsterdam and Rotterdam, the Netherlands 1977-1989	Unscheduled total respiratory, asthma, and COPD admissions in all ages.	1-h max O_3: Amsterdam: Summer: 97 µg/m^3 Winter: 62 µg/m^3 Rotterdam: Summer: 96 µg/m^3 Winter: 54 µg/m^3	SO_2, NO_2, BS	0, 1, 2, 0-1, 0-2, 0-3, 0-4, 0-5	Poisson GLM using APHEA methodology; co-adjustment. No consistent O_3 effects. Concern regarding multiple comparisons.	1-h max O_3 (per 100 µg/m^3): Amsterdam and Rotterdam: Total respiratory, all ages: Summer: Lag 2: 1.051 (1.029, 1.073) Winter: Lag 2: 0.976 (0.951, 1.002)
Hagen et al. (2000) Drammen, Norway Nov 1994-Dec 1997	Total respiratory admissions for all ages.	24-h avg O_3: 44.48 µg/m^3 SD 18.40 IQR 26.29	PM_{10}, NO_2, SO_2, benzene, toluene, formaldehyde	0	Poisson GAM with partial splines; co-adjustment. Single and multipollutant models evaluated. No O_3 effects. Ozone levels low and cycles may not have been adequately controlled.	24-h avg O_3 (per 26.29 µg/m^3): Lag 0: 0.964 (0.899-1.033)
Oftedal et al. (2003) Drammen, Norway 1995-2000	Admissions for respiratory disease.	24-h avg O_3: 44.6 µg/m^3 SD 19.2 IQR 26.9	Benzene, formaldehyde, toluene, PM_{10}, NO_2, SO_2	0	Benzene had the strongest association.	24-h avg O_3 (per 26.9 µg/m^3): 0.996 (0.942, 1.053)
Pönkä and Virtanen (1996) Helsinki, Finland 1987-1989	Asthma admissions for age 0-14 years and 15-64 years.	O_3 (index not specified): 22 µg/m^3	TSP, SO_2, NO_2	0, 1, 2, 3, 4, 5	Poisson GLM using APHEA methodology. Reported significant O_3 effect for age 0-14 years, but also for control (digestive disease) conditions. Ozone levels very low.	Not quantitatively useful.
Ballester et al. (2001) Valencia, Spain 1994-1996	Emergency total cardiovascular admissions for all ages.	8-h max O_3: 23 ppb Range 5-64	SO_2, NO_2, CO, BS	0, 1, 2, 3, 4, 5	Poisson GLM using APHEA methodology. Results stratified by season. No O_3 effects.	8-h max O_3 (per 5 ppb): Lag 2: 0.99 (0.97-1.01)

Table AX7-4 (cont'd). Effects of O_3 on Daily Hospital Admissions

Reference, Study Location and Period	Outcomes and Design	Mean O_3 Levels	Copollutants Considered	Lag Structure Examined	Method, Findings, Interpretation	Effects (Relative Risk and 95% CI)
Latin America						
Gouveia and Fletcher (2000a) São Paulo, Brazil Nov 1992-Sep 1994	Total respiratory, pneumonia, and asthma admissions for age < 5 years.	1-h max O_3: 63.4 µg/m^3 SD 38.1 IQR 50.3	PM_{10}, NO_2, SO_2, CO	0, 1, 2	Poisson GLM with co-adjustment using sine/cosine waves. Significant O3 effects on total respiratory and pneumonia admissions. Ozone effects fairky robust to NO_2 and PM_{10}.	1-h max O_3 (per 119.6 µg/m^3): Total respiratory: Lag 0: 1.054 (1.003, 1.107) Pneumonia: Lag 0: 1.076 (1.014, 1.142) Asthma: Lag 2: 1.011 (0.899, 1.136)
Australia						
Morgan et al. (1998a) Sydney, Australia 1990-1994	Admissions for asthma (age 1-14 years, 15-64 years), COPD (age 65+ years), and heart disease (all ages, 0-64 years, 65+ years).	1-h max O_3: 25 ppb SD 13 IQR 11	$B_{scatter}$, NO_2	0, 1, 2, 0-1, 0-2	Poisson with GEE. No significant effects of O_3 in single or multipollutant models.	1-h max O_3 (per 28 ppb): Asthma, age 1-14 years: Lag 1: 0.975 (0.932, 1.019) Asthma, age 15-64 years: Lag 0: 1.025 (0.975, 1.078) COPD, age 65+ years: Lag 0: 1.010 (0.960, 1.062) Heart disease, all ages: Lag 0: 1.012 (0.990, 1.035)
Petroeschevsky et al. (2001) Brisbane, Australia 1987-1994	Unscheduled asthma, total respiratory and total cardiovascular admissions in several age strata: all ages, 0-4, 5-14, 15-64, 65+ years.	1-h max O_3: 25.3 ppb Range 2.5-107.3 8-h avg O_3 (10 a.m.-6 p.m.): 19.0 ppb Range 1.7-64.7	$B_{scatter}$, SO_2, NO_2	0, 1, 2, 3, 0-2, 0-4	Poisson GLM using APHEA co-adjustment methodology. Results stratified by season. Ozone significantly related to asthma and total respiratory admissions, not for cardiac admissions. Effects varied by age group. Ozone effects robust to copollutants.	8-h avg O_3 (per 10 ppb): All ages: Total respiratory: Lag 2: 1.023 (1.003, 1.043) Asthma: Lag 0-4: 1.090 (1.042, 1.141) Total cardiovascular: Lag 3: 0.987 (0.971, 1.002)

Table AX7-4 (cont'd). Effects of O_3 on Daily Hospital Admissions

Reference, Study Location and Period	Outcomes and Design	Mean O_3 Levels	Copollutants Considered	Lag Structure Examined	Method, Findings, Interpretation	Effects (Relative Risk and 95% CI)
Asia						
Wong et al. (1999a) Hong Kong 1994-1995	Total and cause-specific respiratory and cardiovascular admissions in several age strata: all ages, 0-4, 5-64, 65+ years.	8-h max O_3: 20.2 µg/m^3 Median 24.15 IQR 31.63	NO_2, SO_2, PM_{10}	0, 1, 2, 3, 4, 5, 0-1, 0-2, 0-3, 0-4, 0-5	Poisson GLM using APHEA methodology. Ozone significantly associated with total and cause specific respiratory and cardiac outcomes. Ozone results robust to adjustment for high PM_{10}, but not high NO_2. Effects of O_3 persisted in cold season.	8-h max O_3 (per 10 µg/m^3): All ages: Total respiratory: Lag 0-3: 1.022 (1.015, 1.029) Total cardiovascular: Lag 0-5: 1.013 (1.005, 1.021)
Wong et al. (1999b) Hong Kong Jan 1995-Jun 1997	Total and cause-specific cardiovascular admissions in all ages.	O_3 (index not specified): Warm season: 31.2 µg/m^3 Cool season: 34.8 µg/m^3	NO_2, SO_2, respirable PM	0, 1, 2, 3, 4, 5, 0-1, 0-2, 0-3, 0-4, 0-5	GLM with sinusoids; co-adjustment. Ozone significantly associated with total and cause-specific cardiovascular admissions in cool season only, when O_3 levels are higher in Hong Kong. Details missing in brief report.	O_3 (per 50 µg/m^3): O_3 with NO_2 models: Total cardiovascular: All year: Lag 0-1: 1.03 (1.00, 1.07) Warm season: Lag 0-1: 1.01 (0.95, 1.07) Cool season: Lag 0-1: 1.08 (1.02, 1.14)

Table AX7-5. Effects of Acute O_3 Exposure on Mortality

Reference, Study Location and Period	Outcome Measure	Mean O_3 Levels	Copollutants Considered	Lag Structure Reported	Method	Effect Estimates
United States						
Bell et al. (2004) 95 U.S. communities 1987-2000	All cause; cardiopulmonary; all ages; age < 65 years; age 65-74 years; age 75+ years	24-h avg O_3: 26 ppb	PM_{10}, $PM_{2.5}$; 2-pollutant models	0, 1, 2, 3, 0-6	Poisson GLM; Bayesian hierarchical model	24-h avg O_3 (per 10 ppb): Posterior means: All cause, all ages: All year: Lag 0: 0.25% (0.12, 0.39) Lag 0-6: 0.52% (0.27, 0.77) Warm season: Lag 0: 0.22% (0.08, 0.38) Lag 0-6: 0.39% (0.13, 0.65)
Samet et al. (2000; reanalysis Dominici et al., 2003) 90 U.S. cities 1987-1994	All cause; cardiopulmonary	24-h avg O_3: Mean range: Approximately 12 ppb (Des Moines, IA) to 36 ppb (San Bernardino, CA)	PM_{10}, NO_2, SO_2, CO; 2-pollutant models	0, 1, 2	Poisson GAM (reanalyzed with stringent convergence criteria); Poisson GLM	24-h avg O_3 (per 10 ppb): Posterior means: All cause (results given in graphic format): All year: Lag 0: approximately 0.4% Lag 1: approximately 0.2% Summer: Lag 1: approximately 0.5% Winter: Lag 1: approximately −0.5%

Table AX7-5 (cont'd). Effects of Acute O_3 Exposure on Mortality

Reference, Study Location and Period	Outcome Measure	Mean O_3 Levels	Copollutants Considered	Lag Structure Reported	Method	Effect Estimates
United States (cont'd)						
Huang et al. (2004) 19 U.S. cities Jun-Sept 1987-1994	Cardiopulmonary	24-h avg O_3: 26 ppb	PM_{10}, $PM_{2.5}$; 2-pollutant models	0, 1, 2, 0-6	Poisson GLM; Bayesian hierarchical model	24-h avg O_3 (per 10 ppb): Posterior means: O_3 only model: Lag 0: 0.73% (0.27, 1.19) O_3 with PM_{10} model: Lag 0: 0.74% (−0.33, 1.72) O_3 only model: Lag 0-6: 1.25% (0.47, 2.03) Model adjusted for heat waves: Lag 0-6: 1.11% (0.38, 1.86)
Schwartz (2004) 14 U.S. cities 1986-1993	All cause	1-h max O_3: Median range: 35.1 ppb (Chicago, IL) to 60.0 ppb (Provo, UT)	PM_{10}; 2-pollutant models	0	Case-crossover analysis; controlled for temperature using nonlinear regression splines and matching	1-h max O_3 (10 ppb): Analysis with temperature regression splines: All year: 0.19% (0.03, 0.35) Warm season: 0.26% (0.07, 0.44) Cold season: 0% (−0.27, 0.27) Analysis with temperature matched controls: All year: 0.23% (0.01, 0.44) Warm season: 0.37% (0.11, 0.62) Cold season: −0.13% (−0.53, 0.28)

Table AX7-5 (cont'd). Effects of Acute O_3 Exposure on Mortality

Reference, Study Location and Period	Outcome Measure	Mean O_3 Levels	Copollutants Considered	Lag Structure Reported	Method	Effect Estimates
United States (cont'd)						
Kinney and Özkaynak (1991) Los Angeles County, CA 1970-1979	All cause; respiratory; circulatory	1-h max total oxidants (O_x): 75 ppb	KM (particle optical reflectance), NO_2, SO_2, CO; multipollutant models	1	OLS (ordinary least squares) on high-pass filtered variables	All cause: Multipollutant model: Slope estimate: 0.03 deaths/ppb (SE 0.009), p = 0.0005
Kinney et al. (1995) Los Angeles County, CA 1985-1990	All cause	1-h max O_3: 70 ppb	PM_{10}, NO_2, SO_2, CO; 2-pollutant models	1	Linear, log-linear, and Poisson	1-h max O_3 (per 143 ppb): O_3 only model: 2% (0, 5) O_3 with PM_{10} model: 0% (−6, 6)
Ostro (1995) San Bernardino County and Riverside County, CA 1980-1986	All cause	1-h max O_3: 140 ppb	$PM_{2.5}$	0	Autoregressive linear; Poisson	1-h max O_3 (per 100 ppb): Warm season: 2.0% (0.0, 5.0)
Fairley (1999; reanalysis Fairley, 2003) Santa Clara County, CA 1989-1996	All cause; respiratory; cardiovascular	8-h max O_3: 29 ppb 24-h avg O_3: 16 ppb O_3 ppb-hours > 60 ppb: Levels not reported.	PM_{10}, $PM_{2.5}$, $PM_{10-2.5}$, SO_4^{2-}, coefficient of haze, NO_3^-, NO_2, SO_2; 2-pollutant models	0	Poisson GAM (reanalyzed with stringent convergence criteria); Poisson GLM	GLM: All cause: 8-h max O_3 (per 33 ppb): 3.3% (−0.3, 7.0) O_3 ppb-hours > 60 ppb (increment not reported): 4.1% (1.5, 6.7)

Table AX7-5 (cont'd). Effects of Acute O_3 Exposure on Mortality

Reference, Study Location and Period	Outcome Measure	Mean O_3 Levels	Copollutants Considered	Lag Structure Reported	Method	Effect Estimates
United States (cont'd)						
Gamble (1998) Dallas, TX 1990-1994	All cause; respiratory; cardiovascular; cancer; other	24-h avg O_3: All year: 22 ppb Summer: 30 ppb Winter: 12 ppb	PM_{10}, NO_2, SO_2, CO; 2-pollutant models	1-2	Poisson GLM	All cause: All year: 24-h avg O_3 (per 14.7 ppb): 2.7% (0.6, 4.8) Summer: 24-h avg O_3 (per 13.1 ppb): 3.5% ($p < 0.05$) Winter: 24-h avg O_3 (per 7.7 ppb): 2.4% ($p > 0.05$)
Dockery et al. (1992) St. Louis, MO and Eastern Tennessee 1985-1986	All cause	24-h avg O_3: St. Louis, MO: 22.5 ppb Eastern Tennessee: 23.0 ppb	PM_{10}, $PM_{2.5}$, SO_4^{2-}, H^+, NO_2, SO_2	1	Poisson with GEE	24-h avg O_3 (per 20 μg/m^3): St. Louis, MO: 0.6% ($t = 0.38$) Eastern Tennessee: −1.3% ($t = -0.37$)
Ito and Thurston (1996) Cook County, IL 1985-1990	All cause; respiratory; circulatory; cancer; race/gender subcategories	1-h max O_3: 38.1 ppb	PM_{10}, NO_2, SO_2, CO; 2-pollutant models	0-1	Poisson GLM	1-h max O_3 (per 100 ppb): All cause: O_3 only model: 10% (6, 15) O_3 with PM_{10} model: 7% (1, 12)

Table AX7-5 (cont'd). Effects of Acute O_3 Exposure on Mortality

Reference, Study Location and Period	Outcome Measure	Mean O_3 Levels	Copollutants Considered	Lag Structure Reported	Method	Effect Estimates
United States (cont'd)						
Lippmann et al. (2000; reanalysis Ito, 2003) Detroit, MI 1985-1990 1992-1994	All cause; respiratory; cardiovascular; cause-specific	24-h avg O_3: 25 ppb	PM_{10}, $PM_{2.5}$, $PM_{10-2.5}$, SO_4^{2-}, H^+, NO_2, SO_2, CO; 2-pollutant models	0, 1, 2, 3, 0-1, 0-2, 0-3	Poisson GAM (reanalyzed with stringent convergence criteria); Poisson GLM	24-h avg O_3 (per 5th to 95th % increment): GAM with stringent convergence criteria: For all lags and outcomes during both study periods (n = 140): Median 1.6% Range −1.8-2.6
Lipfert et al. (2000a) Seven counties in Philadelphia, PA area 1991-1995	All cause; respiratory; cardiovascular; all ages; age 65+ years; age < 65 years; various subregional boundaries	1-h max O_3: 44.76 ppb 24-h avg O_3: 23.44 ppb	PM_{10}, $PM_{2.5}$, $PM_{10-2.5}$, SO_4^{2-}, NO_3^-, other PM indices, NO_2, SO_2, CO; 2-pollutant models	0-1	Linear with 19-day weighted average Shumway filters	1-h max O_3 (per 45 ppb less background, not reported): All cause, all ages: O_3 only model: 3.19%, $p < 0.055$ O_3 with $PM_{2.5}$ model: 3.34%, $p < 0.055$
Moolgavkar et al. (1995) Philadelphia, PA 1973-1988	All cause	24-h avg O_3: Spring: 25.9 ppb Summer: 35.5 ppb Fall: 16.2 ppb Winter: 11.9 ppb	TSP, SO_2; multipollutant models	1	Poisson; GEE and nonparametric bootstrap methods	24-h avg O_3 (per 100 ppb): O_3 with TSP and SO_2 models: Spring: 2.0% (−6.7, 11.5) Summer: 14.9% (6.8, 23.6) Fall: −4.5% (−13.9, 5.9) Winter: 0.4% (−15.6, 19.4)

Table AX7-5 (cont'd). Effects of Acute O_3 Exposure on Mortality

Reference, Study Location and Period	Outcome Measure	Mean O_3 Levels	Copollutants Considered	Lag Structure Reported	Method	Effect Estimates
United States (cont'd)						
Chock et al. (2000) Pittsburgh, PA 1989-1991	All cause; age < 74 years; age 75+ years	1-h max O_3: Levels not reported.	PM_{10}, NO_2, SO_2, CO; 2-, 5-, and 6-pollutant models	0	Poisson GLM	1-h max O_3 (per 40 ppb): Age <74 years: O_3 only model: −1.5% (t = −0.68) O_3 with PM_{10} model: −2.0% (t = −0.93) Age 75+ years: O_3 only model: −1.8% (t = −0.82) O_3 with PM_{10} model: −2.2% (t = −0.98)
De Leon et al. (2003) New York City 1985-1994	Circulatory and cancer with and without contributing respiratory causes	24-h avg O_3: 21.59 ppb	PM_{10}, NO_2, SO_2, CO; 2-pollutant models	0 or 1	Poisson GAM; Poisson GLM	Quanititative results not given. Circulatory deaths: Larger O_3 effect estimates with contributing respiratory causes than without (RR non-significant). Cancer deaths: Smaller O_3 effect estimates with contributing respiratory causes than without (RR non-significant).

Table AX7-5 (cont'd). Effects of Acute O_3 Exposure on Mortality

Reference, Study Location and Period	Outcome Measure	Mean O_3 Levels	Copollutants Considered	Lag Structure Reported	Method	Effect Estimates
United States (cont'd)						
Klemm and Mason (2000); Klemm et al. (2004) Atlanta, GA Aug 1998-July 2000	All cause; respiratory; cardiovascular; cancer; other; age < 65 years; age 65+ years	8-h max O_3: 47.03 ppb	$PM_{2.5}$, $PM_{10-2.5}$, EC, OC, NO_2, SO_4^{2-}, NO_3^-, SO_2, CO	0-1	Poisson GLM using quarterly, monthly, or biweekly knots for temporal smoothing	All cause, age 65+ years: Quarterly knots: Slope estimate: 0.00079 (SE 0.00099), t = 0.80 Monthly knots: Slope estimate: 0.00136 (SE 0.00111), t = 1.22
Canada						
Vedal et al. (2003) Vancouver, British Columbia, Canada 1994-1996	All cause; respiratory; cardiovascular	1-h max O_3: 27.3 ppb	PM_{10}, NO_2, SO_2, CO	0, 1, 2	Poisson GAM	1-h max O_3 (per 10.2 ppb): All cause: Summer: Lag 0: 4.2% (1.4, 7.0) Winter: Lag 0: 0.5% (−1.9, 3.0)
Goldberg et al. (2003) Montreal, Quebec, Canada 1984-1993	Congestive heart failure as underlying cause of death versus those classified as having congestive heart failure one year prior to death	24-h avg O_3: 29 µg/m^3	TSP, coefficient of haze, PM_{10}, SO_4^{2-}, SO_2, NO_2, CO	0-2	Poisson GLM	24-h avg O_3 (per 21.3 µg/m^3): Congestive heart failure as underlying cause of death: 4.54% (−5.64, 15.81) Having congestive heart failure one year prior to death: 2.34% (−1.78, 6.63)

Table AX7-5 (cont'd). Effects of Acute O_3 Exposure on Mortality

Reference, Study Location and Period	Outcome Measure	Mean O_3 Levels	Copollutants Considered	Lag Structure Reported	Method	Effect Estimates
Europe						
Gryparis et al. (2004) 23 European cities Study periods vary by city, ranging from 1990-1997	All cause; respiratory; cardiovascular	1-h max O_3: Median range: Summer: 44 ppb (Tel Aviv, Israel) to 117 ppb (Torino, Italy) Winter: 11 ppb (Milan, Italy) to 57 ppb (Athens, Greece) 8-h max O_3: Median range: Summer: 30 ppb (Rome, Italy) to 99 ppb (Torino, Italy) Winter: 8 ppb (Milan, Italy) to 49 ppb (Budapest, Hungary)	PM_{10}, NO_2, SO_2, CO; 2-pollutant models	0-1	Poisson GAM; Bayesian hierarchical model	8-h max O_3 (per 10 μg/m^3): Weighted mean effect across 21 cities with 8-h max O_3 concentrations: Random effects model: All cause: All year: 0.03% (−0.18, 0.21) Summer: O_3 only model: 0.31% (0.17, 0.52) O_3 with PM_{10} model: 0.27% (0.08, 0.49) Winter: O_3 only model: 0.12% (−0.12, 0.37) O_3 with PM_{10} model: 0.22% (−0.08, 0.51)
Touloumi et al. (1997) Four European cities: London, Paris Barcelona, Athens Study periods vary by city, ranging from 1986-1992	All cause	1-h max O_3: London: 41.2 μg/m^3 Paris: 46.1 μg/m^3 Barcelona: 72.4 μg/m^3 Athens: 93.8 μg/m^3	BS, NO_2; 2-pollutant models	0, 1, 2, 3, 0-1, 0-2, 0-3	Poisson autoregressive	1-h max O_3 (50 μg/m^3): Weighted mean effect across four cities (best lag selected for each city): Random effects model: O_3 only model: 2.9% (1.0, 4.9) O_3 with BS model: 2.8% (0.5, 5.0)

Table AX7-5 (cont'd). Effects of Acute O_3 Exposure on Mortality

Reference, Study Location and Period	Outcome Measure	Mean O_3 Levels	Copollutants Considered	Lag Structure Reported	Method	Effect Estimates
Europe (cont'd)						
Zmirou et al. (1998) Four European cities: London, Paris, Lyon, Barcelona Study periods vary by city, ranging from 1985-1992	Respiratory; cardiovascular	8-h avg O_3 (9 a.m.-5 p.m.): London: Cold: 21.0 µg/m^3 Warm: 40.8 µg/m^3 Paris: Cold: 11.5 µg/m^3 Warm: 42.7 µg/m^3 Lyon: Cold: 21.0 µg/m^3 Warm: 40.8 µg/m^3 Barcelona: Cold: 51.5 µg/m^3 Warm: 89.7 µg/m^3	BS, TSP, SO_2, NO_2	0, 1, 2, 3, 0-1, 0-2, 0-3	Poisson GLM	8-h avg O_3 (per 50 µg/m^3): Weighted mean effect across four cities (best lag selected for each city): Random effects model: Respiratory: 5% (2, 8) Cardiovascular: 2% (0, 3)
Anderson et al. (1996) London, England 1987-1992	All cause; respiratory; cardiovascular	1-h max O_3: 20.6 ppb 8-h avg O_3 (9 a.m.-5 p.m.): 15.5 ppb	BS, NO_2, SO_2; 2-pollutant models	0	Poisson GLM	All cause: All year: 1-h max O_3 (per 31 ppb): 2.59% (1.30, 3.89) Warm season: 1-h max O_3 (per 34 ppb): 3.49% (1.81, 5.20) Cool season: 1-h max O_3 (per 26 ppb): 0.99% (−0.80, 2.81)

Table AX7-5 (cont'd). Effects of Acute O_3 Exposure on Mortality

Reference, Study Location and Period	Outcome Measure	Mean O_3 Levels	Copollutants Considered	Lag Structure Reported	Method	Effect Estimates
Europe (cont'd)						
Bremner et al. (1999) London, England 1992-1994	All cause; respiratory; cardiovascular; all cancer; all others; all ages; age specific (0-64, 65+, 65-74, 75+ years)	1-h max O_3: 22.6 ppb 8-h max O_3: 17.5 ppb	BS, PM_{10}, NO_2, SO_2, CO; 2-pollutant models	Selected best from 0, 1, 2, 3, (all cause); 0, 1, 2, 3, 0-1, 0-2, 0-3 (respiratory, cardiovascular)	Poisson GLM	8-h max O_3 (per 26 ppb): All ages: All cause: Lag 2: −0.7% (−2.3, 0.9) Respiratory: Lag 2: −3.6% (−7.7, 0.8) Cardiovascular: Lag 2: 3.5% (0.5, 6.7)
Prescott et al. (1998) Edinburgh, Scotland 1992-1995	All cause; respiratory; cardiovascular; all ages, age < 65 years, age 65+ years	24-h avg O_3: 14.5 ppb	BS, PM_{10}, NO_2, SO_2, CO; 2-pollutant models	0	Poisson GLM	24-h avg O_3 (per 10 ppb): All cause, all ages: −4.2% (−8.1, −0.1)
Dab et al. (1996) Paris, France 1987-1992	Respiratory	1-h max O_3: 23.2 µg/m^3 8-h max O_3: 11.5 µg/m^3	BS, PM_{13}, NO_2, SO_2, CO	0	Poisson autoregressive	1-h max O_3 (per 100 µg/m^3): 1.074 (0.934, 1.235) 8-h max O_3 (per 100 µg/m^3): 1.040 (0.934, 1.157)
Zmirou et al. (1996) Lyon, France 1985-1990	All cause; respiratory; cardiovascular; digestive	1-h max O_3: 15.23 µg/m^3 8-h avg O_3 (9 a.m.-5 p.m.): 9.94 µg/m^3	PM_{13}, SO_2, NO_2	Selected best from 0, 1, 2, 3	Poisson GLM	8-h avg O_3 (per 50 µg/m^3): All cause: Lag 0: 3% (−5, 12) Respiratory: Lag 1: 1% (−8, 10) Cardiovascular: Lag 1: 0% (−11, 12)

Table AX7-5 (cont'd). Effects of Acute O_3 Exposure on Mortality

Reference, Study Location and Period	Outcome Measure	Mean O_3 Levels	Copollutants Considered	Lag Structure Reported	Method	Effect Estimates
Europe (cont'd)						
Sartor et al. (1995) Belgium Summer 1994	All cause; age < 65 years; age 65+ years	24-h avg O_3: During heat wave (42 day period): 72.4 µg/m^3 Before heat wave (43 day period): 52.4 µg/m^3 After heat wave (39 day period): 38.6 µg/m^3	TSP, NO, NO_2, SO_2	0, 1, 2	Log-linear regression	No individual regression coefficient for O_3 alone; interaction with temperature suggested. 24-h avg O_3 (from 18.8 to 111.5 µg/m^3) and temperature (from 10.0 to 27.5°C): Age < 65 years: Lag 1: 16% increase in mortality (5.3% expected) Age 65+ years: Lag 1: 36.5% increase in mortality (4% expected)
Hoek et al., (2000; reanalysis Hoek, 2003) The Netherlands: entire country, four urban areas 1986-1994	All cause; COPD; pneumonia; cardiovascular	8-h avg O_3 (12 p.m.-8 p.m.): Median: 47 µg/m^3	PM_{10}, BS, SO_4^{2-}, NO_3^-, NO_2, SO_2, CO; 2-pollutant models	1, 0-6	Poisson GAM (reanalyzed with stringent convergence criteria); Poisson GLM	GLM: All cause: 8-h avg O_3 (per 150 µg/m^3): Lag 1: 4.3% (2.4, 6.2) 8-h avg O_3 (per 120 µg/m^3): Lag 0-6: 5.9% (3.1, 8.7)

Table AX7-5 (cont'd). Effects of Acute O_3 Exposure on Mortality

Reference, Study Location and Period	Outcome Measure	Mean O_3 Levels	Copollutants Considered	Lag Structure Reported	Method	Effect Estimates
Europe (cont'd)						
Hoek et al. (2001; reanalysis Hoek, 2003) The Netherlands 1986-1994	Total cardiovascular; myocardial infarction; arrhythmia; heart failure; cerebrovascular; thrombosis-related	8-h avg O_3 (12 p.m.-8 p.m.): Median: 47 µg/m^3	PM_{10}, NO_2, SO_2, CO	1	Poisson GAM (reanalyzed with stringent convergence criteria); Poisson GLM	8-h avg O_3 (per 150 µg/m^3): GLM: Total cardiovascular: 6.2% (3.3, 9.2) Myocardial infarction: 4.3% (0.1, 8.6) Arrhythmia: 11.4% (−1.2, 25.5) Heart failure: 10.2% (1.2, 19.9) Cerebrovascular: 9.1% (2.9, 15.7) Thrombosis-related: 16.6% (2.8, 32.2)
Roemer and van Wijinen (2001) Amsterdam, the Netherlands 1987-1998	All cause	8-h max O_3: Background sites: 43 µg/m^3 Traffic sites: 36 µg/m^3	BS, PM_{10}, NO_2, SO_2, CO	1, 2, 0-6	Poisson GAM (default convergence criteria but with only one smoother)	8-h max O_3 (per 100 µg/m^3): Total population using background sites: Lag 1: −0.3% (−4.1, 3.7) Total population using traffic sites: Lag 1: 0.2% (−3.6, 4.2)
Verhoeff et al. (1996) Amsterdam, the Netherlands 1986-1992	All cause; all ages; age 65+ years	1-h max O_3: 43 µg/m^3	PM_{10}, NO_2, SO_2, CO; multipollutant models	0, 1, 2	Poisson	1-h max O_3 (per 100 µg/m^3) All ages: Lag 0: 1.8% (−3.8, 7.8) Lag 1: 0.1% (−4.7, 5.1) Lag 2: 4.9% (0.1, 10.0)

Table AX7-5 (cont'd). Effects of Acute O_3 Exposure on Mortality

Reference, Study Location and Period	Outcome Measure	Mean O_3 Levels	Copollutants Considered	Lag Structure Reported	Method	Effect Estimates
Europe (cont'd)						
Peters et al. (2000b) NE Bavaria, Germany and coal basin in Czech Republic 1982-1994	All cause for Czech Republic; all cause and cardiovascular for Bavaria, Germany	24-h avg O_3: Czech Republic: 40.3 µg/m^3 Bavaria, Germany: 38.2 µg/m^3	TSP, PM_{10}, NO_2, SO_2, CO	0, 1, 2, 3	Poisson GLM	24-h avg O_3 (per 100 µg/m^3): All cause: Czech Republic: Lag 2: 7.8% (−1.8, 18.4) Bavaria, Germany: Lag 0: 8.2% (0.4, 16.7)
Pönkä et al. (1998) Helsinki, Finland 1987-1993	All cause; cardiovascular; age < 65 years, age 65+ years	24-h avg O_3: Median: 18 µg/m^3	TSP, PM_{10}, NO_2, SO_2	0, 1, 2, 3, 4, 5, 6, 7	Poisson GLM	24-h avg O_3 (per 20 µg/m^3): All cause, age < 65 years: Not significant, values not reported. Cardiovascular, age < 65 years: Lag 5: -11.7% (-18.9, -3.9) Lag 6: 9.9% (1.1, 19.5)
Garcia-Aymerich et al. (2000) Barcelona, Spain 1985-1989	All cause; respiratory; cardiovascular; general population; patients with COPD	1-h max O_3: Levels not reported.	BS, NO_2, SO_2,	5 (general population); 3 (COPD cohort)	Poisson GLM	1-h max O_3 (per 50 µg/m^3): General population: 2.4% (0.6, 4.2) COPD patients: 4.0% (−4.7, 13.4)
Saez et al. (1999) Barcelona, Spain 1986-1989	Asthma mortality; age 2-45 years	1-h max O_3: Levels not reported.	BS, NO_2, SO_2,	0-2	Poisson with GEE	Slope estimate: 0.021 (SE 0.011), p = 0.054
Sunyer et al. (1996) Barcelona, Spain 1985−1991	All cause; respiratory; cardiovascular; all ages; age 70+ years	1-h max O_3: Summer: 86.5 µg/m^3 Winter: 55.2 µg/m^3	BS, SO_2, NO_2	0, 1, 5	Autoregressive Poisson	1-h max O_3 (per 100 µg/m^3): All cause, all ages: All year: Lag 0: 4.8% (1.2, 8.6) Summer: Lag 0: 5.8% (1.7, 10.1) Winter: Lag 0: 2.6% (−3.5, 9.1)

Table AX7-5 (cont'd). Effects of Acute O_3 Exposure on Mortality

Reference, Study Location and Period	Outcome Measure	Mean O_3 Levels	Copollutants Considered	Lag Structure Reported	Method	Effect Estimates
Europe (cont'd)						
Sunyer and Basagana (2001) Barcelona, Spain 1990-1995	Mortality in a cohort of patients with COPD	1-h max O_3: Mean not reported IQR 21 µg/m^3	PM_{10}, NO_2, CO	0-2	Conditional logistic (case-crossover)	1-h max O_3 (per 21 µg/m^3): Odds ratio: 0.979 (0.919, 1.065)
Sunyer et al. (2002) Barcelona, Spain 1986-1995	Mortality in a cohort of patients with severe asthma	1-h max O_3: Median: 69.3 µg/m^3 8-h max O_3: Median: 54.4 µg/m^3	PM_{10}, BS, SO_2, NO_2, CO, pollen	0-2	Conditional logistic (case-crossover)	1-h max O_3 (per 48 µg/m^3): Odds ratios: Patients with only one admission: 1.096 (0.820, 1.466) Patients with more than one admission: 1.688 (0.978, 2.643)
Díaz et al. (1999) Madrid, Spain 1990-1992	All cause; respiratory; cardiovascular	24-h avg O_3: Levels not reported.	TSP, NO_2, SO_2, CO	1, 4, 10	Autoregressive linear	24-h avg O_3 (per 25 µg/m^3): For O_3 levels higher than 35 µg/m^3: All cause: Lag 4: 12% ($p < 0.01$) U-shaped (quadratic) O_3-mortality relationship with a minimum of 35 µg/m^3.
Latin America						
Borja-Aburto et al. (1997) Mexico City 1990-1992	All cause; all ages; age < 5 years; age > 65 years	1-h max O_3: Median 155 ppb 8-h max O_3: Median 94 ppb 10-h avg O_3 (8 a.m.-6 p.m.): Median 87 ppb 24-h avg O_3: Median 54 ppb	TSP, SO_2, CO; 2-pollutant models	0, 1, 2	Poisson iteratively weighted and filtered least-squares method	1-h max O_3 (per 100 ppb): All ages: O_3 only model: Lag 0: 2.4% (1.1, 3.9) O_3 with TSP model: Lag 0: −1.8% (−10.0, 6.4)

Table AX7-5 (cont'd). Effects of Acute O_3 Exposure on Mortality

Reference, Study Location and Period	Outcome Measure	Mean O_3 Levels	Copollutants Considered	Lag Structure Reported	Method	Effect Estimates
Latin America (cont'd)						
Borja-Aburto et al. (1998) SW Mexico City 1993-1995	All cause; respiratory; cardiovascular; other; all ages; age > 65 years	1-h max O_3: 163 ppb 24-h avg O_3: 44 ppb	$PM_{2.5}$, NO_2, SO_2; 2-pollutant models	0, 1, 2, 3, 4, 5, 1-2	Poisson GAM (default convergence criteria but with only one smoother)	24-h avg O_3 (per 10 ppb): All cause, all ages: Lag 1-2: 0.6% (−0.3, 1.5) All cause, age > 65 years: Lag 1-2: 0.8% (−0.4, 2.0) Respiratory, all ages: Lag 1-2: −0.7% (−3.6, 2.1) Cardiovascular, all ages: Lag 1-2: 1.8% (0.1, 3.5) Other noninjury, all ages: Lag 1-2: 0.3% (−0.9, 1.4)
O'Neill et al. (2004) Mexico City 1996-1998	All cause; all ages; age 65+ years; SES gradient	24-h avg O_3: 35.3 ppb	PM_{10}	0-1	Poisson GAM	24-h avg O_3 (per 10 ppb): All ages: 0.65% (0.02, 1.28) Age 65+ years: 1.39% (0.51, 2.28) SES gradient did not show any consistent pattern.
Téllez-Rojo et al. (2000) Mexico City 1994	Respiratory; COPD mortality; age 65+ years; within medical unit; outside of medical unit	1-h max O_3: 134.5 ppb	PM_{10}, NO_2, SO_2	1, 2, 3, 4, 5, 1-3, 1-5, 1-7	Poisson, iteratively weighted and filtered least-squares method	1-h max O_3 (per 40 ppb): Outside medical unit: Respiratory: Lag 1-5: 14.0% (4.1, 24.9) COPD mortality: Lag 1-5: 15.6% (4.0, 28.4)
Gouveia and Fletcher (2000b) São Paulo, Brazil 1991-1993	All ages (all cause); age < 5 years (all cause, respiratory, pneumonia); age 65+ years (all cause, respiratory, cardiovascular)	1-h max O_3: 67.9 μg/m^3	PM_{10}, NO_2, SO_2, CO	0, 1, 2	Poisson GLM	1-h max O_3 (per 106 μg/m^3): All cause, all ages: Lag 0: 0.8% (−1.1, 2.7) All cause, age 65+ years: Lag 0: 2.3% (0, 4.6)

Table AX7-5 (cont'd). Effects of Acute O_3 Exposure on Mortality

Reference, Study Location and Period	Outcome Measure	Mean O_3 Levels	Copollutants Considered	Lag Structure Reported	Method	Effect Estimates
Latin America (cont'd)						
Pereira et al. (1998) São Paulo, Brazil 1991-1992	Intrauterine mortality	1-h max O_3: 67.5 µg/m^3	PM_{10}, NO_2, SO_2, CO	0	Poisson, linear with M-estimation	Slope estimate: 0.0000 (SE 0.0004)
Saldiva et al. (1994) São Paulo, Brazil 1990-1991	Respiratory; age < 5 years	24-h avg O_3: 12.14 ppb	PM_{10}, NO_2, SO_2, CO; multipollutant models	0-2	OLS of transformed data	Slope estimate: 0.01048 deaths/day/ppb (SE 0.02481), p = 0.673
Saldiva et al. (1995) São Paulo, Brazil 1990-1991	All cause; age 65+ years	1-h max O_3: 38.3 ppb 24-h avg O_3: 12.5 ppb	PM_{10}, NO_2, SO_2, CO; 2-pollutant models	0-1	OLS; Poisson with GEE	Slope estimate: 1-h max O_3: 0.0280 deaths/day/ppb (SE 0.0213), p > 0.05 24-h avg O_3: 0.0093 deaths/day/ppb (SE 0.0813), p > 0.05
Cifuentes et al. (2000) Santiago, Chile 1988-1966	All cause	1-h max O_3: Summer: 108.2 ppb	$PM_{2.5}$, $PM_{10-2.5}$, CO, SO_2, NO_2	0, 1, 2, 3, 4, 5, 1-2, 1-3, 1-4, 1-5	Poisson GAM (default convergence criteira); Poisson GLM	1-h max O_3 per (108.2 ppb): GLM: Summer: O_3 only model: Lag 1-2: 0.3% (t = 0.3) Multipollutant model: Lag 1-2: −0.1% (t = −0.1)
Ostro et al. (1996) Santiago, Chile 1989-1991	All cause	1-h max O_3: 52.8 ppb	PM_{10}, NO_2, SO_2; 2-pollutant models	1	OLS, several other methods	All year: 1-h max O_3 (per 52.8 ppb): −3% (−4, −2) Summer: 1-h max O_3 (per 100 ppb): 4% (0, 9)

Table AX7-5 (cont'd). Effects of Acute O_3 Exposure on Mortality

Reference, Study Location and Period	Outcome Measure	Mean O_3 Levels	Copollutants Considered	Lag Structure Reported	Method	Effect Estimates
Australia						
Morgan et al. (1998b) Sydney, Australia 1989-1993	All cause; respiratory; cardiovascular	1-h max O_3: 24 ppb	PM by nephelometer, NO_2; multipollutant models	0	Poisson with GEE	1-h max O_3 (per 28 ppb): All cause: 2.04% (0.37, 3.73) Respiratory: −0.84% (−7.16, 5.91) Cardiovascular: 2.52% (−0.25, 5.38)
Simpson et al. (1997) Brisbane, Australia 1987-1993	All cause; respiratory; cardiovascular; all ages; age < 65 years; age 65+ years	8-h avg O_3 (10 a.m.-6 p.m.): All year: 18.1 ppb Summer: 20.2 ppb Winter: 16.1 ppb	PM_{10}, PM by nephelometer, NO_2, SO_2, CO	0	Autoregressive Poisson with GEE	8-h avg O_3 (per 10 ppb): All cause, all ages: All year: 2.4% (0.8, 4.0) Summer: 3.0% (1.0, 5.0) Winter: 1.3% (−1.4, 4.1)
Asia						
Kim et al. (2004) Seoul, Korea 1995-1999	All cause	1-h max O_3: All year: 35.16 ppb Summer: 46.87 ppb Winter: 21.26 ppb	PM_{10}, NO_2, SO_2, CO; 2-pollutant models	1	Poisson GAM (linear model); GLM with cubic natural spline; GLM with B-mode spline (threshold model)	1-h max O_3 (per 21.5 ppb): All year: Linear model: 2.6% (1.7, 3.5) Threshold model: 3.4% (2.3, 4.4) Summer: Linear model: 1.9% (0.5, 3.3) Threshold model: 3.8% (2.0, 5.7)

Table AX7-5 (cont'd). Effects of Acute O_3 Exposure on Mortality

Reference, Study Location and Period	Outcome Measure	Mean O_3 Levels	Copollutants Considered	Lag Structure Reported	Method	Effect Estimates
Asia (cont'd)						
Lee et al. (1999) Seoul and Ulsan, Korea 1991-1995	All cause	1-h max O_3: Seoul: 32.4 ppb Ulsan: 26.0 ppb	TSP, SO_2	0	Poisson with GEE	1-h max O_3 (per 50 ppb): Seoul: 1.5% (0.5, 2.5) Ulsan: 2.0% (−11.1, 17.0)
Lee and Schwartz (1999) Seoul, Korea 1991-1995	All cause	1-h max O_3: Seoul: 32.4 ppb	TSP, SO_2	0	Conditional logistic (case-crossover with bidirectional control sampling)	1-h max O_3 (per 50 ppb): Two controls, plus and minus one week: 1.5% (−1.2, 4.2) Four controls, plus and minus two weeks: 2.3% (−0.1, 4.8)
Tsai et al. (2003) Kaohsiung, Taiwan 1994-2000	All cause; respiratory; cardiovascular; tropical area	24-h avg O_3: 23.6 ppb	PM_{10}, SO_2, NO_2, CO	0-2	Case-crossover analysis	24-h avg O_3 (per 19.2 ppb): Odds ratios: All cause: 0.994 (0.995, 1.035) Respiratory: 0.996 (0.848, 1.169) Cardiovascular: 1.005 (0.919, 1.098)
Yang et al. (2004) Taipei, Taiwan 1994-1998	All cause; respiratory; cardiovascular; subtropical area	24-h avg O_3: 17.18 ppb	PM_{10}, SO_2, NO_2, CO	0-2	Case-crossover analysis	24-h avg O_3 (per 9.34 ppb): Odds ratios: All cause: 0.999 (0.972-1.026) Respiratory: 0.991 (0.897-1.094) Cardiovascular: 1.004 (0.952-1.058)

Table AX7-6. Effects of Chronic O_3 Exposure on Respiratory Health

Reference, Study Location, and Period	Mean O_3 Levels	Study Description	Results and Comments
United States			
Galizia and Kinney (1999; exposure data Kinney et al., 1998) U.S. nationwide 1995	1-h max O_3: 10-year mean Jun-Aug: 61.2 ppb SD 15.5 Range 13-185	Nationwide sample of 520 young adults. Subjects were nonsmokers, aged 17-21 years, 50% males. Each subject provided one spirometric lung function measurement in the spring of their 1st year at Yale College in New Haven, CT, and completed a questionnaire addressing residential history, respiratory diseases, and activity patterns. Long-term O_3 exposure was treated as a high/low dichotomous variable, with subjects assigned to the high O_3 category if they lived for 4+ years in counties with 10-year summer mean O_3 levels greater than 80 ppb. Four lung function variables (FVC, FEV_1, FEF_{25-75}, FEF_{75}) were regressed on O_3 exposure, controlling for age, height, height squared, sex, race, parental education, and maternal smoking history. Respiratory symptom histories (cough, phlegm, wheeze apart from colds, and composite index for any of the three symptoms) were logistically regressed on O_3 exposure, controlling for sex, race, parental education, and maternal smoking.	Significant decrements in FEV_1 and FEF_{25-75} in relation to O_3 exposure were observed for all subjects and for males alone, but not for females alone. Similar patterns observed for FVC and FEF_{75}, but not with statistical significance. % difference in lung function for high versus low O_3 exposure groups: FEV_1: All subjects: −3.07% (−0.22, −5.92) Females: −0.26% (3.79, −4.31) Males: −4.71% (−0.66, −8.76) FEF_{25-75}: All subjects: −8.11% (−2.32, −13.90) Females: −1.96% (6.39, −10.30) Males: −13.02% (−4.87, −21.17) Wheeze and respiratory symptom index were significantly elevated for high O_3 exposure group. Odds ratios for symptoms: Wheeze: 1.97 (1.06, 3.66) Respiratory symptom index: 2.00 (1.15, 3.46)
Goss et al. (2004) U.S. nationwide 1999-2000	1-h max O_3: 51.0 ppb SD 7.3	11,484 cystic fibrosis patients over the age of 6 years. Exposure to O_3, $PM_{2.5}$, PM_{10}, NO_2, SO_2, and CO assessed by linking Aerometric Information Retrieval System with patients' home zip code. Studied exacerbation and lung function. Mortality was also of interest, but study was underpowered to examine this outcome. Logistic regression models were used to analyze the exacerbations and multiple linear regression was used to study lung function. O_3 monitoring season and regional effects also were examined.	Ozone may increase the risk for pulmonary exacerbations in cystic fibrosis patients. Odds ratios for two or more exacerbations (per 10 ppb increase in 1-h max O_3): O_3 only model: 1.10 (1.03, 1.17) O_3 with $PM_{2.5}$ model: 1.08 (1.01, 1.15) $PM_{2.5}$, but not O_3, was significantly associated with declines in lung function in these patients.

Table AX7-6 (cont'd). Effects of Chronic O_3 Exposure on Respiratory Health

Reference, Study Location, and Period	Mean O_3 Levels	Study Description	Results and Comments
United States (cont'd)			
Kinney and Lippmann (2000) Fort Sill, OK; Fort Leonard Wood, MO; Fort Dix, NJ; Fort Benning, GA; West Point, NY Apr-Sep 1990	1-h max O_3: Mean during 5-week summer training period: Fort Benning, GA: 55.6 ppb (0 hours O_3 > 100 ppb) Fort Dix, NJ: 71.3 ppb (23 hours O_3 > 100 ppb) Fort Leonard Wood, MO: 55.4 ppb (1 hours O_3 > 100 ppb) Fort Sill, OK: 61.7 ppb (1 hours O_3 > 100 ppb)	72 nonsmoking students (mean age 20.25 years) at the U.S. Military Academy at West Point, NY were measured for lung function and respiratory symptoms before (Apr) and after (Aug-Sep) taking part in an intensive, largely outdoor, summer training over five weeks (Jul 11-Aug 15) at four U.S. military bases. Ozone levels in the Fort Dix, NJ area were consistently higher than at the three remaining three locations. Analysis assessed change in lung function and respiratory symptoms measured before and soon after the summer training, and examined whether adverse trends would be more pronounced in students exposed to higher O_3 levels during summer training.	Mean FEV_1 declined significantly over the two measurement points for all subjects combined, which may reflect combined effects of O_3 with exposures to dust, vehicle exhaust, and environmental tobacco smoke as reported by subjects from all four locations in the post-summer questionnaire. However, a larger mean decline was seen at the higher O_3 site, Fort Dix, than at the remaining three sites, suggesting an influence of O_3 exposures. A larger decline was observed in subjects with post-summer measurements in the 1st two weeks after returning from training compared to those measured in the 3rd and 4th weeks, which is consistent with the lung function effects being somewhat transient. Change in lung function over the summer: FEV_1: All locations: −44 mL (SE 21), p = 0.035 Fort Dix: −78 mL (SE 41), p = 0.07 Forts Sill, Leonard Wood, and Benning combined: −31 mL (SE 24), p = 0.21
Greer et al. (1993) California 1973-1987	Annual mean O_3: Levels not reported.	3,914 nonsmoking adults aged 25+ years at enrollment in 1977 completed questionnaires at two time points, 1977 and 1987. To be eligible, subjects had to have lived 10 or more years within 5 miles of current residence. Residential histories used to interpolate air pollution levels to zip centroids over a 20-year period (1966-1987). New asthma cases defined as answering yes to doctor diagnosed asthma at 1987 followup among those answering no at enrollment in 1977. Multiple logistic regression used to test for associations between new-onset asthma and long-term exposures to air pollution, controlling for age, education, pneumonia or bronchitis before age 16 years, and years worked with a smoker through 1987. All models stratified by gender.	There were 27 incident cases of asthma among 1,305 males and 51 incident cases among 2,272 females. In logistic regression analyses, long-term O_3 exposures were associated with increased risk of incident asthma among males but not females. Relative risks for incident cases of asthma (per 10 ppb increase in annual mean O_3): Males: 3.12 (1.61, 5.85) Females: 0.94 (0.65, 1.34)

Table AX7-6 (cont'd). Effects of Chronic O_3 Exposure on Respiratory Health

Reference, Study Location, and Period	Mean O_3 Levels	Study Description	Results and Comments
United States (cont'd)			
McDonnell et al. (1999) California 1973-1992	8-h avg O_3 (9 a.m.-5 p.m): 20-year mean: 46.5 ppb SD 15.3	This study continued the work of Greer et al. (1993). 3,091 nonsmoking adults completed questionnaires at one additional time point, 1992. Residential histories used to interpolate air pollution levels to zip centroids over the period 1973-1992, yielding annual mean exposure estimates for O_3, PM_{10}, SO_2, and NO_2. New asthma cases defined as answering yes to doctor diagnosed asthma at either 1987 or 1992. Multiple logistic regression used to test for associations between new-onset asthma and long-term exposures to air pollution, controlling for age, education, pneumonia or bronchitis before age 16, and ever smoking. All models run separately for males and females.	There were 32 incident cases of asthma among 972 males and 79 incident cases among 1,786 females. In logistic regression analyses, long-term O_3 exposures were associated with increased risk of incident asthma among males but not females. Other pollutants were neither associated with asthma incidence nor were confounders of the O_3 association in males. Relative risks for incident cases of asthma (per 27 ppb increase in annual mean 8-h avg O_3): Males: 2.09 (1.03, 4.16) Females: 0.86 (0.58, 1.26)
Peters et al. (1999a,b) 12 Southern California communities 1993-1994	1-h max O_3: Mean range: 1986-1990: 30.2 ppb (Santa Maria) to 109.2 ppb (San Dimas) 1994: 35.5 ppb (Santa Maria) to 97.5 ppb (Lake Gregory)	3,676 children aged 9-16 years enrolled into the 1st cohort of the Children's Health Study in 1993. Subjects provided questionnaire data on respiratory disease histories and symptoms. 3,293 subjects also underwent pulmonary function testing, of which 2,781 were used in air pollution regressions. Air pollution data for O_3, PM_{10}, $PM_{2.5}$, NO_2, and inorganic acid vapors analyzed from 1986-1990 and 1994. For cross-sectional analysis of respiratory diseases, individual pollutants were tested for associations with ever asthma, current asthma, bronchitis, cough, and wheeze after controlling for covariates. For analysis of lung function, individual pollutants and pairs of pollutants were regressed with FVC, FEV_1, FEF_{25-75}, and PEF, controlling for usual demographic and anthropometric covariates.	Acids and NO_2, but not O_3, were associated significantly with prevalence of wheeze. No associations of O_3 with any of the respiratory diseases or symptoms. Decreased lung function was associated with multiple pollutants among females but not males. For O_3 exposure in females, all four lung function variables declined with increasing exposure. Associations were stronger for current (1994) exposure compared to previous (1986-1990) exposure. In males who spent more time outdoors, FVC and FEV_1 declined significantly with higher current exposure to O_3. Change in lung function (per 40 ppb 1-h max O_3 from 1986-1990): Females: PEF: −187.2 mL/s (SE 50.1), $p < 0.005$ FEF_{25-75}: −102.2 mL/s (SE 28.8), $p < 0.01$ Males: PEF: 31.1 mL/s (SE 48.8), $p > 0.05$ FEF_{25-75}: 11.7 mL/s (SE 26.7), $p > 0.05$

Table AX7-6 (cont'd). Effects of Chronic O_3 Exposure on Respiratory Health

Reference, Study Location, and Period	Mean O_3 Levels	Study Description	Results and Comments
United States (cont'd)			
Gauderman et al. (2000, 2004a,b) 12 Southern California communities 1993-2001	8-h avg O_3 (10 a.m.-6 p.m.): Mean range: Approximately 28 ppb (Long Beach) to 65 ppb (Lake Arrowhead)	Analysis of longitudinal lung function change in relation to long-term air pollution levels in the Children's Health Study. Children from 4th (n = 1,498), 7th (n = 802), and 10th (n = 735) grade enrolled in 1993. Children enrolled in 7th and 10th grade were followed until 1997; 4th graders were followed until 2001. Baseline questionnaires completed and annual pulmonary function tests (FVC, FEV_1, FEF_{25-75}, FEF_{75}) performed. Air pollution monitoring stations established in the 12 study communities beginning in 1994 to measure O_3, NO_2, PM_{10}, $PM_{2.5}$, and inorganic acid. Analysis using adjusted linear regression models.	In the 7th and 10th grade cohorts, difference in lung function growth from the least to the most polluted community was not associated with any of the air pollutants, including O_3. Among the 4th graders, decreased lung growth was associated with exposures to PM and NO_2, but not with O_3.
Gauderman et al. (2002) 12 Southern California communities 1996-1999	8-h avg O_3 (10 a.m.-6 p.m.): Mean range: Approximately 27 ppb (Long Beach) to 67 ppb (Lake Gregory)	Second cohort of the Children's Health Study. 2,081 4th graders (mean age 9.9 years) enrolled in 1996. Baseline questionnaires were completed and annual pulmonary function tests (FVC, FEV_1, FEF_{25-75}, FEF_{25-75}/FVC, PEF) were performed. 1,672 children had at least two pulmonary function test data. Air pollutants examined include O_3, NO_2, PM_{10}, $PM_{2.5}$, inorganic acid, elemental carbon, and organic carbon. Adjusted linear regression model was used.	In this cohort, a significant association between O_3 and PEF and FVC was noted in children spending more time outdoors. % difference in lung function growth from least to most polluted community (per 36.6 ppb increase in annual mean 8-h avg O_3): PEF: All children: −1.21% (−2.06, −0.36) Children more outdoors: −1.62% (−2.93, −0.29) Children less outdoors: −0.87% (−2.09, 0.37)
McConnell et al. (1999) 12 Southern California communities 1993	1-h max O_3: Estimated annual daily mean: 65.5 ppb Range 35.5-97.5	First cohort of the Children's Health Study. Association of O_3 with prevalence of chronic lower respiratory tract symptoms among children with a history of asthma was examined in a cross-sectional study in 12 communities. Questionnaires were completed by parents of 3,676 4th, 7th, and 10th graders, of which 493 had asthma. Exposure data (O_3 NO_2, PM_{10}, $PM_{2.5}$, and inorganic acid vapors) collected in 1994 used to estimate exposure. Analysis using logistic regression method.	Children with asthma were much more likely to have bronchitis or related symptoms than children without such history. Among the asthmatic children, significant relationship were observed between phlegm and all pollutants studied, with the exception of O_3.

Table AX7-6 (cont'd). Effects of Chronic O_3 Exposure on Respiratory Health

Reference, Study Location, and Period	Mean O_3 Levels	Study Description	Results and Comments
United States (cont'd)			
McConnell et al. (2002) 12 Southern California communities 1993-1998	1-h max O_3: Four-year mean (1994-1997): Low pollution communities (n = 6): 50.1 ppb Range 37.7-67.9 High pollution communities (n = 6): 75.4 ppb Range 69.3-87.2	3,535 children (age 9-16 years) without a history of asthma recruited in 1993 and 1996, and followed with annual surveys through 1998 to determine incidence of new onset asthma. Participation in sports assessed at baseline. Copollutants included PM_{10}, $PM_{2.5}$, NO_2, and inorganic acid vapors. Asthma incidence was examined as a function of number of sports played in high and low pollution communities, controlling for age, sex, and ethnic origin.	Asthma incidence was not higher in the high pollution communities as compared with the low pollution communities, regardless of the pollutant used to define high/low. In fact, the high O_3 communities had generally lower asthma incidence. However, in high O_3 communities, there was an increased risk of asthma in children playing three or more sports compared to those playing no sports; no such increase was observed in the low O_3 communities. No other pollutant showed this association. These results suggest that high levels of physical activity is associated with risk of new asthma development for children living in communities with high O_3 levels. Relative risk of developing asthma in children playing three or more sports compared to those playing no sports: Low pollution communities: 0.8 (0.4, 1.6) High pollution communities: 3.3 (1.9, 5.9)
Ritz et al. (2000) Southern California 1989-1993	8-h avg O_3 (9 a.m.-5 p.m.): Six weeks before birth: 36.9 ppb SD 19.4 Range 3.3-117 ppb	Data on 97,158 singleton births over period 1989-1993 linked to air monitoring data during different periods of pregnancy to determine associations between pollution exposures and preterm birth. Besides O_3, pollutants of interest included PM_{10}, NO_2, and CO. Multiple regression analysis used, controlling for maternal age, race, education, parity, and other factors.	Both PM_{10} and CO during early or late pregnancy were associated with increased risk for preterm birth. No associations observed with O_3.

Table AX7-6 (cont'd). Effects of Chronic O_3 Exposure on Respiratory Health

Reference, Study Location, and Period	Mean O_3 Levels	Study Description	Results and Comments
United States (cont'd)			
Künzli et al. (1997); Tager et al. (1998) Los Angeles and San Francisco, CA; Berkeley, CA 1995	8-h avg O_3 (10 a.m.-6 p.m.): Range of lifetime mean: Los Angeles: 25-74 ppb San Francisco: 16-33 ppb	In a pilot study, 130 freshman students (age 17-21 years) at the University of California at Berkeley measured for lung function and histories of residential locations and indoor/outdoor activity patterns and levels. By design, students had previously resided in one of two metropolitan areas that differed greatly in O_3 concentrations, San Francisco or Los Angeles. A key goal was to test whether measures of small airways function (e.g., nitrogen washout, FEF_{25-75}, FEF_{75}) were sensitive measures of long-term O_3 impacts. Lifetime exposures to O_3, PM_{10} and NO_2 assigned by interpolation to sequence of residence locations from available monitoring stations. Multiple exposure measures were derived with varying degrees of incorporation of time-activity information, going from ecological concentration to individual time-activity weighted exposure. Performed linear regression analysis of lung function on O_3 exposures, controlling for height, ethnicity, gender, and region.	Decreased FEF_{25-75} and FEF_{75} were associated with long-term O_3 exposures. Results were similar whether O_3 exposure was purely ecologic or incorporated time-activity information. FVC, FEV_1, and nitrogen washout were generally not associated with O_3 levels. No evidence for PM_{10} or NO_2 main effects or confounding of O_3. Similar patterns results using O_3 hours > 60 ppb as exposure metric instead of daily 8-h avg O_3 (10 a.m.-6 p.m.). Surprisingly, region of residence was a major negative confounder as lung function was lower on average among students from the low O_3 city, San Francisco, than among those who had lived in Los Angeles. Ozone exposures were significant predictors only after controlling the regional effect. Change in lung function (per 20 ppb increase in lifetime mean 8-h avg O_3): FEF_{25-75}: −420 mL/s (−886, 46); 7.2% of population mean FEF_{75}: −334 mL/s (−657, −11); 14% decline of population mean
Sherwin et al. (2000) Los Angeles, CA and Miami, FL 1995-1997	Levels not reported.	Lungs obtained from autopsies of young residents (age 11-30 years) of Miami (n = 20) and Los Angeles (n = 18) who died suddenly from homicide, vehicular accident, or other violence. Semiquantitative measurements of centriacinar region alterations were compared between the two cities.	A greater extent ($p < 0.02$) and severity ($p < 0.02$) of centriacinar region alterations were observed in lungs of the Los Angeles residents than the Miami residents. These differences could not be attributed to smoking history. The higher O_3 levels in Los Angeles might be responsible for the greater centriacinar region alterations, however correlations could not be performed due to the relatively small number of cases available.

Table AX7-6 (cont'd). Effects of Chronic O_3 Exposure on Respiratory Health

Reference, Study Location, and Period	Mean O_3 Levels	Study Description	Results and Comments
United States (cont'd)			
Gong et al. (1998b) Glendora, CA 1977-1987	1-h max O_3: Annual means range (1983-1987): 109 ppb to 134 ppb	164 adults (mean age 45 years; 34% males) from a high O_3 community underwent lung function testing in 1986-1987 (T3). Subjects were recruited from a cohort of 208 nonsmoking adults who had been tested on two previous occasions: 1977-1978 (T1) and 1982-1983 (T2). Analyzed changes in lung function at three time points. Subjects were also asked to undergo controlled exposures to 0.40 ppm O_3 over 2 hours with intermittent exercise. 45 subjects agreed to participate. Investigators hypothesized that acutely responsive subjects would show more rapid declines in function over the study period.	Mean FVC and FEV_1 showed nonsignificant increase from T2 to T3, whereas an earlier analysis of the T1 to T2 change had found a significant decline in function (Detels et al., 1987). There was evidence for 'regression to the mean,' in that subject with larger declines in function from T1 to T2 tended to have larger increases in function from T2 to T3. A consistent decline in FEV_1/FVC ratio was observed at all three time points ($p < 0.0001$ by ANOVA). Acute changes in lung function, determined using controlled O_3 exposures, were not associated with chronic lung function changes.
Chen et al. (2002) Washoe County, NV 1991-1999	8-h max O_3: 27.23 ppb SD 10.62 Range 2.76-62.44	Birth weight for 36,305 single births analyzed in relation to mean PM_{10}, O_3, and CO levels in trimesters 1, 2, and 3.	PM_{10} was the only air pollutant associated with decreased birth weights. Ozone levels quite low throughout study.
Kinney et al. (1996) New York City 1992-1993	1-h max O_3: Summer (Jul-Sep 1992): 58 ppb Maximum 100 Winter (Jan-Mar 1992): 32 ppb Maximum 64 Summer (Jul-Sep 1993): 69 ppb Maximum 142	19 healthy adult joggers (age 23-38 years; 18 males) from the Governors Island U.S. Coast Guard facility in New York harbor underwent a series of two bronchoalveolar lavages, first in the summer of 1992 and then again in the winter of 1992. Because the summer of 1992 had lower than average O_3 levels, six subjects underwent a third bronchoalveolar lavage in the summer of 1993. Study tested whether inflammatory markers in bronchoalveolar lavage fluid were elevated during the summer O_3 season among adults who regularly exercised outdoors. Outcomes included cell differentials, release of interleukin-8 (IL-8) and tumor necrosis factor-alpha (TNF-a) in bronchoalveolar lavage cells supernatants, release of reactive oxygen species by macrophages, and concentrations of protein, lactate dehydrogenase, IL-8, fibronectin, a1-antitrypsin (a1-AT), complement fragments (C3a), and prostaglandin E_2 (PGE_2) in bronchoalveolar lavage fluids.	There was no evidence of acute inflammation in the summer of 1992 compared to the winter; i.e., neutrophil differentials, IL-8 and TNF-a showed no significant differences. However, a measure of cell damage, lactate dehydrogenase, was elevated in the summer, suggesting possible O_3-mediated damage to the lung epithelium with repeated exposures to O_3 while exercising. O_3 levels during the summer of 1992 were atypically low for New York City. Among six subjects who agreed to undergo a third bronchoalveolar lavage test in the summer of 1993, lactate dehydrogenase was again elevated compared to winter. In addition, IL-8 was elevated in the summer of 1993, suggesting acute inflammation.

Table AX7-6 (cont'd). Effects of Chronic O_3 Exposure on Respiratory Health

Reference, Study Location, and Period	Mean O_3 Levels	Study Description	Results and Comments
Europe			
Charpin et al. (1999) Seven towns in SE France Jan-Feb 1993	8-h max O_3: Range of means: 30.2-52.1 µg/m^3 24-h avg O_3: Range of means: 20.1-42.1 µg/m^3	2,073 children (age 10-11 years) from 7 towns tested for atopy based on skin prick testing (house dust mite, cat dander, grass pollen, cypress pollen, and *Alternaria*). Towns represented a range of ambient O_3 and other pollutant (NO_2 and SO_2) levels. Tested hypothesis that atopy is greater in towns with higher photochemical pollution levels. To be eligible, subjects must have resided in current town for at least 3 years. Authors stated that Jan to Feb pollution levels correlated with levels observed throughout the year, though no data was given to support this.	In this cross-sectional analysis, no differences in atopy levels were seen across the seven towns. Authors concluded that long-term exposures to oxidant pollution do not favor increased allergy to common allergens. The very low winter O_3 levels monitored and lack of long-term exposure data make it impossible to reach this conclusion in a definitive manner.
Ramadour et al. (2000) Seven towns in SE France Jan-Feb 1993	8-h max O_3: Range of means: 30.2-52.1 µg/m^3	2,445 children (age 13-14 years) who had lived at their current residence for at least three years were recruited from schools in seven towns in SE France. This region has highest O_3 levels in France. Subjects completed ISAAC survey of asthma and respiratory symptoms. In addition to O_3 also collected data on SO_2 and NO_2. Analyzed relationships between asthma and other respiratory conditions with mean air pollution levels across the seven towns using logistic regression, controlling for family history of asthma, personal history of early-life respiratory diseases, and SES. Also did simple univariate linear regressions.	In logistic regressions, no significant associations seen between O_3 and 12-month history of wheezing, history of asthma attack, exercise induced asthma and/or dry cough in last 12 months. In simple bivariate scatterplots of respiratory outcomes versus mean O_3 levels in the seven towns, there appeared to be strong positive relationships ($r = 0.71$ for wheezing in last 12 months and $r = 0.96$ for asthma attacks). No data on slope estimates given. Concerns about potential confounding across towns limits the interpretation of this study.

Table AX7-6 (cont'd). Effects of Chronic O_3 Exposure on Respiratory Health

Reference, Study Location, and Period	Mean O_3 Levels	Study Description	Results and Comments
Europe (cont'd)			
Ihorst et al. (2004) Nine communities in Lower Austria Apr 1994-Oct 1997 Six communities in Germany Feb 1996-Oct 1999	½-h avg O_3: Quartile ranges: Summer: 1st quartile: 22-30 ppb 2nd quartile: 30-38 ppb 3rd quartile: 38-46 ppb 4th quartile: 46-54 ppb Winter: 1st quartile: 4-12 ppb 2nd quartile: 12-20 ppb 3rd quartile: 20-28 ppb 4th quartile: 28-36 ppb	2,153 children (median age 7.6 years) were studied for the effects of semi-annual and 3½-year mean O_3 concentrations on FVC and FEV_1. As a measure of lung growth, the difference between two consecutive values for each child was divided by the number of days between tests. The effect of O_3 exposure on lung growth was analyzed by linear regression models, after adjusting for sex, age, height at start of the time period, and passive smoking exposure.	Higher semi-annual mean O_3 levels were associated with diminished lung function growth during the summer, but increased lung function growth in the winter. Change in lung function (4th quartile compared to 1st quartile semi-annual O_3 mean): Summer: FEV_1 (mL/100 days): −18.5 (−27.1, −9.8) FVC (mL/100 days): −19.2 (−27.8, −10.6) Winter: FEV_1 (mL/100 days): 10.9 (2.1, 19.7) FVC (mL/100 days): 16.4 (8.3, 24.6) No associations between longer term O_3 exposure (mean summer O_3 over a 3½-year period) and lung function growth was found.
Kopp et al. (2000) Ten communities in Austria and SW Germany Mar 1994-Nov 1995	½-h avg O_3: Stratified by low, medium, high exposure: Low: 24-33 ppb Medium: 35-38 ppb High: 44-52 ppb	797 children with a mean age of 8.2 years. Four pulmonary function tests (FVC, FEV_1) performed on each child over two summers. Examined association between average daily lung function growth and exposure to O_3, PM_{10}, NO_2, and SO_2. Analysis using linear regression models.	Lower FVC and FEV_1 increases were observed in children exposed to high ambient O_3 levels compared to those exposed to lower O_3 levels during the summer. During the winter, children in higher O_3 areas showed a slightly greater increase in FVC and FEV_1 than those in the low O_3 areas, which might reflect that children catch up in lung function deficits during the winter season. Change in lung function for high versus low O_3 exposure groups (per ppb O_3): FEV_1: Summer of 1994: −0.303 mL/day, p = 0.007 Winter of 1994/1995: 0.158 mL/day, p = 0.006 Summer of 1995: −0.322 mL/day, p = 0.001

Table AX7-6 (cont'd). Effects of Chronic O_3 Exposure on Respiratory Health

Reference, Study Location, and Period	Mean O_3 Levels	Study Description	Results and Comments
Europe (cont'd)			
Frischer et al. (1999) Nine communities in Austria 1994-1996	24-h avg O_3: Summer: 34.8 ppb SD 8.7 Winter: 23.1 ppb SD 7.7	Communities from two counties chosen to represent a broad range of O_3 concentrations; a two-fold range in mean levels was observed. 1,150 children (mean age 7.8 years; 52% males) from grades 1 and 2 performed spirometry in spring and fall over three years (total of six measurements per child) to determine if seasonal exposure to O_3 would be associated with diminished lung function growth, especially over the summer seasons. Ozone levels were low during lung function testing periods. Participation rates were high. At baseline, respiratory histories were collected and subjects were tested for allergy by skin prick. Examined association between O_3 levels and change in lung function (FVC, FEV_1, and MEF_{50} [maximal expiratory flow at 50% of vital capacity]) over each season, controlling for baseline function, atopy, gender, site, environmental tobacco smoke exposure, season, and change in height. Other pollutants studied included PM_{10}, SO_2, and NO_2.	Seasonal mean O_3 exposures were associated with reductions in growth in all three lung function measures. Inconsistent results seen for other pollutants. Summer season lung function growth decrements per unit O_3 were larger when data restricted to children who spent whole summer in their community. No evidence for nonlinear O_3 effect. No confounding of O_3 effect by temperature, ETS, or acute respiratory illnesses. Change in lung function (per ppb O_3): FEV_1 (mL/day): All subjects: Summer: −0.029 (SE 0.005), $p < 0.001$ Winter: −0.024 (SE 0.006), $p < 0.001$ Restricted to subjects who stayed in community: Summer: −0.034 (SE 0.009), $p < 0.001$ FVC (mL/day): All subjects: Summer: −0.018 (SE 0.005), $p < 0.001$ Winter: −0.010 (SE 0.006), $p = 0.08$ Restricted to subjects who stayed in community: Summer: −0.033 (SE 0.007), $p < 0.001$
Frischer et al. (2001) Nine communities in Austria Sep-Oct 1997	½-h avg O_3: 30-day mean: 31.57 ppb IQR 20.61	A cross-sectional study of 877 school children (mean age 11.2 years). Analyzed for urinary eosinophil protein as a marker of eosinophil activation determined from a single spot urine sample using linear regression models.	Log-transformed urinary eosinophil protein-X concentrations were found to be significantly associated with O_3 levels, after adjusting for gender, site, and atopy. Change in log urinary eosinophil protein-X (per ppb O_3): 0.007 µg/mmol creatinine (SE 0.02), $p < 0.001$

Table AX7-6 (cont'd). Effects of Chronic O_3 Exposure on Respiratory Health

Reference, Study Location, and Period	Mean O_3 Levels	Study Description	Results and Comments
Europe (cont'd)			
Horak et al. (2002a,b) Eight communities in Austria 1994-1997	Seasonal mean O_3: Summer: 31.8 ppb Range 18.7-49.3 Winter: 19.8 ppb Range 12.7-35.9	This study continued the work of Frischer et al., 1999 by including one additional year of data, 1997. The major hypothesis considered PM_{10}. For this study, 80.6% of the 975 children (mean age 8.11 years) performed all six lung function tests. A total of 860 children were included in the GEE analysis. Multipollutant analysis for PM_{10}, SO_2, and NO_2.	Seasonal mean O_3 showed a negative effect on lung function growth, confirming the previous shorter study. Ozone effects were robust to inclusion of PM_{10} into the model. However, for FEV_1 in winter, the O_3 effect slightly diminished after including PM_{10}. Taking into account only children who stayed at home the whole summer period did not affect the results. Change in lung function (per ppb O_3): FEV_1 (mL/day): O_3 only models: Summer: −0.021, $p < 0.001$ Winter: −0.020, $p < 0.001$ O_3 with PM_{10} models: Summer: −0.020, $p < 0.001$ Winter: −0.012, $p = 0.04$
Palli et al. (2004) Florence, Italy 1993-1998	24-h avg O_3: Range of monthly means from 1993-1998: Approximately 25-125 ppb	320 residents (age 35-64 years) in the metropolitan area of Florence enrolled in a study investigating the correlation between levels of DNA bulky adducts and cumulative O_3 exposure. One blood sample was collected for each subject. Various time windows of exposure were examined, ranging from 0-15 days to 0-90 days prior to the blood draw. Simple Spearman correlations between DNA adduct levels and different O_3 exposure time windows were calculated after stratifying by smoking history, area of residence, and population type (random sample or exposed workers).	Consistent relationships between O_3 exposure and DNA adduct levels were observed only among never smokers. Correlations were highest among never smokers who resided in the urban area and were not occupationally exposed to vehicle traffic pollution. Associations were significant up to a time window of 0-60 days prior to the blood draw in the subgroup of never smokers, with strongest relationships observed between 30-45 days prior.

Table AX7-6 (cont'd). Effects of Chronic O_3 Exposure on Respiratory Health

Reference, Study Location, and Period	Mean O_3 Levels	Study Description	Results and Comments
Latin America			
Calderón-Garcidueñas et al. (1995) SW Mexico City Nov 1993 Manzanillo, Mexico Jan 1994	SW Mexico City (urban): 1-h avg O_3 > 120 ppb: 4.4 hours/day Maximum 307 ppb Manzanillo, Pacific port (control): No detectable air pollutants.	Nasal lavage samples collected from 38 urban (mean age 12.2 years) and 28 control (mean age 11.7 years) children. Samples analyzed for polymorphonuclear leukocyte counts, expression of human complement receptor type 3 (CD11b) on nasal polymorphonuclear leukocytes, and nasal cytologies.	Nasal cytologies revealed that children from Mexico City had abnormal nasal mucosae, including mucosal atrophy, marked decreases in the numbers of ciliated-type cells and goblet cells, and squamous metaplasia. Exposed children had significantly higher nasal polymorphonuclear leukocyte counts ($p < 0.001$) and nasal CD11b expression ($p < 0.001$) compared to controls. However, the inflammatory response did not seem to correlate with the previous day's O_3 exposure in a dose-dependent manner, suggesting that there might be a competing inflammatory mechanism at the brochioalveolar level. Overall, these results suggest that ambient O_3 produces an inflammatory response in chronically exposed children.
Calderón-Garcidueñas et al. (1997) SW Mexico City Sep-Nov 1995 Manzanillo, Mexico Jan 1995	SW Mexico City (urban): 1-h avg O_3 > 120 ppb: 82 hours/month Maximum 286 ppb Manzanillo, Pacific port (control): No detectable air pollutants.	129 urban and 19 control children aged 6-12 years old with no history of smoking or environmental tobacco smoke exposure and no current medication use for atopy or asthma. Three nasal biopsies obtained at 4-week intervals and analyzed for DNA damage based on the presence of DNA fragments.	Urban children had significantly more DNA fragments than did control children ($p < 0.0001$). Percentage of damaged cells was 82.2% (SE 6.4) in urban children and 17.0% (SE 6.1) in control children. Among urban children, more upper respiratory symptoms and DNA damage was seen with increasing age. Older children spent more time outdoors and engaged in physical activities ($p < 0.001$). Urban children were exposed to a complex pollution mix, making it difficult to attribute effects to O_3 specifically. However, authors noted that O_3 was the pollutant with most exceedences of air quality standard.

Table AX7-6 (cont'd). Effects of Chronic O_3 Exposure on Respiratory Health

Reference, Study Location, and Period	Mean O_3 Levels	Study Description	Results and Comments
Latin America (cont'd)			
Calderón-Garcidueñas et al. (1999) SW Mexico City May-June 1996 Manzanillo, Mexico May 1996	SW Mexico City (urban): 1-h avg O_3 > 80 ppb: May: 161 hours/month Maximum 232 ppb June: 98 hours/month Maximum 261 ppb Manzanillo, Pacific port (control): Mean < 10 ppb	86 urban and 12 control children aged 6-13 years old with no history of smoking or environmental tobacco smoke exposure and no use of medication for atopy or asthma. Urban children stratified into five groups by school grade level (1st through 5th). Nasal epithelial biopsies obtained from inferior nasal turbinates, and analyzed for single strand DNA breaks and for 8-OHdG (8-hydroxy-2'-deoxyguanosine), a mutagenic lesion produced by G→T mutations. These outcomes relate to possible carcinogenic effects of air pollution exposures. Multiple air pollutants monitored in SW Mexico City within 3 miles of urban subject residences.	No respiratory symptoms reported by control children; urban children reported multiple nasal and lung symptoms, including cough and chest discomfort among 46% of urban children, with higher rates for 5th versus 1st graders. 8-OHdG was approximately 3-fold higher in biopsies from urban children ($p < 0.05$), however, no differences by school grade. Single strand DNA breaks were more common in urban versus control children, with an age-dependent increase in the urban children ($p < 0.05$). These results suggest that DNA damage is present in the nasal epithelial cells of children living in highly polluted SW Mexico City and may reflect enhanced risk of cancer later in life. Though O_3 represents an important component of the pollution mix, it is not possible to attribute effects solely to O_3.
Fortoul et al. (2003) Mexico City May 1997	9-h avg O_3 (9 a.m.-6 p.m.): South: 121 ppb North: 89 ppb	Estimated DNA strand breaks on nasal epithelial cells and leucocytes sampled from asthmatic (n = 15) and nonasthmatic (n = 224) medical students aged 18-28 years using a single-cell gel electrophoresis assay.	Greater genotoxic damage in asthmatics' nasal epithelial cells ($p < 0.05$) may reflect their higher vulnerability for DNA damage, or a decreased ability to repair it, compared with nonasthmatic subjects.
Gouveia et al. (2004) São Paulo, Brazil 1997	1-h max O_3: 63.0 ppb SD 33.5	Birth weight for 179,460 single births analyzed in relation to PM_{10}, SO_2, CO, NO_2, and O_3 levels in trimester 1, 2, and 3. GAM and logistic regression models used for analysis.	Exposures to PM_{10} and CO during 1st trimester were found to have significant negative associations with birth weight. No associations observed for the other air pollutants, including O_3.

Table AX7-6 (cont'd). Effects of Chronic O_3 Exposure on Respiratory Health

Reference, Study Location, and Period	Mean O_3 Levels	Study Description	Results and Comments
Asia			
Kuo et al. (2002) Central Taiwan 1996	1-h max O_3: Annual mean range across 7 of 8 schools: 18.6-27.3 ppb	Respiratory questionaire administered to 12,926 children aged 13-16 years at eight junior high schools in central Taiwan, to determine asthma prevalence. The association between asthma prevalence and air pollution exposure analyzed by simple Pearson correlations of prevalence with annual mean air pollution levels (O_3, SO_2, PM_{10}, and NO_2), and by multiple logistic regression. The 775 asthmatics who were identified then provided follow-up data on symptoms and exacerbations over a one-year period. Simple Pearson correlations were computed between monthly hospital admissions and air pollution levels, not controlling for covariates such as season or weather.	Asthma prevalence ranged from 5.5% to 14.5% across the 8 schools. Based on simple Pearson's correlations, mean O_3 ($r = 0.51$) and NO_2 ($r = 0.63$) levels were correlated with variations in asthma prevalence. However, only NO_2 remained significant in multiple logistic regression analyses after adjusting for various potential confounding factors. Longitudinal hospital admissions results are inconclusive due to analytical limitations. Monthly correlations of hospital admissions for asthmatics yielded variable results, all of which would be confounded by temporal factors.

Table AX7-7. Effects of Chronic O_3 Exposure on Mortality

Reference, Location, Study Period	Mean O_3 Levels	Study Description	Results and Comments
United States			
Pope et al. (2002) U.S. nationwide 1982-1998	1-h max O_3: 59.7 ppb SD 12.8 24-h avg O_3: 45.5 ppb SD 7.3	Approximately 500,000 members of American Cancer Society cohort enrolled in 1982 and followed through 1998 for all cause, cardiopulmonary, lung cancer, and all other cause mortality. Age at enrollment was 30+ years. Air pollution concentrations in urban area of residence at time of enrollment assessed from 1982 through 1998. Other pollutants considered include TSP, PM_{15} , PM_{10}, $PM_{2.5}$, $PM_{15\text{-}2.5}$, SO_4^{2-}, SO_2, NO_2, and CO.	No significant effect of O_3 on mortality risk, though the association of Jul-Sep O_3 concentrations with all cause and cardiopulmonary mortality were positive and nearly significant. Residential location was known only at enrollment to study in 1982. Thus, exposure misclassification is likely to be high.
Lipfert et al. (2000b, 2003) 32 Veterans Administration hospitals nationwide in the U.S. 1976-1996	95th % O_3: 1960-1974: 132 ppb 1975-1981: 140 ppb 1982-1988: 94 ppb 1989-1996: 85 ppb	Approximately 50,000 U.S. veterans (all males) diagnosed with hypertension. Mean age at recruitment was 51 years. Exposure to O_3 during four periods (1960-1974, 1975-1981, 1982-1988, 1989-1996) associated with mortality over three periods (1976-1981, 1982-1988, 1989-1996). Long-term exposures to TSP, PM_{15} , PM_{10}, $PM_{2.5}$, $PM_{15\text{-}2.5}$, SO_4^{2-}, NO_2, and CO also analyzed. Used Cox proportional hazards regression, adjusting for race, smoking, age, systolic and diastolic blood pressure, body mass index, and socioeconomic factors.	Positive average concurrent responses for TSP, SO_4^{2-}, NO_2, O_3 in individual period analyses, but only O_3 was significant for overall. Two-pollutants analyses indicate that responses to peak O_3 are robust. Relative risks (per mean 95th % O_3 less estimated background level, value not reported): Averaged over all four periods: Exposure concurrent with mortality: O_3 only model: 1.094 (SE 4.6), $p < 0.05$ O_3 with NO_2 model: 1.122, $p < 0.05$ Exposure before mortality: O_3 only model: 0.998 (SE 6.3) $p > 0.05$ Analyses were robust to the deletion of diastolic blood pressure in the models, indicating that the association between mortality and O_3 was not mediated through blood pressure.

Table AX7-7 (cont'd). Effects of Chronic O_3 Exposure on Mortality

Reference, Location, Study Period	Mean O_3 Levels	Study Description	Results and Comments
United States (cont'd)			
Abbey et al. (1999) Three California air basins: San Francisco, South Coast (Los Angeles and eastward), San Diego 1977-1992	24-h avg O_3: 26.11 ppb SD 7.65 IQR 12.0 O_3 h/year > 100 ppb: 330 h/year SD 295 IQR 551	Prospective cohort study of 6,338 nonsmoking non-Hispanic white adult members of the Adventist Health Study followed for all cause, cardiopulmonary, nonmalignant respiratory, and lung cancer mortality. Participants were aged 27-95 years at enrollment in 1977. 1,628 (989 females, 639 males) mortality events followed through 1992. All results were stratified by gender. Used Cox proportional hazards analysis, adjusting for age at enrollment, past smoking, environmental tobacco smoke exposure, alcohol use, education, occupation, and body mass index. Analyzed mortality from all natural causes, cardiopulmonary, nonmalignant respiratory, and lung cancer. Ozone results were presented for both metrics.	Of 16 regressions involving O_3 exposures (two genders; four mortality causes; two O_3 metrics), 11 were positive and one was statistically significant, for lung cancer in males for O_3 h/year > 100 ppb. Relative risks for lung cancer mortality in males: 24-h avg O_3 (per 12.0 ppb): 2.10 (0.99, 4.44) O_3 h/year > 100 ppb (per 551 hours/year): 4.19 (1.81, 9.69) Inconsistency across outcomes and genders raises possibility of spurious finding. The lack of cardiopulmonary effects raises plausibility concerns.
Beeson et al. (1998) Three California air basins: San Francisco, South Coast (Los Angeles and eastward), San Diego 1977-1992	Annual mean O_3: 26.2 ppb SD 7.7 O_3 h/year > 100 ppb: 333 h/year SD 297	6,338 nonsmoking non-Hispanic white adult members of the Adventist Health Study aged 27-95 years at time of enrollment. 36 (20 females, 16 males) histologically confirmed lung cancers were diagnosed through 1992. Extensive exposure assessment, with assignment of individual long-term exposures to O_3, PM_{10}, SO_4^{2-}, and SO_2, was a unique strength of this study. All results were stratified by gender. Used Cox proportional hazards analysis, adjusting for age at enrollment, past smoking, education, and alcohol use.	Males, but not females, showed moderate association for O_3 and incident lung cancer risk. Relative risks for lung cancer incident in males: O_3 h/year > 100 ppb (per 556 hours/year): All males: 3.56 (1.35, 9.42) Never smokers: 4.48 (1.25, 16.04) Past smokers: 2.15 (0.42, 10.89)

8. INTEGRATIVE SYNTHESIS

8.1 INTRODUCTION

This integrative synthesis (Chapter 8) aims to provide a coherent framework for the assessment of health risks associated with human exposures to ambient ozone (O_3) in the United States. The main goal of this chapter is to integrate newly available scientific information with that discussed in the 1996 O_3 AQCD, to address issues central to the EPA's assessment of scientific information needed to support the current review of the primary O_3 NAAQS. Other scientific information concerning ambient O_3 welfare effects (i.e., effects on vegetation /ecosystems, surface-level solar UV flux/climate changes, and man-made materials) and pertinent to review of secondary O_3 standards is assessed in ensuing Chapters 9, 10, and 11. The integrated assessment of scientific findings provided here and elsewhere in this document will be used and their policy implications considered in an Ozone Staff Paper to be prepared by EPA's Office of Air Quality Planning and Standards (OAQPS). The scientific and technical assessments provided in that Staff Paper will "bridge the gap" between scientific assessments in this criteria document and judgments required of the EPA administrator in evaluating whether to retain or, possibly, to revise the current O_3 NAAQS.

Ozone found in the earth's atmosphere generally originates from photochemical reactions that are predominantly catalyzed by the interaction of sunlight with other pollutants, especially nitrogen oxides (NO_x) and hydrocarbons such as volatile organic compounds (VOCs). Other photochemical oxidants, such as peroxyacetyl nitrate (PAN) and hydrogen peroxide (H_2O_2), are also generated along with O_3 by such atmospheric interactions. In addition to the tropospheric O_3 generated by these interactions, some O_3 is found near the earth's surface as the result of its downward transport from the stratosphere, even in the absence of photochemical reactions in the troposphere. However, in contrast to stratospheric O_3, which plays an important role in maintaining the habitability of the planet by shielding the surface from harmful solar ultraviolet (UV) radiation, tropospheric O_3 at the surface can exert adverse effects on humans, animals, and vegetation. This criteria document is mainly focused on assessment of health and welfare effects resulting from exposures to surface level concentrations of tropospheric O_3, with only relatively limited attention begin accorded to other photochemical oxidants such as PAN or H_2O_2.

8.1.1 Chapter Organization

This integrative synthesis chapter is divided into several major sections. This first section (Introduction) not only aims to orient the reader to the organization and content of the chapter, but also provides background information on the current O_3 NAAQS and important types of human responses to O_3 exposure that were considered as key bases for the 1997 EPA revision of the O_3 NAAQS. The next section (Section 8.2) focuses on air quality trends and current ambient O_3 concentrations to provide context for ensuing discussions of ambient O_3 exposures and its effects on human health and welfare.

The subsequent sections (8.3, 8.4, and 8.5) then build upon the integrative synthesis presented in Chapter 9 of the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996) to integrate newly available key scientific information assessed in Chapters 4 through 7 of this document. This includes integration of information on dosimetry, as well as toxicological, human clinical, and epidemiological studies.

These sections collectively address the following key issues: (1) ambient exposures, personal exposures, and dosimetric considerations; (2) experimental studies on toxicological responses to acute O_3 exposures in humans (clinical studies) and both acute and chronic effects in animals; (3) assessment of epidemiological evidence for associations between O_3 exposure in human populations and health effects and the robustness of these associations; (4) integration of the experimental data with epidemiological assessments; (5) biological mechanisms and other evidence useful in judging the plausibility of adverse health effects being associated with human exposures to ambient O_3 levels encountered in the United States; and (6) identification of susceptible and vulnerable populations potentially at increased risk for O_3-related health effects and potential public health impacts of human exposure to ambient O_3 in the United States.

The present chapter mainly focuses on discussion of new scientific information that has become available since the 1996 O_3 criteria review that supported EPA's revision of the O_3 NAAQS in 1987. However, it also highlights important data gaps and uncertainties that still exist with regard to various key issues and notes important research needs in a number of key areas. Detailed evaluation of such research needs is beyond the scope of this document, but will be undertaken as part of later EPA efforts focused on identification of O_3 research needs and development of research planning documents.

8.1.2 Current Standards

The NAAQS for ambient O_3 were revised in 1997 by adding an 8-h standard (Table 8-1) in addition to the 1979 1-h standard, which is met if the fourth highest daily maximum 1-h O_3 over a 3-year period is < 0.12 ppm. The 8-h standard is met when the 3-year average of the annual fourth highest daily maximum 8-h average concentration is < 0.08 ppm. The 1997 standards were based on various scientific supportive data from human exposure and epidemiological studies as assessed in the 1996 O_3 AQCD. The gradations of individual responses observed with short-term exposure to O_3 in healthy persons (Table 8-2) and in persons with impaired respiratory systems (Table 8-3) are representative of the critical information used in these evaluations, as summarized in Tables 9-1 and 9-2 of the 1996 O_3 AQCD and reproduced herein Tables 8-2 and 8-3, respectively. Detailed assessments of the scientific information and supportive data used in generating these tables can be found in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996). Key findings from health studies that have become newly available since the 1996 criteria review are discussed below in later sections of this chapter and any important consequent reaffirmations or modifications of findings of the types summarized in Tables 8-2 and 8-3 are highlighted.

Table 8-1. Current National Ambient Air Quality Standards (NAAQS) in the United States

Pollutant	Date of Promulgation	Primary NAAQS	Averaging Time	Secondary NAAQS
Ozone	7/18/97 (62FR38856)	0.08 ppm (157 µg/m^3)	8-h [a]	Same as primary
	3/9/94 (58FR52852)	0.12 ppm (235 µg/m^3)	1-h [b]	Same as primary

[a] Based on the 3-year average of the annual fourth-highest daily maximum 8-h average O_3 concentration measured at each monitor within an area.

[b] The standard is attained when the expected number of days per calendar year with maximum hourly average concentrations above 0.12 ppm is ≤ 1.

Table 8-2. Gradation of Individual Responses to Short-Term Ozone Exposure in Healthy Persons [a]*

Functional Response	None	Small	Moderate	Large
FEV_1	Within normal range (±3%)	Decrements of 3 to ≤ 10%	Decrements of > 10 but < 20%	Decrements of ≥ 20%
Nonspecific bronchial responsiveness [b]	Within normal range	Increases of < 100%	Increases of ≤ 300%	Increases of > 300%
Duration of response	None	< 4 hours	> 4 hours but ≤ 24 hours	> 24 hours
Symptomatic Response	**Normal**	**Mild**	**Moderate**	**Severe**
Cough	Infrequent cough	Cough with deep breath	Frequent spontaneous cough	Persistent uncontrollable cough
Chest pain	None	Discomfort just noticeable on exercise or deep breath	Marked discomfort on exercise or deep breath	Severe discomfort on exercise or deep breath
Duration of response	None	< 4 hours	> 4 hours but ≤ 24 hours	> 24 hours
Impact of Responses	**Normal**	**Normal**	**Mild**	**Moderate**
Interference with normal activity	None	None	A few sensitive individuals choose to limit activity	Many sensitive individuals choose to limit activity

[a] See text for discussion; see Appendix A for abbreviations and acronyms.
[b] An increase in nonspecific bronchial responsiveness of 100% is equivalent to a 50% decrease in PD_{20} or PD_{100} (see Chapter 7, Section 7.2.3).
*This table is reproduced from the 1996 O_3 AQCD (Table 9-1, page 9-24) (U.S. Environmental Protection Agency, 1996).

8.2 TRENDS IN UNITED STATES OZONE AIR QUALITY

8.2.1 Ozone Concentrations, Patterns

Ozone is monitored in the United States during "O_3 seasons," which vary in length from geographic region to region. The O_3 season extends all year in the Southwest, but in most other areas of the country, O_3 is typically monitored from April to October. However, O_3 is present year-round, not only in polluted areas, but in clean areas as well. The median O_3 concentration in the United States from 1996 to 2000, averaged over the appropriate O_3 season, was 33 ppb for "urban" monitors located in Metropolitan Statistical Areas (MSAs); and it was 37 ppb for

Table 8-3. Gradation of Individual Responses to Short-Term Ozone Exposure in Persons with Impaired Respiratory Systems [a]*

Functional Response	None	Small	Moderate	Large
FEV_1 change	Decrements of < 3%	Decrements of 3 to ≤ 10%	Decrements of > 10 but < 20%	Decrements of ≥ 20%
Nonspecific bronchial responsiveness [b]	Within normal range	Increases of < 100%	Increases of ≤ 300%	Increases of > 300%
Airway resistance (SR_{aw})	Within normal range (±20%)	SR_{aw} increased < 100%	SR_{aw} increased up to 200% or up to 15 cm H_2O/s	SR_{aw} increased > 200% or more than 15 cm H_2O/s
Duration of response	None	< 4 hours	> 4 hours but ≤ 24 hours	> 24 hours
Symptomatic Response	**Normal**	**Mild**	**Moderate**	**Severe**
Wheeze	None	With otherwise normal breathing	With shortness of breath	Persistent with shortness of breath
Cough	Infrequent cough	Cough with deep breath	Frequent spontaneous cough	Persistent uncontrollable cough
Chest pain	None	Discomfort just noticeable on exercise or deep breath	Marked discomfort on exercise or deep breath	Severe discomfort on exercise or deep breath
Duration of response	None	< 4 hours	> 4 hours, but ≤ 24 hours	> 24 hours
Impact of Responses	**Normal**	**Mild**	**Moderate**	**Severe**
Interference with normal activity	None	Few individuals choose to limit activity	Many individuals choose to limit activity	Most individuals choose to limit activity
Medical treatment	No change	Normal medication as needed	Increased frequency of medication use or additional medication	Physician or emergency room visit

[a] See text for discussion; see Appendix A for abbreviations and acronyms.
[b] An increase in nonspecific bronchial responsiveness of 100% is equivalent to a 50% decrease in PD_{20} or PD_{100} (see Chapter 7, Section 7.2.3).
*This table is reproduced from the 1996 O_3 AQCD (Table 9-2, page 9-25) (U.S. Environmental Protection Agency, 1996).

monitors located outside MSAs. Median daily maximum 8-h concentrations between 10:00 a.m. and 6:00 p.m. were 46 and 47 ppb for monitors located in and outside MSAs, respectively. Median daily maximum 1-h concentrations were 56 ppb for monitors located in MSAs and 55 ppb for monitors located outside of them. The daily maximum 1-h concentrations tended to be much higher in some large urban areas or in areas downwind of them, e.g., they were 202 ppb in Houston, TX in 1999 and 161 ppb in 2000. Daily 1-h maximum O_3 concentrations were lower in nonurban areas of the country but still above 120 ppb in many locations. Eight-hour daily maximum concentrations were not as high as 1-h daily maxima, but they also tended to be highly correlated with the 1-h maxima.

Within individual MSAs, O_3 concentrations tend to be well correlated across monitoring sites, although variations in concentrations can be substantial. In many city centers, O_3 concentrations tend to be lower than in either upwind or downwind areas, largely due to NO emitted by motor vehicles. Thus, although emissions of nitrogen oxides and VOCs from motor vehicles contribute to O_3 formation, the relationship to O_3 concentrations is not straightforward in terms of proximity to mobile sources. In urban areas with high traffic density or near highways, emissions of NO from traffic react with ozone, thereby reducing its concentration. For example, much lower ozone concentrations overall are found in downtown Los Angeles (e.g., in Lynwood) than at sites located further downwind (e.g., in San Bernadino). The much higher levels are formed from photochemical reactions involving the urban emissions, including products produced as the result of reactions titrating ozone in the urban core. Thus, ozone concentrations tend to be higher downwind of urban centers, and they decrease again in going to areas that are remote from precursor sources.

8.2.2 Seasonal Variations

Ozone concentrations tend to peak in early to mid-afternoon in areas where there is strong photochemical activity and to peak later in the afternoon or early evening in areas where transport is more important in determining the O_3 abundance. Summertime maxima in O_3 concentrations occur in U.S. areas where substantial photochemical activity acts on O_3 precursors emitted as the result of human activities. Monthly maxima can occur anytime from June through August. However, springtime maxima are observed in National Parks, mainly in the western United States and at a number of other relatively unpolluted monitoring sites

throughout the Northern Hemisphere. For example, the highest O_3 concentrations at Yellowstone National Park tend to occur during April and May. Typically, monthly minima tend to occur from November through February at polluted sites and during the fall at relatively remote sites.

8.2.3 Long-Term Trends

Nationwide, 1-h O_3 concentrations decreased ~29% from 1980 to 2003 and by ~16% from 1990 to 2003; and, for the 8-h standard, O_3 levels decreased ~21% since 1980 and ~9% from 1990 to 2003. Note that 1-h and 8-h O_3 levels continue to decrease nationwide, but the rate of decrease has slowed since 1990. These trends have not been uniform across the United States. In general, O_3 reductions have been largest in New England and in states along the West Coast and smallest in the Midwest. Downward trends in O_3 in California have been driven mainly by reductions in Southern California, with reductions in other areas not being as large.

8.2.4 Ozone Interactions with Other Ambient Pollutants

Data for other oxidants (e.g., H_2O_2, PAN) and oxidation products (e.g., HNO_3, H_2SO_4) in the atmosphere are not as abundant as they are for O_3. Because data for these species are usually obtained only as part of specialized field studies, it is difficult to relate O_3 concentrations to ambient levels of other species. In general, these secondary species are expected to be at least moderately positively correlated with O_3. On the other hand, primary species are expected to be more highly correlated with each other than with secondary species, provided that the primary species originate from common source areas. Relationships between ambient O_3 and $PM_{2.5}$ concentrations are complex, because particulate matter (PM) is not a single distinct chemical species, but rather a mix of primary and secondary species. As an example of the subject complexity, $PM_{2.5}$ concentrations were positively correlated with O_3 during the summer, but negatively correlated with O_3 during the winter at Ft. Meade, MD. More data are needed before this result can be applied to other areas; and the degree of positive or negative correlation between O_3 and PM or other pollutants may vary to a greater or lesser extent by season.

8.3 AMBIENT OZONE EXPOSURE ASSESSMENTS

Exposure to O_3 and related photochemical oxidants varies with time due to changes in ambient concentration and because people move between locations with different O_3 concentrations. The amount of O_3 delivered to the lung is not only influenced by the ambient concentration but also by the individual's breathing rate. Thus, activity level is an important consideration in determining the potential exposure and dose received.

The use of ambient air monitoring stations is still the most common surrogate for assigning exposure estimates in epidemiological studies. Since the primary source of O_3 exposure is the ambient air, monitoring concentration data should provide a relative assignment of exposure with time if: concentrations were uniform across the region; time-activitys pattern were the same across the population; and housing characteristics, such as ventilation rates and O_3 sinks contributing to its indoor decay rates, were constant for the study area. Because these factors vary by population and location, there tend to be errors not only in estimating the magnitude of the exposure but also in relative exposure assignments based solely on ambient monitoring data. Still, such data can be used to evaluate health outcomes associated with chronic O_3 exposure.

8.3.1 Personal Exposure

Personal O_3 concentrations have been measured for children, outdoor workers, and individuals with COPD, populations potentially susceptible to respiratory irritants. Children and outdoor workers have somewhat higher exposures than other individuals, because they spend more time outdoors engaged in moderate and heavy exertion. Children are also more active outside and, therefore, have a higher breathing rate than most adults. However, the available exposure studies are not sufficient to allow for confident generalization of differences in exposure between the general population and potentially susceptible subpopulations.

8.3.2 Indoor Concentrations

There are few indoor sources of O_3. Generally, O_3 enters indoor environments through infiltration from outdoors and through building components such as windows, doors, and ventilation systems. The concentration of O_3 in indoor environments is primarily dependent on the outdoor O_3 concentration and the air exchange rate (AER) or outdoor infiltration. Ozone concentrations indoors are higher during the outdoor O_3 season.

Ozone reacts indoors with other contaminants, possibly producing compounds with greater toxicity. Ozone concentrations are typically lower indoors than outdoors, in part due to gas phase reactions that produce other oxidants analogous to the production of photochemical smog. The production of these species indoors is a function of the indoor O_3 concentration and the presence of the other necessary precursors, volatile organic compounds (VOCs) and nitrogen dioxide (NO_2), along with an optimal AER.

Several studies have measured O_3 concentrations in residences, schools, office buildings and museums, and concentrations varied at all locations. However, indoor concentrations were generally associated with the AER in the indoor environment (increasing with higher AER) and generally tend to be notably lower than outdoor ambient O_3 levels. For example, one study examining the relationship between O_3 concentrations indoors and outside of a school in New England reported averaged O_3 concentrations of 20 ppb indoors and 40 ppb outdoors. With regard to mobile source microenvironments, as is the case for other enclosed environments, ozone exposures depend on the extend of mixing of outdoor air into the vehicle cabin. If windows are kept open, ozone in the vehicle may be expected to approach outdoor values; however, if windows are kept closed and there is air conditioning, then interior values could be much lower than those outside, especially if recirculated air is used. For example, in one N.C. study involving police cars with air conditioning and recirculated air, O_3 concentrations in the vehicle cabin (11.7 ppb average) were less than half those outside (28.3 ppb average at outdoor monitoring sites in the area).

8.4 SYNTHESIS OF AVAILABLE INFORMATION ON OZONE-RELATED HEALTH EFFECTS

The integrated synthesis of the latest available information on O_3-related health effects poses large challenges, especially in view of the emergence of important new information generated since the 1996 O_3 AQCD, which adds greatly to the complexity of any integrative assessment. Such information includes new findings from:

- Epidemiological studies, reflecting progress in addressing many research recommendations from the last review as well as raising new issues and reevaluating previously addressed issues that remain important in interpreting the body of epidemiological evidence and characterization of its strengths and limitations;

- Experimental toxicological studies using laboratory animals and controlled human exposures aimed at understanding the potential biochemical mechanisms underlying toxic effects, pathology, and susceptibility.

Thus despite substantial progress, challenges remain in integrating the new scientific information on O_3 health effects, including newly reported epidemiological evidence for associations between ambient O_3 exposures and increased mortality risks among human populations.

8.4.1 Assessment of Epidemiological Evidence

Based on the O_3 epidemiological evidence available at the time, the 1996 O_3 AQCD arrived at the following conclusions:

> An association between daily mortality and O_3 concentration for areas with high O_3 levels (e.g., Los Angeles) has been suggested, although the magnitude of such an effect is unclear. Increased O_3 levels are associated with increased hospital admissions and emergency department visits for respiratory causes. Analyses from data in the northeastern United States suggest that O_3 air pollution is associated with a substantial portion (on the order of 10 to 20%) of all summertime respiratory hospital visits and admissions. Pulmonary function in children at summer camps in southern Ontario, Canada, in the northeastern United States, and in Southern California is associated with O_3 concentration." (U. S. EPA, 1996, p1-29).

The 1996 O_3 AQCD further stated that only suggestive epidemiologic evidence existed for health effects of chronic ambient O_3 exposure in the population, and this was partly due to an inability to isolate potential effects related to O_3 from those of other pollutants, especially PM (U.S. Environmental Protection Agency, 1996).

The scientific strength and limitations of the growing body of epidemiological evidence for associations between exposure to O_3 and health effects discussed in this section is based primarily on Chapter 7 evaluations. The following criteria were considered in assessing the relative scientific quality of the epidemiologic studies: (1) quality of *exposure metrics* to evaluate credible exposure indicators; (2) *quality and size* of the study groups/population to arrive at meaningful analysis of health effects; (3) *robustness* of reported associations (based on defined health endpoint criteria), potential confounding by copollutants; (4) the *strength* of reported associations, in terms of magnitude, statistical significance and statistical power of effects estimates; (5) *temporality,* in terms of lag periods between exposure and observed effects; and (6) *biological plausibility*, consistency and coherency of the reported findings. The body of epidemiological evidence is further considered in terms of its coherence within itself and in

relation to findings derived from controlled human exposure studies which, overall, provide insights into the plausibility of reported O_3 human health effects reflecting causal relationships.

Many newly available epidemiological studies have provided additional evidence for O_3-related health effects beyond that which was known previously. Significant statistical associations have been observed by various investigators between acute O_3 exposure and several respiratory health endpoints, including: mortality; hospital admissions; emergency department visits; respiratory illness and symptoms; and changes in pulmonary function. Similarly, long-term exposure to O_3 has been associated with: increased morbidity; development of respiratory disease; and declines in lung function and lung function growth. The epidemiological studies that have been conducted in areas across the United States and Canada, as well as in Europe, Latin America, Australia and Asia, are summarized in Annex 7. Based on evidence extracted from the full body of epidemiologic studies that have been carried out and reviewed since the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996), it has been well demonstrated that deleterious human health outcomes are positively associated with ambient O_3 concentrations currently encountered in the United States and elsewhere.

8.4.2 Strength of Epidemiological Associations

As quoted above, assessments in the 1996 O_3 AQCD supported a consistent relationship between O_3 concentration and respiratory illness, hospital visits and reduced lung function. However, due to insufficient evidence examining O_3-mortality associations and uncertainties regarding weather model specification, the 1996 O_3 AQCD was limited to only a very qualitative assessment of O_3-mortality associations. Since then, generalized Additive Models (GAMs) have become widely utilized for epidemiologic analysis of health effects attributable to air pollution, making quantitative estimation of O_3-mortality risks much more meaningful. On the other hand, certain statistical issues raised with regard to use of default convergence criteria in applications of commercially available software employed for GAM analyses in many newly available air pollution epidemiologic studies led to a reanalyses of previously published studies and revised estimation of reported PM — mortality/morbidity risks. The impacts of the GAM-related statistical issues were thoroughly discussed in the 2004 PM AQCD (U.S. Environmental Protection Agency, 2004). Of most importance here, the reanalyses of a number of studies, comparing results using default GAM convergence criteria to results from analyses using

stringent GAM convergence criteria and/or from GLM analyses, found little difference among the O_3 effect estimates obtained (as discussed in detail in Chapter 7 of this document). Furthermore, the magnitude of the effect-size estimates observed from O_3-mortality relationships tend to be relatively consistent across the newly available studies and to compare well with those obtained for O_3-morbidity endpoints.

8.4.3 Acute Exposure Studies

Numerous epidemiological studies carried out over the past decade have added evidence to the knowledge base that was assessed in the 1996 O_3 AQCD, which included both (a) individual-level camp and exercise studies that established a relationship between human lung function decline with ambient O_3 exposure and (b) aggregate time-series studies that suggested positive relationships for O_3-related respiratory morbidity. The new studies reviewed in Chapter 7 in this document included numerous field/panel studies and time-series studies from various regions. In field studies on the effects of air pollution exposure, the most common health outcomes measured were lung function and respiratory symptoms. Time-series studies examined daily hospital admissions, emergency department visits, and mortality data.

8.4.3.1 Panel Studies

Many of the new field/panel studies reviewed in Chapter 7 and the controlled human exposure studies reviewed in Chapter 6 of this document provide additional data supporting two major findings reported in the 1996 O_3 AQCD, i.e.: (1) O_3-related lung function decrements and (2) respiratory symptoms in exercising healthy subjects and asthmatic subjects. Pulmonary function was determined by either spirometry (forced expiratory volume in 1 s [FEV_1] and forced vital capacity [FVC]) or by peak expiratory flow (PEF) meters. While the spirometric parameter, FEV_1 is a stronger and more consistent measure of lung function, PEF is more feasibly performed in field studies.

In a number of newly available field/panel studies, FEV_1 was measured in panels of exercising children, outdoor workers, and adult hikers exposed to ambient O_3 while experiencing elevated exertion levels. Collectively, the results of the new studies (discussed in Section 7.2.3.1) confirm and extend those from analogues field/panel studies assessed in the 1996 O_3 AQCD and findings from experimental controlled human exposure studies indicating that acute

O_3 exposures prolonged over several hours and combined with elevated levels of exertion or exercise magnify O_3 effects on lung function, as evaluated in terms of FEV_1.

For example, six field studies by three different research groups of 7- to 17-year-old, healthy (nonasthmatic) children exposed for several hours to ambient O_3 during increased physical exertion in summer camp activities were assessed in the 1996 O_3 AQCD. When analyzed together by consistent statistical methods, the data from those studies showed an average relationship between afternoon FEV_1 and concurrent 1-h O_3 concentrations of −0.50 mL/ppb, with individual slopes ranging from −0.19 to −1.29 mL/ppb (likely reflecting, in part, the multi-hour O_3 exposures preceding the pulmonary function tests). Four new filed/panel studies assessed in Section 7.2.3.1 of this document that evaluated pulmonary function in healthy school-aged children exposed to mean 1-h O_3 concentrations ranging from ~20 to 120 ppb found exposure-response functions of approximately −0.23 to −1.42 mL/ppb. Also, two other studies assessed in the 1996 document that measured lung function before and after well-defined exercise events (0.5-h long) in adults during exposures to ambient O_3 across 4 to 135 ppb found exposure-response slopes of −0.4 mL/ppb. In comparison, four new studies of healthy adult workers (street workers, berry pickers) and hikers engaged in prolonged (≥ 6 to 8 h) strenuous physical exertion while exposed to mean ambient O_3 concentrations of ~26 to 70 ppb (l-h maximum) or 40 ppb (8-h average) reported exposure-response slopes of −1.13 to −3.8 mL/ppb (as assessed in Chapter 7 of this document). The most representative data is that of Korrick et al. (1998) from a U.S. study of adult hikers that provided outcome measures stratified by gender, age, smoking-status, and presence of asthma within a population capable of above-normal exertion.

8.4.3.2 Asthma Panels

Several studies assessed in the 1996 O_3 AQCD that evaluated elevated respiratory symptoms and/or pulmonary function decrements in asthmatic children showed greater responses in asthmatic than nonasthmatic subjects, suggesting that asthmatic individuals might constitute a sensitive population group in oxidant epidemiologic studies.

Additional panel studies carried out over the past decade to understand the effect of acute exposure to O_3 in asthmatics evaluated either (a) lung function by PEF and/or (b) respiratory symptoms (i.e. cough, wheeze, shortness of breath and medication use) ascertained by

questionnaire. Several regional studies, typically consisting of children with asthma, collectively tend to confirm O_3-induced decrements in pulmonary function both in the United States and in other countries (see Section 7.2.3.2). One U.S. multicity study (Mortimer et al., 2002), featuring the largest panel of asthmatic children from eight urban areas, observed a statistically significant decrement in PEF with a cumulative lag of 1 to 5 days (−1.18% per 30 ppb increase in 8-h average O_3). Overall, these studies suggest that ambient O_3 exposures may be associated with enhanced decreases in lung function in asthmatics.

Most studies evaluating respiratory symptoms (i.e. cough, shortness of breath, and wheeze) and the increased use of asthma medications related to O_3 exposure also focused on asthmatic children. Several U.S. studies observed significant associations between O_3 ambient concentrations and increased symptoms or asthma medication use that appeared to be fairly robust to adjustment for copollutants. Analyses by Mortimer et al. (2002), conducted in eight U.S. urban areas, and Gent et al. (2003), conducted in the New England region, have used data from large sample populations likely to be representative of U.S. data. Odds ratios from six new studies for prevalence of cough among asthmatic children mainly varied from ~1.05 to 1.5 standardized per 40 ppb increase in 1-h max O_3 (or equivalent) or ~1.35 for all symptoms per 30 ppb increase in 8-h O_3 concentration.

8.4.3.3 School Absences

Two new U.S. studies (Chen et al., 2000; Gilliland et al., 2001) investigated the relationship between ambient O_3 concentrations and school absenteeism. Both studies were carried out during a period when O_3 levels were mostly below the highest levels typically observed in the summer season. In the Chen et al. study, with a distributed lag of 1 to 14 days, a 10.4% excess rate of school absences was found per 40 ppb increase in daily 1-h max O_3 concentrations. The study by Gilliland et al. (2001), which was able to distinguish specific illness-related absence, found significant O_3 effects on school absences due to respiratory causes with a lag period ranging from 2 to 4 weeks. A notably higher respiratory-related absence rate increase (147% increase per 30 ppb increase in 8-h O_3) was seen versus that seen for non-respiratory causes (61% increase per 30 ppb).

8.4.3.4 Field Studies on Cardiovascular Effects

A limited number of air pollution studies have examined cardiac physiologic endpoints, including heart rate variability, arrhythmia, and risk of myocardial infarction. One large U.S. study (Liao et al., 2004) found that ambient O_3 concentrations were inversely associated with ECG high-frequency power readings among whites. However, consistently more pronounced associations were suggested between PM_{10} and heart rate variability among persons with a history of hypertension. While these results may somewhat be supportive of hypothesized air pollution-heart rate variability-cardiovascular disease pathways at the population level, a lack of consistency within or across the limited available studies indicates that additional studies are needed before any clear conclusions can be made.

8.4.4 Emergency Department Visits and Hospital Admissions

Many time-series studies reviewed in the 1996 O_3 AQCD indicated positive associations between O_3 air pollution and increased hospital admissions. Strong evidence establishing a correlation between O_3 exposure and increased exacerbations of preexisting respiratory disease in the general public were reported at 1 h-maximum O_3 concentrations < 0.12 ppm. Several studies have been published over the past decade examining the temporal associations between O_3 exposures and emergency department visits for respiratory diseases (see Table AX7-2 in Annex 7). Among studies with adequate controls for seasonal patterns, many reported at least one significant positive association involving O_3. Overall, the analyses of data for asthma-related emergency room visits clearly indicate increased respiratory morbidity during warm seasons when ambient O_3 concentrations are high. These studies are summarized in Figure 8-1, showing both yearly and seasonal results from U.S. and Canadian studies.

Studies reviewed in Chapter 7 (Section 7.3.3) reported a significant O_3 effect on respiratory hospital admissions. While some inconsistencies are noted across studies, the evidence is supportive of significant and robust O_3 effects on hospitalizations for various respiratory diseases. Large multicity studies, as well as many studies from individual cities, have reported significant O_3 associations with total respiratory asthma and chronic obstructive pulmonary disease (COPD) hospitalizations, especially in studies analyzing the O_3 effects during the summer or warm season (Figure 8-2). The most robust and informative results on the effects of O_3 on respiratory hospital admissions are from muticity studies that used a consistent analytical

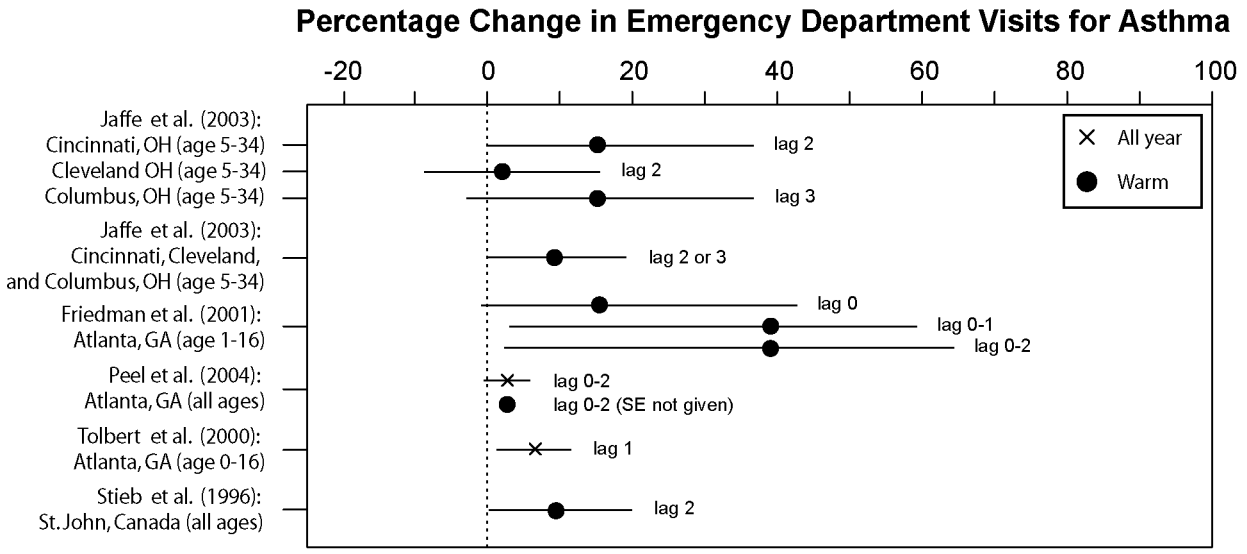

Figure 8-1. Ozone-associated percent change (95% CI) in emergency room visits for asthma. Percent change effects are per 40 ppb increase in 1-h maximum O_3 or equivalent. Analysis includes all age unless otherwise noted, and only studies conducted in the United States and Canada are presented.

methodology across a broad geographic area, such as the 16 Canadian cities study by Burnett et al. (1997a). Of the few studies that examined the relationship between O_3 and hospital admissions for cardiovascular diseases, most did not find any consistent positive associations.

8.4.5 Acute Effects of Ozone on Mortality

Due to the limited number of studies and uncertainties regarding weather model specifications, no meaningful quantitative assessment of O_3-mortality associations were possible in the 1996 O_3 AQCD. However, newly available large multicity studies designed specifically to examine the effect of O_3 on mortality have provided much more robust and credible information. The results from two key studies carried out in 95 U.S. communities (U.S. National Morbidity, Mortality Air Pollution Study [NMMAPS]; Bell et al., 2004) and in 23 European cities (Air Pollution on Health: European Approach [APHEA]; Gryparis et al., 2004) showed positive and significant O_3 effect estimates for all cause (nonaccidental) mortality (Figure 8-3; see Section 7.4 of Chapter 7 for complete discussion). The influence of season on O_3-mortality risk estimates from various U.S. and Canadian time-series studies is also shown in Figure 8-3. In the APEHA

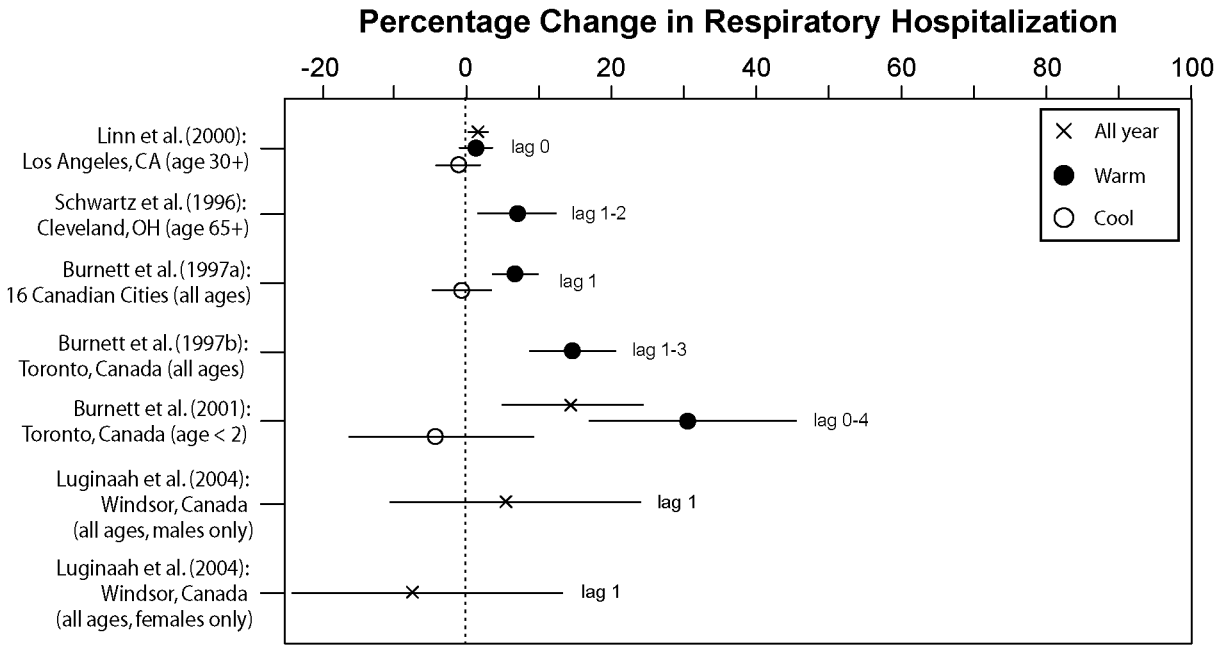

Figure 8-2. Ozone associated percent change (95% CI) in total respiratory hospitalizations (95% CI) for <u>all year</u> and for <u>by season</u>. Percent change effects are per 40 ppb increase in 1-h maximum O_3 or equivalent. Analysis includes all age unless otherwise noted, and only studies conducted in the United States and Canada are presented.

study (Gryparis et al., 2004), significant O_3 effects were observed only during the warm season. With the exception of one study (Chock et al., 2000), all risk estimates from warm-season-only analyses were positive, with the majority indicating statistical significance at $p < 0.05$.

The effect of PM on mortality was thoroughly discussed in the 2004 PM AQCD. Because PM indices correlate highly with O_3 levels in some areas, confounding of the O_3-mortality association by PM is of great concern. Figure 8-4 shows O_3-mortality risk estimates with and without adjustment for PM indices. Collectively, the results indicate that the O_3 risk estimates were not substantially affected with the addition of PM in the various reported analyses.

The effect estimates presented in Figures 8-3 and 8-4 lead to the following findings: (1) O_3-mortality associations from several U.S. and Canadian studies reported fairly consistent and positive combined estimates of 0.4 to 4.8% excess risk of total nonaccidental mortality per 40 ppb increase in 1-h maximum O_3 (excluding the Vedal et al., 2003 study, which examined the

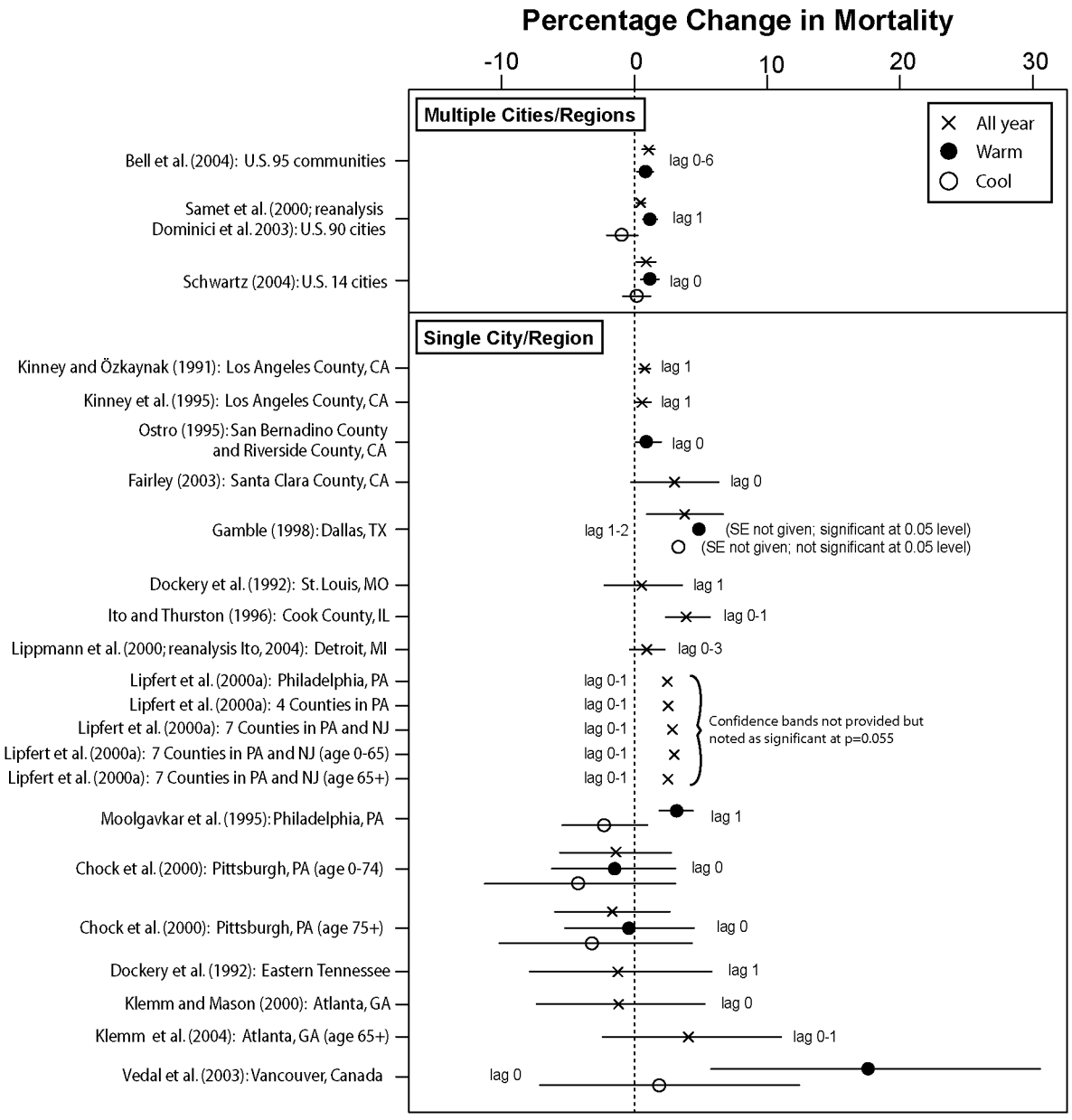

Figure 8-3. All cause (nonaccidental) O_3 excess mortality risk estimates (95% CI) for <u>all year</u> and for <u>by season</u> per 40 ppb increase in 1-h maximum O_3 or equivalent. Analysis includes all age unless otherwise noted, and only studies conducted in the United States and Canada are presented.

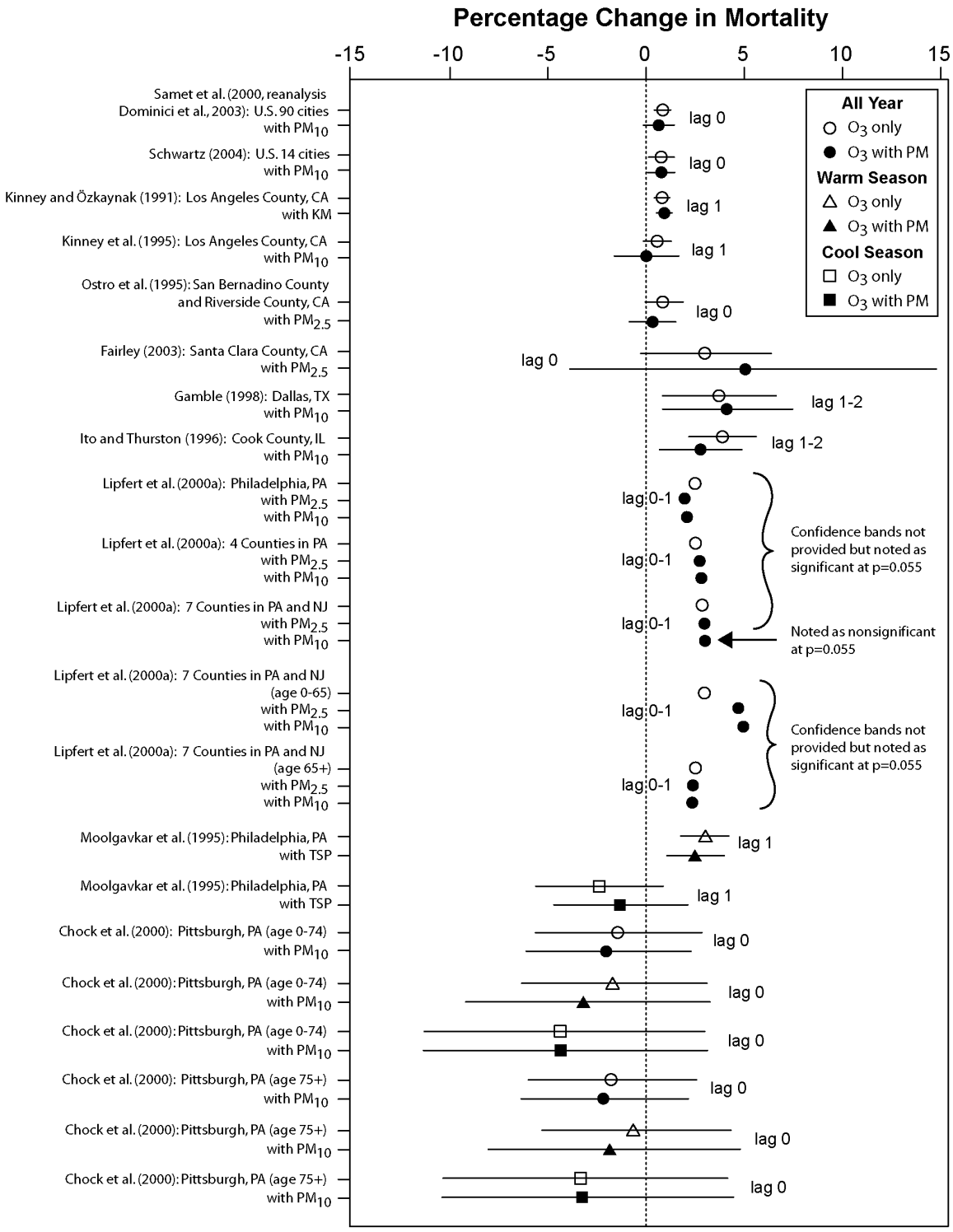

Figure 8-4. All cause (nonaccidental) O_3 excess mortality risk estimates (95% CI) with adjustment for PM indices for all analyses. Percent change effects are per 40 ppb increase in 1-h maximum O_3 or equivalent, and adjusted for PM indices. Only U.S. and Canada studies are presented.

O_3-mortality association in a region with very low O_3 concentrations); (2) season-stratified analyses indicated that the O_3-mortality effect estimates were significant and positive in the warm season, with larger effects observed compared to the year-round and cool-season analyses; (3) the risk estimates were robust to adjustment for PM indices, indicating that O_3 effect on mortality is independent of PM.

The results from the U.S. studies are generally consistent with those from other regions of the world. The results from all available studies indicate substantial strength in the epidemiological evidence for the association between exposure to O_3 and excess risk of total nonaccidental mortality. The overall range of estimates were relatively narrow with the positive estimates between 0 and 7% per 40 ppb increase in 1-h maximum O_3 or equivalent.

8.4.6 Chronic Ozone Exposure Studies

There were a limited number of studies reported in the 1996 O_3 AQCD that provided insufficient evidence to consider potential health effects of long-term ambient O_3 exposures. Several longitudinal epidemiological studies carried out in the past decade evaluated the potential effects of chronic exposure (several weeks to many years) to O_3 on lung function, respiratory symptoms, lung inflammation, asthma prevalence, and mortality.

Evidence from recent long-term morbidity studies indicates that chronic exposure to O_3 may have negative effects on inflammation, respiratory symptoms, and development of asthma; however, the evidence is limited and, at times, lacks consistency. The strongest evidence for an effect from chronic O_3 exposure is derived from studies examining lung function measurements. Seasonal decrements or reduced growth in lung function measures have been reported in several studies; however, the changes appear to be transient. Studies of lung function decrements with longer-term or annual data are not as conclusive.

Very few studies have investigated the effect of long-term O_3 exposure on mortality. Uncertainties regarding the exposure period of relevance and inconsistencies across mortality outcomes and gender raise concerns regarding plausibility. The most representative U.S. study by Pope et al. (2002) observed positive but non-significant associations between O_3 exposure and all cause mortality. Thus, the current evidence is inconclusive for a relationship between chronic O_3 exposure and increased mortality risk.

8.4.7 Robustness of Epidemiological Associations

In evaluating the strength of the epidemiological evidence, the magnitude of observed O_3 effect estimates and their statistical significance is important; however, consideration must be given to the precision of the effect estimates and the robustness of the effects associations. Examining the robustness of the associations includes the impact of alternative models, model specifications for temporal trends and meteorological factors, and potential confounding by copollutants. Also of interest are issues related to exposure assessment and measurement error. A detailed discussion on each of these topics can be found in Chapter 7 (Section 7-6). The following sections focus on the extent to which the current epidemiological findings can be considered robust.

8.4.7.1 Exposure Issues: Ambient versus Personal

In time-series studies, or other large-scale epidemiology studies of long duration, it is often impractical and unfeasible to monitor the personal exposure of each subject. Thus, the ambient concentrations of O_3 and other air pollutants at central monitoring sites are often used as a proxy for individual exposure measurements. The relationship between ambient O_3 concentrations and personal O_3 exposure levels varies depending on factors such as time spent outdoors, ventilation conditions, personal factors, and air quality indices. Because ambient concentrations often overestimate true personal O_3 exposures, the use of ambient data likely tends to underestimate the effect of the air pollutant on health.

Comparisons between ambient concentrations and personal exposures to O_3 have indicated that ambient concentrations do not reflect the variability of individual exposures. However, daily ambient O_3 concentrations have been shown to be well-correlated to daily-averaged personal exposures obtained by aggregating the personal measurements from all subjects. Therefore, though unresolved issues remain, the evidence suggests that ambient O_3 levels measured at central monitors may serve as valid surrogate measures for aggregate personal exposures in time-series studies investigating mortality and hospitalization outcomes.

Ambient O_3 measurements from the three main exposure indices (1-h maximum O_3, 8-h maximum O_3, and 24-h average O_3) used were highly correlated. As such, the excess health risk estimates and significance of associations appear to be comparable for the same distributional increment. The commonly used 8-h maximum O_3 or 8-h average O_3 index continue to be

appropriate choices, as no other exposure index has been demonstrated to offer a better advantage.

8.4.7.2 Confounding by Temporal Trends and Meteorologic Effects

The effect of seasonal differences in the health outcomes and O_3 exposure levels were recognized in the 1996 O_3 AQCD; and this issue is discussed in detail in Section 7.6.5 of this document. Two important factors, i.e., temporal trends and meteorological factors must be considered in evaluating O_3 health effects estimates. In the U.S. 95 communities study (Bell et al., 2004), sensitivity analyses indicated that the O_3 risk estimates were robust to tripling the degrees of freedom for smoothing terms used to control for temporal trends. In a case-crossover study by Schwartz (2004), the O_3-mortality risk estimates from an analysis using nonlinear regression splines to control for temperature were similar to those from an analysis that matched on temperature, indicating that the effect estimates were not sensitive to methods used to control confounding by temperature.

Analysis of O_3 health effects is further complicated in view of the fact that the relationship of O_3 with temperature and with other pollutants appears to change across seasons. As shown in Figures 8-4, the O_3 effect estimates from warm season data were consistently larger compared to those calculated using all-year data and cool-season data. In a study of daily hospital admissions (Burnett et al., 2001), season-stratified analyses appeared to effectively control confounding by season.

In summary, adjusting for temporal trends and meteorological factors is critical to obtaining meaningful O_3-effect estimates. Seasonal analyses indicate that mortality and morbidity data computed using year-round data need to be interpreted with caution. Air pollution epidemiological studies that integrate sensitivity analyses for seasonal stratification, meteorological factors, and multipollutant models may provide a better and more comprehensive understanding of the health effects estimates.

8.4.7.3 Assessment of Confounding by Copollutants

The presence and influence of PM and other gaseous copollutants have to be considered in assessing O_3-health effects associations found by observational studies. The potential for copollutant confounding in the epidemiological time-series studies was assessed in some detail

in Section 7.6.6. Multipollutant modeling is the most common method used to test for potential confounding in epidemiological studies; however, interpretation of the results is often complicated by the high degree of correlation among air pollutants. The O_3 mortality risk estimates from two-pollutant models adjusted for PM are presented in Figure 8-4 (U.S. and Canadian studies only). In the two multicity studies analyzed here, the addition of PM_{10} did not substantially change the risk estimates (Samet et al., 2000; Dominci et al., 2003; Schwart, 2004). The O_3-mortality effects in single-city studies also were robust after adjusting for PM_{10} indices, both in all-year and season-stratified analyses data.

In summary, assessing the health effects attributable to O_3 is very challenging, even with well-designed studies. Definitive partitioning out of the individual pollutant-specific health outcomes from among an ambient mixture of multiple components is very difficult due to the dynamic nature of their interactions over time. However, the new limited time-series studies that made an exhaustive survey using populations from multiple U.S. cities do provide substantial epidemiological evidence indicating that associations for O_3 with mortality and morbidity are robust to confounding by copollutants.

8.4.7.4 Lag Period between Ozone Exposure and Health Response

The lag times between causes and effects depend on underlying biological mechanism involved in the process as well as the hypotheses tested. Different lag periods are appropriate for assessing different health outcomes. As discussed in Section 7.6.4, examining longer lag periods may be needed to understand more fully the O_3-related health outcomes. The most significant associations between O_3 concentrations and mortality and respiratory hospitalization were observed with 0-day and 1-day lags. These associations generally diminished with increased lag days. In the 95 U.S. communities studies (Bell et al., 2004), the mortality risk estimated over multiple days (cumulative lag of 0 to 6 days) using distributed lag models indicated an effect of O_3 that was twice as large as the effect estimated using 1-day lags. It should be noted that when there is a pattern of effects across lag periods, selecting the 1-day lag effect estimate is likely to underestimate the overall effect size and does not fully capture the risk distributed over adjacent days. Longer averaging periods may aid in characterizing cumulative O_3-related effects over several days; however, interpreting these results may not be straightforward.

8.4.7.5 Concentration-Response Functions and Threshold

Ozone concentration-response relationships have been explored in several studies with various health outcomes, including mortality, hospitalizations, emergency department visits, lung function, and respiratory symptoms. While some studies found no threshold for O_3 health effects, others have found that a low-level threshold may be present. Note that an absence of a detectable threshold in population studies does not necessarily indicate an absence of individual thresholds and, conversely, evidence of a threshold for individuals does not necessarily indicate a population threshold due to variability in response among individuals in the population. With the current evidence, no definitive conclusion can be made regarding the threshold issue; however, the limited evidence suggests that the possible threshold level may be well below the current O_3 standard level. The distribution of potential thresholds, particularly around the NAAQS value of 80 ppb for 8-h maximum O_3, needs to be further investigated.

8.4.7.6 Summary and Conclusions for Epidemiology Findings

Discussions presented in the previous sections evaluated the merits of the epidemiological studies to derive judgments about the potential causal relationship between O_3 exposures and health outcomes. These evaluations were carried out in the context of the criteria listed in Section 8.2.1. Information with regard to one of the criteria, i.e., coherence and biological plausibility, is discussed in the section following the next one, which undertakes to provide an integrated analysis of the biological evidence from human and animal toxicology studies with the epidemiological evidence.

The results from the new field/panel studies evaluated in this document provided additional evidence for likely causal relationships being reflected by significant associations between acute O_3 exposure and (a) decrements in lung function, (b) respiratory symptoms, and (c) increased use of asthma medication in children and, in some cases, adults. Similarly, significant positive associations can be inferred between acute O_3 exposure and respiratory morbidity indexed by hospital admissions and emergency visits, especially based on season-stratified data. The results from large multicity studies suggest an elevated risk of mortality for acute exposure to O_3; however, the magnitudes of these estimates are small. Analysis of the data from chronic mortality and morbidity studies indicate some significant associations between O_3 and seasonal

changes in lung function; but, overall, the strength of the data does not allow establishment of a conclusive relationship to O_3 as a causal factor for other observed health outcomes.

Issues regarding strengths of models used in air pollution epidemiology were carefully considered. There have been improvements in the modeling to adjust for potential confounding variables, including temporal trends, meteorological factors, and copollutants. However, more sensitivity analyses would still be useful to examine the extent of adequate adjustment for confounding by these factors. Results from multipollutant models indicate that copollutants, e.g., PM, generally do not confound the association between O_3 and acute health outcomes, suggesting an independent effect of O_3.

In conclusion, the epidemiological evidence continues to support likely causal associations between O_3 and acute respiratory morbidity and mortality, based on the assessment of strength, robustness, and consistency of results reported from numerous studies reviewed in Chapter 7. Substantial evidence is lacking, however, by which to convincingly establish a positive association between chronic O_3 exposure and respiratory morbidity and mortality. Additional investigations are needed to further understand the health effects resulting from long-term O_3 exposure.

The positive associations for increased morbidity and mortality risk estimates during warmer seasons (when O_3 concentrations tend to be high) support a causal role for O_3 in affecting human health. Though seasonality in O_3-related health effects was observed in both time-series and longitudinal cohort studies, no clear evidence of a threshold for O_3 effects has yet been found.

8.4.8 Integration of Experimental and Epidemiologic Evidence

In this section, effects are made to integrate the epidemiological evidence discussed above with results of human and animal experimental studies carried out in vivo and in vitro, to understand O_3-induced alterations at the physiological, pathological, and biochemical levels of importance for the assessment of human health effects due to ambient O_3 exposure. Also, the influence of O_3-induced changes at cellular and molecular levels are integrated to elucidate scientific bases for the observed physiological and pathological alterations. These research reports will be evaluated to assess (1) the scientific merit pertaining to the biological plausibility of the health outcome associations observed in the epidemiological studies and (2) the coherence

of the overall body of evidence relevant to O_3-related health outcomes supporting conclusions regarding the attribution of observed effects of ambient O_3 exposure.

The 1996 O_3 AQCD, based on the limited number of controlled human exposure studies and the animal toxicology data available to that date, arrived at the following conclusions regarding potential health effects of ambient O_3 exposure:

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

As mentioned above, many questions remained unanswered, and the 1996 O_3 AQCD neither identified clear biological bases nor provided convincing experimental evidence supporting the biological plausibility of reported O_3 effects and/or the mechanisms of action underlying potential O_3 toxicity.

The available new epidemiological research reports on the health effects of O_3 and controlled human exposure studies using novel and refined models indicate certain positive O_3-health effect associations. This section focuses on interpreting the overall meaning of the epidemiological findings and evaluates their bearing in the context of obtaining evidence for the biological plausibility and possible mechanism(s) of action. This section also addresses the complexities involved in extrapolating the extent of coherence observed from epidemiological

studies to specific health outcomes and related toxicological and biochemical mechanisms for observed pathological and physiological changes in controlled human, animal, and in vitro exposure studies. Experimental in vitro and in vivo studies using novel molecular technologies that predict new hypotheses regarding the mechanisms of action are also included and discussed as appropriate.

Several criteria listed in Section 8.2.1 are used in evaluating the available scientific support for conclusions regarding potential causal relationships between O_3 exposure and specific types of health outcomes. In addition to those criteria addressed in the preceding discussion of epidemiological evidence, certain other critical evaluation measures must be considered to ensure that these observations are biologically relevant and consistent with experimentally demonstrated biological mechanisms of action. For this assessment, the ensuing discussion on biological plausibility and coherence considers (a) the extent to which available epidemiological evidence shows associations with a range of logically linked health endpoints and (b) whether available toxicological and biochemical evidence provides support for the observed epidemiological associations reflecting causal relationships.

8.4.8.1 Background on Cross-Cutting Issues

Discussion of several cross-cutting issues that will facilitate a clear understanding of the ensuing assessment is provided here to enhance an integrated and comprehensive understanding of the experimental and epidemiological studies on O_3 health effects. An important issue to be considered is the extrapolation of observed effects from the perspective of dosimetry and animal-to-human extrapolation models used and their strength. The most challenging issue is the interpretation of the epidemiological observations in light of physiological and toxicological endpoints derived from the experimental studies, wherein the data derived for O_3-specific effects have to be used to interpret and evaluate possible causative roles for O_3 in contributing to health outcomes observed in the air pollution epidemiology studies.

8.4.8.2 Approaches to Experimental Evaluation of Ozone Health Effects

Three chapters in the current document provide detailed discussion of various experimental approaches utilized to evaluate O_3-related health effects. Chapter 4 discusses dosimetry issues in both animal and human exposure scenarios. Chapter 5 discusses the experimental studies of

physiological, biochemical (cellular and molecular changes) and pathological observations in laboratory animals (including nonhuman primates, dogs, and rodent species) and in vitro studies using cell culture systems (in certain cases, on humans cells recovered from BALF postexposure to O_3). Chapter 6 evaluated controlled human experimental studies that investigated various physiological and biochemical endpoints. Many of the experimental animal toxicology studies have been carried out using relatively high O_3 exposure concentrations/doses that do not reflect "real-world" exposure scenarios. These approaches have been used mainly to test hypotheses to understand potential mechanism(s) of action implicated with the health outcomes identified in epidemiological studies. In interpreting the results from the experimental approaches, one must consider the following three issues: (1) controlled animal exposures studies use high concentration to elicit biochemical/ physiological changes in healthy animals; (2) the roles of other confounding pollutants that commonly occur with ambient exposures cannot be fully reflected in the controlled exposure studies, and (3) the differences between human and rodents with regard to O_3 inhalability, deposition, clearance, and retention profiles (see Chapter 4 and 5 for details). Note that most of the in vitro toxicological studies were aimed at hypothesis generation to predict mechanism(s) of action based on cellular and molecular endpoints and, therefore, also generally used high O_3 concentrations. The following discussion attempts to integrate the experimental and epidemiological evidence to develop a holistic understanding of O_3 health effects in keeping with the points discussed above.

8.4.8.3 Interspecies Comparison of Experimental Effects — Dosimetry Considerations

As discussed in the previous section, the most important factor to consider while attempting to integrate experimental studies across the species is the exposure-dose relationship. Animal studies, particularly rats, have been valuable in developing mathematical dosimetry models and useful for dosimetric extrapolation to humans in the strict sense of dose-response basis. For example, dose-dependent increases in breathing frequency and decrease in tidal volume were observed in both animals and humans with little effect on uptake. The O_3-uptake efficiency data from human and animals were consistent without much bearing on the mode of breathing.

Experimental O_3 dosimetry studies include response to bolus dose and general uptake. Although the former approach is of limited relevance to environmental exposures, it has indicated that prior exposure to O_3 limits uptake of bolus dose. New uptake studies (Ultman et al., 2004) carried out in controlled human clinical studies observed gender-specific differences in the uptake of O_3, but these differences do not correlate well with spirometric responses. Bolus dose studies demonstrated that the uptake and regional respiratory tract distribution of O_3 is sensitive to the mode of breathing (nasal or oral) and to air flow rate. The change in breathing due to exercise can cause a shift in distribution, allowing deeper penetration with resultant damage to bronchiolar and alveolar tissue. This observation of an inverse relationship between uptake and air flow is in agreement with animal studies. Additional uptake studies carried out in humans using environmentally relevant O_3 concentrations demonstrated the significance of incorporating intersubject variability in dose-response relationship predictions and extrapolation.

The high degree of consistency observed in O_3 uptake in animal and human experimental exposure studies provided increased confidence in the use of theoretical dosimetry modeling (see Chapter 4 for detailed discussion). The early models computed dose-response relationships based on the assumption of O_3 as the only active toxicant responsible for the observed respiratory injury. Newer models have taken into consideration various factors such as age, as well as anatomical, physiological, and biochemical alterations. The identification of conducting airways as the primary site of acute cell injury, the site of O_3 reaction/diffusion in the epithelial lining fluid, the roles of intermediate reactive oxygen species (ROS) and lipid-ozonation products in oxidative injury, and the roles of metabolic enzyme profiles in developing lung tissue, when incorporated, will lead to refined novel models. The PBPK models were developed using some of the refinements listed above and indicated the difference in dose metrics between adult and infant with no difference projected after the age of five.

8.4.8.4 Integrated Critical Analysis of Physiological, Biochemical Effects

In the following subsections, research results generated from experimental studies on humans and animals during the past decade are assessed (keeping in view the interspecies differences discussed in the preceding section) in evaluating experimental evidence for epidemiological studies to lead to the discussion of the biological plausibility and coherence for O_3 health effects in the later sections.

8.4.8.4.1 Pulmonary Function

The 1996 O_3 AQCD reported decreases in FVC, FEV_1, decreased tidal volume, increased breathing frequency, and increased resistance on short-term exposure to O_3, based on research reviewed from epidemiological, controlled human exposure, and animal studies. Inhalation of O_3 for several hours while physically active elicits both subjective respiratory tract symptoms and acute pathophysiologic changes. The typical symptomatic response consistently reported is that of tracheobronchial airway irritation. This is accompanied by decrements in lung capacities and volumes, bronchoconstriction, airway hyperresponsiveness, airway inflammation, immune system activation, and epithelial injury. The severity of symptoms and the magnitude of response depend on inhaled dose, individual O_3 sensitivity, and the extent of tolerance resulting from previous exposures.

The development of effects is time-dependent during both exposure and recovery periods, with considerable overlap of evolving and receding effects. In healthy human subjects exposed to typical ambient concentrations (i.e., < 0.2 ppm O_3), spirometric responses largely resolve within a few hours (4 to 6 h) postexposure, however cellular effects persist for longer periods (~24 h). Persisting small residual lung function effects are almost completely resolved within 24 hours. In hyperresponsive individuals, the recovery takes longer, as much as 48 h, to return to baseline values.

It has also been observed that there is a large amount of intersubject variability and, furthermore, the majority of these symptoms are attenuated after repeated exposure, but such tolerance to O_3 is lost within a week postexposure. Recent controlled human exposure studies on prolonged O_3 exposure using healthy subjects (reviewed in Chapter 6) found statistically significant changes in pulmonary function during or after exposure to O_3 concentrations $\geq$ 0.08 ppm.

New controlled human exposure studies (reviewed in Chapter 6) clearly indicate that FEV_1 decrements and symptom responses decrease with age beyond young adulthood (18 to 20 years). Hazucha et al. (2003) also examined gender differences along with age in O_3 responsiveness and observed that young females lose O_3 sensitivity faster than young males, but that the rate is about the same for both genders by middle age.

Human studies consistently report that inhalation of O_3 alters the breathing pattern without significantly affecting minute ventilation. A progressive decrease in tidal volume and a

"compensatory" increase in frequency of breathing to maintain steady minute ventilation during exposure suggests a direct modulation of ventilatory control. These changes parallel a response of many animal species exposed to O_3 and other lower airway irritants (Tepper et al., 1990). Earlier human studies (Coleridge et al., 1993; Hazucha and Sant'Ambrogio, 1993) reported a role for bronchial C-fibers and rapidly adapting receptors are the primary vagal afferents responsible for O_3-induced changes in ventilatory rate and depth. A new study by Passannante et al. (1998) observed that the primary mechanism of O_3-induced reduction in inspiratory lung function is an inhibition of inspiration elicited by stimulation of the C-fibers and suggest a role for nociceptive mechanisms in modulating O_3-induced inhibition of inspiration. This neurogenic mechanism also has an effect on airway responsiveness and lung inflammation.

Lung function changes evaluated in patients with preexisting respiratory diseases, under controlled experimental exposure regimens with or without physical exertion in the form of intermittent exercise, indicated minimal O_3-induced effects in COPD patients. However, newer studies (see Chapter 6) indicate that pulmonary function deficiencies detected by spirometric analyses in asthmatics augment the observations made in the 1996 O_3 AQCD. More specifically, Gong et al. (1997a) exposed nine COPD patients (0.24 ppm O_3 for 4 h with intermittent exercise) and observed a nonsignificant FEV_1 decrement of −8% in COPD patients, which was not statistically different from the decrement of −3% in healthy subjects. In contract, studies of between 4- and 8-h duration with O_3 concentrations of ≤ 0.2 ppm, suggest a tendency for increased O_3-induced pulmonary function responses in asthmatics relative to healthy subjects (Scannell et al., 1996). Similarly, Alexis et al. (2000) observed statistically significant O_3-induced decreases in FEV_1 in mild atopic asthmatics compared to healthy controls. Though controlled human exposure studies may not provide the required statistical power (due to the limited number of subjects compared to panel or field studies), they do suggest that asthmatics are at least, if not more, sensitive than healthy subjects.

8.4.8.4.2 Airway Responsiveness

Increased responsiveness of the pulmonary airways, or "airway hyperresponsiveness" (AHR), is usually analyzed in response to a bronchoconstrictor challenge. An extensive animal studies database (using rats, mice, guinea pigs, and rabbits) exploring the effects of acute, long-term, and repeated exposures to O_3, indicates that induction of AHR occurs at high O_3

concentrations. These studies provide clues to the roles of physiological and biochemical components involved in this process. Experimental human exposure studies also reported that acute O_3-induced AHR, independent of pulmonary function changes or lung inflammation, resolves to normalcy within 24 h. Airway hyperresponsiveness, as well as O_3-induced airway-antigen reactivity, were observed in asthmatics in several controlled exposure studies and found to persist several hours postexposure (Chapter 6). Gong et al. (1997b) found that subjects with asthma developed tolerance to repeated O_3 exposures in a manner similar to normal subjects; however, subjects with asthma had more persistent effects of O_3 on airway responsiveness, which was only partially attenuated when compared to filtered-air (FA) control subjects. These observations suggest that O_3 may act as a cofactor in response to airborne allergens or other bronchoconstrictor agents in people with allergic asthma. Ozone-mediated modulation of airway responsiveness may be a plausible link between ambient O_3-exposure- related increased use of asthma medication and the increased hospital admissions and emergency department visits observed in epidemiological studies. Biochemical alterations observed in humans and animals with exposure to O_3 and discussed in the following sections may provide additional insights into their roles in mechanistic aspects underlying the observed AHR.

8.2.8.4.3 Morphological and Biochemical Abnormalities

Most of the research results alluded to the ensuing discussion come from toxicology studies using various laboratory animal species that were usually exposed to higher, non-ambient concentrations of O_3. However, these exploratory and mechanistic studies may provide important and useful hypotheses to consider in integrating various health outcomes observed or predicted by epidemiological studies. Controlled human exposure studies evaluated a few cellular and biochemical parameters, mostly from BALF analyses. These studies have yielded some limited evidence supporting the observations made in animal toxicology studies. Again, caution should be exercised in extrapolating these observations to humans, due to species-specific differences, as outlined earlier (see Section 8.2.7.3).

Lung Injury and Morphological Changes

Most animal species tested, including primates, exhibit similar morphological alterations dependent on the exposure dose and the regional specificity. Differences in the distribution of

antioxidants in the centriacinar region (CAR) of the lung were responsible for the differences in injury and morphological changes observed between nonhuman primates and rodents. Cells in the CAR are the primary targets of O_3, but ciliated cells in the nasal cavity, airways, and Type I epithelial cells in the gas-exchange region are also targets. Though acute O_3 exposure induces structural changes such as fibrosis in CAR, these structural alterations appear to be transient with recovery shortly postexposure, but the time for recovery is dependent on species and the dose of O_3. The remodeling of lung tissue indicated in a simulated seasonal-exposure scenario in primates suggests the development of possible stable structural alterations as compared to continuous-exposure scenarios. In an autopsy pathologic study, a significantly greater extent and severity of centriacinar region alterations were observed in lungs of Los Angeles residents than Miami residents, independent of a smoking effect (Sherwin et al., 2000). The results suggest that the greater extent and severity of centriacinar region alterations may be related to the higher O_3 levels in Los Angeles. Similar observations of CAR thickening and deposition of collagen in the rat suggests that long-term O_3 exposure may cause a progressive structural lung injury that can evolve into a more chronic form, such as fibrosis. Ozone-induced mucous membrane cell metaplasia observed in rodents appears to be mediated by inflammation. Again, one must be cautious in extrapolating these observations in animals to humans, given the exposure regimens and doses used.

Lung Inflammation and Permeability

Ozone has long been recognized to cause lung inflammation and increased permeability in the rat lung. These distinct, independent biological events have been observed in all species studied, including humans, in response to acute exposure to O_3. Increased lung inflammation and permeability have been observed at levels as low as 0.12 ppm exposure for 6 h in rats and 24 h in mice. Both the inflammatory response and increased lung permeability have been observed as early as 1 h and found to persist for at least 18 h in humans on exposure to O_3 at 0.2 to 0.6 ppm. Subchronic exposures in animals suggest that permeability changes are transient (and species-dependent) and return to control levels even with continuing exposure. Repeated exposures in humans also indicate ongoing cellular damage irrespective of attenuation of the inflammatory responses and lung function. Several studies have analyzed bronchioalveolar

lavage (BAL) and nasal lavage (NL) fluid and cells from O_3-exposed humans for markers of inflammation and lung damage (see Tables AX6-12 and AX6-13 in the Annex to Chapter 6).

The presence of neutrophils (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. It is apparent, however, that inflammation within airway tissues may persist beyond the point that inflammatory cells are found in BAL fluid. Soluble mediators of inflammation such as the cytokines IL-6 and IL-8 as well as arachidonic acid metabolites (e.g., PGE_2, $PGF_{2\alpha}$, thromboxane, and leukotrienes [LTs] such as LTB_4) have been measured in the BAL fluid of humans exposed to O_3. In addition to their role in inflammation, many of these compounds have bronchoconstrictive properties and may be involved in increased airway responsiveness following O_3 exposure. Inflammation and cellular responses associated with acute O_3 exposure were also attenuated after 5 consecutive days of O_3 exposure (compared to historical data for responses after a single-day exposure). Even though indicators of epithelial cell damage were not seen immediately after acute exposure, they were present in BALF after the fifth day of exposure. When reexposed 2 weeks later, changes in BALF indicated that epithelial cells appeared to be fully repaired (Devlin et al., 1997). Similar adaptive response was also observed in an epidemiological study by Kopp et al. (1999). The analysis of BALF in human subjects after first O_3 peak in summer indicated increased levels of protein and leukocytes and no such increase was observed later in summer even after exposure to higher levels of O_3.

Interaction of O_3 with the constituents of the extracellular lining fluid and the induction of oxidative stress is implicated in injury and inflammation. Animal toxicological and a few in vitro studies analyzed cells recovered from BALF for many biochemical mediators implicated in injury and inflammation and found alterations in the expression of cytokines, chemokines, and adhesion molecules, indicative of an active stress response as well as injury repair and regeneration processes. Both animal and human studies indicate cellular and biochemical changes associated with inflammation and increased permeability, but the relationship between these changes and their role in lung function and airway responses is not known.

Host Defense

A number of closely integrated defense mechanisms exist that offer protection to respiratory tract cells from the adverse effects of inhaled pollutants and microbes. Acute O_3

exposure had been found to impair host defense capabilities both in humans and animals by depressing alveolar macrophage functions and by decreasing the mucocilliary clearance of inhaled particles and microbes. Interference by O_3 exposure with the clearance process has been found to be dose-dependent, whereby low doses accelerate clearance, but high doses slow clearance. Some respiratory tract regional- and species-specific differences have also been observed. Though acute O_3 exposures were found to suppress alveolar phagocytosis and immune functions, these alterations appeared to be transient and were attenuated with continuous or repeated exposures. Continuous exposures to O_3 impairs immune responses, followed by adaptation and recovery to normalcy. Studies using various genetically sensitive or susceptible strains indicated a possible interaction between innate and acquired immune system components, possibly through Toll-like receptor-4 and downstream pathways; but these studies were carried out using high O_3 doses that may not be relevant to ambient exposures.

Biochemical Alterations

An extensive experimental database, including the research presented in 1996 O_3 AQCD, suggests that potential biochemical alterations in various intermediary metabolic pathways are involved in lung injury, inflammation, and functional alterations. Recent experimental evidence still points to the importance of initial interactions of O_3 with the lipid constituents of the ELF and to the generation of ozonation products and secondary redox mediators in the initiation of site-specific cell-injury response cascades. One such ozonation product, 4-hydroxynonenal, has been found to bind to proteins and increase protein adducts in human alveolar macrophages, suggesting a possible role in acute cell toxicity. Species- and region-specific increases in lung xenobiotic metabolism has been observed in response to both short- and long-term O_3 exposure. Antioxidants in the ELF react with O_3 and confer protection from toxicity, and even with environmentally relevant exposures, the reactivity of O_3 was not quenched. Species-specific and age-dependent changes in the antioxidant metabolism add another dimension to their role in this process. Carefully controlled studies of dietary antioxidant supplementation (Samet et al., 2001; Trenga et al., 2001) have found some protective effects of α-tocopherol and ascorbate for O_3-induced spirometric lung function decrements but not for the intensity of subjective symptoms and inflammatory responses (including cell recruitment, activation, and a release of mediators). Dietary antioxidants have also afforded partial protection to asthmatics by

attenuating postexposure bronchial hyperresponsiveness (Trenga et al., 2001). Also, two epidemiologic studies of street workers and asthmatic children in Mexico City found that subjects taking antioxidant supplements containing vitamins E and C were protected from O_3-induced changes in lung function (Romieu et al., 1998, 2002).

Based on the above, it is evident that the extensive experimental database accumulated from animal toxicology studies (including nonhuman primate studies) and limited controlled human exposure studies, has provided insights into various biochemical, cellular, and molecular alterations in lung tissue exposed to O_3. The majority of these studies used acute exposure regimens and high concentrations, and provide hypotheses regarding potential molecular mechanisms implicated in O_3 toxicity. Utilizing this information in relevant rodent-to-human extrapolation models with appropriate species-specific adjustments may well provide useful information on initial biochemical alterations that may aid in the development of suitable biomarkers for O3 exposures/effects.

Systemic Effects

A number of rodent toxicology studies that investigated the effects of acute O_3 exposure on extrapulmonary systems have reported neurobehavioral, neuroendocrine, developmental, and skin effects, albeit typically at much higher than ambient O_3 concentrations. Ultrastructural, cellular, and biochemical parameters evaluated in these studies indicate a role for O_3 in mediating biochemical and functional alterations through interactions with the redox systems. An increasing body of animal toxicology evidence suggests that thermoregulatory and hematological alterations (in heart rate variability and/or core body temperature) may mediate acute cardiovascular effects. Limited human exposure studies have also explored O_3-induced cardiovascular effects, but did not observe acute cardiovascular effects in normal and hypertensive subjects.

Susceptibility Factors

Many factors such as age, gender, disease, nutritional status, smoking, and genetic variability may contribute to the differential effects of environmental pollutants, including O_3. Genetic factors, such as single nucleotide polymorphisms (SNPs) and developmental defects, can contribute to innate susceptibility, while acquired susceptibility may develop due to personal

habits (smoking, diet, exercise) and other risk factors such as age, gender, pregnancy, and copollutants. However, the available information from animal toxicologic and epidemiologic studies does provide clear scientific evidence by which to identify and/or associate any specific factor as contributing to adverse health effects of O_3 (U.S. Environmental Protection Agency, 1996).

New animal toxicology studies using various strains of mice and rat have identified O_3-sensitive and resistant strains and illustrate the importance of genetic background in determining O_3 susceptibility. Biochemical and molecular parameters extensively evaluated in these experiments were used to identify specific loci on the chromosomes and, in some cases, to relate the differential expression of specific genes to biochemical and physiological differences observed among these species. Utilizing O_3-sensitive and O_3-resistant species, it has been possible to identify the involvement of AHR and inflammation processes in O_3 susceptibility. However, most of these studies were carried out using high doses of O_3, making the relevance of these studies questionable in human health effects assessment. No doubt, the molecular parameters identified in these studies may serve as useful biomarkers with the availability of suitable technologies and, ultimately, can likely be integrated with epidemiological studies. Interindividual differences in O_3 responsiveness have been observed across a spectrum of symptoms and lung function responses but do no yet allow identification of important underlying factors, except a significant role for age.

8.4.9 Preexisting Disease as a Potential Risk Factor

People with preexisting pulmonary disease may be at increased risk from O_3 exposure. Altered physiological, morphological and biochemical states typical of respiratory diseases like asthma, COPD and chronic bronchitis may render people sensitive to additional oxidative burden induced by O_3 exposure. Based on studies assessed in the 1996 criteria document (U.S. Environmental Protection Agency, 1996), asthmatics appear to be at least as, or more, sensitive to acute effects of O_3 as healthy nonasthmatic subjects. The new results reviewed in Chapters 6 and 7 from controlled exposure and epidemiological studies also suggest that asthmatics are a potentially sensitive subpopulation for O_3 health effects.

A number of time-series epidemiological studies have reported increased risk in study subsets of individuals with preexisting lung diseases and tend to implicate asthmatics as

potentially susceptible individuals. The epidemiological studies of acute exposure to O_3 discussed in Section 8.2.3 indicate increased risk for exacerbation of disease symptoms during the warm season.

Newly available human exposure studies by Stenfors and coworkers have shown differences regarding PMN influx in BALF between asthmatics and healthy human subjects. In vitro studies (Stenfors et al., 2002) using nasal mucosal biopsies from atopic and nonatopic subjects exposed to 0.1 ppm O_3 found significant difference in the induced release of IL-4, IL-6, IL-8, and TNF-α. A subsequent study by the same group (Schierhorn et al., 2002) found a significant difference in the O_3-induced release of the neuropeptides neurokinin A and substance P from allergic patients, compared to nonallergic controls, suggesting increased activation of sensory nerves by O_3 in the allergic tissues. Another report from Bayram et al. (2002) using in vitro culture of bronchial epithelial cells recovered from atopic and nonatopic asthmatics indicated the existence of a significant difference in permeability by measuring the paracellular flux of ^{14}C-BSA. Additional controlled O_3 exposure studies in human subjects with intermittent asthma (Hiltermann et al., 1999), and asthmatics (Basha et al., 1994; Scannell et al., 1996) reported increased secretion of IL-8 suggesting increased neutrophilic inflammation in those subjects.

The observation of increased pathological symptoms in long-term animal exposure studies in the absence of observable physiological changes also suggests that chronic exposure may increase susceptibility to adverse health effects, but this needs to be validated via long-term epidemiological studies.

8.4.10 Biological Plausibility and Coherence of Evidence for Adverse Respiratory Health Effects

The research evidence discussed in the preceding section indicates that injury to lung tissue is the initial step in mediating deleterious health effects of O_3, and, in turn, activates a cascade of events starting with inflammation, altered permeability of the epithelial barrier, impaired clearance mechanisms (including host defense), and pulmonary structural alterations that can potentially exacerbate a preexisting disease status. Although many or all of the above proposed mechanisms are hypothesized to be implicated in O_3 toxicity, scientific evidence is still lacking for clearly establishing a role for one or a group of mechanistic pathways underlying O_3 health

effects observed in epidemiologic studies. Most of these mechanisms of action were predicted based on animal toxicology studies, with some support from human exposure studies.

In this section, the new scientific information reviewed on animal toxicology studies (Chapter 5) and human exposure studies (Chapter 6) are used to evaluate plausible biological bases for the health effects observed in epidemiological (Chapter 7) studies. The interpretations provided in this section are, in the majority of situations, based on theoretical extrapolations, while being mindful (based on our understanding in the post-genome era) of the existence of common biochemical and molecular pathways that operate or function across different species. In order to help interpret health endpoints (hospital admissions, mortality, and disease exacerbations) purported to be associated epidemiologically with either acute or long-term ambient exposure to O_3, this section is organized into two subsections, based on the physiological observations presented in the first section of (a) O_3 effects on pulmonary function as supported by cellular and molecular biological observations discussed in the second section, and (b) O_3-related lung injury, inflammation and host defense effects.

As exposure to O_3 progresses, lung injury and inflammation begin to develop and initiate cellular and subcellular changes. Airway hyperreactivity develops more slowly than pulmonary function effects, while inflammation develops even more slowly and reaches its maximum 3 to 6 h postexposure. Cellular responses, such as release of inflammatory mediators or cytokines, appear to be active as late as 20-h postexposure (Jörres et al., 2000). Although the following discussion is divided into two subsection, there may be cross-references between the sections to better establish meaningful biological plausibility, as physiological and biochemical changes overlap. Each subsection summarizes pertinent key information and then arrives at conclusions as to the plausibility of effects attributable to O_3 exposure.

8.4.10.1 Pulmonary Function

Ozone-induced critical respiratory functional deficiencies were monitored by measuring changes in pulmonary function. Studies detailing alterations in pulmonary function discussed in Chapters 5, 6, and 7 from animal, human toxicology, and epidemiology studies are summarized in Sections 8.2.2, 8.2.3, and 8.2.7.4. Evaluation of pulmonary function on acute O_3 exposure in animals show a positive association with increased breathing frequency, decreased tidal volume (rapid and shallow breathing), increased resistance, and altered breathing mechanics (compliance

and resistance). A similar increased breathing frequency observed in human subjects suggests modulation of ventilatory control on acute O_3 exposure. Direct or indirect stimulation of lung receptors and bronchial C-fibers by O_3 and/or its oxidative products have been implicated in modulating breathing pattern changes. Acute O_3-induced biochemical changes suggest potential interactions of O_3 with the extracellular lining fluid and the generation of lipid ozonation products and reactive oxygen species, ultimately leading to lung injury and/or inflammation. These reactive species cause inhibition or decrease in the maximal inspiration capacity by neurogenic mechanisms acting via the C-fiber afferents. Recent work by Passannante et al. (1998) implicates stimulation of nociceptive receptors on bronchial C-fibers as the primary mechanism for O_3-induced inhibition of inspiration. Alternately, inhibition in maximal inspiration may also be mediated by mediators such as prostaglandin E2 released due to lung epithelial injury. This hypothesis gains strength from the observation of the blocking of spirometric response in human subjects who were pretreated with nonsteroidal anti-inflammatory agents such as indomethacin and ibuprofen. While recovery from pulmonary function decline and airway hyperreactivity had been found to be rapid (4 to 6 h) in moderately responsive individuals, persistent small residual lung function effects were found to take more than 48 h to return to baseline values in hyperresponsive individuals (Nightingale et al., 2000). Such an extended recovery from lung function decline, airway hyperresponsiveness, and increased O_3-induced pulmonary function decline in asthmatics (Scannell et al., 1996) compared to normal subjects may be responsible for the increased emergency room visits or hospital admission and the increased use of asthma medication in asthmatics reported in recent time-series epidemiological studies. The contribution of morphological alterations in the decline of lung function on chronic exposure is not known.

Airway hyperresponsiveness (AHR) due to O_3 exposure is another important factor involved in the observed decline in pulmonary function. Intermittent airway obstruction and increased airway responsiveness to physical or chemical stimuli is also the hallmark of asthma. Asthma-related AHR includes both physiological and morphological components such as inner-wall thickening and mucus secretion. Airway hyperresponsiveness in response to chemical challenge is found to be predominant in children compared to adults and older children. Several controlled human O_3 exposure studies reported increased airway responsiveness at baseline both in normal and asthmatic subjects. The mechanisms mediating AHR are not yet

fully understood, but it appears to be mediated by multiple pathways. Involvement of AHR in pulmonary function decline appears to be mediated by neurogenic mechanisms, as pretreatment of neonatal rats with capsaicin prevented O_3-induced release of neuropetides, suggesting a role for C-fibers in AHR. Significant reduction in the immunoreactivity for substance P in the bronchial biopsies from human subjects 6 h postexposure and its negative correlation with FEV_1 decrements suggests involvement of similar neurogenic mechanisms to be persistent in O_3-induced bronchoconstriction (Krishna et al., 1997). Studies carried out in human subjects using cyclooxygenase inhibitors to block the influx of PMNs and the inhibition of neutrophilic inflammation by probenecid in dogs (Freed et al., 1999) indicated that O_3-induced inflammation and AHR occur as two independent events. Many of the inflammatory mediators that exhibit bronchoconstrictive properties may also play a significant role in the persistent spirometry changes observed on exposure to O_3 (Blomberg et al., 1999). Either the inflammation or AHR may be the underlying biological mechanism responsible for the observed lung function decline in children and male human subjects reported in epidemiological studies.

Repeated-exposure studies in monkeys with a house dust mite antigen-sensitization regimen (Schelegle et al., 2003; Chen et al., 2003) associated lung function changes to the adaptation of respiratory motor responses. Similarly, controlled human exposure studies using asthmatic subjects exposed to house dust mite indicated an immediate increase in airway-antigen reactivity that persisted longer (18 to 20 h) in asthmatics than in normal subjects (Kehrl et al., 1999). The enhanced decline in pulmonary function in subjects with allergic rhinitis (Jörres et al., 1996) suggests slow recovery from O_3-induced pulmonary function declines. These observations suggest that O_3 exposure, therefore, may be a clinically important cofactor in the response to airborne bronchoconstrictor substances in individuals with preexisting allergic asthma. This phenomenon could plausibly contribute to increased symptom exacerbations and, even increased consequent physician or ER visits and possible hospital admissions. However, even a small decline in lung function and its persistence in sensitive populations such as asthmatics will have substantial effects and can lead to increased frequencies in emergency room hospital visits or in medication use.

8.4.10.2 Lung Injury, Inflammation, and Host Defense

An extensive biochemical database collected from animal toxicology studies using varied species indicates that the interaction of O_3 with the ELF in the lung leads to the generation of a series of reactive radical species, including lipid ozonation products and hydroperoxy radicals, which contribute to the increased injury and inflammatory response. Using in vitro and ex vivo studies on isolated ELF, it has been increasingly recognized that ozonation of polyunsaturated fatty acids (PUFA) and its distribution profiles in the respiratory tract plays a critical role in the extent of site-specific injury (Postlewait et.al., 1998; Connor et al., 2004). Ozone-induced lung injury was also found to cause persistent fibrotic changes with the accumulation of collagen and the thickening of CAR postexposure, suggesting progressive structural changes in the lung tissue. Bronchial mucosal biopsies after repeated O_3 exposure over 5 days in human subjects indicated that inflammation of the bronchial mucosa persisted after repeated O_3 exposure, despite attenuation of some inflammatory markers in BALF and attenuation of lung function responses and symptoms. Along with this, the persistent, although small, decrease in baseline FEV_1 observed by Jörres et al. (2000) suggested a difference in timescales among the functional responses to O_3. Elevated protein levels remaining after repeated exposures confirm the findings of others (Christian et al., 1998; Devlin et al., 1997) and suggest that cellular damage is ongoing irrespective of the attenuation of cellular inflammatory responses. The results of chronic-exposure studies in animal toxicology evaluating fibrotic changes are inconsistent. Simulated seasonal-exposure studies in infant rhesus monkey (0.5 ppm O_3) indicate possible injury-repair processes as observed with chronic exposure studies described in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996).

Lung inflammation is a host response to injury, which in turn triggers various biochemical and physiological responses such as epithelial permeability, PMN influx, and the release of pro- and anti-inflammatory mediators. The inflammatory response is a transient phenomenon and resolves entirely postexposure in all the species studied, including humans. Subchronic exposure to O_3 has been found to induce inflammation after a few days (depending upon species studied and exposure dose), which resolves to a normal state, even with continuing exposure; adaptation on repetitive exposures has also been observed. Biochemical and molecular analysis of BALF from various animal species and human subjects exposed to O_3 clearly suggest the participation of various inflammatory mediators, which in turn can activate multiple cascades of

physiological events. The adaptive response to repetitive exposure to O_3 indicates resolution of many of these inflammatory markers to different extents, suggesting that persistent mild inflammation may compromise the abilities of the lung tissue to handle various host defense functions and allergic responses.

Ozone-induced dysfunction in the barrier function of lung epithelium and subsequent permeability changes also can impact lung host defense functions. Altered mucocilliary and alveolar clearance of inhaled particles or microorganisms observed on acute and subchronic exposure to O_3 in animal toxicology studies indicate compromise in this important host defense function. Similar compensation observed in the in vitro studies using cells recovered in BALF from normal and asthmatic human subjects (Newson et al., 2000; Bosson et al., 2003; Bayram et al., 2002) on acute O_3 exposure suggest an additional burden on the host defense functions.

The new information obtained in the past decade on the morphological, biochemical, cellular, and molecular aspects of O_3 toxicology have increased our understanding of the intricate biochemical and molecular mechanisms involved in lung tissue pathology. Combining basic toxicology approaches with the sophisticated molecular technologies and using various O_3-sensitive and -resistant animal strains in these investigations have provided additional knowledge in understanding the possible biochemical bases for the adverse health effects. Newer studies that examined various inflammatory parameters, such as PMN influx (Stenfors et al., 2002) and molecular changes in the nasal or bronchial biopsies from atopic asthmatics and normal human subjects, indicated significant differences. Nichols et al., (2001) observed a role for oxidative stress in O_3-induced inflammation and increased release of TNF-α from nasal epithelial cells. Increased neurogenic involvement in the O_3-induced inflammatory response was observed by Schierhorn et al. (1999). Taken together, the new science gathered from an extensive animal toxicology database and limited human controlled exposure studies on pulmonary function, lung defense, and biochemistry provide evidence consistent and coherent with health outcomes endpoints observed in human subjects in clinical or field studies. These biological and toxicological observations will gain additional value and support with future research efforts, particularly those using controlled human exposure studies including subjects with preexisting disease. Such research inquiries will aid in reducing the data gaps and in the development of better extrapolation models that can be used in interpreting the health endpoints monitored in epidemiological studies.

8.4.11 Coherence Between Epidemiological and Experimental Evidence for Respiratory Health Effects

Recent epidemiological studies (collected from various metropolitan cities in the United States, Canada, and Europe) reported associations between short-term O_3 exposure for various indices such as respiratory-related mortality, hospital admissions, and emergency department visits for respiratory diseases. Three U.S. multicity studies of 95 communities and 90 cities reported positive associations between acute O_3 exposure and daily mortality. Data from four European multicity studies have also reported similar positive associations to daily mortality on acute O_3 exposure. Several epidemiological individual and multicity studies reported significant O_3 effects on hospital admission. Some of the epidemiological studies from Europe reported a strong relationship between unscheduled hospitalizations for COPD and O_3 exposure. In addition, new evidence exists for O_3 effects on lung function decline/respiratory symptoms from controlled human exposure (exercising) studies and recent exercise panel (field) studies. Recent time-series studies reported excess risk for emergency department visits, particularly in summer seasons with relatively high O_3 concentrations. Epidemiological studies also reported positive associations between the onset of asthma and asthma prevalence due to long-term O_3 exposure.

The respiratory health effects observed in epidemiological studies gain relevance from controlled human exposure studies. The health responses observed in these studies were indicative of deleterious health effects due to exposure to O_3. The studies indicate an acute O_3-induced decline in lung function with inflammatory changes in the lung such as increased levels of infiltrating PMNs, release of inflammatory mediators, lung injury, permeability changes, and altered host defense mechanisms. These observations gain further support from similar biochemical, morphological, and immunological changes in animal toxicology studies that suggest perturbations in lung tissue physiology. This body of evidence provides coherent links between the results of large multicity epidemiological studies reporting increases in hospitalizations and unscheduled emergency department visits with toxicologic evidence of acute O_3-induced lung tissue injury and inflammation in humans and animals.

8.4.12 Summary and Conclusions for Ozone Health Effects

This section discussed the development of a coherent understanding of O_3 health effects through considering the plausibility and coherence of information derived from epidemiological evidence and human and animal toxicology studies. As discussed in Sections 8.4.2 to 8.4.5, considerations related to the epidemiologic evidence alone appear to support likely causality for observed associations between O_3 and health outcomes. This section further described evidence from both epidemiologic and toxicologic studies for health effects that are logically linked together.

Epidemiological studies have reported positive associations between O_3 exposure and health effects across a range of endpoints, from respiratory-related mortality, increased respiratory-related hospital admissions, and emergency department visits, to more subtle effects such as decrements in lung function, lung inflammation, airway responsiveness, and altered mucocilliary clearance. The new toxicologic and physiological evidence suggest links to potential molecular pathways that may provide reasonable explanations for the observed epidemiological findings, but as described earlier, caveats must be considered in interpreting these studies. The new toxicological information confirms the earlier findings and presents important new evidence supporting the plausibility of associations between O_3 and adverse respiratory health effects. While many research questions remain, the convergence of epidemiologic and toxicologic evidence related to respiratory health effects for ambient O_3 exposure argues for coherence and plausibility for this body of evidence.

Controlled human exposure studies have provided new information indicating that age at time of exposure is a major susceptibility factor for observed decrements in lung function. Epidemiological studies and some preliminary supportive data from toxicological studies suggest that asthmatics are a potential sensitive subpopulation although additional scientific evidence is needed. However, little experimental evidence is available by which to judge the plausibility of any chronic O_3 exposure effects observed in epidemiologic studies. Thus, further study is required on the potential toxicologic or pathologic mechanisms that may underly chronic effects of ambient O_3 exposure to relate to observed respiratory health effects in epidemiological studies.

Analysis of the body of toxicologic studies suggests plausible mechanisms for epidemiologic findings. The newly available epidemiological studies on positive associations

between acute exposure to O_3 and a range of health outcomes support the general conclusion that O_3 is causally related to respiratory-related mortality and morbidity. A very limited database (epidemiologic and toxicologic) is available on the long-term effects of O_3 on respiratory-related mortality and morbidity, but given our understanding of the plausible biological mechanisms implicated in acute response and differential recovery, O_3 may also be causally related to long-term respiratory-related health risks. Additional scientific information to support the predicted sensitivity of asthmatics reported in epidemiological studies is still needed. Substantial scientific evidence gathered in the past decade provides additional support for the conclusions stated in the 1996 O_3 AQCD with regard to health effects shown to be associated with ambient O_3 exposure. The recent epidemiological studies provide further strong evidence supporting potential morbidity health risks associated with exposure to ambient O_3. Furthermore, newly available epidemiological data linking acute O_3 exposure to respiratory-related mortality and to the exacerbation of respiratory-related disease symptoms suggest even larger health impacts and costs to society than previously demonstrated, warranting additional research.

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